UNIVERSITY OF BIRMINGHAM

GULLS (LARIDAE) AS BIOINDICATORS OF FLAME RETARDANT EMISSIONS FROM LANDFILL: A SPECIES-ASSEMBLAGE INVESTIGATION

by

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ABSTRACT

The contamination of free-living avifauna via chemical pollution resulting from anthropogenic activity is globally ubiquitous and poses a threat for avian conservation. Waste streams are important reservoirs of several persistent organic pollutants (POPs), including legacy brominated flame retardants (BFRs). However, landfill is often an important foraging substrate for birds such as gulls (Laridae). Some BFRs are known to exert deleterious effects on birds. Given that lipophilic pollutants sequester in the yolk compartment of avian eggs, this tissue can be an important non-invasive biomonitoring matrix. The primary aim of this thesis was to assess whether gulls breeding in proximity to a UK landfill constitute effective bioindicators of BFR emissions, with an additional aim being the identification of a suitable bioindicator species for future biomonitoring purposes. Polybrominated diphenyl ethers (PBDEs) were detected in substantial concentrations (up to 7,000 ng/g lipid weight) in the eggs of five larid taxa of UK / European conservation concern (black-headed gulls *Chroicocephalus ridibundus*, common gulls *Larus canus*, great black-backed gulls *L. marinus*, European herring gulls *L. argentatus* and lesser black-backed gulls *L. fuscus*) breeding in proximity to a municipal solid waste landfill compared to reference conspecifics breeding away from landfill. Mean $\sum_8$ PBDE concentrations in the eggs of landfill-breeding gulls followed lesser black-backed gulls > great black-backed gulls > herring gulls > common gulls > black-headed gulls. The novel brominated flame retardant, DBDPE, was detected in the highest concentrations reported in biota to date globally in the eggs of landfill-breeding great black-backed gulls and herring gulls (up to 8,000 ng/g lw) but was not detected in reference eggs. Given their numerical superiority at the landfill and colonies, the most statistically robust data was obtained for herring gulls. The eggs of landfill-breeding herring gulls exhibited significantly higher burdens of $\sum_8$ PBDEs compared to reference conspecifics ($P = 0.02$). A significant negative relationship between BDE-209 and $\delta^{13}C$ enrichment in eggs indicated that the more terrestrial diets of landfill-breeding herring gulls resulted in them being more exposed to this PBDE congener, formerly widely used in the UK. However, behavioural observations indicated that ingestion of food was unlikely to be the primary pathway of BFR contamination in gulls using landfill, and that dermal contact, respiration and preening may be more important routes of exposure. Notwithstanding the potential conservation implications of herring gull BFR exposure at such sites, this species can be considered an important bioindicator of BFR emissions from municipal solid waste landfill in north-west Europe.

DEDICATION

This thesis is dedicated to my wonderful daughter Grace, who is well on her way to becoming an outstanding scientist.

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ABBREVIATIONS

ASE Accelerated Solvent Extraction

AOP Adverse Outcome Pathway

BDE Brominated Diphenyl Ether

BDE-209 Decabromodiphenyl Ether

BFR Brominated Flame Retardant

BH Black-headed Gull (*Chroicocephalus ridibundus*)

BSEF Bromine Science and Environmental Forum

BTBPE (1,2-bis(2,4,6-tribromophenoxy) ethane

BTO British Trust for Ornithology

CM Common (Mew) Gull (*Larus canus*)

DCM Dichloromethane

DBDPE Decabromodiphylethane

ECHA European Chemicals Agency

EHTBB 2-ethylhexyl-2,3,4,5-tetrabromobenzoate

GB Great Black-Backed Gull (*Larus marinus*)

GC Gas Chromatography

GC-EI/MS Gas Chromatography Electron Ionisation Mass Spectrometry

GFF Glass Fibre Filter

GLHGMP Great Lakes Herring Gull Monitoring Program

GMT Greenwich Mean Time

HBCDD Hexabromocyclododecane

HG European Herring Gull (*Larus argentatus*)

HPLC High Pressure Liquid Chromatography

IAEA International Atomic Energy Agency

ICC Intraclass correlation coefficient

IS Internal Standard

IUCN International Union for Conservation of Nature

LB Lesser Black-Backed Gull (*Larus fuscus*)

LC Liquid Chromatography

LC-MS/MS Liquid Chromatography combined with two mass analysers

LOD Limit of Detection

LOQ Limit of Quantification

lw Lipid Weight

M2 Methionine/alanine/glycine/gelatine

mg Milligrams

MS Mass Spectrometry

MSAG2 Methanesulfonamide/Gelatine

MSW Municipal Solid Waste

NBFR Novel Brominated Flame Retardant

NERC Natural Environment Research Council (UK)

OSPAR Oslo and Paris Conventions for the Protection of the Marine Environment of the North-East Atlantic

PAS Passive Air Sampler

PBB Pentabromobenzene

PBDE Polybrominated Diphenyl Ether

PBEB Pentabromoethylbenzene

PLE Pressurised Liquid Extraction

POP Persistent Organic Pollutant

PUF Polyurethane Foam

QA/QC Quality Assurance / Quality Control

RDS Recovery Determination Standard

SAAG2 Sulfanilamide/alanine/gelatine

SD Standard Deviation

SEPA Scottish Environment Protection Agency

SIA Stable Isotope Analysis

SNH Scottish Natural Heritage

SPE Solid Phase Extraction

TBBPA Tetrabromobisphenol A

ww Wet Weight

U.S. EPA United States Environmental Protection Agency

CHAPTER I

GENERAL INTRODUCTION†

Accidental fires lead to human mortality, injury and property damage and can be financially costly for individuals and municipalities (Guerra and Barcelo, 2010)†. In England (UK), during the period April 2014 to March 2015, for example, there was a total of 258 human fatalities and 7,546 non-fatal human casualties due to fire, 63 % of which related to accidents in the home and other dwellings (UK Department of Communities and Local Government, 2015). Use of flame retardants can be traced back to antiquity, with the first early attempts to reduce the combustibility of natural cellulosic materials, such as cotton and wood. The Greek historian Herodotus noted that Egyptians in approximately 450 BC were imparting some fire retardance to wood by soaking it in alum (potassium aluminium sulphate). Later, the Romans in approximately 200 BC also used alum, mixed with vinegar, for the same purpose (Hindersinn, 1990). The dramatic increase in the global production and use of polymeric items, coupled with increasingly rigorous health and safety considerations, has led to the establishment of a multi-billion US dollar international flame-retardant chemical industry, with over 175 chemicals classed as flame retardants (Alaee, 2003; Besis and Samara, 2012; Darnerud, 2003). Four classes of flame retardant are generally recognised, comprising i. inorganic compounds (e.g., aluminium trihydroxide), ii. nitrogen-based compounds (e.g., melamine), iii. organophosphorus products (mainly phosphate esters), and iv. halogenated compounds based on bromine or chlorine (Van Esch, 1997).

Brominated flame retardants (BFRs) are among the most widely used halogenated flame retardants and have been incorporated into many products, including circuit boards, wire and cable

† This chapter contains some material that is taken from Tongue et al. (2019).

insulation, electronic and electrical goods, as well as textiles, soft furnishings, building materials and paper and wood-based products (De Wit, 2002; Jenssen et al., 2007; Stubbings and Harrad, 2014; Wu et al., 2008). The most widely employed BFRs in recent decades are detailed in Table 1.1.

Table 1.1 Most recent (as of 2001) industry-published figures for global brominated flame retardant total market demand (in metric tonnes) by region. (Source: BSEF, 2003) .

BFR	Americas	Europe	Asia	Rest of World	World total
TBBPA	18,000	11,600	89,400	600	119,700
HBCDD	2,800	9,500	3,900	500	16,700
Deca–BDE	24,500	7,600	23,000	1,050	56,100
Penta–BDE	7,100	150	150	100	7,500
Octa–BDE	1,500	610	1,500	180	3,790
Total PBDEs	33,100	8,360	24,650	1,330	67,440
Total BFRs	53,900	29,460	117,950	2,430	203,740

They comprise tetrabromobisphenol-A (TBBPA), hexabromocyclododecane (HBCDD) and three technical mixtures of polybrominated diphenyl ethers (PBDEs): *penta*-BDE, *octa*-BDE and *deca*-BDE (Alaee, 2003; BSEF, 2003; Leonards et al., 2007; Stubbings and Harrad, 2014). Despite their attractive properties to industry, environmental concerns over PBDEs emerged in the late 1990s followed by HBCDD in the mid-2000s. These concerns centered around the toxicity of these chemicals, their resistance to degradation, capacity for long-range atmospheric transport and bioaccumulative properties (Chen and Hale, 2010; Darnerud, 2003; De Wit, 2002; Law et al., 2003).

The lower brominated *tri*- to *octa*-BDEs were designated as persistent organic pollutants (POPs) by the United Nations Environment Programme (UNEP) under the Stockholm Convention in 2009 (ECHA, 2015; UNEP, 2009). They have been banned in the European Union (EU) since 2004, at which time in the United States (US), producers agreed to a voluntary phase-out (La Guardia et al.,

2006; Renner, 2004). HBCDD was listed as a POP in November 2014 (Stockholm Convention Secretariat, 2017), with manufacturers discontinuing its production shortly thereafter (Edser, 2015). The fully brominated *deca*-BDE (composed predominantly of the PBDE congener BDE-209) undergoes debromination and degradation during waste treatment and disposal and via metabolism in organisms (Huwe and Smith, 2007; Stubbings and Harrad, 2014). BDE-209 has been banned in the EU since 2008 (European Court of Justice, 2008). In the US, producers of *deca*-BDE ceased production, importation and sale for all uses domestically in 2013 (United States Environmental Protection Agency, 2010). Deca-BDE was listed under the Stockholm Convention in May 2017. PBDEs and HBCDD have acquired the prefix of 'legacy' BFRs, although they are yet to be phased out in certain jurisdictions. For example, Shi et al. (2018) reported that *deca*-BDE and HBCDD were still produced in China.

One important group of flame retardants (FRs) that have entered widespread use in response to these developments, is the 'novel brominated flame retardants' (NBFRs). These chemicals are attractive in that they retain many of the superior fire retardant qualities of legacy BFRs but are ostensibly less toxic, bioavailable or persisent. Harju et al. (2009) estimated that approximately 100,000 metric tonnes per year of 21 different NBFRs were produced globally. However, despite recent advances in our understanding, several authors have identified knowledge gaps in terms of the impacts of NBFRs on biota (Covaci et al., 2011; Ezechiáš et al., 2014).

Most FRs are applied to products via one of two methods: 'additive', where the commercial FR mixture is blended with the molten polymer, and 'reactive' where FRs are covalently-bound. PBDEs, HBCDD and NBFRs fall into the former category (Stubbings and Harrad, 2014). Additive FRs have been demonstrated to leach into the environment from the products containing them (Hutzinger and Thoma, 1987). Examples of reactive FRs include TBBPA (not investigated in this thesis).

1.1 Birds as bioindicators

The contamination of free-living avifauna by chemical pollution resulting from anthropogenic activity is globally ubiquitous (Brown et al., 1997; Chen and Hale, 2010; Giesy et al., 2003; Peck et al., 2016). Birds are among the more vulnerable animal classes in terms of the general toxicity of POPs (Fernie et al., 2017; Guigueno and Fernie, 2017). Compared to mammals, for example, birds possess generally less effective detoxification mechanisms for many pollutants, including organohalogens (Marteinson et al., 2011a; Newton, 1986). As a result, birds are important bioindicators of wider environmental pollution (Furness and Greenwood, 1993; LeBlanc and Bain, 1997; O'Brien et al., 1993). Being highly lipophilic, organohalogens sequester in the yolk compartment of eggs and therefore the analysis of egg contents is a relatively convenient and non-invasive method of exposure assessment and environmental monitoring (Chen and Hale, 2010; Furness and Greenwood, 1993; Guigueno and Fernie, 2017). Several long-running egg-sampling ('specimen-banking') studies exist globally. Examples include the North American Great Lakes Herring Gull Monitoring Program (Hebert, 1999), the UK's Predatory Bird Monitoring Scheme (Walker et al., 2008) and the German Environmental Specimen Bank (Koschorreck et al., 2015; Paulus, 2010). More recently (i.e., between 2010 and 2015), European raptor sampling was coordinated via the EURAPMON initiative (Gómez-Ramírez et al., 2014).

The impacts of organohalogens on birds became widely publicised in Europe and North America in the mid-20th Century when it was demonstrated that chlorinated hydrocarbon pesticides, most notably dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE), had multiple negative effects on bird-eating raptors via trophic biomagnification, especially that of eggshell thinning, which significantly reduced their breeding performance through dramatic reductions in hatching success. Raptors were also shown to be at risk of poisoning via the bioaccumulation of cyclodiene pesticides (i.e., aldrin, dieldrin and heptachlor epoxide) in their prey (Newton and Bogan, 1974; Ratcliffe, 1967). The toxic effects of

BFRs on birds have been demonstrated via investigations of various endpoints, including reproduction, behaviour and growth in free-living and captive avian subjects (reviewed by Guigueno and Fernie, 2017).

Waste streams constitute an important reservoir of legacy BFRs given that they are repositories of obsolete goods treated with these formerly widely used chemicals (Danon-Schaffer et al., 2013; Eguchi et al., 2013b; Gavilan-Garcia, 2017). The presence of NBFRs in waste streams has also been reported (McGrath et al., 2017a; Nyholm et al., 2013; Olukunle and Okonkwo, 2015). In this thesis, 'landfill' is defined as those sites designated for the disposal of domestic and commercial waste and serving a municipality (i.e., municipal solid waste [MSW]). As a result of the quantities of human food refuse that it may receive, landfill is often an important resource for birds (Oro et al., 2013; Plaza and Lambertucci, 2017). To date, limited attention has been paid by toxicologists, ornithologists and wildlife conservationists to landfill as a potentially important source of organohalogen contamination in birds. Figure 1.1 depicts gulls at a landfill facility in the UK.

Figure 1.1 European herring gulls and lesser black-backed gulls at a municipal solid waste landfill facility in the UK (C. Honan).

1.2 Landfill as a source of environmental BFR contamination

Among the routes by which anthropogenic chemicals enter the environment, waste disposal ranks amongst the most important (de Lapuente et al., 2014; Eggen et al., 2010; Masoner, 2015; Teuten et al., 2009; Weber et al., 2011). Landfill has been identified as a source of abiotic environmental FR contamination on various continents, including Africa (Daso et al., 2017; Odusanya et al., 2009), Asia (Eguchi et al., 2013b; Hafeez et al., 2016; Ilyas, 2010; Kwan et al., 2013; Osako et al., 2004), Australasia (Gallen et al., 2016), Europe (Morin et al., 2017; Morris et al., 2004) and North America (Gavilan-Garcia, 2017). McGrath et al. (2017b) compared landfill *vs.* reference soils (i.e., from sites located away from landfill) in five Asian countries, reporting a significantly higher level of PBDE soil contamination at landfill sites. In Vietnam, mean concentrations of $\sum_{14}$ PBDEs in landfill were 95 ng/g compared to 0.22 ng/g at a reference site (Eguchi et al., 2013b). The routes by which BFRs transfer from waste products to the environment were reviewed by Stubbings and Harrad (2014). Two main pathways identified were emissions to air and leaching to groundwater, with both facilitated by abrasion of polymeric waste (releasing particles to air and leachate), and photolytic and/or biodegradation of less volatile and water-soluble high molecular weight FRs (such as BDE-209) to lower brominated products. There are apparently no guidelines at present on minimum soil / air BFR concentrations impacting on human or wildlife health.

Landfill is the most common method of waste disposal worldwide, despite reforms aimed at eliminating it in some jurisdictions such as the EU (European Union, 1999) on account of its atmospheric emissions. Landfill receives annually approximately 59 % of total global MSW (The World Bank, 2012). Global solid waste generation is anticipated to peak later this century (Eggen et al., 2010; European Union, 1999; Hoornweg, 2013). Alcock et al. (2003) estimated that over 80 % of total BFR-containing waste in the UK and North America enters landfill, with the remainder incinerated. Danon-Schaffer et al. (2013) predicted that even an immediate ban on BFR-treated products entering landfill would not result in a decline in landfill PBDE concentrations until 2080.

1.3 Bird utilisation of landfill

The United States Environmental Protection Agency (US EPA) estimated that domestic food waste in 2010 comprised 13.9 % (31.5 million tonnes) of total MSW (United States Environmental Protection Agency, 2011). Parfitt et al. (2010) suggested that 30–40 % of global food designated for human consumption is wasted, with the UK, for example, disposing of 14 million tonnes of food and drink annually. Such waste is regularly available in space and time, thereby constituting a predictable anthropogenic food source for wildlife, including birds (Oka, 2016; Oro et al., 2013; Osterback et al., 2015; Plaza and Lambertucci, 2017). The enclosed fringes of some landfill facilities may also contain relatively undisturbed terrestrial and aquatic habitats that are used as foraging and breeding sites for various avian taxa (Mehra, 2017; Tuljapurkar and Bhagwat, 2007).

The bird species community found on landfill is diverse (Appendix 1) and includes those that forage directly on anthropogenic waste (e.g., gulls), those that utilise aquatic and vegetated habitats within landfill sites (e.g., wildfowl, shorebirds, songbirds), plus those that may target landfill as a source of live prey (e.g., raptors). Eighteen of the species listed in Appendix 1 are designated by the International Union for Conservation of Nature (IUCN) as being of conservation concern. Aside from the foraging opportunities that it affords birds, landfill sites may be important for some groups such as gulls for loafing (i.e., resting or preening). Herring gulls (*Larus argentatus*) have been found to spend considerable proportions of their daily time budgets (i.e., >60 %) loafing at landfill sites (Belant, 1993; Patton, 1988). While some authorities have recently divided this species into the European herring gull *vs.* the American herring gull, in this thesis the general term 'herring gull' refers to either taxon unless specified. Landfill can also be important for gull social interactions, including heterosexual activity (Belant et al., 1993).

The consumption of anthropogenic waste by birds is associated with both costs and benefits for individuals and populations. For example, consuming refuse may correlate with elevated body condition (Steigerwald et al., 2015), breeding performance (Djerdali et al., 2016; Pons, 1992; Weiser

and Powell, 2010) and population size (Bosch, 1994; Mazumdar et al., 2016; Rock, 2005; Smith and Carlile, 1993). The year-round presence of landfill-provided food has been linked with reduced migratory demands for some species, such as the white stork (*Ciconia ciconia*) (Arizaga et al., 2018; Gilbert et al., 2016) and the lesser black-backed gull (*Larus fuscus*) (Barnes, 1961) in Europe. Species of conservation concern, such as various raptors, may benefit from the regular availability of such food (Jaksic, 2001).

However, other studies report negative impacts of foraging at landfill and in other terrestrial anthropogenic environments, such as urban centres. These include reduced breeding performance (Belant, 1998; Katzenberger et al., 2017; O'Hanlon et al., 2017; Pierotti and Annett, 1991; Real et al., 2017) and impaired fitness (Annett and Pierotti, 1989). Stable isotope analysis (SIA) of feathers taken from museum skins by Hobson et al. (2015) suggested that glaucous-winged gulls (*Larus glaucescens*) breeding at the Salish Sea (Canada and US) are increasingly reliant on human refuse and terrestrial invertebrates, instead of marine items. This has correlated with declines in egg volume and clutch size in this species since the early 20th Century (Blight et al., 2015). In addition, birds associating in large aggregations at anthropogenic waste facilities may be at heightened risk of pathogen exposure (Elliott et al., 2006b; Macdonald and Standring, 1978; Nicastro et al., 2018; Ortiz, 1994).

1.4 BFR contamination in bird populations associated with landfill

Fourteen peer-reviewed studies to date have reported BFR concentrations in bird populations associated with landfill (Table 1.2). These are summarised below. They are covered in order of publication date, except for the body of research relating to gulls and starlings, which is grouped separately (ordered by publication date). To aid comparison between studies, concentrations are expressed in wet weight (ng/g ww), unless otherwise stated. Further discussion of these studies are made in Tongue et al. (2019), which also covers those studies reporting concentrations of non-BFR flame retardants (i.e., chlorinated and non-halogenated organophosphate triester flame retardants) in

landfill-associated avifauna. Table 1.3 compares PBDE burdens in landfill and reference birds of the same species, showing substantial PBDE increments for landfill-associated individuals.

Table 1.2 Studies in which BFR tissue concentrations were measured in avian populations associated with landfill in various countries. Entries are listed by advancing date of publication of the study.

Species	**Sampling period**	**Location**	**Tissues sampled (*n* = sample size)**	**Types of flame retardants detected**	**Reference**
African sacred ibis (*Threskiornis aethiopicus*)	2004-2005	South Africa	Egg (*n* = 2)	PBDEs, HBCDDs	(Polder et al., 2008)
White stork (*Ciconia ciconia*)	1999-2000	Spain	Egg (*n*= 33)	NBFRs	(Munoz-Arnanz et al., 2010)
	1999-2000	Spain	Egg (*n*= 33)	PBDEs	(Muñoz-Arnanz et al., 2011)
Ring-billed gull (*Larus delawarensis*)	2010	Canada	Liver(*n*=28); plasma (*n* = 30)	PBDEs, NBFRs	(Gentes et al., 2012)
Herring gull (*L. argentatus*)	2008	Canada	Egg(*n*=~200)	PBDEs	(Chen et al., 2012)
Yellow-legged gull (*L. michahellis*)	2010	Spain	Egg (*n* = 36)	PBDEs	(Morales et al., 2012)
Common starling (*Sturnus vulgaris*)	2009-2011	Canada	Egg (*n*= 259)	PBDEs, NBFRs	(Chen et al., 2013)
	2009-2010	Canada	Egg (*n*= 150)	PBDEs	(Eens et al., 2013)
Ring-billed gull	2010–12	Canada	Plasma(*n*=76)	PBDEs	(Gentes et al., 2015)
Common starling	2012	Canada	Liver microsomes (*n* = 7)	PBDEs	(Erratico et al., 2015)

Species	**Sampling period**	**Location**	**Tissues sampled (*n* = sample size)**	**Types of flame retardants detected**	**Reference**
Black kite (*Milvus migrans*)	2012–2014	Pakistan	Tail feather (*n* = 8)	PBDEs, HBCDD, BTBPE	(Abbasi et al., 2016a)
Yellow-legged gull	2007	Spain	Egg (n = 18)	PBDEs	(Roscales et al., 2016)
Cinereous vulture (*Aegypius monachus*)	2016	Spain	Nestling down (*n* = 16)	PBDEs	(Monclús et al., 2018)
Black kite	2007–2016	Spain	Egg (*n* = 51)	PBDEs	(Blanco et al., 2018)

Table 1.3 A comparison of PBDE burdens reported in landfill-associated birds with reference (i.e., non-landfill-associated) conspecifics in the literature. All concentrations are in ng/g ww, except for Blanco et al., 2018 (ng/g lw).

Species	**Sampling period**	**Tissue**	**Landfill ∑PBDEs (*n* = sample size)**	**Reference ∑PBDEs (*n* =sample size)**	**Source**
White stork	1999–2000	Egg	2.79–20.5 (*n* = 10) §	0.214–9.50 (*n* = 23) §	(Muñoz-Arnanz et al., 2011)
Common starling	2009–2011	Egg	11–805 (*n* =68) §	12–15 (*n*=11) §	(Chen et al., 2013)
Ring-billed gull	2010–2012	Plasma	43.3 ± 10.6 †	36.9 ± 5.7 †	(Gentes et al., 2015)
Black kite	2007–2016	Egg	1,570 (*n* = 28) ‡	13.6 (*n* = 23) ‡	(Blanco et al., 2018)

Entries are listed by advancing date of publication § Range † Mean ‡ Maximum

In addition to the studies identified, it should be noted that a broad suite of FRs has been found in the eggs of herring gulls in the North American Great Lakes Basin (Hebert, 1999) and in Germany (Koschorreck et al., 2015), as well as in Arctic-breeding glaucous gulls (*Larus hyperboreus*) (Verreault et al., 2018). These authors have suggested that use of landfill is one possible source of such contaminants in these birds.

The first study to make an explicit connection between elevated avian FR burdens and an association with landfill was undertaken by Polder et al. (2008). In that study, PBDE and HBCDD concentrations were reported in the eggs of eight species, albeit with several low sample sizes (*n* = 1–20). Σ_8PBDEs and total (i.e., α-, β- and γ-) HBCDD concentrations were highest in African sacred ibis (*Threskiornis aethiopicus*) eggs (*n* = 2) (ranges of 4.03–19.8 ng/g and 0.31– 3.5 ng/g ww, respectively). The authors suggested that elevated BFR concentrations in this species likely resulted

from the birds supplementing their natural diet of fish, amphibians and invertebrates with anthropogenic waste obtained from landfill.

Muñoz-Arnanz et al. (2011) collected addled (failed) eggs of white storks to investigate PBDE profiles and burdens in two contrasting Spanish colonies: one located in urban-industrial Madrid (*n* = 10) where birds fed predominantly on landfill, the other in the rural Coto-Doñana National Park (*n* = 23), where birds had a more natural diet consisting of crayfish (*Decapoda* spp.), terrestrial invertebrates, earthworms (Lumbricidae) and amphibians. Eggs laid by urban breeders had approximately six times higher mean $\sum_{28}$PBDE concentrations (mean: 9.08, range: 2.79–20.5 ng/g ww) than rural eggs (mean: 1.64, range: 0.214–9.50 ng/g ww). Muñoz-Arnanz et al. (2011) measured BDE-202 concentrations in all urban eggs and in approximately 80 % of rural eggs. Since this congener has not been reported in any commercial PBDE mixture, its detection was likely either due to debromination in the environment and subsequent uptake, or as a metabolic product of higher brominated BDE congeners (i.e., BDE-209), as demonstrated in captive American kestrels (*Falco sparverius*) exposed to BDE-209 (Letcher et al., 2014).

It is well documented that ring-billed gulls (*Larus delawarensis*) in North America utilise urban-industrial environments, including landfill and wastewater treatment plants. Use of such facilities by this species near Montreal (Canada) may explain the elevated FR concentrations in birds breeding at a major colony near that city (Ille Deslauriers). Patenaude-Monette (2014) demonstrated the importance of waste as a food source for this colony, with over 40 % of the birds' diet comprised of anthropogenic refuse. Gentes et al. (2012) reported mean $\sum_{45}$PBDEs in livers of these birds of 205 ± 32 ng/g ww; range: 22.4–682 ng/g ww. In a pan-Canadian study of FRs in the eggs of gulls breeding at 26 colonies, Chen et al. (2012) found that the highest BFR burdens were in herring gulls and ring-billed gulls breeding at Ille Deslauriers (median $\sum_{16}$PBDE concentrations of 610 and 144 ng/g ww, respectively). Using GPS telemetry, Gentes et al. (2015) found that ring billed gulls that visited waste management facilities also exhibited a smaller relative plasma percentage of the lower brominated

penta-BDE congeners (i.e., the sum of BDEs -47, -99 and -100) associated in studies of waterbirds with a higher trophic level diet (e.g., Roscales et al., 2016). Ring-billed gulls breeding at Ille Deslauriers exhibited mean Total-HBCDD liver concentrations of 5.22 ± 1.02 ng/g ww; n = 28) (Gentes et al., 2012). Gentes et al. (2015) also demonstrated that only brief visits (<5 % of time away from this colony, lasting approximately 40 minutes) by gulls to landfill and wastewater treatment plants resulted in the uptake of significant concentrations of *deca*-BDE. Conversely, *deca*-BDE concentrations were not related to the percentage of time gulls spent in agricultural fields, urban areas and on the St Lawrence River. Male ring-billed gulls that visited waste management sites contained a greater percentage of BDE-209 in plasma relative to those that did not (38 ± 5 % *vs.* 19 ± 1 %) (Gentes et al., 2015). Among the NBFRs detected in the livers of Ille Deslauriers-breeding ring-billed gulls, Gentes et al. (2012) reported what is believed to be the highest detection frequency (89 % of samples) and highest concentration (max: 17.6 ng/g ww) of bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (BEHTBP), found in free-living birds to date.

Organohalogen burdens are generally understood to be lower in female birds compared to males, likely due to a proportion of contaminant loads being deposited in eggs (Burger, 2007). In landfill-foraging birds, such differences may also be influenced by competition between males and females. For example, in direct competition for food items obtained during terrestrial foraging on landfill, male herring gulls competitively exclude females (Greig et al., 1985; Monaghan, 1980). This may result in males having higher FR burdens. Gentes et al. (2015) found that male ring-billed gulls exhibited greater ∑PBDE plasma concentrations (38.5 ± 5.0 ng/g ww) than females (24.4 ± 3.2 ng/g ww), as well as higher relative *deca*-BDE concentrations, despite GPS telemetry showing that both sexes spent similar amounts of time on landfill (Gentes et al., 2015).

Several Mediterranean-based studies (e.g., Bertolero et al., 2015; Morales et al., 2012; Pastor et al., 1995; Roscales et al., 2016; Zapata et al., 2018) have compared the burdens and profiles of various contaminants, including FRs, in the eggs of the marine piscivore, Audouin's gull (*Ichthyaetus*

audouinii), with those of the generalist (and landfill-associated) yellow-legged gull (*Larus michahellis*). The two species regularly occur in mixed colonies (González-Solís et al., 1997). Morales et al. (2012) found a predominance of BDE-209 in both species breeding in the industrial Ebro Delta (north-east Spain). However, in the south-west Mediterranean Sea, Roscales et al. (2016) reported that the higher trophic level diet of Audouin's gulls was the likely source of a greater percentage of lower brominated BDE congeners (i.e., *tetra*- and *penta*- BDEs) in eggs compared to yellow-legged gulls. Conversely, the latter species laid eggs containing higher percentages of higher brominated congeners (i.e., *hepta*-, *octa*- and *deca*- BDEs). Sardines (*Sardina pilchardus*) are an important prey species for Audouin's gulls (Witt et al., 1981). Parera et al. (2013) reported levels of ∑PBDEs of 5.49 ng/g lipid weight (lw) in sardines, with lower brominated congeners (BDEs -28, -47 and -100) predominating. Fishes appear to be an important dietary source of more bioavailable lower brominated PBDE congeners in both marine and freshwater piscivorous avifauna: elevated BDE burdens (>1000 ng/g ww) were reported in the eggs of ospreys (*Pandion haliaeetus*), an obligate piscivorous raptor, in Oregon and Washington State, US (Henny et al., 2009). As was the case for Audouin's gulls, lower brominated BDE congeners predominated in osprey eggs, with BDE-47 being the dominant congener, followed by BDEs -100, -99, -154 / BB153, -153 and -28.

Common starlings (*Sturnus vulgaris*) breeding at Canadian landfill sites showed orders of magnitude higher ∑PBDE egg concentrations (range: 11–805 ng/g ww) compared to those breeding at adjacent urban centres (range: 4.9–146 ng/g ww), and agricultural locations 10 km and 40 km distant (ranges: 1.9–488 and 2–375 ng/g ww, respectively) (Chen et al., 2013). Starlings routinely forage at landfill sites (Belant et al., 1995; Burger and Gochfeld, 1983b). Chen et al. (2013) reported maximum egg PBDE concentrations laid by landfill-associated starlings that were among the highest reported in free-living passerines and comparable to egg concentrations in some terrestrial raptors (e.g., 616 ng/g lw in liver of Eurasian sparrowhawk (*Accipiter nisus*) in Belgium; Voorspoels et al., 2007). Chen et al. (2013) reported that the median Σ_{14}PBDE egg concentrations of starlings breeding

on landfill and the human population density of the surrounding area were significantly positively correlated (r = 0.99, P < 0.001, n = 7). BEHTBP was also found in the eggs of landfill-breeding starlings (maximum concentration: 26 ng/g ww). In contrast to most other studies described in this chapter, Chen et al. (2013) found that BDE-209 accounted for a small proportion (1–15 %) of ΣPBDE concentrations in starling eggs, with no relationships observed with land use. It should be noted that Van den Steen et al. (2007) concluded that BDE-209 in blood and tissues of captive common starlings was either eliminated or debrominated to lower brominated BDE congeners.

Other studies report elevated BFR concentrations in free-living common starling populations associated with landfill. Comparing PBDE (and other contaminant) egg burdens of common and / or spotless starlings (*Sturnus unicolor*) in 13 countries across three continents, (Eens et al., 2013) found common starling eggs (n = 10) at a municipal landfill in Vancouver, BC, Canada to contain the highest $\sum_7$PBDE concentrations (4400 ± 830 ng/g lw), compared to the lowest of 3.7 ± 1.1 ng/g lw in spotless starling eggs (n = 10) at a rural location in Spain. BDE-99 accounted for 55 % of ∑PBDEs in eggs from the Vancouver landfill, likely due to its presence in the *penta*-BDE commercial formulation, which has been used more extensively in North America than elsewhere (BSEF, 2003; Table 1.1). At the same Vancouver landfill, starling nestlings exhibited $\sum_6$PBDE plasma concentrations approximately 60 times in excess of nestling plasma from a rural reference site 40 km from the city (Erratico et al., 2015).

Black kites (*Milvus migrans*) forage on landfill in various countries, including India (Mazumdar et al., 2016), Italy (De Giacomo, 2008), Spain (Blanco et al., 2018), Turkey (Biricik and Karakaş, 2011) and Uganda (Pomeroy, 1975). Their generalist diet and close association with urban centres was suggested to be the likely reasons for their elevated FR concentrations (n = 8) compared with those of nine other avian taxa in Pakistan (Abbasi et al., 2016a). Black kites had median tail feather $\sum_7$PBDE concentrations of 2.4 (range: 0.70–7.50) ng/g dry weight (dw) and Total-HBCDD concentrations of 1.5 (range: 0.5–8.1) ng/g dw. Abbasi et al. (2016) found that this was the only

species (out of a total of 10 sampled) in which concentrations of the NBFR, 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) were quantifiable, albeit at low levels (median: 0.10 (range: <LOQ-0.1) ng/g dw; BTBPE LOQ 0.02 ng/g dw). Blanco et al. (2018) measured concentrations of PBDEs in addled eggs of black kites breeding in urban Madrid (n = 28) and at Coto-Doñana (n = 23). Madrid-breeding kites are known to forage on landfill, whereas those breeding at the Coto-Doñana have a more natural diet which includes waterbirds (Blanco et al., 2018). Black kite eggs from Madrid contained significantly higher PBDE concentrations than those from Doñana (maximum ∑PBDEs: 1570 ng/g lw *vs.* 13.6 ng/g lw, respectively). Monclús et al. (2018) reported median $\sum_7$PBDE concentrations of 0.51 ng/g dw (n = 16) in the down feathers of nestling cinereous vultures (*Aegypius monachus*) close to Madrid. Adult birds were observed foraging at a nearby landfill. BDE-99 was the individual congener for which the highest concentrations were noted.

1.5 Routes of BFR exposure, toxicokinetics and toxicity

Birds may be exposed to BFRs and other POPs through several routes, including diet, dermal exposure and respiration. Adult birds generally exhibit elevated BFR burdens compared to younger birds primarily as a result of bioaccumulation (Guigueno and Fernie, 2017). More research is required to better evaluate these pathways. Landfill-foraging gulls, for example, are known to ingest not only putrescible organic matter, but also plastics, metals, glass, building material, rubber and fabrics (Basto et al., 2019; Bond, 2016; Seif, 2017). This is likely to result from their highly competitive foraging behaviours in large aggregations, where competition is intense and foraging opportunities are short-lived as a result of their regular displacement by other birds and heavy machinery (Coulson, 2015). Dermal exposure is an area of increasing interest in the field of human BFR toxicokinetics (Abdallah and Harrad, 2018), but it is an under-researched area in avian toxicology (Alharbi et al., 2016; Mineau, 2011). Airborne particulates have been shown to be an important contaminant pathway in birds (Brown et al., 1997; Fernie et al., 2018a) and also warrant further study.

The impacts of BFRs on birds have been reported for both captive and free-living subjects (recently reviewed in Guigueno and Fernie, 2017). However, this remains a nascent field and considerably less is known about the impacts of NBFRs (Ezechiáš et al., 2014). The effects of FRs on landfill-foraging birds could be amplified if birds are exposed to complex mixtures involving not only FRs, but also other anthropogenic chemicals known to occur at waste disposal facilities, such as polychlorinated biphenyls (PCBs), polycyclic aromatic compounds (PACs) (Melnyk et al., 2015), dioxins and furans (Gómez-Lavín et al., 2018) and trace elements (de la Casa-Resino et al., 2014). Recent studies show that landfill-associated common starlings in Canada are also exposed to perfluoroalkyl acids (Gewurtz et al., 2018) and volatile methylsiloxanes (Lu et al., 2017).

1.5.1. Polybrominated diphenyl ethers (PBDEs)

PBDE concentrations in avian taxa appear to be influenced by several factors. These include diet and trophic level, exposure to different commercial mixtures and distance to point sources, metabolic capacity, nutritional state, age, sex, and migratory behaviour (Chen and Hale, 2010; Kelly et al., 2007; Polder et al., 2008; Roscales et al., 2016). Given that the bioaccumulation and biomagnification potential of different BDE congeners is influenced by their octanol-water partition coefficient (K_{OW}), the nature of the food web concerned (i.e., aquatic *vs.* terrestrial) is important. Congeners with log K_{OW} values ranging between ~5.9 and ~7.2 (i.e., *tetra*- and *penta*- BDEs) have the greatest biomagnification potential. Biomagnification potential declines significantly for chemicals such as *octa*- through *deca*- BDEs with log K_{OW} values >7.2 (reduced absorption rates) or <5.9 (efficiently eliminated by respiration) (Chen and Hale, 2010; Kelly et al., 2007). Those avian predators most closely associated with terrestrial ecosystems (i.e., those that predominantly consume terrestrial invertebrates, birds and mammals), tend to exhibit a greater percentage of higher brominated congeners, such as BDE-209 (Voorspoels et al., 2006). Conversely, PBDE profiles of birds at the apices of aquatic food chains are often characterised by a greater relative percentage of more bioavailable lower brominated congeners, particularly in North America, where these congeners have

been subject to more extensive use (Chen and Hale, 2010; Elliott et al., 2005; Henny et al., 2011; Law et al., 2003). For example, from the early 1980s, North America witnessed a continuous release of *penta*-BDE for approximately 20 years that was associated with a relatively rapid increase of lower brominated, more bioavailable BDE congeners in birds and other biota (Alcock et al., 2003; Chen and Hale, 2010). Europe underwent earlier reductions in *penta*-BDE use, but a lengthy period of *octa*- and *deca*-BDE utilisation may account for the comparatively higher percentages of congeners associated with these commercial formulations reported in European wildlife (Chen and Hale, 2010).

BDE-209 is regularly reported as the dominant PBDE congener in landfill solid matrices (i.e., soil, sediment and biosolids) (Eguchi et al., 2013b; Ilyas, 2010; Kajiwara et al., 2014; Li et al., 2012; Morin et al., 2017) and this is the case across terrestrial substrates in general (McGrath et al., 2017a; Tokarz et al., 2008; Wei et al., 2016). Gavilan-Garcia et al. (2017) did not detect BDE-209 in landfill sludge (Mexico City, Mexico) and suggested that this may have been due to products containing this congener being yet to enter the waste stream. However, among other congeners, BDE-209 is known to undergo sequential debromination and degradation in abiotic matrices (Danon-schaffer and Mahecha-botero, 2010; Gerecke et al., 2006; Liu et al., 2018; Robrock et al., 2008; Schenker et al., 2008) and has been shown to do so in birds. Examples include free-living ring-billed gulls in Canada (Francois et al., 2016) and captive American kestrels (Letcher et al., 2014) and common starlings (Van den Steen et al., 2007).

Guigueno and Fernie (2017) used an adverse outcome pathway (AOP) approach to synthesise information from 61 *in vitro* and *in vivo* studies of the most commonly reported endpoints in avian FR toxicity research. They reported that the most sensitive of all endpoints was behaviour specifically related to reproduction (Appendix 13), with significant effects identified for all such studies reviewed. Most relevant to this thesis were the reported impacts associated with exposure of ring-billed gulls, glaucous gulls, herring gulls and common starlings. Appendix 2 summarises PBDE burdens reported in landfill-associated species. In ring-billed gulls breeding at Ille Deslauriers (Quebec, Canada), liver

type-1 deiodinase mRNA expression was inversely related to hepatic ∑*octa*-BDE levels (François et al., 2016). Males from this colony had bones that were demineralised as a result of PBDE exposure (Plourde et al., 2013). In male Norwegian Arctic-breeding glaucous gulls, plasma progesterone was positively related to concentrations of several POPs, including PBDEs, suggesting that POPs exposure may interfere with steroidogenesis and affect circulating progesterone homeostasis (Verreault et al., 2006). Similarly, in the eggs of glaucous gulls breeding in Svalbard, positive relationships existed between $\sum_{11}$PBDEs and HBCDD concentrations (among other POPs) and both testosterone and 17β-estradiol (Verboven et al., 2008). In liver and brain tissue samples of glaucous gulls in Norway and of herring gulls in Canada, hydroxylated and non-hydroxylated PBDEs, and selected hydroxylated PCBs (polychlorinated biphenyls), disrupted triiodothyronine (T_3) and thyroxine (T_4) transport via binding interactions with the protein transthyretin (TTR) (Ucán–Marín et al., 2009). Crump et al. (2008) found $\sum_{13}$PBDEs in the brains of herring gulls (n = 5) in the Great Lakes region at concentrations of up to 143 ng/g ww. They also found down-regulation of TTR mRNA in embryonic neuronal cells of birds collected from eastern Canada following treatment with both T_3 and BDE-71, implying a shared mechanism of action.

The effects of PBDE exposure on captive common starlings include disruption of the thyroid system (Eng et al., 2014), elevated egg mass and volume (Van den Steen et al., 2009) and reduced T_3 levels (Van den Steen et al., 2010). Evidence for PBDE debromination has also been reported in Eurasian sparrowhawks (a predator of common starlings; Crosse et al., 2012), white storks (Muñoz-Arnanz et al., 2011) and common kingfishers (*Alcedo atthis*) (Mo et al., 2012). Erratico et al. (2015) concluded that the oxidative metabolism of PBDEs in liver microsomes of juvenile common starlings proceeds at a slow rate, suggesting that certain PBDEs are likely to accumulate in individuals over time.

1.5.2 Hexabromocyclododecane (HBCDD)

The toxic effects of HBCDD in birds have been identified predominantly in captive American kestrels. These include impacts on courtship behaviour, nest temperature and reduced nestling plasma TT4 and FT4 (Guigueno and Fernie, 2017). However, few studies have examined its toxicity in those taxa associated with landfill. In eggs laid by Norwegian-breeding glaucous gulls, concentrations of both α-HBCDD and $\sum_{11}$PBDEs were found to correlate with testosterone and estradiol levels (Verboven et al., 2008). Appendix 3 summarises HBCDD burdens reported in seven landfill-associated species. Of the 3 commonest commercial HBCDD diastereoisomers (i.e., α-HBCDD, β-HBCDD and γ-HBCDD), the first is most commonly reported in biota because of its longer half-life and greater persistence in organisms (Letcher et al., 2015). Landfill-associated species are apparently no exception (Gauthier et al., 2007; Verboven et al., 2008). Global HBCDD concentrations in avifauna have yet to show declines and, indeed, may still be rising, although data remain scarce (Abbasi et al., 2016b; de Wit et al., 2010; Law et al., 2014; Letcher et al., 2010; Wu et al., 2012). Su et al. (2015) reported that α-HBCDD and BDE-209 concentrations in herring gull eggs collected in 2012 under the Great Lakes Herring Gull Monitoring Program (GLHGMP) were significantly higher ($P < 0.05$) than in 2006 and 2008 eggs collected from the same colonies, with the mean concentration of HBCDD in eggs collected in 2012 110 % greater than in those from 2006 for the same colony.

1.5.3 NBFRs

NBFRs are reported at considerably lower concentrations in avian tissues than are legacy BFRs. For example, Chen et al. (2013) reported BEHTBP as the only frequently quantifiable NBFR in common starling eggs (detectable in 47 % of all egg homogenates, with concentrations ranging from <MLOD–26 ng/g ww [LOD: 0.05 ng/g ww]). In contrast, PBDEs were found in 96 % of eggs, ranging in concentration from 2–805 ng/g ww. Such observations may reflect the relatively short time that many NBFRs have been in circulation, although they might hint at limited bioavailability and / or marked

biotransformation (e.g., Greaves et al., 2016). For gulls that use landfill, the toxic effects of NBFRs have been documented *in vitro*. Embryonic hepatocytes from an unspecified number of herring gulls in the Great Lakes region were sensitive to the cytotoxic effects of 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (DBE-DBCH or TBECH) (Porter et al., 2014). In terms of DPDBE (i.e., the most commonly-occurring NBFR reported in this thesis; Chapter III) toxicity in birds, Egloff et al. (2011) reported a significant upregulation in the expression of CYP1A4/5 at 0.1 and 0.2 μM to a maximum of 29- and 53-fold respectively, as well as a significant 1.8 fold increase in DIO1 mRNA in chicken embryonic embryonic hepatocytes treated with 0.1 μM DBDPE.

1.6 Summary of research to date

Undoubtedly, MSW landfill represents an important potential source of BFR contamination in birds that exploit this predictable anthropogenic food subsidy. A total of 14 published studies report elevated BFR burdens across eight bird species (i.e., white storks, African sacred ibis, cinereous vultures, black kites, ring-billed gulls, herring gulls, yellow-legged gulls and common starlings) regularly found on landfill in four countries (i.e., Canada, Pakistan, South Africa and Spain; Table 1.2). These studies analysed samples for legacy BFRs and / or NBFRs. Other studies have documented temporal trends in FR burdens of three gull species (i.e., American herring gulls breeding in the North American Great Lakes region, European herring gulls in the German North Sea and Baltic coasts and Arctic-breeding glaucous gulls) that frequently use landfill. Several of these studies report FR burdens in eggs at concentrations close to the highest reported in free-living biota to date. However, concentrations in liver samples are below the maximum ever recorded in biota (a maximum hepatic concentration of 197,000 ng/g lw in an individual Coopers hawk (*Accipiter cooperi*) in Vancouver, Canada; Elliott et al., 2015). Examples of elevated egg concentrations include 2,201 ng/g ww $\sum_{14}$PBDEs in herring gulls in the Great Lakes region (Su et al., 2015), 23 ng/g ww Total-HBCDDs in glaucous gulls in the Norwegian Arctic (Verboven et al., 2008) and 9.79 ng/g ww NBFRs (PBEB) in white storks in Spain (Muñoz-Arnanz et al., 2010). The PBDE concentrations reported by

Su et al. (2015) are either equal to, or higher than the lowest observed effects of PBDEs in captive American kestrels which, compared to control birds, exhibited various responses, including immunomodulation (Fernie et al., 2005a), changes in thyroid, vitamin A, glutathione homeostasis and oxidative stress (Fernie et al., 2005b) and reduced eggshell thickness and reproductive success (Fernie et al., 2009).

1.7 Aims, objectives, working hypotheses and structure of this thesis

There are a number of research gaps related to BFR exposure in birds that breed, forage or rest on or in proximity to landfill sites. No study to date has examined BFR profiles and concentrations across an assemblage of landfill-associated species and few European studies, in particular, have quantified non-PBDE BFR contamination in such birds. Limited use has been made of stable isotopes as dietary tracers in birds using landfill in relation to BFR burdens and research is also required to understand and quantify the different potential pathways of BFR exposure in such birds.

This thesis aims to assess whether gulls breeding in proximity to a UK landfill constitute effective bioindicators of BFR emissions. The two primary working hypotheses were that: i. gulls breeding close to landfill exhibit elevated BFR egg concentrations compared to reference conspecifics, and ii., that European herring gulls, by virtue of their abundance and foraging ecology on landfill, represent the most effective bioindicator species for future work on contaminants in landfill-associated gulls in north-west Europe. In order to test these hypotheses, work was undertaken to meet the following two objectives: i., to compare BFR burdens in the eggs of five gull species breeding in proximity to a landfill and ii., to assess the BFR increment in such eggs by comparing their concentrations and profiles with the eggs of reference conspecifics breeding away from the study landfill. In addition, stable isotopes of carbon, nitrogen and sulphur ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) were measured in the same eggs to test a third additional hypothesis, i.e., that the trophic level at which gulls forage differs between landfill and reference conspecifics and will influence the concentrations and relative abundance of BFRs in their eggs. Furthermore, observations of gulls frequenting the

study landfill were undertaken, with a focus on behaviour likely to influence BFR exposure in order to test a fourth additional hypothesis, i.e., that the behaviour of birds on landfill will influence BFR egg concentrations and profiles.

This thesis is structured as follows: Chapter II details study design, sampling and analytical methodology. Chapter III discusses BFR concentrations in the eggs of landfill-breeding and reference gulls. Chapter IV concerns stable isotope analysis of eggs. Chapter V relates to behavioural observations and Chapter VI marshalls the findings of this thesis into a summary and conclusions, identifying research gaps and future perspectives.

CHAPTER II

STUDY DESIGN, SAMPLING AND ANALYTICAL METHODOLOGY

This study comprised seven key steps: 1. the identification of suitable field locations, 2. the undertaking of fieldwork (egg sampling and collection of behavioural data), 3. the analysis of eggs of five species of gull for legacy BFR and NBFR concentrations / profiles (including extensive quality assurance / quality control [QA/QC] procedures), 4. analysis of egg morphometrics, 5. the determination of dietary tracers in egg content via SIA, 6. analysis of video recordings of foraging birds and 7. interpretation of results, including statistical analyses. This chapter outlines each of these steps in detail.

2.1 Field sampling

2.1.1 Study area

A desk study was undertaken to identify the locations of colonies of five widespread UK-breeding gull (Laridae) species (black-headed gull *Chroicocephalus ridibundus*, common gull *Larus canus*, great black-backed gull *Larus marinus*, European herring gull *Larus argentatus* [hereafter referred to as 'herring gull'] and lesser black-backed gull *Larus fuscus*) in terms of, i. their proximity to active municipal solid waste landfill facilities in England, Scotland, Northern Ireland and Wales (UK) and ii. their remoteness from major centres of human population, which are known to be sources of environmental BFR contamination (Drage et al., 2016; Harrad and Hunter, 2006). This necessitated consultation of recent UK county bird reports (accessed at the British Trust for Ornithology [BTO], Thetford, UK) and communication with designated voluntary 'County Bird Recorders' (i.e., individuals involved in the production of annual local bird reports). Information on active landfill sites in England and Wales was obtained from the UK government's 'Gov.uk' website (https://www.gov.uk/government/publications/landfill-tax-site-operators). In Scotland, the equivalent source of information was the Scottish Environmental Protection Agency (SEPA) Scottish

Waste Sites and Capacity Tool (https://www.sepa.org.uk/data-visualisation/waste-sites-and-capacity-tool/). In Northern Ireland, the Northern Ireland Environment Agency Authorised Landfill Sites database (https://www.opendatani.gov.uk/dataset/niea-authorised-landfill-sites) was used. An operationally active municipal landfill in western Scotland (identity withheld), in operation since 1958 (Argyll and Bute Council, 2003) was selected. This was due to the site i. receiving, amongst other items, human food refuse (therefore being a potential avian food source), ii. being located within 2 km of documented breeding colonies (hereafter 'landfill colonies') of black-headed gulls, great black-backed gulls, herring gulls and lesser black-backed gulls, iii. having reference populations of all species breeding between 50–110 km distant and iv. being in a rural location away from large centres of human population and therefore likely to be an important source of local BFR emissions. Exploratory fieldwork undertaken during 2016 confirmed i. and ii. and led to the discovery of a previously undocumented colony of a fifth species (i.e., common gulls), located approximately 0.9 km from the landfill. Fieldwork in 2017 confirmed iii. Figure 2.1 displays the location of the study landfill in relation to the gull colonies from which eggs were collected. Reference colonies of all five species were from the same Scottish Natural Heritage (SNH)-designated 'Natural Heritage Zone' (Argyll West & Islands; SNH, 2002), located in the Inner Hebrides archipelago.

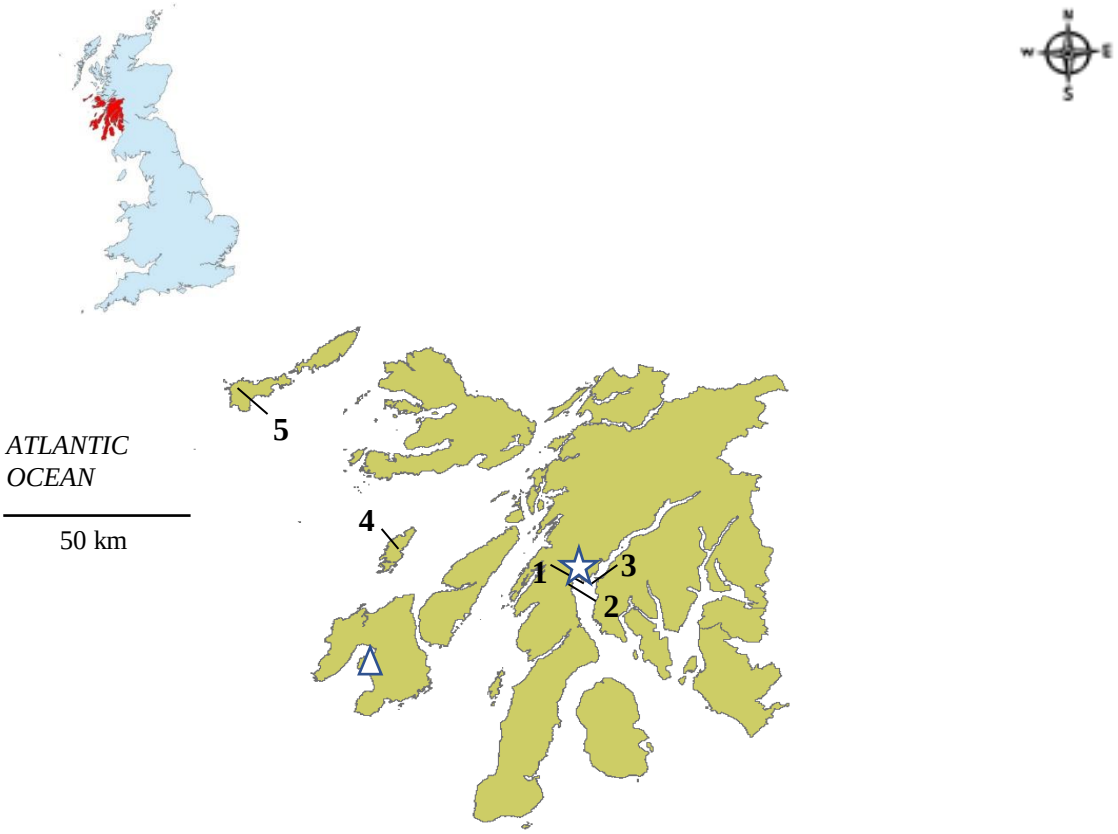

Figure 2.1 The location of the study landfill (star) and gull breeding colonies in western Scotland where five gull species were investigated. Colonies 1–3 were within 0.9–2 km of the landfill. Sites 4 and 5 contained reference colonies located 50 and 110 km, respectively, from the landfill. The location of the breeding colonies of each species from which eggs were collected, were: 1. black-headed gulls, 2. common gulls, 3. great black-backed gulls, herring gulls and lesser black-backed gulls, 4. Colonsay (common gulls, great black-backed gulls, herring gulls and lesser black-backed gulls) and 5. Tiree (black-headed gulls). Also shown is the Gartbrek landfill on the island of Islay (white triangle).

Table 2.1 provides data on the types of waste entering the study landfill for each year of fieldwork. The majority of this waste derived from three small towns located 3 km (Town 'A'; human population of 2,300), 60 km (Town 'B'; human population of 20,000), and 80 km (Town 'C'; human population of 5,000), respectively, from the landfill. Waste data are also provided for the Gartbrek landfill on the island of Islay, western Scotland, as discussed in Chapters III and IV (Table 2.2). The immediate environs of the study landfill were a tidal sea loch (with exposed mudflats at low tide), moorland and commercial forestry, with two small towns containing human populations of approximately 2,300 and 1,200, located approximately 1 mile to the north and west, respectively (Figure 2.2).

Table 2.1 Waste data returns summary for the study landfill in western Scotland for 2016–18. Data are in metric tonnes.

SEPA Reporting Waste Category Description	2016	2017	2018	Grand total
Animal and mixed food waste	61.00	0.00	5.20	66.20
Common sludges	398.59	418.44	435.82	1,252.85
Household and similar wastes	10,424.98	13,712.40	11,570.63	35,708.01
Mineral waste from construction and demolition	24.28	119.94	58.52	202.74
Mineral wastes from waste treatment and stabilised wastes	ND	7.36	ND	7.36
Mixed and undifferentiated materials	11.08	9.42	7.06	27.56
Other mineral wastes	15.24	28.68	4.54	48.46
Plastic wastes	ND	92.52	ND	92.52
Soils	3,247.92	5,223.82	2,144.92	10,616.66
Grant total	**14,183.09**	**19,612.58**	**14,226.69**	**48,022.36**

Table 2.2 Waste data returns summary for Gartbrek landfill, island of Islay, western Scotland for 2016–18. Data are in metric tonnes.

SEPA Reporting Waste Category Description	2016	2017	2018	Grand total
Animal and mixed food waste	10.22	0.00	0.00	10.22
Common sludges	18.82	4.88	41.72	65.42
Household and similar wastes	1,532.95	1,431.41	1,481.19	4,445.55
Mineral waste from construction and demolition	385.04	199.60	653.08	1,237.72
Mixed and undifferentiated materials	250.72	303.00	343.38	897.10
Soils	895.00	1,005.00	1,785.00	3,685.00
Sorting residues	19.01	37.17	17.74	73.92
Grand total	**3,111.76**	**2,981.06**	**4,322.11**	**10,414.93**

ND: No data available.

Source: Scottish Environmental Protection Agency (SEPA) information request.

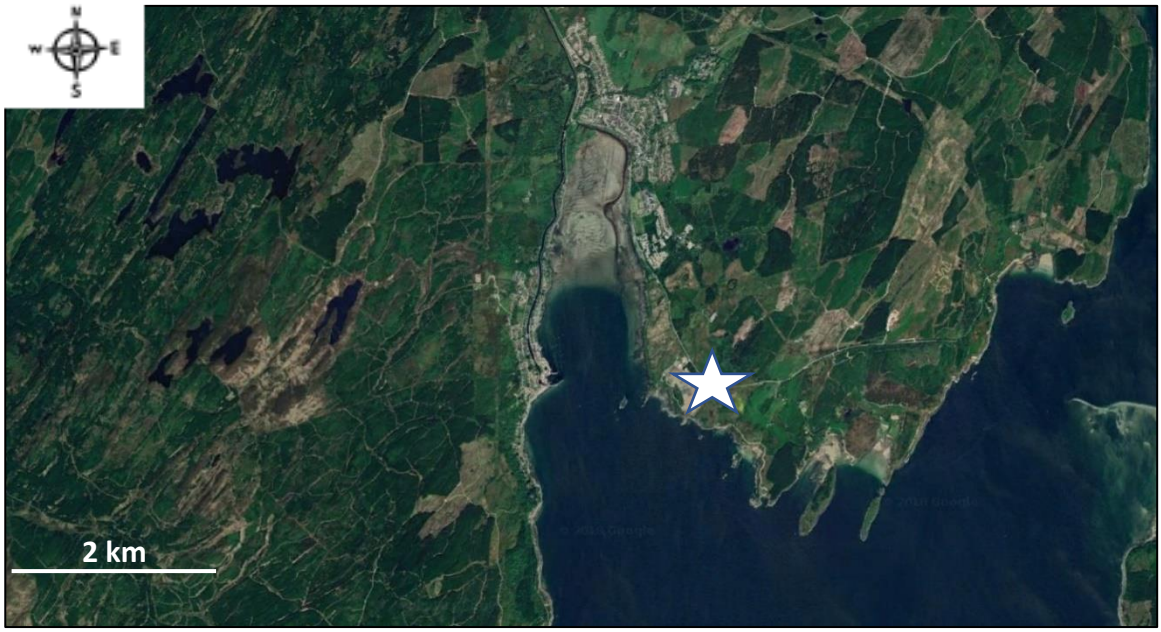

Figure 2.2 Satellite image of the study landfill (denoted by white star) (western Scotland, UK) and surrounding land-use (https://earth.google.com/web/).

2.1.2 Vantage point surveys of gull flightlines

A total of 25 hours was spent undertaking vantage point surveys of gull flightlines in the vicinity of the study landfill and the colony of large gulls (great black-backed gulls, herring gulls and lesser black-backed gulls) during May 2016. These were performed using 8 × 42 binoculars (Carl Zeiss, Wetzlar, Germany) and a 20-60 × 83 zoom telescope (Kowa, Aichi, Japan) mounted on a tripod (Manfrotto, Cassola, Italy). Observation points provided all-round visibility of the surrounding landscape (Figure 2.3) and surveys were undertaken only during periods of greatest visibility (i.e., dry and bright weather). Each observed gull was identified to species and its flight direction recorded. Vantage point surveys were explanatory only, providing initial insight as to the extent to which birds commuted between the colony and landfill.

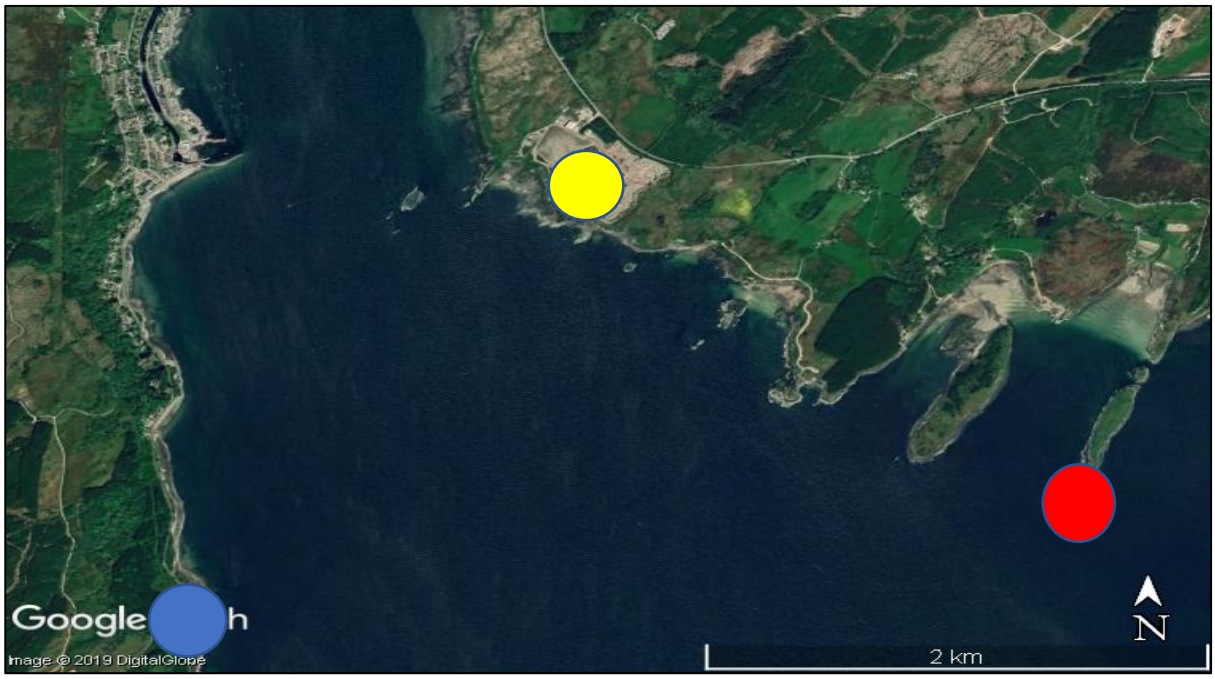

Figure 2.3 Vantage point viewshed for surveying gull flightlines in the vicinity of the study landfill and landfill colonies. Circles: blue - vantage point; yellow - landfill; red – colony of large gulls. Great black-backed gulls, herring gulls and lesser black-backed gulls were observed to commute regularly between the study landfill and the colony (Source: Google Earth).

2.1.3 Egg sampling

Freshly-laid eggs of each species were collected under SNH licences during late April and early May in the 2016 (licence number 77830), 2017 (licence number 92331) and 2018 (licence number 112381) breeding seasons. In 2016, eggs of five species were collected from landfill-adjacent colonies only (hereafter 'landfill colonies'), while in 2017, eggs were collected from both landfill and reference colonies. In 2018, only eggs of herring gulls, great black-backed gulls and lesser black-backed gulls were collected from both landfill and reference colonies. No eggs of black-headed gulls or common gulls were collected in 2018 as neither species had been observed frequenting the landfill during the previous two field seasons. Table 2.3 details single eggs analysed for BFR concentrations in this study. Egg collection was undertaken following GLHGMP methodology.

Table 2.3 Totals of single eggs of five gull species collected from colonies located in proximity (up to 2 km) to the study landfill and from reference sites (at distances of 50–110 km from landfill) analysed for BFRs following fieldwork conducted in western Scotland between 2016–18. Figures in brackets show total number of eggs collected.

Species	**Sampling year**	**No. of eggs (*n*) analysed from:**	
		landfill colonies	**reference**
Black-headed gull	2016	12 (13)	0 (0)
	2017	0 (6)	5 (5)
Common gull	2016	14 (14)	0 (0)
	2017	0 (12)	6 (15)
Great black-backed gull	2016	4 (4)	0 (0)
	2017	2 (4)	4 (4)
	2018	1 (3)	4 (6)
Herring gull	2016	16 (16)	0 (0)
	2017	17 (17)	14 (14)
	2018	15 (15)	11 (13)
Lesser black-backed gull	2016	11 (11)	0 (0)
	2017	0 (6)	0 (0)
	2018	0 (12)	2 (2)
Total		**92 (133)**	**46 (59)**
Grand total number of single eggs analysed for BFRs		**138 (192)**	

(i.e., the collection of one pre-embryonated egg per nest from 10–14 individual nests per species per site; de Solla et al., 2016). However, if an insufficient number of nests containing full clutches (i.e., three eggs) were available at a colony, eggs were taken from nests containing one or two eggs. In addition, for herring gulls, a total of 10 full clutches (consisting of three eggs each) were obtained during 2016–18 in order to obtain insights into intra-clutch BFR burdens and profiles. In the case of single eggs taken from a clutch, the 'largest looking' egg was obtained for each nest based upon a visual inspection. In order to avoid collecting embryonated eggs, OSPAR (Oslo / Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) guidelines (OSPAR, 2000) were observed: each egg was placed into a one litre capacity plastic measuring jug pre-filled with bottled freshwater. The egg was collected if it was between approximately one and six days of incubation, as indicated by the egg either laying on the bottom of the jug with the long axis parallel

to the bottom, or resting with the pointed end on the bottom of the jug with the long axis forming an angle of no more than 30–45° (Figure 2.4).

Figure 2.4 Photograph to illustrate the field method to check eggs for embryonation and therefore inform whether suitable for laboratory analysis, western Scotland, 2018 (ADWT).

Eggs which floated or stood vertically were considered to be of more than seven days of incubation and were not selected. At the landfill large gull colony (i.e. that containing herring gulls, great black-backed gulls and lesser black-backed gulls), different species were generally confined to different micro-habitats: herring gulls were widely distributed across the islet approximately 250 pairs), generally occupying lower-lying less-vegetated areas; great black-backed gulls (3–4 pairs) tended to use the most elevated points of the islet, nesting at low densities, and a discrete lesser black-backed gull colony (approximately 30 pairs) was confined to the north-west section of the islet in an area of greater ground vegetation dominated by bracken *Pteridium aquilinum* and bluebells *Hyacinthoides*

non-scripta. To eliminate the possibility of egg misidentification, nests were marked with colour-coded garden canes and incubating birds were observed at a distance of 10–50 m with 8 × 42 binoculars using a portable chair hide (Bushwear.co.uk, Stirling, UK) prior to flotation testing and collection. The same was undertaken for reference birds in mixed colonies, such as at the Ardskenish peninsula, Colonsay, which comprised approximately 30 pairs of herring gulls, three pairs of great black-backed gulls and two pairs of lesser black-backed gulls. Conversely, common gulls and black-headed gulls tended to breed in single-species colonies which made the specific identification of nests straightforward. However, when they nested in close proximity, it was important to differentiate between nests of common gulls (i.e., eggs have darker background colour, are larger, have blurred maculation and are laid in a well-constructed nest bowl) and Eurasian oystercatchers *Haematopus ostralegus* eggs (paler, have fine maculation, are somehwat more pyriform and are laid in a rudimentary nest). The landfill black-headed gull and common gull colonies each contained approximately 20 pairs, with the reference colonies containing approximately 17 and 60 pairs, respectively). It was important to be cognisant of the phenomenon of intra-and interspecific nest parasitism. Yom-Tov (1980) defined intraspecific nest parastism (also known as 'egg dumping') as the laying of eggs in a conspecific nest without incubating eggs or caring for the offspring. Interspecific nest parasitism is the same except an egg is laid in the nest of a different species (Davies, 2010). Either form has the potential to mislead fieldworkers as to the individual or specific identity of a sampled egg. It can result in multiple clutches in a colony containing eggs laid by the same individual and has implications for certain techniques undertaken in this study, such as stable isotope analysis and intraclutch BFR analyses). Intraspecific nest parasitism is prevalent in colonial waterbirds that have precocial young, including gulls (Rohwer and Freeman, 1989; Yom-Tov, 2001) although this is apparently not commonly discussed in the avian toxicological literature relating to larids. Duda et al. (2008) analysed 160 black-headed gull clutches using protein finger printing of egg albumen and reported that over 30 % of clutches contained extra-pair eggs. The incidence of

interspecific nest parasitism has been documented in detail in the west of Scotland study area (Craik, 2010), most commonly involving Eurasian oystercatchers laying eggs in the nests of common gulls. The egg of a common eider *Somateria mollissima* was found in the nest of a lesser black-backed gull at the landfill large gull colony in the 2016 breeding season but was obviously laid by a different species and was not collected for sampling purposes (Figure 2.5). In the present study, the greatest challenge presented by interspecific nest parasitism woud be differentiating between the eggs of herring gulls and lesser black-backed gulls because their eggs are virtually indistinguishable, although the eggs of the latter tend to be relatively smaller and more pyriform (Craik, 2010; Ferguson-Lees et al., 2011). No evidence of interspecific nest parasitism between these two species, or any gull study species, was seen during fieldwork, although the likelihood of it taking place cannot be ruled out.

Eggs were individually-labelled using standard BTO two-letter species codes combined with the first three letters of the site name and the collection date using a black ink Sharpie™ (Shelbyville, TN, USA) permanent marker. Whole, unbroken eggs were then wrapped in aluminium foil and

Figure 2.5 Egg of a common eider laid in a lesser black-backed gull nest at the large gull colony in proximity to the study landfill, western Scotland, 2016 (ADWT).

placed into an individually labelled 18 ounce sealable Whirlpak™ (Nasco, Fort Atkinson, WI, USA) sample bag and stored securely in foam-lined Peli Storm iM2300™ cases (Pelican Products, Torrance, CA, USA) (shown in laboratory tests at Birmingham not to contain BFRs) until laboratory analysis. On the day of collection, the length and breadth of eggs were recorded (to nearest 0.1 mm) using digital Vernier calipers (MachineMart, Nottingham, UK) and the fresh weight of eggs was recorded (to nearest 0.1 g) using a table top scale (On Balance, Liverpool, UK). Eggs were stored in Peli Storm cases away from sunlight until transportation to the Public Health Laboratories at the University of Birmingham, where they were stored in a walk-in cold room at 3–7°C for approximately one to five days before processing.

2.2 Egg processing

The albumen and yolk fractions were collected by puncturing the eggshell across the equator with a stainless steel scalpel (Scientific Laboratory Supplies, Nottingham, UK) rinsed in HPLC-grade *n*-hexane and HPLC-grade dichloromethane (DCM) and the egg contents (yolk and albumen) emptied into a hexane and DCM-rinsed glass jar (Smith Scientific, Edenbridge, UK). Jars ranged in volume from 60 mL for black headed gull and common gull eggs, to 120 mL for herring gull and lesser black-backed gull eggs and 250 mL for great black-backed gull eggs. Eggs were then manually homogenised using a stainless steel *n*-hexane and DCM-rinsed stainless steel spatula (Fisher Scientific, Loughborough, UK) and frozen at -70°C. A small minority of eggs (< 5 %) contained small embryos; embryos were discarded prior to homogenisation. The inner shell membrane and any remaining egg content were gently removed using a clean stainless steel scalpel under running tap water. Eggshells were dried in a 60°C oven (LEEC, Nottingham, UK) for 4 hours and stored in individually-labelled 18 ounce Whirlpak™ sample bags prior to eggshell thickness measurements being made using a digital micrometer with a ballpoint tip (Mitutoy, Kawasaki, Japan). Eggshell thickness was measured to the nearest 0.01 mm. For each eggshell, nine thickness measurements were

obtained (3 at each of the pointed end, blunt end and equator). This was used for calculating mean shell thickness.

2.3 Laboratory analysis of BFR concentrations in eggs

Analysis of egg samples for BFR concentrations was carried out by Dr Daniel Drage and assisted by ADWT in accordance with the standard laboratory procedures of the Persistent Organic Pollutants Research Group at the University of Birmingham (Drage, pers. comm.; Drage, 2013; Appendix 5). The compounds of interest were polybrominated diphenyl ethers (PBDEs) (BDE congeners -28 [2,4,4'-tribromodiphenyl ether], -47, [2,2',4,4'-tetrabromodiphenyl ether] -99 [2,2',4,4',5-pentabromodiphenyl ether], -100 [2,2',4,4',6-pentabromodiphenyl ether], -153 [2,2′,4,4′,5,5′-hexabromodiphenyl ether], -154 [2,2',4,4',5,6'-hexabromodiphenyl ether], -183 [2,2',3,4,4',5',6-heptabromodiphenyl ether] and -209 [decabromodiphenyl ether]), hexabromocyclododecane (HBCDD) (α-, β- and γ- diastereomers), as well as five novel brominated flame retardants (NBFRs): (1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenylethane (DBDPE), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), pentabromobenzene (PBB) and pentabromoethylbenzene (PBEB). Time and resource constraints meant that of the total number of collected eggs (*n* = 192), 138 were analysed for BFRs. For the purposes of egg lipid extraction, aliquots (~1 g wet weight, accurately weighed) of egg sample were spiked with internal standards prior to pressurised liquid extraction using an ASE-350 Accelerated Solvent Extraction System (ThermoFisher,Waltham, MA, USA). Hydromatrix (diatomaceous earth) (Varian, Palo Alto, CA, USA) was pre-cleaned in Dionium™ pressurised liquid extraction PLE cells (ThermoFisher, Waltham, MA, USA). PLE cells were packed from the bottom upwards as follows: two glass fibre filters (GFF) (Thames Restek, High Wycombe, UK), 3 g of pre-cleaned hydromatrix, 2 g of 1 % deactivated silica, 1 GFF, 10 g of 44 % acid silica and 4 g of pesticide-grade florisil (all: Sigma-Aldrich, St Louis, MO, USA). Accelerated Solvent Extraction was undertaken according to the following parameters: Solvents: *n*-hexane / DCM (3:1, v/v ratio); temperature: 90°C; heating time: 5

min.; static time: 4 min.; purge time: 90 sec.; static cycles: 2; flush volume: 40 %; and pressure: 1500 psi. Extract was transferred to a 200 mL Turbovap™ tube (Biotage) and concentrated to near-dryness in a Turbovap™ evaporator (Biotage). The sample was reconstituted in 50 µL of recovery determination standard solution (RDS), sonicated for 10 sec. and transferred to a labelled glass-inserted vial prior to analysis. For analysis of HBCDD concentrations, samples were transferred to a vial and injected onto an LC-MS/MS, a Shimadzu LC-20AB liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) interfaced with an API 2000 (Applied Biosystems, Foster City, CA, USA). In order to determine PBDE and NBFR concentrations, sample extracts required solvent exchange into *n*-hexane, iso-octane or nonane and injection onto a GC-EI/MS (ThermoScientific Trace 1310, (Waltham, MA, USA) coupled to a ThermoScientific Single Quadrupole mass spectrometer.

2.3.1 Validation and QA/QC criteria

All samples would ideally have been analysed in duplicate. However, due to project time restrictions, one sample was used for each analysis presented. The following validation and QA/QC procedures are adapted from Drage (2013).

2.3.2Analyte identifcation and quantification criteria

The elution orders for all compounds on both GC/MS and LC-MS/MS had been established previously from earlier work by the Birmingham POPs research group. Mixtures of solutions containing each individual target compound were injected to perform a full seven-point calibration with a concentration range of 0.5–50 ng/mL in order to determine exact retention times and linearity of the MS response. Calibration curves (and standards) were accepted if R^2 values exceeded 0.995. The peak areas from the seven-point calibration were used to determine relative response factors (RRFs) for each target compound. The RRF is defined as the instrument response for a unit amount of target pollutant relative to the instrument response obtained for the same amount of internal standard (IS). RRFs were calculated using the following equation:

$$RRF = \frac{A_{NAT}}{A_{IS}} \times \frac{C_{IS}}{C_{NAT}} \qquad \text{(Eqn 2.1)}$$

where A_{NAT} is the peak area for the "native" compound in the standard; A_{IS} is the peak area of the internal standard in the standard; C_{NAT} is the concentration of the "native" compound in the standard; and C_{IS} is the concentration of the internal standard in the standard. The relative standard deviation (RSD) of the RRFs calculated for each target compound at the five points of its calibration curve did not exceed 5 %.

A single calibration standard was injected before and after each batch of ten samples and the average RRFs were calculated. To be acceptable these had to be within ±25 % of the average RRFs from the initial calibration. The following equation was used to calculate the concentration of a target analyte in each sample:

$$\text{Concentration} = \frac{A_{NAT}}{A_{IS}} \times \frac{1}{RRF} \times \frac{M_{IS}}{SS} \quad \text{(Eqn 2.2)}$$

where M_{IS} = mass of internal standard added to sample (ng) and SS = sample mass (g).

The following criteria had to be met to ensure that a given peak was a target pollutant in a sample:

i. The signal to noise ratio (S/N) must exceed 3:1.
ii. The relative retention time (RRT) of the peak in the sample must be within ±0.2 % of the average value determined for the same congener in the two calibration standards ran before and after each batch of samples.
iii. The bromine isotope ratios must be within ±20 % of the average for the two calibration standards run before and after each sample batch.

2.3.3 Determination of internal standard recovery

The recoveries of internal standards during sample extraction and clean-up were determined relative to the recovery determination standard (RDS) added to samples prior to MS analysis – PCB-129 for GC/MS analysis and d_{18}-γ-HBCDD for LC-MS/MS analysis. The IS recoveries were calculated using the following equation:

$$\% \text{ IS Recovery} = \left[\left(\frac{A_{IS}}{A_{RDS}} \right)_S \times \left(\frac{A_{RDS}}{A_{IS}} \right)_{STD} \times \left(\frac{C_{IS}}{C_{RDS}} \right)_{STD} \times \left(\frac{C_{RDS}}{C_{IS}} \right)_S \right] \times 100 \text{ (Eqn 2.3)}$$

where $(A_{IS}/A_{RDS})_S$ = ratio of internal standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{IS})_{STD}$ = ratio of recovery determination standard peak area to internal standard peak area in the calibration standard (the average of values obtained for both calibration standards run for a batch of samples is used); $(C_{IS}/C_{RDS})_{STD}$ = ratio of concentration of internal standard to concentration of recovery determination standard in the calibration standard; and $(C_{RDS}/C_{IS})_S$ = ratio of concentration of recovery determination standard to concentration of internal standard in the sample (assuming 100 % recovery). Table 2.4 shows a summary of internal standard recoveries across all samples analysed. All samples analysed were within the acceptance criteria of 35–150 %.

Table 2.4 Summary of internal standard recoveries (%) in egg samples.

Internal standard	Mean	Standard deviation	Median	Min	Max
BDE-77	83	21	85	42	120
BDE-128	75	31	68	40	130
$^{13}C_{12}$-BDE-209	78	24	78	26	130
$^{13}C_6$-BTBPE	77	31	76	36	140
d_{17}-$^{13}C_6$-EH-TBB	62	24	55	36	140
$^{13}C_{12}$-α-HBCDD	69	18	67	45	120
$^{13}C_{12}$-β-HBCDD	69	21	64	44	130
$^{13}C_{12}$-γ-HBCDD	81	29	75	39	140

2.3.4 Validation of method and ongoing accuracy and precision

In the absence of an appropriate certified reference material, the method was validated by replicate analysis of matrix spiked with known concentrations of target compounds. Chicken eggs were purchased from a local supermarket and homogenised into one control sample. Twelve ASE extraction cells were prepared according to the analytical method, and labelled according to Table 2.5. In cells MD_001 to MD_011, 1 g (+/- 0.02 g) was accurately weighed. MD_012 was used as a reagent blank (i.e. no further materials were packed into the cell). Samples were spiked with target compounds according to the below table. All samples were spiked with internal standards and extracted using the same methods as gull egg samples.

Table 2.5 Method validation sample details and spiking levels (pg/g).

		Spiking Level (pg/g)															
	Spiking Level	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	PBB	PBEB	EH-TBB	BTBPE	DBDPE	a-HBCDD	b-HBCDD	g-HBCDD
MD_001	Control (unspiked egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MD_002 MD_003 MD_004 MD_005 MD_006	Low Spike	1000	1000	1000	1000	1000	1000	1000	5000	1000	1000	1000	1000	5000	1000	1000	1000
MD_007 MD_008 MD_009 MD_010 MD_011	High Spike	5000	5000	5000	5000	5000	5000	5000	25000	5000	5000	5000	5000	25000	5000	5000	5000
MD_012	Reagent Blank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Extracts were reconstituted in 50 µL of iso-octane and injected for PBDEs and NBFRs on GC/MS. Extracts were solvent exchanged into methanol and injected onto LC-MS/MS for determination of HBCDD diastereomers. Tables 2.6 and 2.7 demonstrate that average measurement of all samples was within 80–120 % of the spiked concentrations with a relative standard deviation of <15 % at both spiking levels, demonstrating acceptable accuracy and precision of the method. For ongoing accuracy and precision, a control sample spiked with target compounds was analysed as every twentieth sample (n = 9), and was required to be within 80–120 % of the spiked concentration for the sample batch to be accepted. Table 2.8 provides a summary of the recovery of target analytes from each of these samples.

Table 2.6 Measured concentrations of BFRs in method validation egg samples.

	Concentration (pg/g) in egg															
	Spiked Concentration	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-209**	**PBB**	**PBEB**	**EH-TBB**	**BTBPE**	**DBDPE**	**α-HBCDD**	**β-HBCDD**	**γ-HBCDD**
MD_001	**Control (unspiked egg)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MD_002	**1000 pg/g†**	919	934	994	1030	999	1100	1030	5220	975	1020	1120	1050	5770	958	975	974
MD_003		895	847	969	984	979	1030	1010	4730	960	1020	1120	1120	4840	862	934	869
MD_004		915	983	920	985	984	1070	1010	4970	920	982	1010	1020	5060	908	1010	936
MD_005		869	963	979	992	1010	1060	1010	4950	944	932	1100	984	5550	950	878	1020
MD_006		935	897	1010	1080	1010	1120	1000	5150	948	1080	1160	1110	5930	896	889	1020
MD_007	**5000 pg/g†**	5110	5250	5700	5910	5640	5980	6120	27900	5060	5980	5960	6060	30000	4440	4390	4660
MD_008		4500	4760	4870	5140	5200	5030	5830	24500	4820	4800	5510	5300	25500	3970	4030	4170
MD_009		5160	4890	5430	5990	5810	5450	5940	27500	4950	5860	5960	6010	29900	4390	4310	4950
MD_010		4850	5120	5630	5610	5910	5900	5820	29200	4880	5870	5960	6020	29600	5000	5010	4720
MD_011		4810	4930	5720	5990	5480	5520	5930	27200	5150	6000	5960	5900	30000	4140	4060	4240
MD_012	**Reagent Blank**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

† Spiked concentrations for BDE-209 and DBDPE were five times higher (i.e., 5,000 and 25,000, respectively).

Table 2.7 Recoveries of target compounds in method validation samples.

		Recovery (%)															
	Spiked Concentration	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-209**	**PBB**	**PBEB**	**EH-TBB**	**BTBPE**	**DBDPE**	**α-HBCDD**	**β-HBCDD**	**γ-HBCDD**
MD_002		92	93	99	103	100	110	103	104	98	102	112	105	115	96	98	97
MD_003		90	85	97	98	98	103	101	95	96	102	112	112	97	86	93	87
MD_004	**1000 pg/g†**	92	98	92	99	98	107	101	99	92	98	101	102	101	91	101	94
MD_005		87	96	98	99	101	106	101	99	94	93	110	98	111	95	88	102
MD_006		94	90	101	108	101	112	100	103	95	108	116	111	119	90	89	102
Average	**Average**	91	92	97	101	100	108	101	100	95	101	110	106	109	91	94	96
Precision	**RSD (%)**	2.8	5.9	3.5	4.1	1.4	3.3	1.1	3.8	2.1	5.4	5.1	5.5	8.6	4.3	6.0	6.6
MD_007		102	105	114	118	113	120	122	112	101	120	119	121	120	89	88	93
MD_008		90	95	97	103	104	101	117	98	96	96	110	106	102	79	81	83
MD_009	**5000 pg/g†**	103	98	109	120	116	109	119	110	99	117	119	120	120	88	86	99
MD_010		97	102	113	112	118	118	116	117	98	117	119	120	118	100	100	94
MD_011		96	99	114	120	110	110	119	109	103	120	119	118	120	83	81	85
Average	**Average**	98	100	109	115	112	112	119	109	99	114	117	117	116	88	87	91
Precision	**RSD (%)**	5.4	3.9	6.5	6.4	5.0	6.9	2.0	6.3	2.7	8.9	3.4	5.4	6.8	8.9	9.1	7.3

† Spiked concentrations for BDE-209 and DBDPE were five times higher (i.e., 5,000 and 25,000, respectively).

Table 2.8 Average recoveries of target analytes measured in spiked control samples analysed with sample batches.

Compound	Average	RSD	Min	Max	Median
BDE-28	89	4.3	81	94	90
BDE-47	91	5.8	82	98	91
BDE-99	96	4.5	87	101	97
BDE-100	100	4.8	91	108	99
BDE-153	98	4.2	88	101	98
BDE-154	107	4.4	97	112	107
BDE-183	100	3.8	91	104	101
BDE-209	100	4.5	92	105	100
PBB	94	3.9	86	98	95
PBEB	99	5.5	90	108	100
EH-TBB	109	5.2	99	116	110
BTBPE	103	5.9	92	112	103
DBDPE	109	7.1	97	119	111
α-HBCDD	92	4.7	84	97	92
β-HBCDD	94	5.5	86	101	94
γ-HBCDD	95	6.1	86	102	95

2.3.5 Analysis of blanks, LODs and LOQs

Instrumental limits of detection (LODs) were calculated for all compounds of interest based on a 3:1 signal to noise ratio. The sample limits of quantification (LOQ) were then calculated using the following equation:

$$LOQ = \frac{LOD \times FEV}{VFEI \times SS} \times \frac{100}{\%\ IS\ Recovery} \quad \text{(Eqn 2.4)}$$

where *FEV* is the final extract volume (µL) and *VFEI* is the volume of the final extract injected (µL).

A reagent blank sample was analysed with every batch of nine samples. In the majority of sample batches, none of the target compounds was measured above the LOQ. Therefore in these cases the samples were assigned LOQs based on the above. However in three batches of samples, BDE-209 was detected in the blank concentrations above the LOQ (0.95, 0.90 and 0.95 ng/g). In these cases the LOQ was reported as the average blank plus 3 times its standard deviation (1.0 ng/g). From the three sample batches where BDE-209 was detected in reagent blank samples, the sample was

corrected by subtracting the average blank concentration plus three times its standard deviation. If the blank concentration was ≥50 % of the sample concentration the sample was reported as <1.0 ng/g.

2.4 Stable isotope analysis (SIA) of egg contents

A successful application (proposal no. EK290-13/17) was made to the Natural Environment Research Council (NERC) Life Sciences Mass Spectrometry Core Facility at the Scottish Universities Environmental Research Centre (SUERC), East Kilbride, UK, to undertake carbon ($\delta^{13}C$), sulphur ($\delta^{34}S$) and nitrogen ($\delta^{15}N$) SIA of egg contents (albumen and yolk, homogenised) of herring gulls, great black-backed gulls and lesser black-backed gulls (i.e., the species known to frequent the study landfill). Funding was provided to allow stable isotope analysis of a subset (n = 86 eggs) of eggs, each from a separate clutch (i.e, not comprising SIA analyses of full clutches). This comprised 63 herring gull eggs (40 landfill *vs.* 23 reference), 11 great black-backed gull eggs (5 landfill *vs.* 6 reference) and 9 lesser black-backed gull eggs (7 landfill *vs.* 2 reference) (Table 2.9).

Prior to SIA, eggs were freeze-dried using a Christ Beta 1–8 LSCplus freeze-dryer (Martin Christ, Osterode am Harz, Germany). Approximately 2 mL of homogenised egg material was measured into a 15 mL conical bottom centrifuge tube (KeL Scientific, Las Vegas, NV, USA). The lid of each tube was ventilated to facilitate the required vacuum conditions. Egg material was prevented from exiting the tube during freeze-drying by placement of a 1 cm × 1 cm square of Kimwipe (Kimberley Clarke, Irving, CA, USA) medical wipe in the opening of the tube. Prior to freeze-drying, tubes containing samples were frozen overnight at -70°C along with the freeze-dryer plates. Tubes were then loaded into glass 250 mL laboratory beakers (Thermofisher, Waltham, MA, USA) in groups of 20. Samples were freeze-dried overnight until all egg material was dry. Since eggs were to be analysed for carbon, sulphur and nitrogen, two aliquots (lipid-extraced *vs.* non lipid-extracted) were prepared for each egg. Given the elevated carbon content of lipids (Post et al., 2007), lipid- extracted aliquots were required for carbon SIA. For the analysis of nitrogen and sulphur isotopes, non-lipid extracted aliquots were used. Aliquot weights were measured using an electronic

Table 2.9 Division of the numbers of single eggs laid by three gull species breeding at landfill and reference colonies in western Scotland collected during 2016–18 which were the subject of stable isotope analysis (SIA).

Species	Year	No. of eggs (*n*) for which SIA undertaken:	
		landfill	reference
Great black-backed gull	2016	2	0
	2017	2	2
	2018	1	4
Herring gull	2016	14	0
	2017	13	12
	2018	13	11
Lesser black-backed gull	2016	7	0
	2017	0	0
	2018	0	2
Total:		**52**	**31**

balance (Mettler Toledo, Columbus, OH, USA) and packed into pressed tin capsules (Elemental Microanalysis, Okehampton, UK).

Laboratory analyses were undertaken following standard SUERC procedures (R.A.R. McGill, pers. comm.), i.e, a Pyrocube elemental analyser (Elementar Analysensysteme, Langenselbold, Germany) was coupled to a VisION mass spectrometer (Elementar UK, Cheadle Hulme, Stockport, UK). Laboratory standards methanesulfonamide/gelatine (MSAG2), methionine/alanine/glycine/gelatine (M2) and sulfanilamide/alanine/gelatine (SAAG2) were repeated with every 10 samples to correct for instrument drift and linearity. Stable isotope ratios are all reported in delta (δ) per mil (‰) notation relative to international standards: "Vienna PDB" (VPDB; replacement for original Pee Dee Belemnite standard) ($\delta^{13}C$), air ($\delta^{15}N$), and Vienna - Canyon Diablo Troilite (VCDT) ($\delta^{34}S$). Silver sulphide (Ag_2S) was used to ensure accuracy and calculate content with respect to $\delta^{34}S$ ratios. Using nitrogen as an example, isotopic ratios are expressed according to:

$$\delta X = [(R\text{sample}/R\text{standard}) - 1] \qquad \text{(Eqn 2.5)}$$

where $X = {}^{15}N$ and R = the ratio of ${}^{15}N/{}^{14}N$ isotopes in a given sample compared with VCDT.

2.5 Foraging and loafing behaviours of gulls at the study landfill

Between 9th and 20th April 2018 (inclusive) video recordings of gull foraging behaviour and observations of gull loafing / preening activity were undertaken at the study landfill during weekday (Monday to Friday) operational hours (09.00–16.00 hrs BST approximately) from a portable chair hide positioned on an embankment approximately 400 m from the active tip face (i.e., the locus of waste dumped by bin lorries and other trucks and where birds foraged and 200 m. from the loafing area where preening took place. (Figure 2.6).

The period of yolk formation in large gulls is approximately 14 days (Roudybush et al., 1979) and locally-breeding birds are known to commence laying in late April (Kim et al., 2010). Hence, the behavioural observations took place at the time during which eggs were being formed as well as the time during which sequestration of BFRs into developing yolks in the ovary and oviduct would have occurred (Fernie et al., 2009). This is particularly relevant in the case of those bird species such as larids, that can generally be categorised as 'income breeders', i.e., tending to be more reliant on exogenous, as opposed to endogenous (stored) nutrient reserves for reproduction (Roscales et al., 2016). A compactor vehicle was intermittently in operation at the active tip face (i.e., the locus of waste dumped by visiting bin lorries and other trucks) from approximately 09.00 hrs to 16.00 hrs each week-day. The compactor was idle during 13.00 hrs to 14.00 hrs, when the driver

Figure 2.6 Portable chair hide, used for video recording and observations of gulls at the study landfill, western Scotland, April 2018 (ADWT).

took a lunch break. The hide was occupied from 07.30 to 16.30 BST , with a one hour break during 13.00–14.00. Trucks (daily mean of 12) delivered an assortment of waste (including household, commercial and building waste) over the course of the day, depositing waste from a raised track running above the active tip face. Following dumping of waste, the compactor would flatten and redistribute the newly-delivered material. Tipping and subsequent compacting operations were associated with the greatest gull activity at the active tip face: gulls appeared to benefit from the actions of the compactor in breaking open refuse sacks, exposing edible organic material. Video recording of foraging behaviour was undertaken using a colour video recorder (Sony Digital HD HDR-PJ10E, Sony Corporation, Minato, Japan) mounted on a tripod (Velbon Corporation, Tokyo, Japan) (Figure 2.7).

Figure 2.7 Still from video footage of herring gulls, great black-backed gulls and lesser black-backed gulls foraging at the active tip face of the study landfill, western Scotland, April 2018 (ADWT).

On each occasion that aggregations of birds (single birds foraged alone very rarely) alighted on the active tip face, video recording commenced and continued until most or all foraging birds had flown. Recorded foraging bouts lasted from ~30 sec. to ~12 min. The median number of video recordings taken per day was 27 (range: 21–35), with 267 video recordings made in total, comprising approximately 56 hours of footage. For each recording, the following details were noted on pre-printed field data sheets: date of recording, recording number and time of day, estimated Beaufort wind speed and direction, cloud cover, precipitation (Yes/No), approximate number of foraging birds on the landfill and the approximate percentage of foraging birds by species Upon review of video footage, a total of 2,329 observations of foraging birds was made, consisting of 255 observations of great black-backed gulls, 1,961 of herring gulls and 113 of lesser black-backed gulls. At no point were black-headed gulls and common gulls observed on the landfill. No marked (i.e., colour-marked, ringed or tagged) gulls of any species were seen at the landfill and no birds were marked in any way for the purposes of this study. Over the course of the day, gulls regularly loafed on a plastic and

gravel-covered former waste pile on site (Figures 2.8 and 2.9). Counts and observations of loafing birds were made at 30-min. intervals.

Figure 2.8 Aerial view of the study landfill in western Scotland to show the approximate areas and relative positions of: i. the active tip face (where rubbish was dumped and compacted and birds foraged; black circle) and ii., the loafing area on an embankment (where birds also preened; white circle). Arrow indicates North (Google Earth).

Figure 2.9 Loafing herring, great black-backed and lesser black-backed gulls at the study landfill site, western Scotland, April 2018 (ADWT).

In terms of loafing by gulls, the following were recorded on pre-printed field data sheets: the total numbers of birds of each species loafing, the incidence and duration of preening bouts performed by individual adult-plumaged (i.e., potentially-breeding) gulls, and the approximate number and percentage of loafing *vs.* foraging birds on site. This provided a total of 129 'snapshot' observations of the loafing behaviour of the three species using the landfill.

2.5.1 Data extraction of foraging behaviour from video recordings

Video recordings were analysed using 'Power Media Player' software (Cyberlink Corporation, New Taipei City, Taiwan). Analysis of video data followed Greig et al. (1985, 1986): An observation period of 15 sec. per bird was defined during which the number of (i.) pecks made into the substrate, (ii.) occasions when food items were swallowed, and (iii.) paces made (scored from 0 to 3: 0 = 0 paces, 1 = 1–4 paces, 2 = 5–10 paces, and 3 = > 10 paces) were recorded. Mean number of paces (per 15 sec.) was estimated and scored from 1 to 3: 1 = 2.5 paces, 2 = 7.5 paces, 3 = 15 paces (approx.). The length of time that each bird stood stationary during foraging was also measured (scored from 0 to 3: 0 = 0 sec., 1 = 1–5 sec., 2 = 6–10 secs, and 3 = 11–15 sec.). Mean time spent stationary was estimated by assigning the following number of seconds to each rank: 1 = 2.5 sec., 2 = 7.5 sec. and 3 = 12.5 sec. These behaviours were identified as being the most straightforward to quantify in terms of those which were most likely to expose foraging birds to FRs. The decision was made to analyse both the number of paces made, as well as the time spent stationary, since either behaviour may potentially expose birds to FRs via dermal contact (Alharbi et al., 2016; Henderson et al., 1994; Mineau, 2011). Herring gulls were the most numerous species at the landfill, comprising on average 91.5 % of all gulls observed. The remainder of gulls were either great black-backed gulls (arithmeric mean: 6 %) or lesser black-backed gulls (arithmetic mean: 2.5 %). Given the abundance of herring gulls, a random selection of a maximum of 10 individuals from each recording was made using a transparent acetate grid containing individually-numbered squares mounted on a laptop screen used alongside an online random number generator (www.numbergenerator.org). Conversely, due to their

comparative scarcity, the behaviour of all great black-backed gulls and lesser black-backed gulls was analysed to obtain behavioural data for those species. For each species, the mean, median and range were calculated for each variable measured.

2.6 Statistical analyses

All data analyses were undertaken in the R statistical environment (R Core Team, 2018). Data were checked for normality using visualisation (histograms, quantile-quantile plots), skewness / kurtosis and the Shapiro-Wilk Test. Homogeneity of variance was assessed using Levene's Test. The majority of data were established to be non-normally distributed. In some cases (e.g., aspects of the behavioural data in Chapter V, as well as BFR concentrations in Chapter III), data were $\log_{10}$-transformed. Box and whisker plots (showing median values, 10^{th}, 25^{th} , 75^{th} and 90^{th} percentiles and outliers) were used to compare behavioural, BFR and isotopic data visually between species and colonies. Other visualisation tools used to interrogate data included simple linear regression scatterplots with fitted lines, the corrplot package (Wei and Simko, 2017) and stacked barplots. Descriptive statistics (including measures of frequency, central tendency and variation) were used when required. Compounds for which detection frequencies were <30 % were excluded from analyses. Repeatability of eggshell thickness measurements was calculated using the intraclass correlation coefficient (ICC) (Fanson and Biro, 2019; Lessells and Boag, 1987; Middleton-Welling et al., 2018) using the ICC package in R (Wolak et al., 2012). Given the non-normal distribution of the majority of data, non-parametric tests were used for statistical comparisons. The Kruskal-Wallis test was used when three or more groups were compared (e.g., making comparisons across the five study taxa), whereas the Mann-Whitney *U*-test was performed to test for differences between two groups (e.g., between landfill and reference data). Where appropriate, differences in means between datasets were subsequently evaluated using the Pairwise Wilcoxon Rank Sum Test with a Holm adjustment. All confidence limits were set to 95 % and an alpha threshold of 0.05 was used for all statistical comparisons except for behavioural observations, for which the alpha threshold was adjusted to 0.01

(after Grant and Grant 2001; Portugal et al., 2010) because statistical independence of data could not be guaranteed given that none of the gulls was individually identifiable.

CHAPTER III

BIRDS AS BIOINDICATORS OF BROMINATED FLAME RETARDANT EMISSIONS FROM LANDFILL

3.1 Synopsis

Landfill sites are likely important reservoirs of legacy BFRs as the products to which such chemicals have been applied enter obsolescence. Landfill is also a foraging substrate for birds such as gulls. The present chapter is the first study to investigate BFR egg burdens and profiles across a species-assemblage of five Laridae taxa (black-headed gulls, common gulls, great black-backed gulls, European herring gulls and lesser black-backed gulls) breeding in proximity (within 2 km) to an operational munipcal solid waste landfill facility. Landfill-breeding gulls of all five species exhibited higher $\sum_8$PBDE including BDE-209 egg concentrations compared to reference conspecifics and exceeded egg $\sum_8$PBDE including BDE-209 concentrations reported in landfill-associated populations of white storks and black kites in Spain and African sacred ibis in South Africa. However, only in the eggs of common gulls and great black-backed gulls was there evidence of elevated HBCDD concentrations for landfill breeders. Concentrations of the NBFR, DBDPE included the highest reported in biota to date (7724 ng/g lw in the egg of a great black-backed gull). Ongoing research into the presence and fate of DBDPE in biota is necessary. Herring gulls were numerically dominant on the study landfill and were the most widespread species in terms of egg sampling. As a consequence, this species is recommended as a biomonitoring taxon for future assessments of BFR emissions from landfill in a north-west European context.

3.2 Introduction

Municipal solid waste landfill facilities and other dump sites designated for the practice of domestic and commercial waste disposal (hereafter, 'landfill') have been identified as important reservoirs of legacy POP-BFRs (i.e., PBDEs and HBCDD) worldwide as they constitute an accumulation of obsolete manufactured polymeric items from which such organohalogens enter local abiotic matrices, predominantly via emissions to air and leaching to groundwater (Danon-Schaffer et al., 2013; Eguchi et al., 2013a; Gallen et al., 2016; Morris et al., 2004; Odusanya et al., 2009; Stubbings and Harrad, 2014). Landfill sites can also be of considerable importance to bird populations (for example, storks, certain raptors, gulls and corvids) since they may also be a predictable source of abundant human food refuse (Arizaga et al., 2018; Baglione and Canestrari, 2009; Belant et al., 1995; Elliott et al., 2006b) . Other birds, for example wildfowl, shorebirds and songbirds, have also been reported to utilise the semi-natural habitats and waterbodies often present within landfill sites (Tongue et al., 2019).

Among animal classes, birds are particularly susceptible to POPs (Chen and Hale, 2010; Fernie et al., 2017; Guigueno and Fernie, 2017; Marteinson et al., 2011b). The vulnerability of birds to organohalogens, via processes such as eggshell thinning, became a critical avian conservation issue in Europe and North America post-WWII when the introduction of organochlorine pesticides led to dramatic declines in species such as the Eurasian sparrowhawk and peregrine falcon (*Falco peregrinus*). Legacy BFRs have been demonstrated to exert deleterious effects in birds across various endpoints, including at the molecular level, the endocrine system, biochemical concentrations, brain structure, bird behaviour, growth and reproductive measures (reviewed in Guigueno and Fernie, 2017). Avian tissues can be valuable bioindicators of anthropogenic contamination (Furness and Greenwood, 1993). Eggs are a particularly effective non-invasive matrix for BFR analysis given that organohalogens, being lipophilic, sequester in the yolk compartment (Chen and Hale, 2010) and a small number of egg monitoring schemes, covering gulls and raptors, exist globally (Hebert, 1999;

Koschorreck et al., 2015; Walker et al., 2008). Important characteristics of biomonitoring species are: i. that they are known to elicit clear responses to contaminants present in the environment, ii. that they are sufficiently widespread to allow collection of statistically robust data and iii. that the species is relatively sedentary, thereby providing a reliable indication of pollution levels in the geographical area in which it lives (Furness and Greenwood, 1993; LeBlanc and Bain, 1997).

In temperate regions, the association of gulls with landfill is well documented (e.g., Belant et al., 1993; Coulson, 2015; Duhem et al., 2005; Pons, 1992; Steigerwald et al., 2015; Verbeek, 1977c). Elevated FR concentrations have been reported in those gull populations known for their use of anthropogenic environments (Chen et al., 2012; Desjardins et al., 2018; Muñoz-Arnanz et al., 2012; Roscales et al., 2016) and Canadian-breeding ring-billed gulls that spend time in waste facilities have been shown to exhibit higher plasma BDE-209 concentrations compared to conspecifics that do not use such sites (Gentes et al., 2015).

In north-west Europe, herring gulls are typically associated with landfill (Coulson, 2015; Coulson, 2019; Coulson et al., 1987; Greig et al., 1983; Greig et al., 1985). The manner in which herring gulls methodically dig and pull with the bill at natural food items such as marine invertebrates led Verbeek (1977a) to assert that such behaviour has preadapted this species to exploit novel environments such as landfill, where it behaves similarly, digging and pulling at the substrate in order to isolate and obtain human food refuse which may be admixed with other anthropogenic waste and soil. The resourcefulness of herring gulls foraging in anthropogenic environments has been documented elsewhere (e.g., Henry and Aznar, 2006; Holman et al., 2019). Herring gulls were the focal taxon in this study give their numerial dominance on the landfill (constituting over 90 % of birds) and at breeding colonies. Four other gull species commonly use landfill in the UK, these being black-headed gulls, common gulls, great black-backed gulls and lesser black-backed gulls (Bellebaum, 2006; Cristina et al., 1991; Greig et al., 1986; Horton et al., 1983; Scott et al., 2014; Verbeek, 1979).

The diet and foraging range of the five gull species used in this study make them ideal model species for identifying whether landfills are sources of chemical contamination for birds utilising such facilities. Black-headed gulls (UK and Ireland breeding population: 130,000 pairs; Robinson, 2005) have a widespread distribution. This small *Chroicocephalus* species can be encountered foraging in a range of habitats, from urban parks to coastlines. Invertebrates, especially earthworms (Lumbricidae) and beetle (Coleoptera) and fly (Diptera) larvae dominate the diet of black-headed gulls, though they also regularly feed on human refuse at landfill sites in large numbers, particularly in winter (Coulson, 2019). Black-headed gulls have breeding season foraging range of approximately 10 km from the breeding colony (Thaxter et al., 2012). Such information is useful when assessing the foraging habitats available to a study population. Common gulls (UK and Ireland breeding population: 48,000 pairs; Robinson, 2005) have a more restricted distribution during the breeding season compared to black-headed gulls, with the greatest densities in Scotland and western Ireland (Balmer et al., 2013). Although the diet of common gulls is broad, this species is known for its tendency to forage on open grassland, where it feeds on invertebrates (Cramp and Simmons, 1983). Common gulls also use landfill as a foraging substrate, though this is less frequently observed in the UK compared to other gull species (Horton et al., 1983). The mean foraging range of common gulls in the breeding season is 25 km (Thaxter et al., 2012). Great black-backed gulls (UK and Ireland breeding population: 17,000 pairs; Robinson, 2005) are the largest species of gull globally. In the UK and Ireland, its main breeding distribution is concentrated on western coastlines (Balmer et al., 2013). Great black-backed gulls are apex predators, with a broad diet at an elevated trophic level that includes other birds and mammals (Cramp and Simmons 1983). Great black-backed gulls regularly use landfill sites and often obtain food on landfill by stealing it from other species (Verbeek, 1979). No data appear to be currently available in the literature with respect to the breeding season foraging range of great black-backed gulls. Herring gulls (UK and Ireland breeding population: 130,000 pairs; Robinson, 2005) are omnivorous, with a strong connection with landfill as a foraging substrate

(Coulson, 2015, 2019, Cramp and Simmons, 1983). Herring gulls have a breeding season foraging range of approximately 10 km (Thaxter et al., 2012). Lesser black-backed gulls (UK and Ireland breeding population: 110,000 pairs; Robinson, 2015) also have a wide-ranging diet which includes items obtained in anthropgenic environments such as landfill (Coulson 2019). The mean foraging range of breeding lesser black-backed gulls is approximately 70 km (Thaxter et al., 2012). Chapter II provides the numbers of landfill *vs*. reference eggs of each of these five species that were sampled for the purposes of this study along with details of all materials and methods.

Recent assessments have categorised the populations of all five study species as being of unfavourable conservation status (Eaton et al., 2015; Stanbury et al., 2017) (notwithstanding recent questioning of the validity of these designations by Coulson, 2019). Black-headed gulls have undergone a non-breeding (i.e., winter) population decline of 47 % in the UK in the 25 years to 2017, with the UK hosting 60–70 % of the total European wintering population of this species (Eaton et al., 2015). Using International Union for the Conservation of Nature (IUCN) criteria, Stanbury et al. (2017) classified the UK's black-headed gull population as 'Vulnerable' and Eaton et al. (2015) designated this species as 'Amber Listed' in the UK (i.e., as being of moderate conservation concern). Common gulls were also 'Amber Listed' in the UK by Eaton et al. (2015) as a result of the UK hosting 40–50 % of the species' total European wintering population. Great black-backed gulls have undergone a UK breeding population decline of 29 % since 1970 and a non-breeding population decline of 65 % over the 25 years to 2017 (Eaton et al., 2015). This species was classified as 'Endangered' in a UK context by Stanbury et al. (2017) and was accorded UK 'Amber List' status by Eaton et al. (2015). European herring gulls (hereafter 'herring gulls') are 'Red Listed' (i.e., the species is of the highest priority conservation concern) in the UK as a result of a 60 % breeding population decline since 1970, a decline of 79 % in the non-breeding population in the 25 years to 2017 and because the UK hosts 20–30 % of the European wintering population). Stanbury et al. (2017) assigned herring gulls the status of 'Endangered' in the UK. Herring gulls also occupy the

IUCN European Red List as being 'Near-Threatened' (BirdLife International, 2015) and 'Vulnerable' across EU-27 states. Lesser black-backed gulls are 'Amber Listed' in the UK as a result of a localised breeding population (70–80 % of breeding birds are confined to EU-designated 'Important Bird Areas') and because the UK breeding population is internationally significant, comprised of 20–30 % of the total European breeding population (Eaton et al., 2015). Davis et al. (2018) also reported that the UK breeding population of lesser black-backed gulls had declined by more than 30 % since 2002. To date, the unfavourable conservation status of these species has not been addressed in terms of their exposure to legacy POP-BFRs when frequenting landfill. The aim of this chapter was to determine the extent to which gulls breeding in proximity to landfill constitute effective bioindicators of BFR emissions from such sites in the UK. There were two primary objectives: i., to obtain sufficient egg samples from each species in an attempt to identify a suitable bioindicator species for future research and ii., to assess the BFR increment in the eggs of landfill breeders by collecting reference eggs from individuals breeding away from the study landfill. The working hypotheses of this study were: i., birds breeding in proximity to UK landfill are bioindicators of BFR emissions from such sites, and ii., that herring gulls, by virtue of their abundance and foraging ecology on landfill, may represent the most effective species for future work on contaminants in landfill-associated gulls in north-western Europe. Such data may therefore potentially inform conservation strategies for the species concerned. Compounds of interest were eight PBDE congeners (BDEs -28, -47, -99, -100, -153, -154, -183 -209), three HBCDD diastereomers (α-, β- and γ-) and five NBFRs: (1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenylethane (DBDPE), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), pentabromobenzene (PBB) and pentabromoethylbenzene (PBEB).

3.3 Materials and Methods

Site selection, field sampling, determination of BFR egg concentrations and statistical analyses were all undertaken as described in Chapter II.

3.4 Results

Given that herring gulls were the species for which the most egg samples were analysed by a considerable margin (n = 63), it is the eggs of this species which provide the greatest opportunity to address the primary hypothesis of this thesis, i.e., that birds are bioindicators of FR emissions from UK landfill. Results are therefore first presented for herring gulls before those of other species, which are then covered in taxonomic order following Gill and Wright (2006). For the purposes of this chapter and thesis, $\sum_8$PBDE concentrations includes concentrations of the congener BDE-209, whereas $\sum_7$PBDEs excludes BDE-209.

3.4.1 BFR concentrations and profiles in the eggs of landfill and reference herring gulls

Herring gull eggs collected in 2017 and 2018 were pooled for the purposes of statistical analysis given that there was no significant difference between those years in terms of egg concentrations of $\sum_8$PBDEs (Mann Whitney / Wilcoxon U = 312, P = 0.14), Total-HBCDD (U = 300, P = 0.10) or DBDPE (U = 474, P = 0.26). Compounds for which detection frequencies were <30 % (all NBFRS, i.e., BTBPE, DBDPE, EH-TBB, PBB and PBEB) were excluded from statistical analyses. For β-HBCDD and BDE-154, detection frequencies were >30 % only in the case of reference eggs collected in one year, i.e., 2017, which appears to be an artefact and unlikely to be biologically meaningful. As a result, β-HBCDD and BDE-154 were also excluded from analyses. In the case of BDE-100, samples from 2018 were excluded given that their detection frequencies for that year were <30 %. Detection frequencies for BDE-28 in the eggs of reference-only herring gulls were <30 %, although these data were retained for statistical analyses under the assumption that they indicated a genuine difference between site-types in terms of the presence (landfill) / absence (reference) of this congener (Appendix 6). Table 3.1 displays the arithmetic mean (± SE), median and range of the compounds of interest in the eggs of landfill-breeding and reference herring gulls collected in western Scotland during 2017 and 2018, respectively. Appendix 7 shows wet weight data for the same samples.

Table 3.1 The mean (± standard error), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (n = 32) and reference (n = 25) herring gulls collected in western Scotland in the 2017 and 2018 breeding seasons (pooled) (ng/g lipid weight).

Compound	Landfill mean	Reference mean	Landfill median (range)	Reference median (range)	$P^{\dagger}$
$\sum_8$PBDEs (inc. BDE-209)	760.8 ± 226.8	318.7 ± 140.0	252.2 (7.49–4720.9)	108.2 (9.6–3437.5)	0.02*‡
$\sum_7$PBDEs	463.8 ± 196.4	264.7 ± 114.0	44.6 (4.2–4667)	94.1 (0.4–2702.7)	0.69
BDE-28§	3.8 ± 3.3	3.1 ± 1.7	<1.5 (<1.5–106.3)	<1.5 (<1.5–35.1)	0.74
BDE-47	34.6 ± 14.1	41.0 ± 14.6	8.3 (1.25–422.2)	10.2 (<0.7–274.5)	0.75
BDE-99	8.4 ± 2.2	6.5 ± 3.3	2.1 (<0.7–46.0)	0.1 (<0.7–77.1)	0.53
BDE-100§	7 ± 4	<0.70 ± 0.10	<0.70 (<0.70– 120)	<0.70 (<0.70– 3)	<0.001***‡
BDE-153	266.1 ± 133.0	111.2 ± 66.4	1.3 (<0.7–3047.6)	<0.7 (<0.7–1632.8)	0.07
BDE-154§	53 ± 32	56 ± 50	<0.70 (<0.78– 730)	<0.78 (<0.78– 1300)	-
BDE-183	90.17 ± 36.91	46.15 ± 39.07	<0.78 (<0.7–880.4)	<0.7 (<0.7–976.0)	0.15
BDE-209	296.9 ± 132.1	53.9 ± 31.6	104.5 (<7.8–3297.9)	<7.8 (<7.8–734.8)	0.002**‡
∑HBCDD	183.4 ± 60.2	223.8 ± 62.5	35.4 (0.7–1548.2)	100.2 (0.1–1140.1)	0.98
α-HBCDD	111.1 ± 36.7	124.9 ± 40.1	6.0 (<0.9–972.8)	8.2 (<0.9–885.6)	0.78
β-HBCDD§	4.3 ± 4.3	8.7 ± 5.9	<1.2 (<1.2–139.1)	<1.2 (<1.2–142.5)	-
γ-HBCDD	67.8 ± 22.8	90.1 ± 26.0	6.7 (<1.2–575.3)	42.4 (<1.2–496.9)	0.62
BTBPE§	<0.6	<0.6	<0.6 (<0.6–3.5)	<0.6 (<0.6–3.7)	-
DBDPE§	380.9 ± 189.9	<41.4	<41.4 (<41.4–4696.0)	<41.4 (<41.4– <41.4)	-
EH-TBB§	<0.9	<0.9	<0.9 (<0.9–1.7)	<0.9 (<0.9– <0.9)	-
PBB§	<1.5	<1.5	<1.5 (<1.5– <1.5)	<1.50 (<1.5– <1.5)	-
PBEB§	<0.1	<0.1	<0.1 (<0.1–0.15)	<0.1 (<0.1–0.15)	-

† Significance test for difference between landfill and reference egg concentrations derived using Wilcoxon Mann-Whitney U test.
‡ Significantly higher for the eggs of landfill-breeding birds. <LOD: Below limit of detection
§ Section 3.4.1 provides details of those compounds of interest which were excluded from analyses.
Average lipid weight limits of detection (ng/g lw): BDE-28: 1.5; BDE-47: 0.7; BDE-99: 0.7; BDE-100: 0.7; BDE-153: 0.7; BDE-154: 0.7; BDE-183: 0.7; BDE-209: 7.8; α-HBCDD: 0.9; β-HBCDD: 1.2; γ-HBCDD: 1.2; BTBPE: 0.6; DBDPE: 41.4; EH-TBB: 0.9; PBB: 1.5; PBEB: 0.1.

3.4.1.1 Herring gull PBDE concentrations & profiles

Comparing 2017–18 landfill and reference lipid weight data, landfill eggs contained significantly elevated lipid weight concentrations of $\sum_8$ PBDEs ($U = 541$, $P = 0.02$), BDE-100 ($U = 206$, $P < 0.001$; 2017 data only) and BDE-209 ($U = 586$, $P = 0.002$) (Figure 3.1 A, B, C and D respectively).

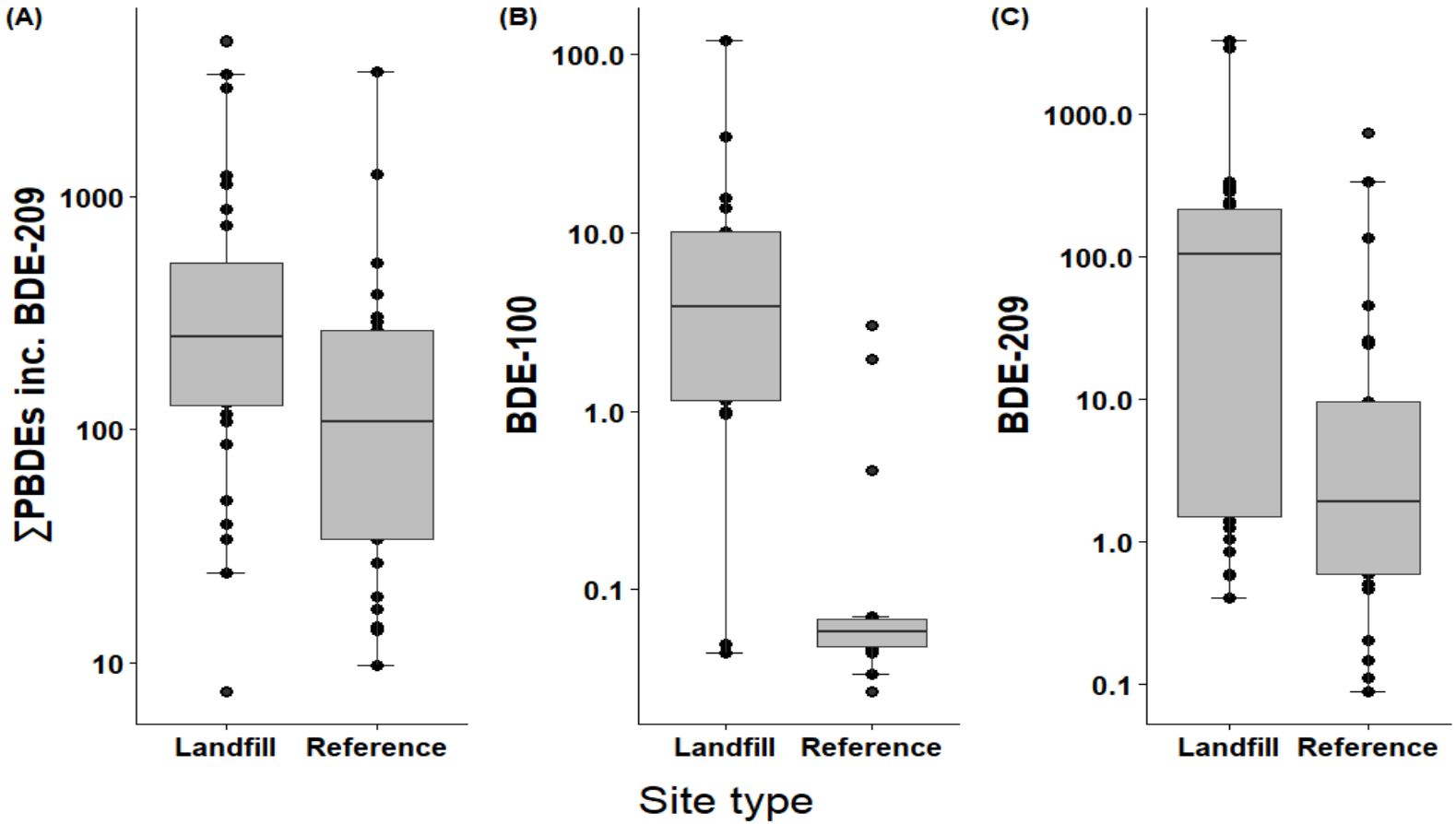

Figure 3.1 Box and whisker plots showing significantly higher log10-transformed concentrations in this study in the case of (A) ∑8PBDEs including BDE-209, (B) BDE-100 and (C) BDE-209 concentrations (ng/g lw) in eggs laid by European herring gulls breeding in proximity to landfill (n = 32) and at a reference site 50 km distant (n = 25) in 2017–18. Black lines are medians, boxes indicate the 25th and 75th percentiles, whiskers show the 10th and 90th percentiles. Data for BDE-100 relates to 2017 only.

3.4.1.2 Herring gull ∑PBDE profiles: landfill vs. reference

The stacked barplot in Figure 3.2 shows the arithmetic mean percentage composition of individual PBDE congeners (as a constituent of $\sum_8$PBDEs) in the eggs of landfill *vs*. reference herring gulls obtained during 2017–18. In landfill breeders, the mean percentages of BDE-28 (2 % *vs*. 0 %, respectively) (U = 800, P < 0.001) and BDE-209 (49 % *vs*. 18 %, respectively) (U = 267; P = 0.03) were significantly higher than for reference birds. Reference eggs contained a significantly higher mean percentage of BDE-47 (44 % *vs*. 15 %, respectively; U = 267, P = 0.03).

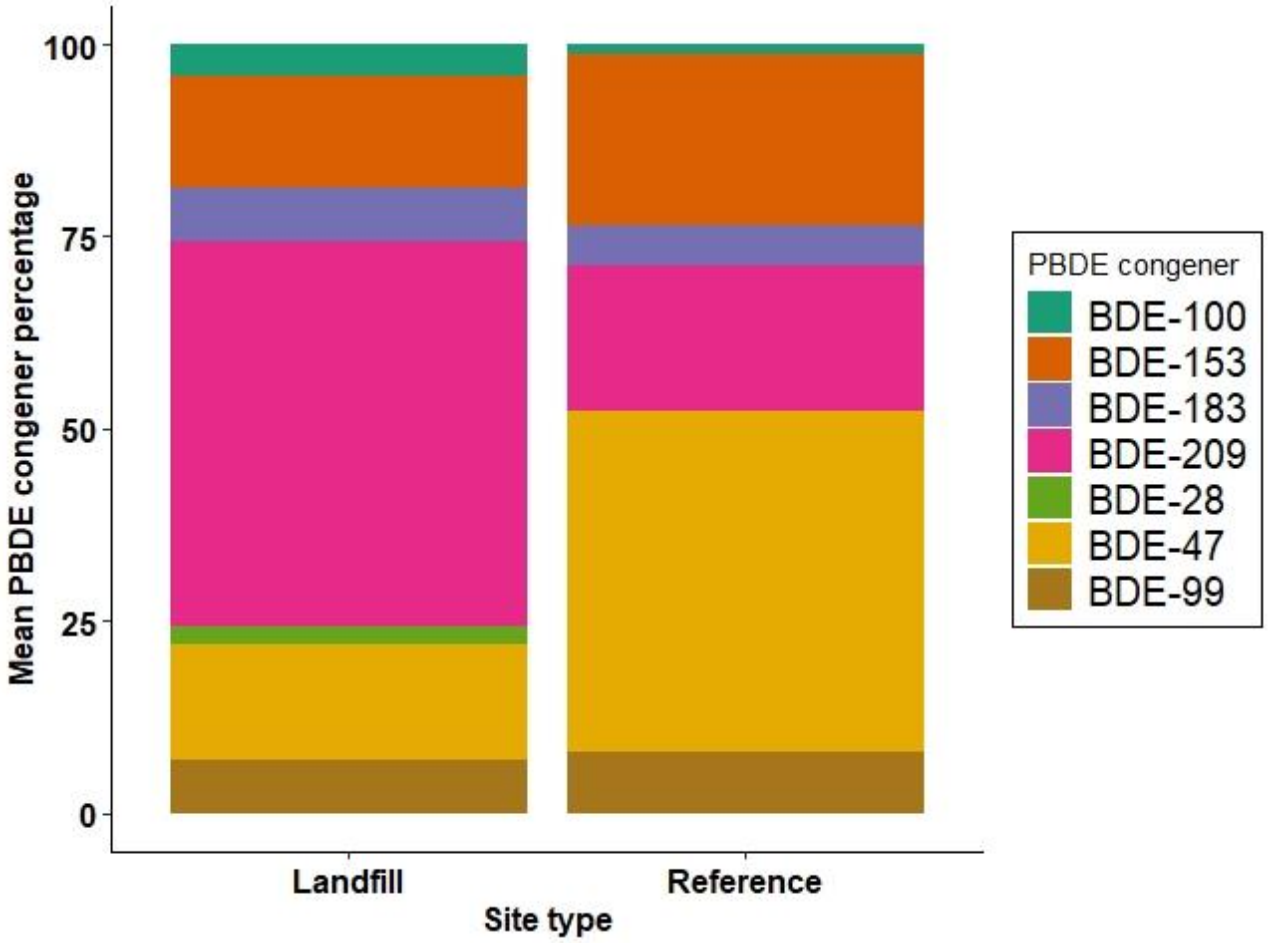

Figure 3.2 Stacked barplot displaying the arithmetic mean percentage composition of eight PBDE congeners in the eggs of landfill (n = 32) and reference (n = 25) herring gulls collected in western Scotland in 2017–18.

3.4.1.3 Herring gull HBCDD concentrations

No significant differences between the eggs of landfill and reference-breeding herring gulls were observed in the case of Total-HBCDD concentrations, as well as for any individual diastereomer (Table 3.1).

3.4.1.4 Herring gull HBCDD diastereomer profiles: landfill vs. reference

The stacked barplot in Figure 3.3 depicts the arithmetic mean percentage composition of α- and γ-HBCDD diastereomers in the eggs of landfill *vs*. reference herring gulls in 2017–18.

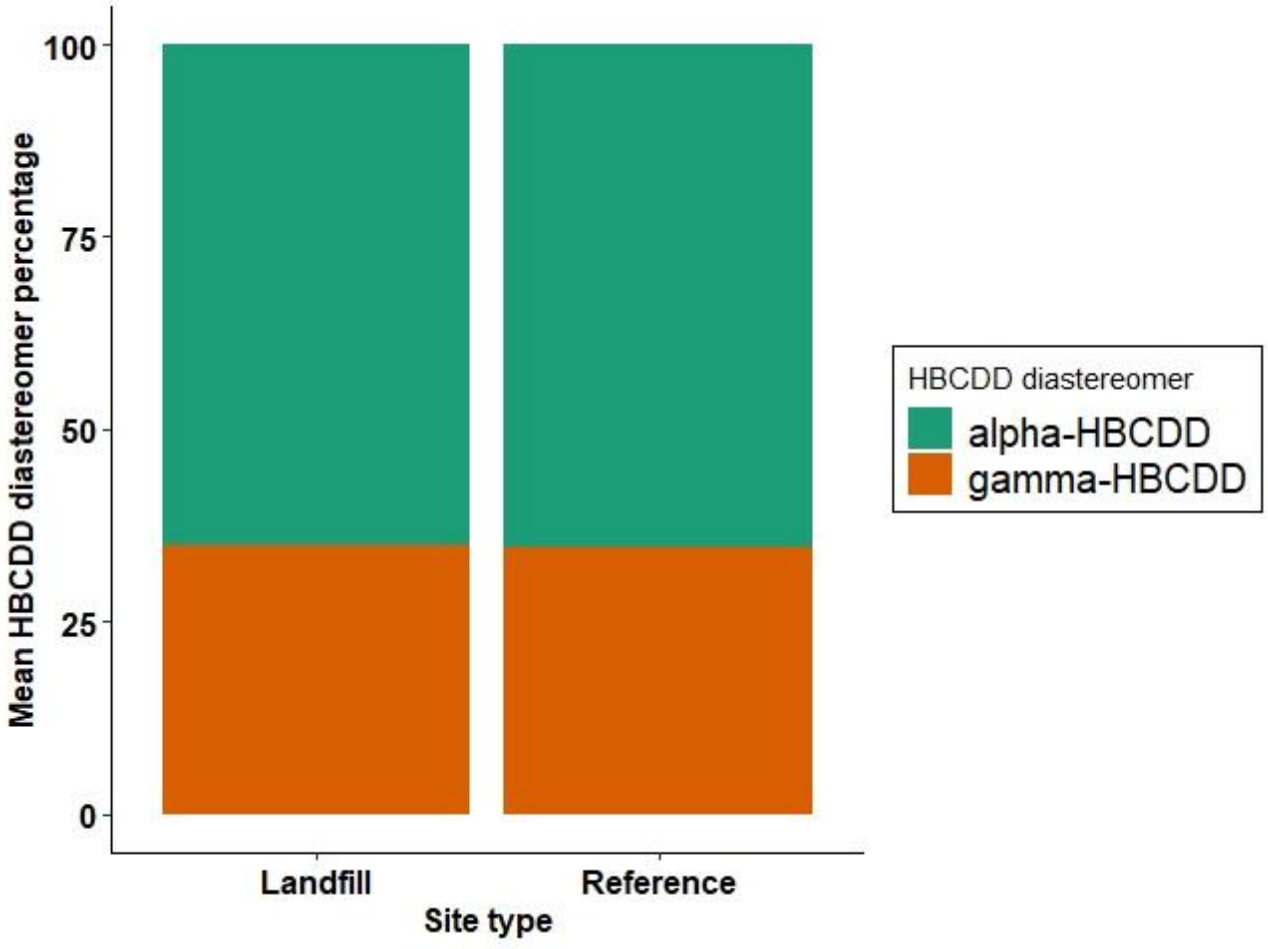

Figure 3.3 Stacked barplot displaying the mean percentage composition of α- and γ- HBCDD diastereomers in the eggs of landfill (n = 32) and reference (n = 25) herring gulls collected in western Scotland during 2017–18.

α-HBCDD was the dominant diastereomer for both colony-types, comprising a mean percentage of 65 % in the eggs of both landfill and reference breeders, respectively.

3.4.1.5 Herring gull NBFR concentrations and profiles

Concentrations of BTBPE, EH-TBB, PBB and PBEB in landfill and reference eggs in both 2017 and 2018 were below lipid weight detection limits (0.6, 41.4, 0.9, 1.5 and 0.1 ng/g lw, respectively; Table 3.1). DBDPE was detected in 31 % of landfill eggs but not in any reference eggs. The highest herring gull DBDPE concentration was 4700 ng/g lw (Table 3.1).

3.4.2 Herring gull egg traits in relation to BFR concentrations and colony type

There was no significant difference between the eggs of landfill (5.2 ± 0.2 %) and reference herring gulls (5.7 ± 0.3 %) in terms of egg lipid fraction, expressed as a percentage of egg wet weight (U = 349.5, P = 0.42). No significant correlation was observed in terms of herring gull egg lipid fraction and the measured BFR concentrations. Mean eggshell thickness for herring gull eggs obtained during 2017–18 was significantly (5 %) higher in the case of reference eggs (mean: 0.35 ± 0.006 mm) compared to those of landfill breeders (0.33 ± 0.004 mm) (U = 253, P = 0.01) (Figure 3.4). For herring gull eggs collected during 2016–18, eggshells were significantly thinner in those eggs containing in excess of 50 ng/g ww $\sum_8$PBDEs (U = 227.5, P = 0.004). This relationship appears to have been driven by concentrations of the environmentally-recalcitrant BDE-153, since this was the only individual PBDE congener to show a significant negative relationship with eggshell thickness (adjusted r-squared = 0.04; d.f. = 71, P = 0.03) (Figure 3.5). Concentrations of none of the other compounds of interest showed a significant relationship with eggshell thickness (for example, in the case of BDE-209, adjusted r-squared = -0.01, d.f. = 71, P = 0.53). There was no significant difference between landfill (86,461.9 ± 1632. mm^3) and reference (90,421.9 ± 1667.5 mm^3) herring gulls in terms of mean egg volume (U = 311, P = 0.15). No significant correlations were found between herring gull egg volume and concentrations of the compounds of interest.

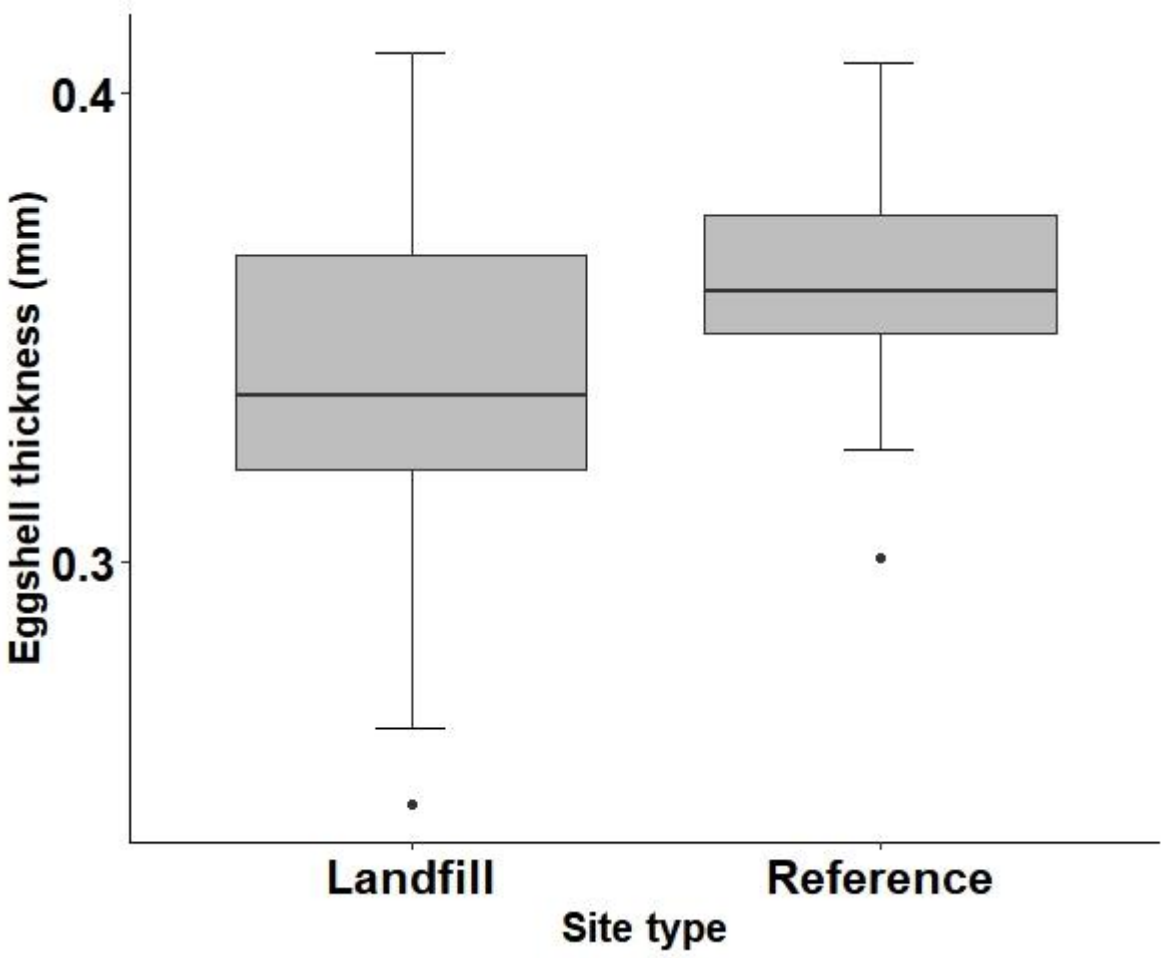

Figure 3.4 Box and whisker plot showing significantly greater eggshell thickness for reference herring gulls (*n* =32) in comparison to landfill-breeding conspecifics (*n* = 25). Eggs collected in western Scotland in 2017–18. Black lines within boxes indicate medians, boxes indicate the 25th and 75th percentiles, whiskers indicate the 10th and 90th percentiles. Points are outliers.

Mean fresh egg weight, dessication-corrected using Hoyt (1979) did not differ significantly between landfill (88308.6 ± 1751.8 mg) and reference (92607.8 ± 1827.4 mg) eggs (*U* =3119, *P* = 0.15). No significant correlation was found between fresh egg weight and concentrations of any target compounds for herring gulls. Mean fresh egg weight, dessication-corrected using Hoyt (1979) did not differ significantly between landfill (88,308.6 ± 1,751.8 mg) and reference (92,607.8 ± 1,827.4 mg) eggs (*U* =3119, *P* = 0.15). No significant correlation was found between fresh egg weight and concentrations of any target compounds for herring gulls.

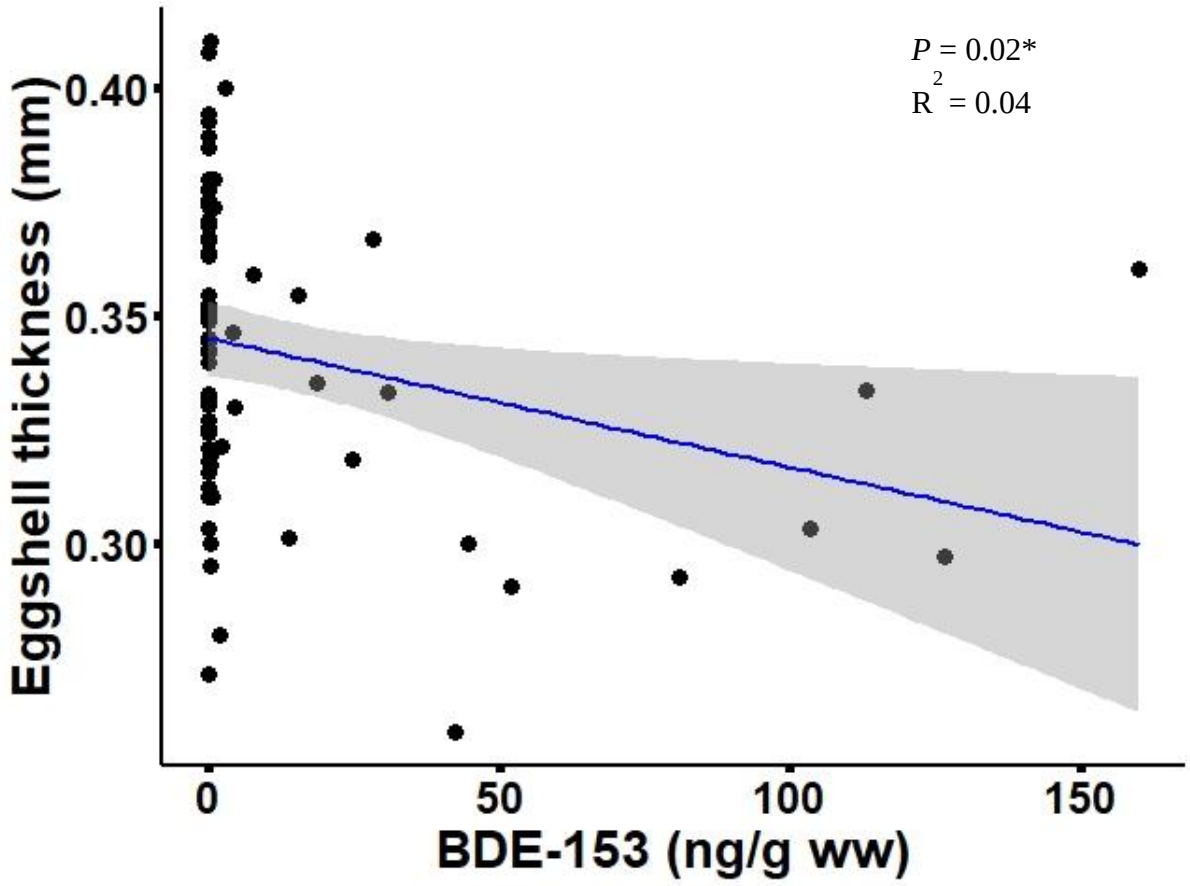

Figure 3.5 Relationship (blue line) between eggshell thickness and concentrations of BDE-153 (ng/g ww) in herring gull eggs collected in western Scotland during 2016-18 (*n* = 73). Grey shading shows standard error of the fitted line.

3.4.3 Intraclutch BFR burdens in herring gulls

Appendix 8 shows the lipid weight concentrations of PBDEs, HBCDD and DBDPE in the eggs of 10 herring gull clutches (3 eggs per clutch; 6 landfill and 4 reference clutches) obtained during 2016–2018. Egg size (expressed via volume) was used to provide a guide as to the first-laid ('A'), second-laid ('B') and third-laid ('C') eggs. 'A' and 'B' eggs are usually the largest eggs in the clutch in large gull species (Nager et al., 2000). There were no significant differences between 'A', 'B' and 'C' eggs in terms of concentrations of all target compounds (all *P* values ≥ 0.09). Similarly, when 'A' and 'B' eggs were combined, no significant differences between 'A'/'B' *vs.* 'C' eggs were found for any compound (all *P* values ≥ 0.1).

3.4.4 BFR concentrations & profiles in other gull species

Tables 3.3–3.6 show the arithmetic mean (± standard error for datasets where $n > 10$), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding and reference black-headed gulls, common gulls, great black-backed gulls and lesser black-backed gulls, respectively, in ng/g lw. Wet weight concentrations for the same samples are provided in Appendices 9–12. Given the disparities between years in terms of the BFR data available for these species, coupled with small sample sizes, it was not possible to undertake meaningful statistical analyses of these data. Any conclusions drawn with regard to differences in BFR contamination between landfill *vs*. reference subjects can therefore only be tentative. For species other than herring gulls, several target compounds were eliminated in the same fashion (i.e., due to detection frequencies being <30 % or where observed concentrations were unlikely to be biologically meaningful). These are displayed in Table 3.2. Detection frequencies for target PBDE congeners and HBCDD diastereomers for all species are shown in Appendix 6.

Table 3.2 Target compounds eliminated for the purposes of statistical analyses in the eggs of black-headed gulls, common gulls, great black-backed gulls and lesser black-backed gulls.

Species	**Target compound(s) eliminated for analyses**
Black-headed gull	BDE-100, BDE-154, β-HBCDD
Common gull	BDE-28, BDE-47 BDE-183, β-HBCDD
Great black-backed gull	β-HBCDD
Lesser black-backed gull	BDE-47, β-HBCDD, γ-HBCDD

Table 3.3 Mean, median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 12; 2016) and reference (*n* = 5; 2017) black-headed gulls collected in western Scotland (ng/g lipid weight).

Compound	Landfill mean ± SE (2016) †	Reference mean (2017)	Landfill median (range) (2016)	Reference median (range) (2017)
$\sum_8$PBDEs inc. BDE-209	125.5 ± 28.5	27.7	107.6 (1.0–394.3)	2.1 (0.9–132.0)
$\sum_7$PBDEs	87.7 ± 15.5	9.3	70.1 (1.0–394.3)	1.9 (0.8–40.7)
BDE-28	10.7 ± 6.7	<1.5	<1.5 (<1.5–95.1)	<1.5 (<1.5– <1.5)
BDE-47	13.7 ± 6.8	2.9	1.8 (<0.7–75.6)	1.7 (<0.7–9.9)
BDE-99	14.3 ± 7.7	5.5	8.7 (<0.7– 56.6)	<0.7 (<0.7–27.3)
BDE-100§	1.3 ± 0.8	<0.7	<0.7 (<0.7–11.6)	<0.7 (<0.7– <0.7)
BDE-153	43.6 ± 27.8	<0.7	2.2 (<0.7– 393.6)	<0.7 (<0.7– <0.7)
BDE-154§	<0.7	<0.7	<0.7 (<0.7– 6.0)	<0.7 (<0.7– <0.7)
BDE-183	3.3 ± 2.3	<0.7	<0.7 (<0.7– 40.0)	<0.7 (<0.7– 3.1)
BDE-209	37.7 ± 10.1	18.3	25.5 (<7.8–131.2)	<7.8 (<7.8–91.2)
∑HBCDD	12.2 ± 3.5	49.7	6.5 (0.7–51.7)	4.1 (0.3–236.6)
α-HBCDD	11.4 ± 3.4	49.6	5.9 (<0.9–50.1)	4.0 (<0.9–236.5)
β-HBCDD§	<1.2	<1.2	<1.2 (<1.2– <1.2)	<1.2 (<1.2– <1.2)
γ-HBCDD	<1.2	<1.2	<1.2 (<1.2–5.7)	<1.2 (<1.2– <1.2)
BTBPE§	<0.6	<0.6	<0.6 (<0.6– <0.6)	<0.6 (<0.6– <0.6)
DBDPE§	41.6 ± 33.6	<41.4	<41.4 (<41.4–482.5)	<41.4 (<41.4– <41.4)
EH-TBB§	<0.9	<0.9	<0.9 (<0.9– <0.9)	<0.9 (<0.9– <0.9)
PBB§	<1.5	<1.5	<1.5 (<1.5– <1.5)	<1.5 (<1.5– <1.5)
PBEB§	<0.1	<0.1	<0.1 (<0.1– <0.1)	<0.1 (<0.1– <0.1)

† Arithmetic mean ± standard error (the latter omitted for reference eggs due to sample size).
<LOD: Below limits of detection.
§ Table 3.2 lists those compounds of interest which were excluded from analyses.
Average lipid weight limits of detection (ng/g lw): BDE-28: 1.5; BDE-47: 0.7; BDE-99: 0.7; BDE-100: 0.7; BDE-153: 0.7; BDE-154: 0.7; BDE-183: 0.7; BDE-209: 7.8; α-HBCDD: 0.9; β-HBCDD: 1.2; γ-HBCDD: 1.2; BTBPE: 0.6; DBDPE: 41.4; EH-TBB: 0.9; PBB: 1.5; PBEB: 0.1.

Table 3.4 The mean (± standard error for landfill), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 14; 2016) and reference (*n* = 6; 2017) common gulls collected in western Scotland (ng/g lipid weight).

Compound	Landfill mean (2016) §	Reference mean (2017)	Landfill median (range) (2016)	Reference median (range) (2017)
$\sum_8$PBDEs inc. BDE-209	586.0 ± 140.4	1.8	271.0 (27.7–1965.0)	1.3 (0.4–4.1)
$\sum_7$PBDEs	457.3 ± 120.6	1.6	264.7 (27.3–1808.68)	1.1 (0.3–3.8)
BDE-28[§]	5.0 ± 2.6	<1.5	<1.5 (<1.5–48.00)	<1.5 (<1.5– <1.5)
BDE-47[§]	18.4 ± 3.5	1.2	19.1 (<0.7–42.2)	<0.7 (<0.7–3.2)
BDE-99	22.3 ± 10.2	<0.7	2.6 (<0.7–132.4)	<0.7 (<0.7– <0.7)
BDE-100	7.0 ± 3.3	<0.7	<0.7 (<0.7–42.6)	<0.7 (<0.7– <0.7)
BDE-153	265.5 ± 88.1	<0.7	142.5 (<0.7–1237.7)	<0.7 (<0.7– <0.7)
BDE-154	138.9 ± 38.5	<0.7	77.0 (<0.7–530.8)	<0.7 (<0.7– <0.7)
BDE-183[§]	<0.7	<0.7	<0.7 (<0.7– <0.7)	<0.7 (<0.7– <0.7)
BDE-209	128.6 ± 59.8	<7.8	25.0 (<7.8– 704.8)	<7.8 (<7.8– <7.8)
∑HBCDD	54.1 ± 16.1	0.06	28.1 (1.0–255.0)	0.06 (0.02–0.09)
α-HBCDD	37.8 ± 9.5	<0.9	23.5 (<0.9–146.6)	<0.9 (<0.9– <0.9)
β-HBCDD[§]	5.3 ± 3.3	<1.2	<1.2 (<1.2–49.7)	<1.2 (<1.2– <1.2)
γ-HBCDD	10.9 ± 4.6	<1.2	<1.2 (<1.2–58.5)	<1.2 (<1.2– <1.2)
BTBPE	<0.62	<0.62	<0.62 (<0.62– <0.62)	<0.62 (<0.62– <0.62)
DBDPE	332.7 ± 271.4	<41.4	<41.4 (<41.4–4211.5)	<41.4 (<41.4– <41.4)
EH-TBB	<0.9	<0.9	<0.9 (<0.9– <0.9)	<0.9 (<0.9– <0.9)
PBB	<1.5	<1.5	<1.5 (<1.5– <1.5)	<1.5 (<1.5– <1.5)
PBEB	<0.1	<0.1	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)

§ Arithmetic mean ± standard error (the latter omitted for reference eggs due to sample size).
n.d.: not detected.
[§] Table 3.2 lists those compounds of interest which were excluded from analyses.
[†] Average lipid weight limits of detection (ng/g lw): BDE-28: 1.5; BDE-47: 0.7; BDE-99: 0.7; BDE-100: 0.7; BDE-153: 0.7; BDE-154: 0.7; BDE-183: 0.7; BDE-209: 7.8; α-HBCDD: 0.9; β-HBCDD: 1.2; γ-HBCDD: 1.2; BTBPE: 0.6; DBDPE: 41.4; EH-TBB: 0.9; PBB: 1.5; PBEB: 0.1.

Table 3.5 The mean, median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 7; 2016–18) and reference (*n* = 8; 2017 and 2018) great black-backed gulls collected in western Scotland (ng/g lipid weight).

Compound	Landfill mean (2016–18) †	Reference mean (2017–18) †	Landfill median (range) (2016–18)	Reference median (range) (2017–18)
$\sum_8$PBDEs inc. BDE-209	641.4	202.9	248.8 (45.8–2106.2)	139.0 (24.4–705.6)
$\sum_7$PBDEs	505.6	178.3	184.0 (45.7–1791.7)	118.0 (18.6–574.8)
BDE-28	27.9	<1.5	<1.5 (<1.5–109.1)	<1.5 (<1.5– <1.5)
BDE-47	57.0	113.8	45.4 (6.0–127.0)	62.8 (9.7–363.1)
BDE-99	304.2	13.2	56.3 (3.5–1210.8)	2.9 (<0.7–52.7)
BDE-100	72.8	24.8	17.4 (<0.7–261.5)	21.9 (<0.7–61.4)
BDE-153	27.9	12.8	22.2 (6.3–59.0)	4.7 (<0.7–46.4)
BDE-154	6.7	11.7	5.9 (1.5–15.4)	3.8 (<0.7–58.1)
BDE-183	9.1	1.8	9.8 (1.4–15.5)	<0.78 (<0.78–14.9)
BDE-209	135.7	24.5	69.3 (<7.8–314.4)	<7.8 (<7.8–130.8)
∑HBCDD	163.2	24.1	179.1 (43.0–251.5)	18.0 (4.8–49.4)
α-HBCDD‡	86.3	24.0	92.0 (15.8–145.3)	17.9 (4.7–49.3)
β-HBCDD§‡	<1.2	<1.2	<1.2 (<1.2–<1.2)	<1.2 (<1.2–<1.2)
γ-HBCDD‡	76.9	<1.2	4.4 (2.5–6.6)	<1.2 (<1.2– <1.2)
BTBPE	<0.04	<0.04	<0.04 (<0.04– <0.04)	<0.04 (<0.04– <0.04)
DBDPE	1103.6	<41.4	<41.4 (<41.4–7724.2)	<41.4 (<41.4–<41.4)
EH-TBB	<0.9	<0.9	<0.9 (<0.9– <0.9)	<0.9 (<0.9– <0.9)
PBB	<1.5	<1.5	<1.5 (<1.5– <1.5)	<1.5 (<1.5– <1.5)
PBEB	<0.1	<0.1	<0.1 (<0.1– <0.1)	<0.1 (<0.1– <0.1)

† Arithmetic mean.

§ Table 3.2 lists those compounds of interest which were excluded from analyses.

Average lipid weight limits of detection (ng/g lw): BDE-28: 1.5; BDE-47: 0.7; BDE-99: 0.7; BDE-100: 0.7; BDE-153: 0.7; BDE-154: 0.7; BDE-183: 0.7; BDE-209: 7.8; α-HBCDD: 0.9; β-HBCDD: 1.2; γ-HBCDD: 1.2; BTBPE: 0.6; DBDPE: 41.4; EH-TBB: 0.9; PBB: 1.5; PBEB: 0.1. ‡ HBCDD data obtained for 7 eggs, comprised of 4 landfill (from 2016) and three reference eggs (from 2017).

Table 3.6 The mean (± standard error), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 11; 2016) and reference (*n* = 2; 2018) lesser black-backed gulls collected in western Scotland (ng/g lipid weight).

Compound	Landfill mean (± SE) (2016) †	Reference mean 2018) †	Landfill median (range)	Reference median (range)
$\sum_8$PBDEs inc. BDE-209	1109.5 ± 483.1	160.0	395.0 (52.4–6804.0)	160.0 (1.4–318.6)
$\sum_7$PBDEs	966.4 ± 474.4	13.0	219.7 (37.1–6573.2)	13.0 (1.0–25.0)
BDE-28	3.1 ± 1.1	<1.5	<1.5 (<1.5–14.1)	<1.5 (<1.5– <1.5)
BDE-47§	17.1 ± 3.4	12.5	12.4 (1.0–36.9)	12.5 (<0.7–24.5)
BDE-99	117.6 ± 42.0	<0.7	35.6 (<0.7– 565.9)	<0.7 (<0.7– <0.7)
BDE-100	22.2 ± 6.2	0.2	8.4 (<0.7–76.0)	0.2 (0.2–0.2)
BDE-153	512.3 ± 301.7	<0.7	9.4 (<0.7–3976.2)	<0.7 (<0.7– <0.7)
BDE-154	229.9 ± 155.1	<0.7	2.2 (<0.7–2127.9)	<0.7 (<0.7– <0.7)
BDE-183	63.9 ± 32.1	<0.7	<0.7 (<0.7–430.6)	<0.7 (<0.7– <0.7)
BDE-209	143.0 ± 48.5	147.0	16.7 (<7.8–582.1	147.0 (<0.7–293.5)
∑HBCDD	36.8 ± 11.8	249.9	7.9 (1.1–141.1)	249.9 (163.7–336.2)
α-HBCDD	28.6 ± 9.2	106.1	7.7 (1.0–124.8)	106.1 (86.9–125.3)
β-HBCDD§	<1.2	20.6	<1.2 (<1.2–<1.2)	20.6 (<1.2–41.2)
γ-HBCDD§	8.1 ± 4.7	123.1	<1.2 (<1.2–48.3)	123.1 (76.78–169.53)
BTBPE	<0.6	<0.6	<0.6 (<0.6–<0.6)	<0.6 (<0.6–<0.6)
DBDPE	<41.4	<41.4	<41.4 (<41.4– 68.4)	<41.4 (<41.4–<41.4)
EH-TBB	<0.9	<0.9	<0.9 (<0.9–<0.9)	<0.9 (<0.9–<0.9)
PBB	<1.5	<1.5	<1.5 (<1.5–<1.5)	<1.5 (<1.5–<1.5)
PBEB	<0.1	<0.1	<0.1	<0.1

† Arithmetic mean ± standard error (the latter omitted for reference eggs due to sample size).
<LOD: Below limit of detection.
§ Table 3.2 lists those compounds of interest which were excluded from analyses.
Average lipid weight limits of detection (ng/g lw): BDE-28: 1.5; BDE-47: 0.7; BDE-99: 0.7; BDE-100: 0.7; BDE-153: 0.7; BDE-154: 0.7; BDE-183: 0.7; BDE-209: 7.8; α-HBCDD: 0.9; β-HBCDD: 1.2; γ-HBCDD: 1.2; BTBPE: 0.6; DBDPE: 41.4; EH-TBB: 0.9; PBB: 1.5; PBEB: 0.1.

3.4.4.1 PBDE concentrations & profiles: landfill vs. reference

The stacked barplots in Figure 3.6 show the arithmetic mean percentage composition of individual PBDE congeners (as a constituent of $\sum_8$PBDEs) in the eggs of landfill *vs.* reference black-headed gulls (A), common gulls (B), great black-backed gulls (C) and lesser black-backed gulls (D) collected during 2016–18. For black-headed gulls, the three most prevalent congeners for landfill breeders were BDE-209 (43 %), BDE-99 (18 %), BDE-153 (16 %), with BDE-47 (55 %), BDE-209 (21%) and BDE-183 (12 %) being the equivalent for reference birds. In the case of common gulls, the top three congeners were BDE-153 (34 %), BDE-209 (24 %) and BDE-154 (23 %) for landfill birds, compared to BDE-209 (43 %), BDE-153 (17 %) and BDEs -99, -100 and -154 (all 13 %) for reference conspecifics. In landfill-breeding great black-backed gulls, BDE-99 (32 %), BDE-209 (23 %) and BDE-47 (17 %) were the three most important congeners, with the equivalent congeners in the case of reference birds being BDE-47 (52 %), BDE-100 (19 %) and BDE-209 (11 %). For lesser black-backed gulls, the top three congeners in landfill breeders were BDE-99 (34 %), BDE-209 (25 %) and BDE-153 (14 %), with those for reference birds being BDE-209 (73 %), BDE-100 (12 %) and BDEs -28, -99 and -153 (all 3 %).

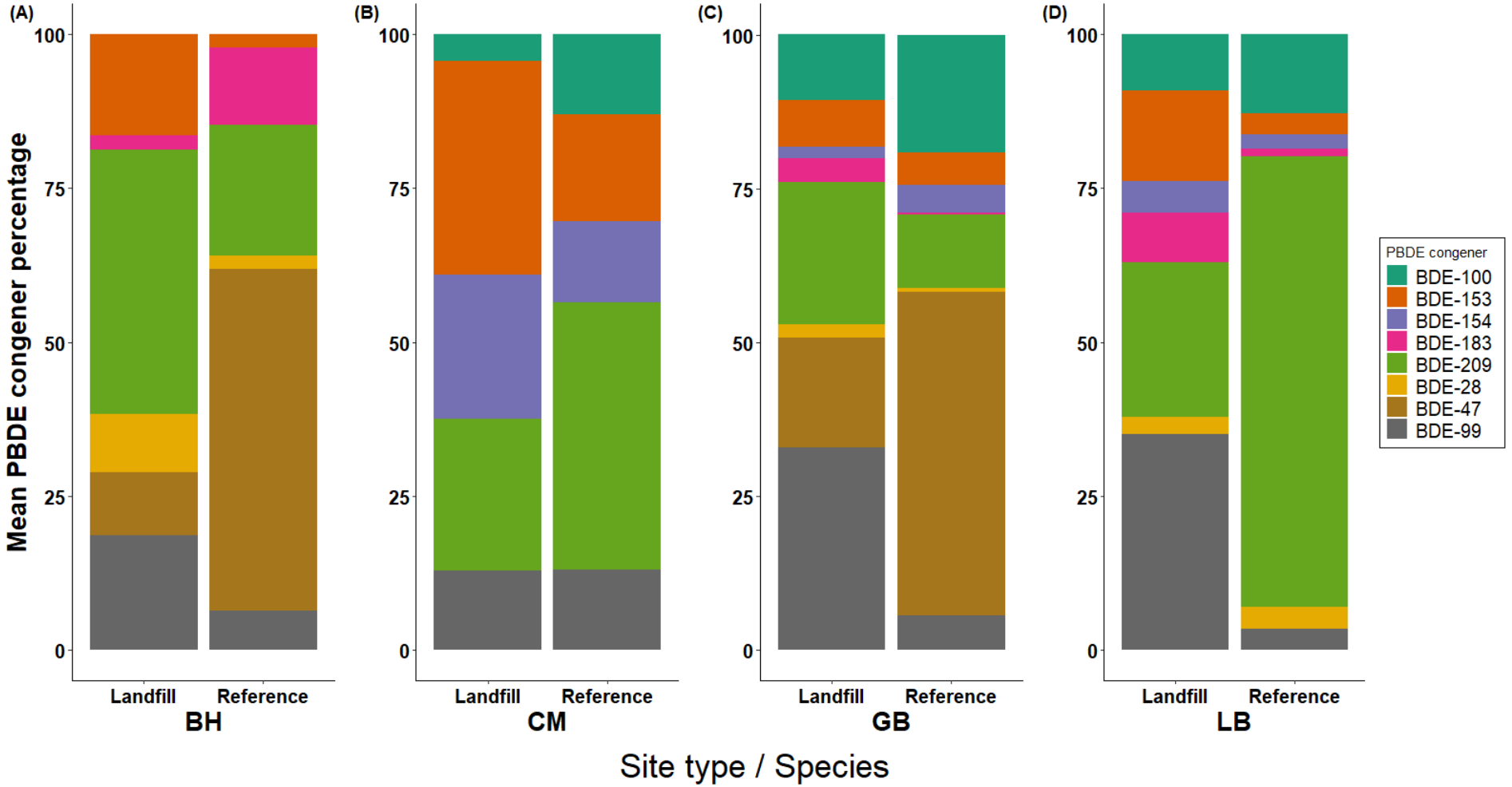

Figure 3.6 Stacked barplot displaying the mean percentage composition of eight PBDE congeners in the eggs of landfill and reference breeding black-headed gulls during (BH; *n* = 12 and 5, respectively), common gulls (CM; *n* = 14 and 6, respectively), great black-backed gulls (GB; *n* = 7 and 8, respectively) and lesser black-backed gulls (LB; *n* = 11 and 2, respectively) collected in western Scotland (UK) during 2016–18.

3.4.4.2 HBCDD diastereomer profiles: landfill vs. reference

The stacked barplots in Figure 3.7 depict the arithmetic mean percentage composition of α-, β- and γ- HBCDD diastereomers (as a constituent of Total-HBCDD) in the eggs of landfill *vs.* reference black-headed gulls (A), common gulls (B), great black-backed gulls (C) and lesser black-backed gulls (D) collected during 2016–18. In terms of great black-backed gulls, HBCDD data were available for only seven (four landfill *vs.* three reference) eggs. In black-headed gulls, α-HBCDD was the predominant diastereomer, comprising on average 85–96 % of Total-HBCDD. In common gulls, α-HBCDD was dominant in the eggs of landfill breeders, but in reference eggs, γ-HBCDD comprised on average 100% of Total-HBCDD. Reference great black-backed gull eggs were dominated by α-HBCDD (99% of Total-HBCDD, on average) but landfill eggs contained similar mean proportions of α-HBCDD (48 %) and γ-HBCDD (51 %). In lesser black-backed gulls, α-HBCDD dominated both landfill and reference eggs (comprising on average 100 % of Total-HBCDD).

3.4.4.3 NBFR concentrations and profiles

In terms of the measured NBFRs, only DBDPE was detected, being present only in the eggs of landfill breeding black-headed gulls, common gulls, great black-backed gulls and lesser black-backed gulls.

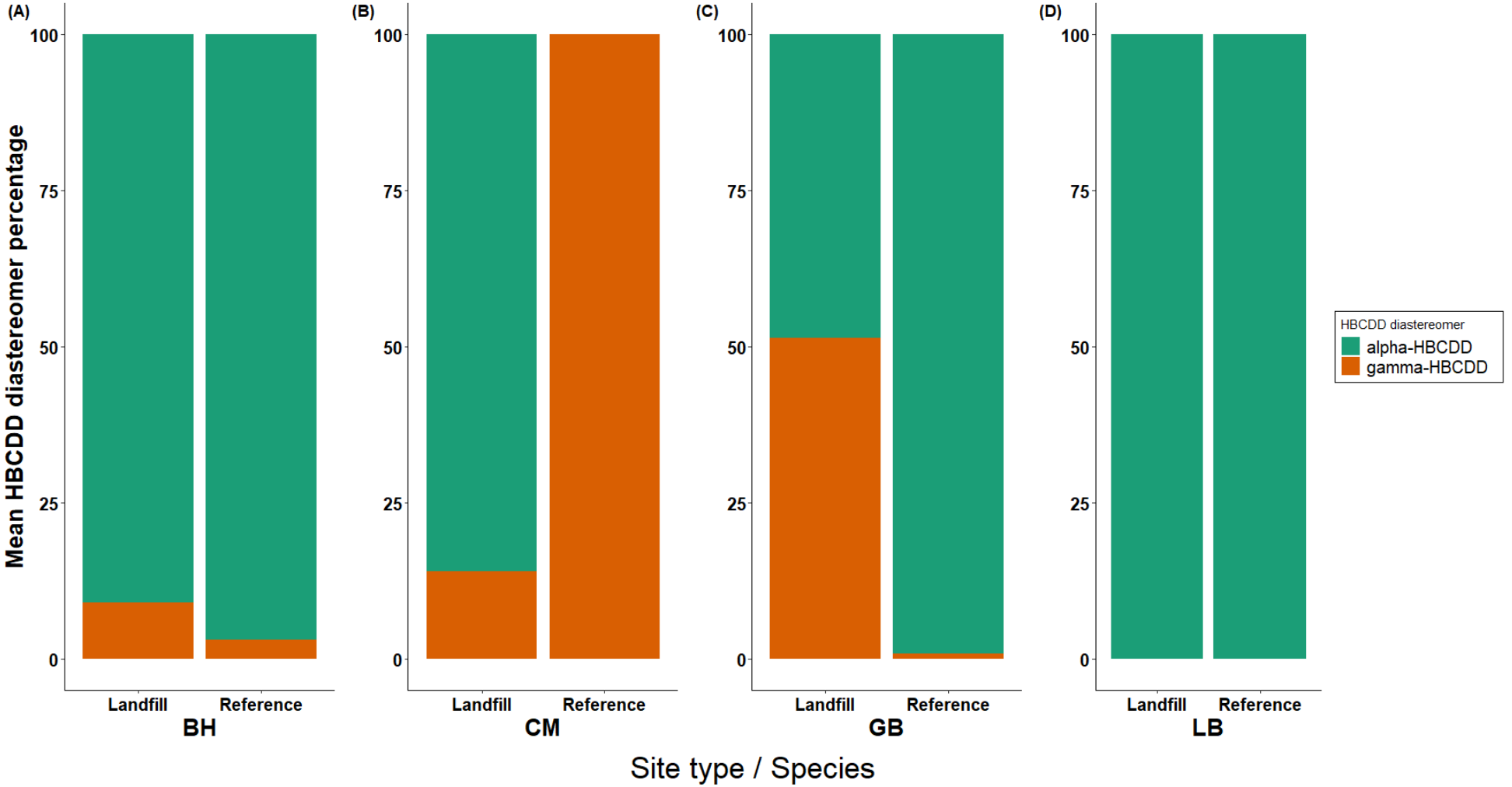

Figure 3.7 Stacked barplot displaying the mean percentage composition of α- and γ- HBCDD diastereomers in the eggs of landfill and reference breeding black-headed gulls (BH; *n* = 12 and 5, respectively), common gulls (CM; *n* = 14 and 6, respectively), great black-backed gulls (GB; *n* = 4 and 3, respectively) and lesser black-backed gulls (LB; *n* = 11 and 2, respectively) collected in western Scotland (UK) during 2016–18.

3.4.5 Interspecies comparisons of BFR egg concentrations in landfill-breeding gulls

Interspecies differences in BFR egg concentrations for landfill-only colonies were analysed using 2016 data (i.e., the year for which the most complete data were available). Sample sizes were: black-headed gulls n = 12, common gulls n = 14, great black-backed gulls n = 4, herring gulls n = 16 and lesser black-backed gulls n = 11) (Table 3.7). The output of statistical analyses should be interpreted with caution given the small sample sizes for some species.

3.4.5.1 PBDE concentrations in landfill-breeding gulls (2016)

Notwithstanding the small sample sizes for great black-backed gulls, there were significant overall interspecies differences in terms of egg concentrations of $\sum_8$PBDEs for landfill breeding gulls (χ^2 = 9.78, d.f. = 4, P = 0.04) (Figure 3.8), although there were no significant differences in $\sum_8$PBDE levels among individual species (post-hoc test P values ≥ 0.052). In terms of individual PBDE congeners, significant overall interspecies differences existed in terms of BDE-28 (χ^2 = 33.9, d.f. = 4, P <0.001), BDE-47 (χ^2 = 47.97, d.f. = 4, P <0.001), BDE-99 (χ^2 = 10.99, d.f. = 4, P = 0.02) , BDE-100 (χ^2 = 18.28, d.f. = 4, P = <0.001), BDE-154 (χ^2 = 30.35, d.f. = 4, P <0.009) and BDE-183 (χ^2 = 38.61, d.f. = 4, P <0.001) (Figure 3.9 A–F), with significant differences among species evident for these congeners. Post-hoc tests showed that for BDE-28, black-headed gulls, great black-backed gulls, herring gulls and lesser black-backed gulls all had significantly higher egg concentrations compared to common gulls (all P values < 0.001). For BDE-47, black-headed gulls, great black-backed gulls and herring gulls had significantly higher egg concentrations than common gulls (all P values < 0.001), with great black-backed gulls and lesser black-backed gulls also having significantly higher levels than black-headed gulls (P values ≤ 0.03). Great black-backed gulls exhibited significantly elevated egg concentrations of BDE-99 compared to common gulls (P = 0.04). For BDE-100, common gulls, great black-backed gulls, herring gulls and lesser black-backed gulls all showed significantly higher concentrations compared to black-headed gulls (all P values ≤ 0.01). In the case of BDE-154, common gulls, great black-backed gulls, herring gulls and lesser black-backed gulls all

showed significantly higher levels than black-headed gulls (all P values ≤ 0.004). For BDE-183, concentrations in common gull eggs were significantly below those in the eggs of black-headed gulls, great black-backed gulls, herring gulls and lesser black-backed gulls (all P values < 0.001), with herring gulls also showing higher concentrations than black-headed gulls ($P = 0.01$) (Table 3.8).

Table 3.7 Concentrations (arithmetic mean ± SE and range[§]) in ng/g lw of the BFR compounds of interest in the eggs of five species of gull breeding within 2 km of a landfill in western Scotland in 2016.

BFR compound	Black-headed gull	Common gull	Great black-backed gull	Herring gull	Lesser black-backed gull
	***n* = 12**	***n* = 14**	***n* = 4**	***n* = 16**	***n* = 11**
$\sum_8$PBDEs inc. BDE-209	123.5 ± 33.8 (0.9–394.2)	562.5 ± 154.6 (0.6–1942.8)	997.7 (45.8–2106.2)	783.3 ± 227.5 (17.7–2933.8)	1092.3 ± 600.9 (50.3–6796)
$\sum_7$PBDEs exc. BDE-209	85.7 ± 32.5 (0.3–393.8)	433.9 ± 133.1 (0.1–1786.4)	799.1 (45.7–1791.7)	616.7 ± 213.7 (3.3–2932.6)	949.3 ± 590.2 (0.1–6565.1)
BDE-28	10.7 ± 8.0 (<1.5–95.1)	-	48.5 (<1.5–109.1)	7.3 ± 2.3 (<1.5–24.4)	3.1 ± 1.4 (<1.5–14.1)
BDE-47	13.7 ± 8.1 (<0.7–75.6)	-	63.5 (6.0–127.0)	21.6 ± 7.3 (<0.7–112.3)	-
BDE-99	14.3 ± 5.2 (<0.7–56.6)	22.3 ± 11.3 (<0.7–132.4)	512.3 (21.2–1210.8)	147.8 ± 72.0 (<0.7–1114.2)	117.6 ± 52.2 (<0.7–565.9)
BDE-100	-	7.0 ± 3.7 (<0.7–42.6)	120.2 (8.8–261.5)	29.5 ± 11.6 (<0.7–155.9)	22.2 ± 7.8 (<0.7–76.0)
BDE-153	43.6 ± 33.1 (<0.7–393.6)	265.5 ± 97.1 (<0.7–1237.7)	37.9 (6.3–59.0)	307.5 ± 157.7 (<0.7–2049.6)	512.3 ± 375.1 (<0.7–3976.2)
BDE-154	-	138.9 ± 42.4 (<0.7–530.8)	9.0 (2.0–15.4)	33.3 ± 19.8 (<0.7–285.5)	229.9 ± 192.8 (<0.7–2127.9)
BDE-183	3.3 ± 3.3 (<0.7–40.0)	-	7.5 (1.1–10.3)	69.4 ± 40.4 (<0.7–573.4)	63.9 ± 39.9 (<0.7–430.6)
BDE-209	37.7 ± 12.1 (<7.8–131.2)	128.6 ± 65.9 (<7.8–704.8)	198.6 (<7.8–314.4)	166.6 ± 61.6 (<7.8–930.7)	143.0 ± 60.3 (<7.8–582.1)
Total-HBCDD	12.2 ± 4.2 (0.7–51.7)	54.1 ± 17.7 (1.0–255.0)	163.2 (43.0–251.5)	374.6 ± 239.6 (1.0–3753.4)	36.8 ± 14.7 (1.1–141.1)
α-HBCDD	11.3 ± 4.0 (<0.9–50.1)	37.8 ± 10.1 (<0.9–146.6)	86.3 (15.8–145.3)	163.2 ± 102.6 (<0.9–1660.4)	28.6 ± 11.5 (1.0–124.8)
β-HBCDD	<1.2 (<1.2–<1.2)	5.3 ± 3.6 (<1.2–49.7)	<1.2 (<1.2–<1.2)	38.6 ± 38.6 (<1.2–618.3)	<1.2 (<1.2–<1.2)
γ-HBCDD	<1.2 (<1.2–5.7)	10.9 ± 5.1 (<1.2–58.5)	76.9 (27.2–123.3)	172.8 ± 104.6 (<1.2–1474.5)	8.1 ± 2.4 (<1.2–48.3)
BTBPE[†]	<0.6 (<0.6–<0.6)	<0.6 (<0.6–<0.6)	<0.6 (<0.6–<0.6)	<0.6 (<0.6–<0.6)	<0.6 (<0.6–<0.6)
DBDPE[†]	41.6 ± 40.0 (<41.4–82.5)	332.7 ± 299.1 (<41.4–4211.5)	1931.1 (<41.4–7724.2)	<41.4 (<41.4–57.2)	<41.4 (<41.4–68.4)
EH-TBB[†]	<0.9 (<0.9–<0.9)	<0.9 (<0.9–<0.9)	<0.9 (<0.9–<0.9)	<0.9 (<0.9–<0.9)	<0.9 (<0.9–<0.9)
PBB[†]	<1.5 (<1.5–<1.5)	<1.5 (<1.5 –<1.5)	<1.5 (<1.5 –<1.5)	<1.5 (<1.5 –<1.5)	<1.5 (<1.5 –<1.5)
PBEB[†]	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)

[§] standard error omitted where *n* = <10.

<LOD: Below limit of detection.

Dashes indicate compounds which were eliminated for purposes of analysis; see Table 3.2.

[†] Average lipid weight limits of detection (ng/g lw): BDE-28: 1.5; BDE-47: 0.7; BDE-99: 0.7; BDE-100: 0.7; BDE-153: 0.7; BDE-154: 0.7; BDE-183: 0.7; BDE-209: 7.8; α-HBCDD: 0.9; β-HBCDD: 1.2; γ-HBCDD: 1.2; BTBPE: 0.6; DBDPE: 41.4; EH-TBB: 0.9; PBB: 1.5; PBEB: 0.1.

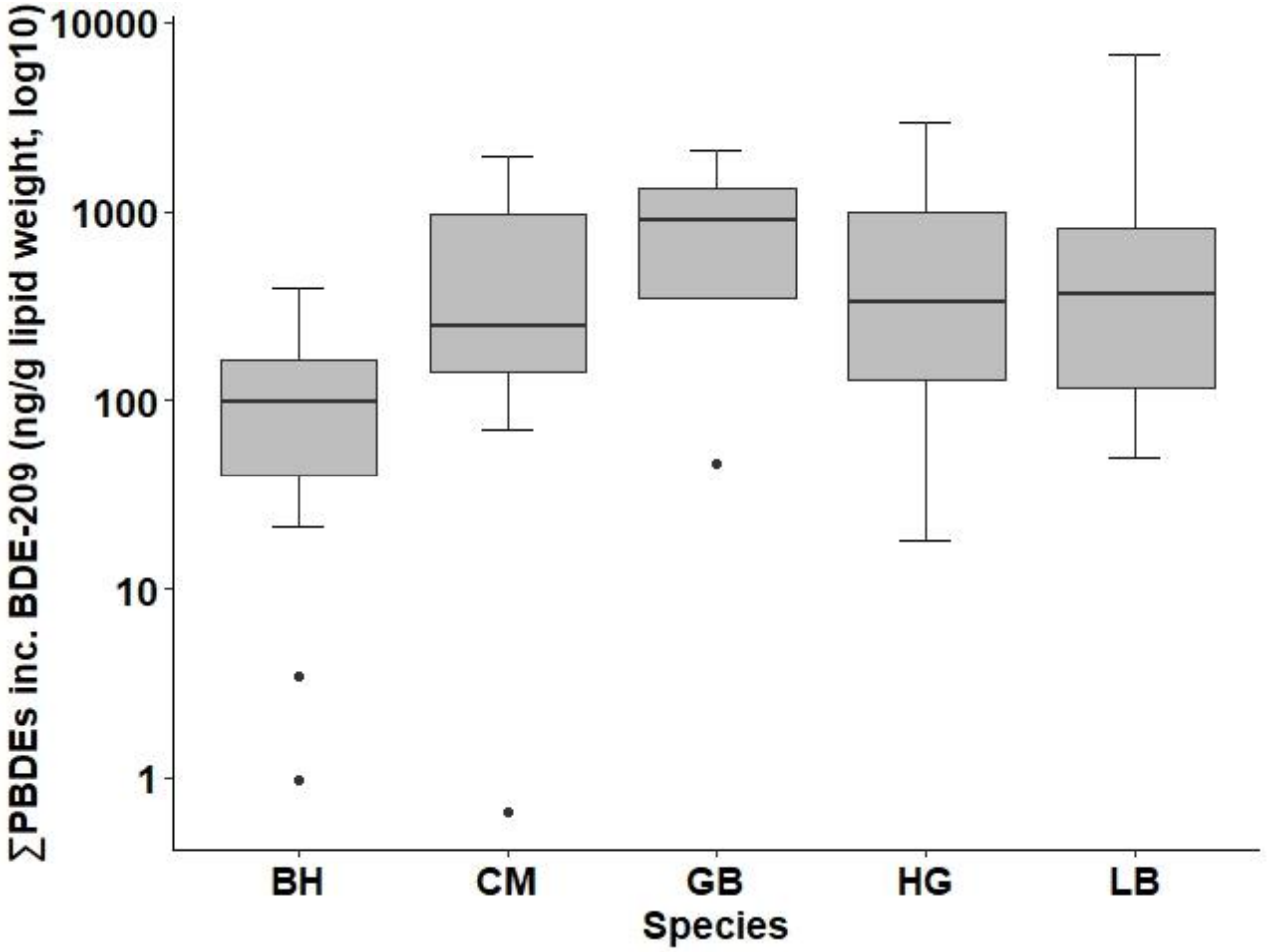

Figure 3.8 Box and whisker plots showing $\sum_8$PBDEs (log_{10}-transformed) in the eggs of five gull species breeding in proximity to landfill in Scotland (2016). Black lines within boxes indicate median values, boxes indicate the 25th and 75th percentiles, whiskers indicate the 10th and 90th percentiles. Points are outliers. BH: black-headed gulls (*n* = 12); CM: common gulls (*n* = 14); GB: great black-backed gulls (*n* = 4); HG: herring gulls (*n* = 16); LB: lesser black-backed gulls (*n* = 11).

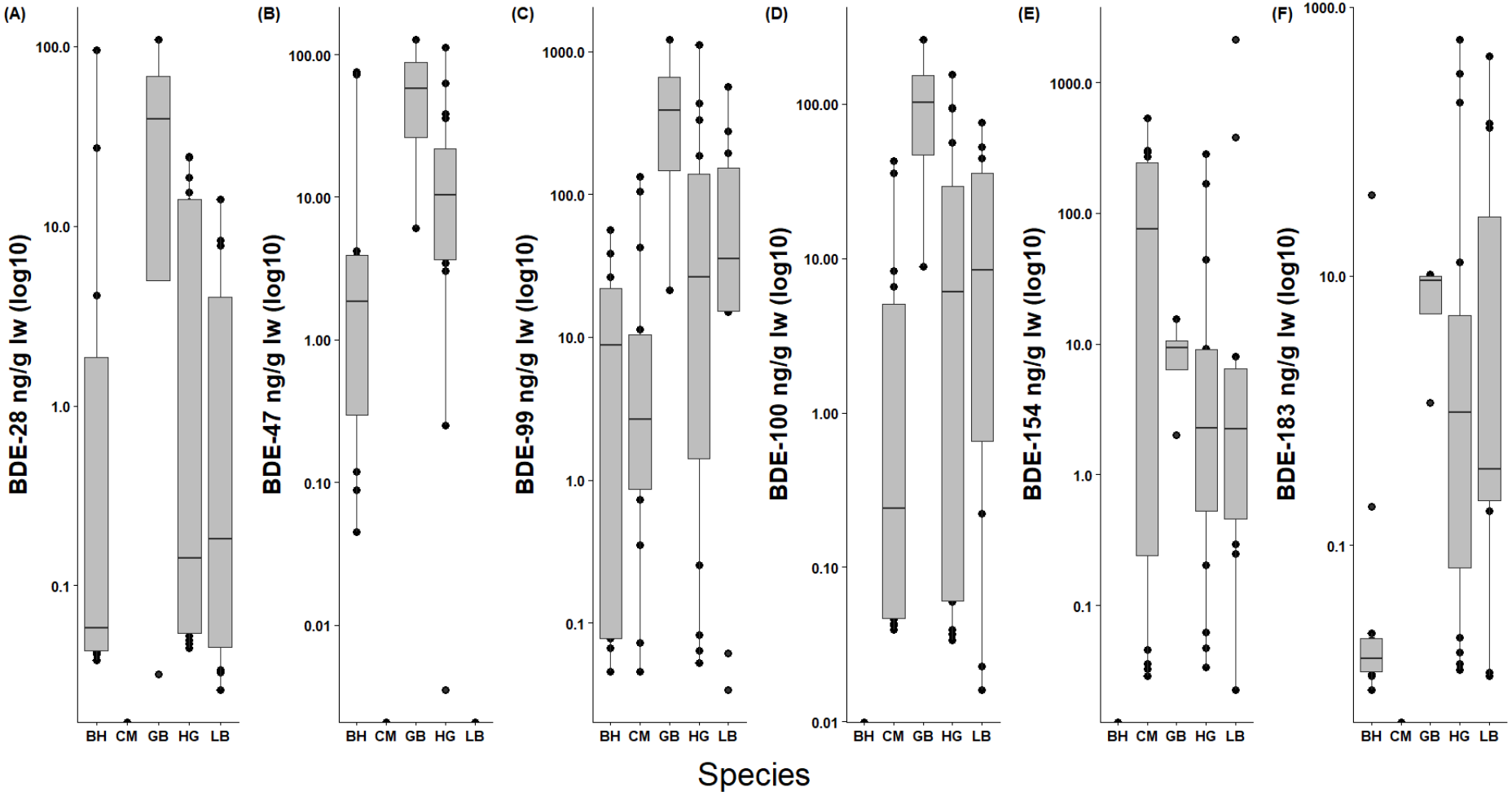

Figure 3.9 Box and whisker plots showing those PBDE congeners (log_{10}-transformed) for which there were significant differences in concentrations between species in the eggs of landfill-breeding gulls collected in Scotland during 2016 (A: BDE-28, B: BDE-47, C: BDE-99, D: BDE-100, E: BDE-154, F: BDE-183). Black lines within boxes indicate the median values, boxes indicate the 25th and 75th percentiles; whiskers indicate the 10th and 90th percentiles. BH: black-headed gulls (*n* = 12); CM: common gulls (*n* = 14); GB: great black-backed gulls (*n* = 4); HG: herring gulls (*n* = 16); LB: lesser black-backed gulls (*n* = 11).

Table 3.8 Post-hoc comparisons using Pairwise Wilcoxon Rank Sum test, Holm-adjusted, following significant interspecific comparisons of concentrations of BDE-99, BDE-100, BDE-154 and BDE-183 in the eggs of five gull species breeding in proximity to a landfill in western Scotland in 2016 (BH: black-headed gull, CM: common gull, GB: great black-backed gull, HG: herring gull, LB: lesser black-backed gull).

PBDE congener	**Pairwise comparison**	***P***
BDE-28	BH *vs*. CM	<0.001
	GB *vs*. CM	<0.001
	HG *vs*. CM	<0.001
	LB *vs*. CM	<0.001
BDE-47	BH *vs*. CM	<0.001
	GB *vs*. BH	0.03
	LB *vs*. BH	<0.001
	GB *vs*. CM	<0.001
	HG *vs*. CM	<0.001
	GB *vs*. LB	0.001
	LB *vs*. HG	<0.001
BDE-99	GB *vs*. CM	0.04
BDE-100	CM *vs*. BH	<0.001
	GB *vs*. BH	0.01
	HG *vs*. BH	<0.001
	LB *vs*. BH	<0.001
BDE-154	CM *vs*. BH	0.004

	GB *vs*. BH	0.001
	HG *vs*. BH	<0.001
	LB *vs*. BH	<0.001
BDE-183	BH *vs*. CM	<0.001
	HG *vs*. BH	0.01
	GB *vs*. CM	<0.001
	HG *vs*. CM	<0.001
	LB vs. CM	<0.001

3.4.5.2 PBDE congener composition in the eggs of landfill-breeding gulls (2016)

The stacked barplot in Figure 3.10 displays the mean percentage composition relative contribution of eight PBDE congeners in the eggs of landfill-breeding black-headed gulls, common gulls great black-backed gulls, herring gulls and lesser black-backed gulls (2016 only). The dominant congeners were BDE-99, BDE-153 and BDE-209, although with a substantial average proportion of BDE-154 (20 %) in the eggs of common gulls.

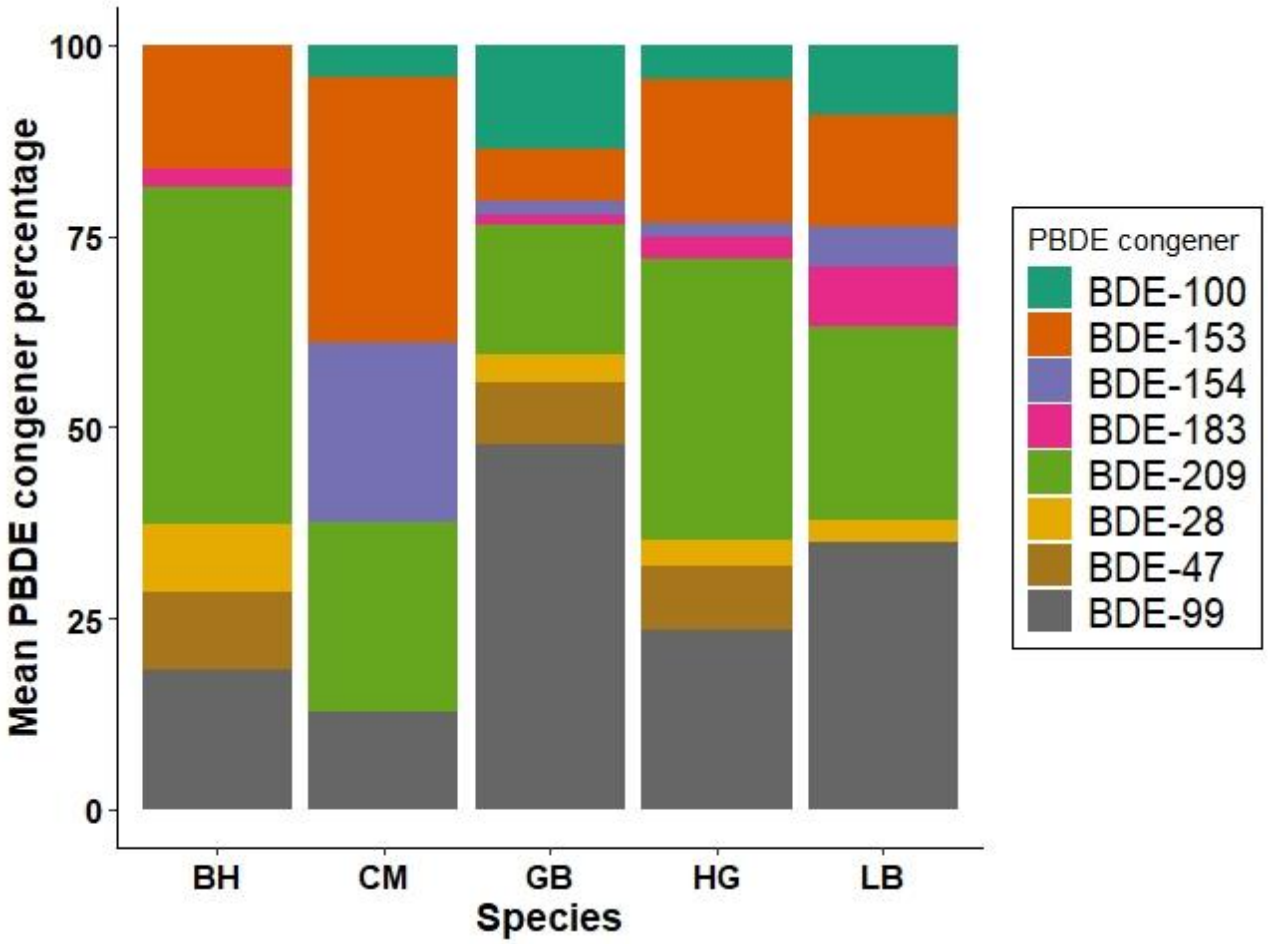

Figure 3.10 Stacked barplot displaying the mean percentage relative contribution of eight PBDE congeners in the eggs of landfill-breeding only gulls of five species collected in western Scotland in the same year, i.e., 2016. BH: black-headed gull (*n* = 12), CM: common gull (*n* = 14), GB: great black-backed gull (*n* = 4), HG: herring gull (*n* = 16), LB: lesser black-backed gull (*n* = 11).

3.4.5.3 HBCDD concentrations in landfill-breeding gulls (2016)

In 2016, there were significant interspecies differences in terms of Total-HBCDD egg concentrations in landfill-breeding birds (χ^2 = 15.19, d.f. = 4, *P* = 0.004) (Figure 3.11). Post-hoc testing identified a significant difference between great black-backed gulls and black-headed gulls in terms of Total-HBCDD egg concentrations (*P* = 0.02). Significant interspecies differences were identified for concentrations of α-HBCDD (χ^2 = 10.50, d.f. = 4, *P* = 0.03) and γ-HBCDD (χ^2 = 40.06, d.f. = 4, *P* <0.001) (Figure 3.12). Removing great black-backed gulls (due to their small sample size) resulted

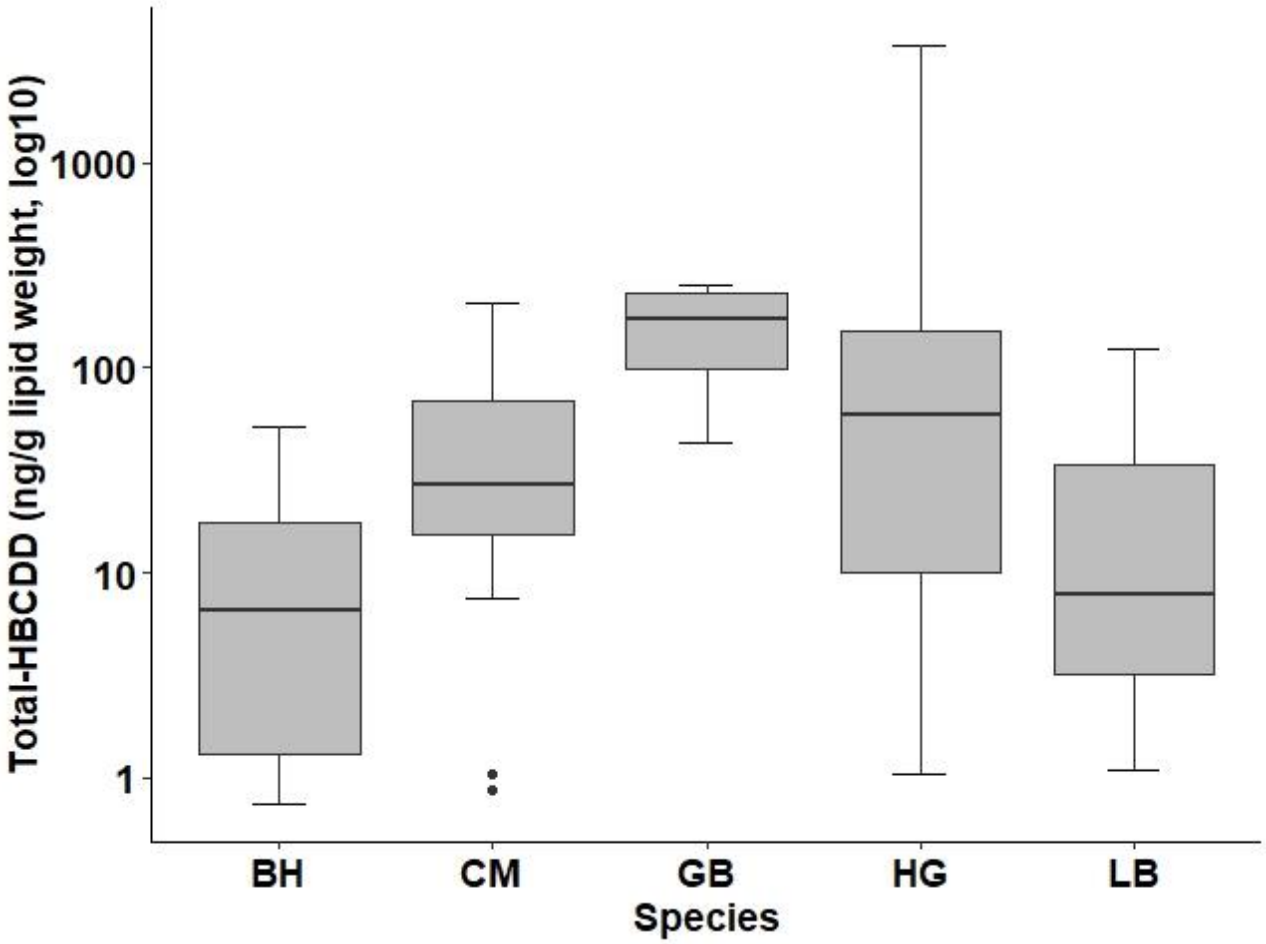

Figure 3.11 Box and whisker plots showing Total-HBCDD (log_{10} - transformed) concentrations in the eggs of five gull species breeding in proximity to landfill in Scotland (2016). Black lines within boxes indicate median values, boxes the 25th and 75th percentiles, whiskers the 10th and 90th percentiles. Points are outliers. BH: black-headed gulls (*n* = 12); CM: common gulls (*n* = 14); GB: great black-backed gulls (*n* = 4); HG: herring gulls (*n* = 16); LB: lesser black-backed gulls (*n* = 11).

in there being significant interspecies differences for Total- ($P = 0.02$) and γ-HBCDD ($P < 0.001$), but no significant difference for α-HBCDD ($P = 0.06$). Post-hoc testing of data that included great black-backed gulls identified significant pairwise differences in respect to γ-HBCDD concentrations. Great black-backed gulls and herring gulls had significantly higher levels compared to black-headed gulls and common gulls ($P \leq 0.04$), whilst all other species showed significantly higher γ-HBCDD compared to lesser black-backed gulls ($P \leq 0.001$) (Table 3.9).

Table 3.9 Significant post-hoc comparisons using Pairwise Wilcoxon Rank Sum test, Holm-adjusted, following significant interspecific comparisons of concentrations of γ-HBCDD in the eggs of five gull species breeding in proximity to a landfill in western Scotland in 2016 (BH: black-headed gull, CM: common gull, GB: great black-backed gull, HG: herring gull, LB: lesser black-backed gull).

HBCDD diastereomer	**Pairwise comparison**	***P***
γ-HBCDD	GB *vs*. BH	0.005
	GB *vs*. CM	0.01
	HG *vs*. BH	<0.001
	BH *vs*. LB	<0.001
	HG *vs*. CM	0.04
	CM *vs*. LB	<0.001
	GB *vs*. LB	<0.001
	HG *vs*. LB	<0.001

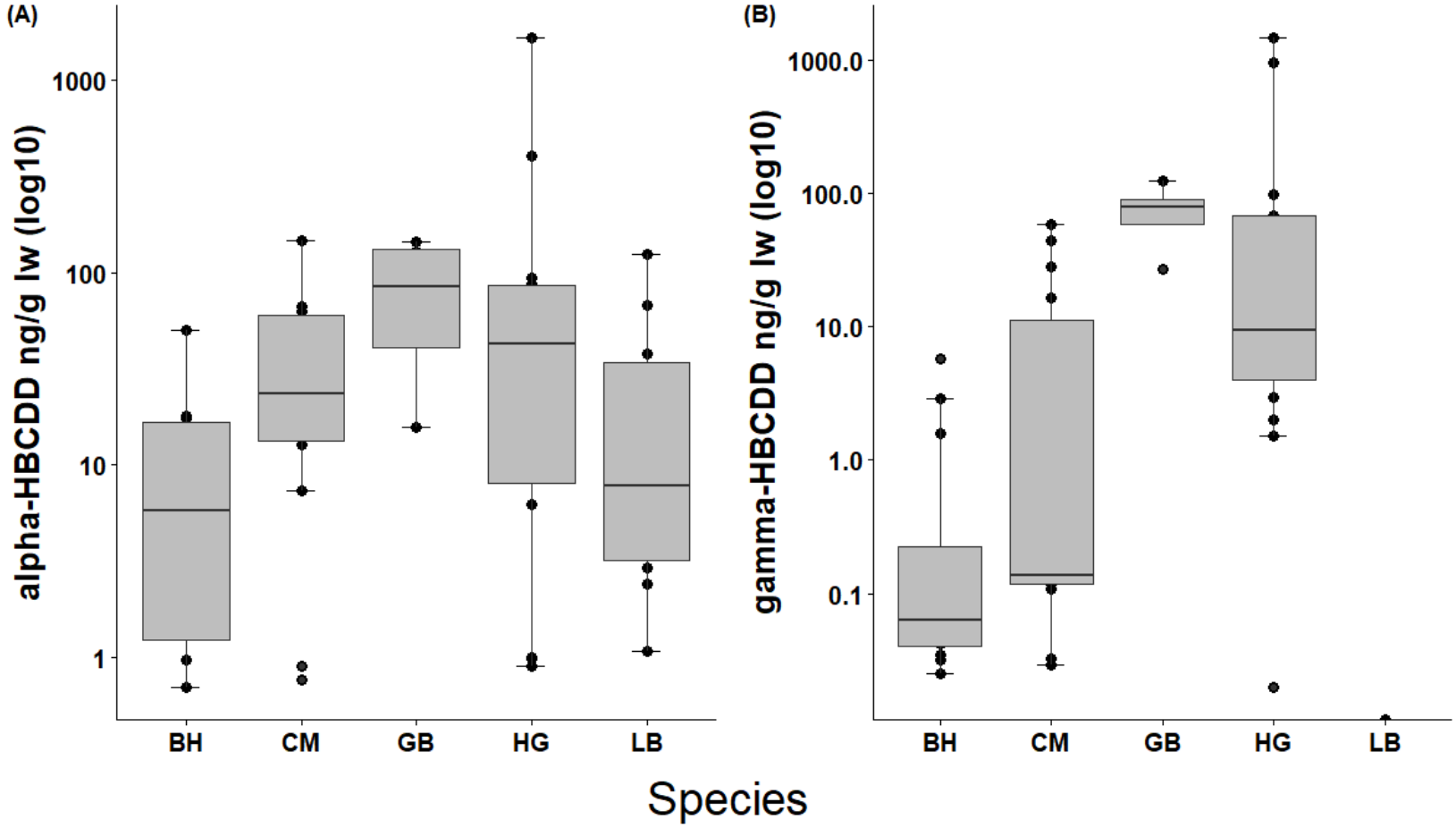

Figure 3.12 Box and whisker plots showing concentrations of α-HBCDD and γ-HBCDD diastereomers (log_{10}-transformed) in the eggs of landfill-breeding gulls collected in western Scotland during 2016. Black lines within boxes indicate the median values, boxes indicate the 25th and 75th percentiles; whiskers indicate the 10th and 90th percentiles. BH: black-headed gulls (*n* = 12); CM: common gulls (*n* = 14); GB: great black-backed gulls (*n* = 4); HG: herring gulls (*n* = 16); LB: lesser black-backed gulls (*n* = 11).

3.4.5.4 HBCDD diastereomer composition in the eggs of landfill-breeding gulls (2016)

The stacked barplot in Figure 3.13 shows the arithmetic mean percentage relative contribution of α- and γ-HBCDD diastereomers (as constituents of Total-HBCDD) in the eggs of black-headed gulls, common gulls great black-backed gulls, herring gulls and lesser black-backed gulls breeding in proximity to the study landfill in 2016. For all species except great black-backed gulls, α-HBCDD was the dominant diastereomer, comprising on average, 61–90 % of Total-HBCDD.

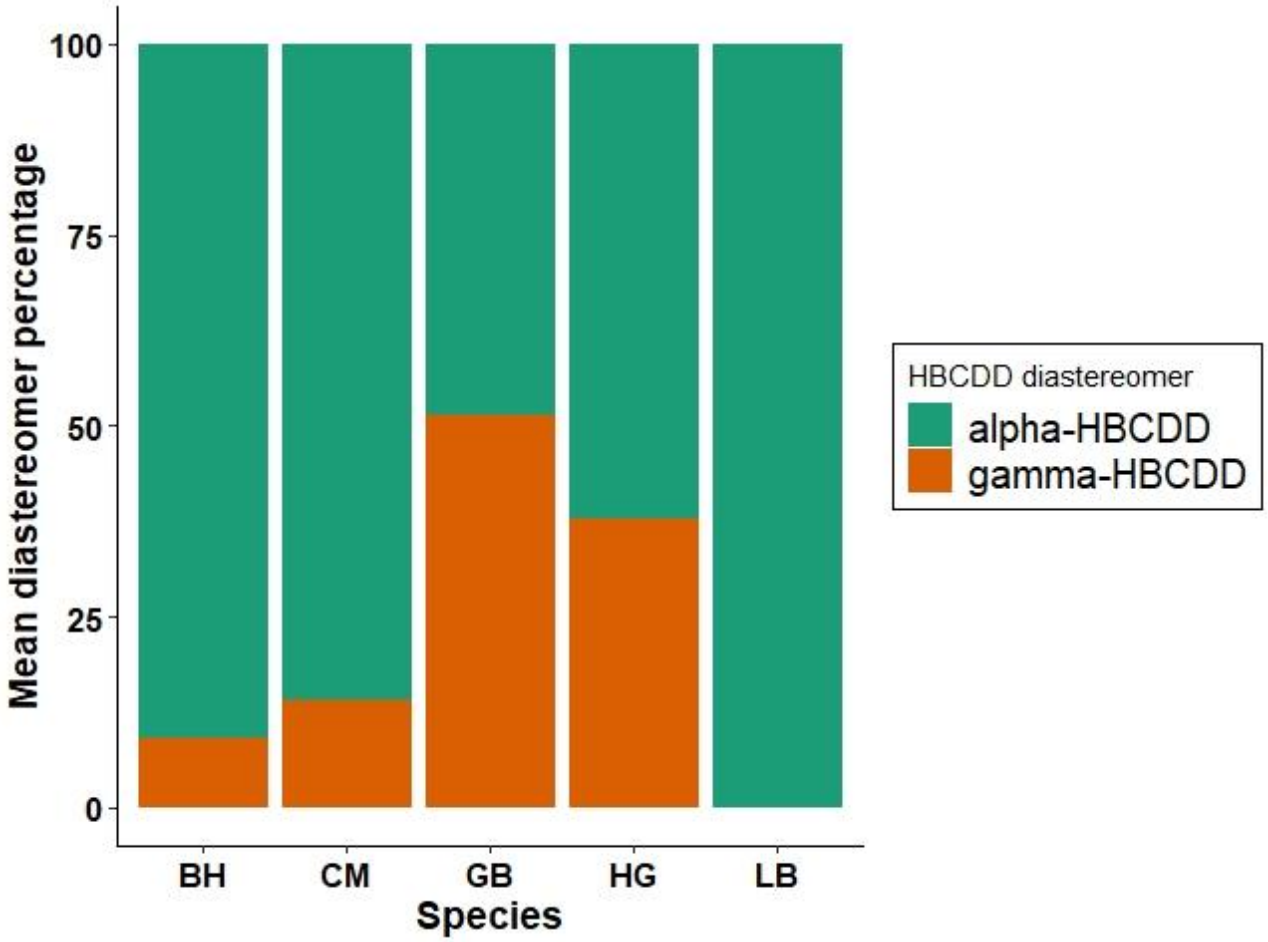

Figure 3.13 Stacked barplot displaying the mean percentage relative contribution of α- and γ-HBCDD diastereomers in the eggs of landfill-breeding gulls of five species collected in western Scotland in the same year, i.e., 2016. BH: black-headed gull (*n* = 12), CM: common gull (*n* = 14), GB: great black-backed gull (*n* = 4), HG: herring gull (*n* = 16,) LB: lesser black-backed gull (*n* = 11).

3.4.5.5 NBFR concentrations in landfill-breeding gulls (2016)

DBDPE was the only targeted NBFR detected in 2016 egg samples, where it occurred sporadically (i.e., in 8%, 28%, 25%, 6% and 9% of the eggs of landfill-breeding black-headed gulls, common gulls, great black-backed gulls, herring gulls and lesser black-backed gulls, respectively, at concentrations ranging from 71.40 ng/g lw (common gull) to 7,724.20 ng/g lw (great black-backed gull).

3.5 Discussion

The findings of the present chapter develop our knowledge of BFR concentrations and profiles in landfill-associated gulls in terms of: i. contamination across a species assemblage and ii. identification of a potential bioindicator species for future biomonitoring of BFR emissions from landfill in a north-west European context. This is the first study of FRs in landfill-associated birds in the UK. This is important given that different jurisdictions differ in terms of historic BFR use (Table 1.1), with the implication being that potential toxicological risks to avifauna from BFR exposure may vary regionally.

3.5.1 Herring gull landfill vs. reference comparisons

The focal taxon in the present study was herring gulls. This was as a result of the large numbers of this species at the study landfill and its relative abundance as a breeding bird in the study area. This species, as well as the congeneric American herring gull, are established as bioindicator species for assessment of contaminants in eggs in Germany and North America respectively (Hebert, 1999; Koschorreck et al., 2015). In the present study, the most robust evidence for a BFR increment in gull populations breeding in proximity to landfill is that landfill-breeding herring gulls in 2017–18 exhibited significantly higher $\sum_8$PBDE egg concentrations compared to reference conspecifics (Table 3.1). This was related to the fact that herring gulls were the most numerous species on the landfill, which in turn, may reflect their foraging ecology. This suggests therefore that there is substantial merit in this species being considered a useful bioindicator of BFR emissions from landfill in north-

west Europe given its known relative abundance amongst gull species in this region (Cramp and Simmons, 1983). The fact that BDE-209 concentrations were significantly higher in the eggs of landfill breeders, also comprising a greater arithmetic mean percentage of ∑PBDEs compared to reference birds (Figure 3.2), provides further evidence of the dominance of this fully brominated congener in European landfill and other terrestrial matrices, as previously demonstrated in studies comprising both abiotic (Morin et al., 2017) and avian (Morales et al., 2012; Roscales et al., 2016) research on that continent. This congener has a history of substantially greater use as a FR in Europe (and especially the UK) compared to North America in particular (BSEF, 2003; Söderström et al., 2004), and it is known to undergo sequential metabolic debromination and photocatalytic degradation to lower brominated and more bioavailable congeners in birds (Francois et al., 2016; Letcher et al., 2014; Van den Steen et al., 2007) and abiotic matrices (Gerecke et al., 2006; Robrock et al., 2008), respectively. BDE-100 showed significantly higher concentrations in the eggs of landfill breeders (2017 only). BDE-100 may be a product of BDE-209 debromination. Alternatively, it may reflect BFR profiles in waste at the study landfill or toxicokinetics.

In contrast to the significant differences in PBDE egg concentrations between landfill and reference herring gulls, there was no such difference between colonies in terms of HBCDD. HBCDD was used primarily in extruded polystyrene insulation foam in the construction industry (Alaee, 2003). The fact that the eggs of reference and landfill breeders contained similar mean Total-HBCDD concentrations (223.88 ± 62.57 *vs.* 183.42 ± 60.20 ng/g lw, respectively; Table 3.1) may also suggest that some reference birds utilise landfill. Located approximately 30 km SSW from the reference herring gull colony on Colonsay is a small landfill facility on the island of Islay (Gartbrek). Analysis of Tables 2.1 and 2.2 shows that during 2017–18, the arithmetic mean percentage of total waste comprising construction and demolition materials received at Gartbrek was 11 %, whereas the equivalent figure for the study landfill was 0.4 %. This indicates that birds using the former site may potentially be at greater risk of HBCDD exposure, although the distance from the reference colony

to Gartbrek exceeds the mean breeding season foraging range for herring gulls (approximately 10 km; Thaxter et al., 2012).

The only NBFR regularly found above detection limits in herring gulls was DBDPE. It was detected only in the eggs of landfill breeding gulls, at concentrations of up to 4696 ng/g lw (Table 3.1). This chemical, which is structurally similar to BDE-209, appears to be increasingly employed as a *deca*-BDE replacement (Betts, 2009; de Wit et al., 2019; Guo et al., 2019; Stubbings et al., 2019; Wemken et al., 2019). These results suggest that DBDPE should be focus of future biominotiring work on NBFRs in biotic matrices.

In terms of herring gull egg traits, the reduction (on average 5 % thinner) in eggshell thickness found in landfill breeding birds (only assessed in this species given disparities between years and site-type in other taxa) and in eggs containing in excess of 50 ng/g ww $\sum_8$PBDE may indicate potentially deleterious effects of PBDEs in this species. However, whether this is causative or merely correlative is unknown. Fernie et al. (2009) reported comparable reductions in mean eggshell thickness in American kestrels dosed with environmentally-relevant PBDE concentrations (8 % *vs.* 5 % in the present study), although Zapata et al. (2018) found no signficant correlation between eggshell thickness and PBDEs in Iberian-breeding yellow-legged gulls. Eggshell thickness influences egg viability and therefore also reproductive success (Fox et al., 1980). The percentage reduction in eggshell thinning reported in association with breeding failure and associated dramatic declines in the populations of certain raptors as a result of organochlorine pesticide contamination post-WWII was considerably in excess of the findings of the present study. For example, in the case of UK-breeding peregrine falcons, the average decrease in eggshell thickness during 1947–71 was 16 % (range: 2.2–20.5 %) (Ratcliffe, 1981). The reproductive implications of a 5 % reduction in eggshell thickness in European herring gulls are unknown and require further research.

3.5.2 Other species: landfill vs. reference comparisons

Comparisons between landfill and reference populations for species other than herring gulls in this study were compromised by smaller sample sizes and the different years for which data were available for either site type. Nevertheless, in keeping with the central hypothesis of this study, $\sum_8$PBDE concentrations in the eggs of landfill breeding black-headed gulls, common gulls, great black-backed gulls and lesser black-backed gulls exceeded those for reference conspecifics (Tables 3.3–3.6). The lower brominated BDE-47, associated with a higher trophic level diet in aquatic food webs (Chen and Hale, 2010; Henny et al., 2009; Roscales et al., 2016) formed a substantial arithmetic mean percentage of ∑PBDEs in the eggs of reference breeders in the case of black-headed gulls and great black-backed gulls (Figure 3.6). Despite the admittedly very small sample size (n = 2), the results of this study indicate that Colonsay-breeding lesser black-backed gulls may travel considerable distances inland in order to forage in anthropogenic habitats, as has been demonstrated for this species previously (Gyimesi et al., 2016; Thaxter et al., 2012).

Although black-headed gulls and common gulls were not observed to frequent the study landfill, the eggs of landfill breeding individuals contained substantially higher DBDPE concentrations in comparison to reference conspecifics (Tables 3.3 and 3.4, respectively). Again, given the proximity (within 2 km) of these colonies to the landfill (Figure 2.1), this may represent DBDPE contamination from the landfill as result of particulate-phase air concentrations. It may be the case that those eggs with very high DBDPE concentrations related to individuals which had directly ingested polymeric items treated with this NBFR, perhaps in combination with metabolic differences between individuals.

3.5.3 Interspecies comparisons of egg data for landfill-breeding gulls (2016)

In terms of 2016 data (i.e., interspecies comparison of landfill breeders), it ishould be noted that the significant interspecies differences in egg concentrations of individual PBDE congeners (applying to BDE-28, BDE-47, BDE-99, BDE-100, BDE-154 and BDE-183, via post-hoc testing; Table 3.8)

generally applied between the large white-headed gull taxa (i.e., great black-backed gulls, herring gulls and lesser black-backed gulls) *vs*. the two small gull species (i.e., black-headed gulls and common gulls). Neither of the latter two species were observed to frequent the study landfill, although they both bred within 2 km of the site. This may suggest that use of landfill, as well as general foraging and trophic ecology plays a role in interspecies differences in BFR burdens. Similarly, post-hoc tests identified a significant difference in Total-HBCDD concentrations between great black-backed gulls and black-headed gulls.

The only NBFR present in 2016 landfill samples was DBDPE, which was detected sporadically in all species, reaching a maximum concentration of 7724.20 ng/g lw in a single great black-backed gull egg, understood to be the highest concentration of this NBFR found in biota to date, globally. The relatively high DBDPE concentrations in the eggs of black-headed and common gulls (82.52 and 4211.50 ng/g lw, respectively) (i.e., species not observed to frequent the landfill) is harder to explain. However, the extent to which these individuals may have used landfill in the non-breeding season is unknown, as is the half-life of DBDPE in birds. DBDPE exposure via air for colonies in proximity to the landfill / human settlements may also explain such elevated concentrations. This chemical may potentially occur in these gulls' food webs; more research is required in this area.

Maximum egg $\sum_8$PBDE concentrations in landfill-breeding gulls in 2016 exceed or are equal to those reported for white storks (range: 2.7–20.5 ng/g ww; Muñoz-Arnanz et al., 2011a) associated with landfill in Spain. Maximum egg $\sum_8$PBDE concentrations in landfill-breeding great black-backed gulls, herring gulls and lesser black-backed gulls exceed those in black kites associated with landfill, also in Spain (maxima: 1570 ng/g lw; Blanco et al., 2018) and in landfill-associated African sacred ibis in South Africa (maximum: 315 ng/g ww). Arithmetic mean $\sum_8$ PBDE concentrations in the eggs of landfill-breeding lesser black-backed gulls in the present study also exceed those reported in the eggs of yellow-legged gulls breeding in Spain (mean: 38.3 ng/g ww; Morales et al., 2012). However,

mean ∑PBDE concentrations exceeding those described in the present study have been reported in the eggs of common starlings in Canada (range: 11–805 ng/g ww; Chen et al., 2013). The maximum ∑PBDE egg concentration reported in landfill-associated avifauna to date is 4400 ± 83 ng/g ww, also in Canadian-breeding common starlings (Eens et al., 2013). Any comparisons between such studies are hampered given that they relate to different species in different jurisdictions, with samples (of different sizes) collected during different years. However, it is notable that samples in the present study were obtained from relatively remote locations where there were likely to be fewer competing sources of environmental BFR contamination, unlike the Spanish and Canadian studies, for which eggs were obtained from urban locations. This provides further evidence for the importance of landfill as a source of BFR contamination in birds. *In ovo* PBDE burdens reported in the present thesis are also lower than those reported in American herring gulls. For example, Chen et al. (2012) reported mean ∑PBDE concentrations in eggs of the latter taxon breeding in proximity to urban Montreal (Canada) of 610 ng/g ww. The same study also reported higher mean ∑PBDE egg concentrations for California gulls (199 ng/g ww) and ring-billed gulls (225 ng/g ww) compared to the focal taxa of the present study, although the equivalent figure obtained by Chen et al. (2012) for glaucous-winged gulls (62 ng/g ww) approximates to that for lesser black-backed gulls in the present study. Metabolic differences between species may, to some extent, account for disparities between the present study and others: it has been demonstrated that the sensitivity of the aryl hydrocarbon receptor to the effects of dioxin-like compounds varies significantly even in closely phyllogenetically-related avian taxa (Head et al., 2008).

There are considerably fewer published data in terms of Total-HBCDD concentrations in the eggs of landfill-associated avian species. Arithmetic mean Total-HBCDD egg concentrations for landfill-breeding gulls in the present thesis are below mean HBCDD egg concentrations reported in African sacred ibis eggs (maxima: 71 ng/g ww; Polder et al., 2008). However, mean *in ovo* egg Total-HBCDD concentrations in Laurentian Great Lakes-breeding American herring gulls have been

reported at similar concentrations to European herring gulls in the present study (13.20 ng/g ww; Su et al., 2015). Norwegian-Arctic breeding glaucous gulls have been found to contain mean egg α-HBCDD concentrations of 19.8 ±2.2 ng/g ww (Verreault et al., 2018), comparable with data in the present study.

3.6 Conclusions

This chapter has demonstrated that five larid taxa breeding in proximity to an active municipal solid waste landfill in the UK exhibit elevated egg PBDE burdens compared to reference subjects. This is commensurate with studies elsewhere which have examined PBDE contamination in landfill-associated avifauna. PBDE egg concentrations in the present study exceeded those reported in the eggs of landfill-associated white storks, yellow-legged gulls and black kites in Spain and landfill-associated African sacred ibis in South Africa. Concentrations of BDE-100 and BDE-209 were significantly higher in the eggs of landfill-breeding herring gulls compared to reference conspecifics. The dominance of BDE-209 in anthropogenic and terrestrial biotic and abiotic matrices is well known. Of the NBFRs measured, only DBDPE was detected (in landfill birds only), at up to 7724.20 ng/g lw, the highest concentration reported in biota to date globally. Further monitoring of the presence of DBDPE in biota and research into its potential for debromination, given its structural similarities to BDE-209 (i.e., the chemical it has apparently replaced), should be prioritised. This is the first study globally to examine FR concentrations across an assemblage of bird species breeding in proximity to a landfill. All study species are designated as being of UK conservation concern. Egg BFR concentrations in all species were at levels commensurate with various deleterious effects reported in captive American kestrels, including reduced courtship behaviours and reduced parental care in males, reduced nest temperatures and reductions in total and free thyroxine in the plasma of nestlings (Appendix 13). Landfill-breeding herring gulls laid eggs with significantly thinner shells than reference individuals and eggshell thickness was significantly lower in eggs containing >50 ng/g ww $\sum_8$PBDEs, though it is not known the extent to which either BFR contamination or the potentially

reduced nutritional value of human refuse may be responsible . This chapter has shown that herring gulls breeding in proximity to UK landfill can be considered effective bioindicators of emissions of BFRs (in particular, certain PBDE congeners and DBDPE) from such sites.

By virtue of their association with landfill, numerical abundance and the fact that collection of their eggs can be facile due to their ground-nesting ecology, European herring gulls can be considered an important biomonitoring species for the emission of PBDEs (especially BDE-209) from municipal solid waste landfill in northwestern Europe.

The analysis of stable isotope ratios in tissue compartments has become central to the study of avian trophic ecology in recent decades. Stable isotope analysis (SIA) is regularly used in avian ecotoxicological studies (many of which have involved gulls), allowing an individual bird's contaminant burdens to be analysed in relation to diet. In the following chapter, the relationships between carbon ($\delta^{13}C$), sulphur ($\delta^{34}S$) and nitrogen ($\delta^{15}N$) signatures in the eggs of the three gull species (great black-backed gulls, lesser black-backed gulls and herring gulls) observed foraging at the study landfill are analysed in order to relate the diets of species and individuals to egg concentrations of the compounds of interest.

CHAPTER IV

THE USE OF STABLE ISOTOPES TO ELUCIDATE BROMINATED FLAME RETARDANT EXPOSURE IN LANDFILL-ASSOCIATED GULLS

4.1 Synopsis

Stable isotope analysis (SIA) is central to ecotoxicological research, providing time-integrated quantitative dietary information which can be examined in relation to contaminant burdens. Little is known in terms of the exposure routes of BFRs in landfill-associated avifauna. Such birds are potentially at risk of exposure to these chemicals, some of which are listed as POPs. Landfill facilities are likely important reservoirs of these regulated compounds as the goods to which they were once applied have become obsolete. Likewise, such sites are increasingly likely to contain a range of NBFRs. In the present study, analysis of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) values was undertaken for the eggs of great black-backed gulls (n = 11), herring gulls (n = 63) and lesser black-backed gulls (n = 9) breeding in western Scotland (UK). For each species, one subset of eggs was from a colony breeding in proximity to an active landfill and a second subset was from reference colonies located approximately 50 km distant. Stable carbon isotopes were significantly depleted in the eggs of landfill-breeding herring gulls. A significant negative correlation between $\delta^{13}C$ and BDE-209 egg concentrations indicated that the terrestrial diets of landfill-breeding herring gulls led to them being exposed to this PBDE congener. This chapter provides further evidence of the importance of herring gulls as a potential biomonitoring species in terms of BFR emissions from landfill in north-west Europe.

4.2 Introduction

Chapter III reported egg BFR concentrations and profiles across a species assemblage of five gull species (black-headed gulls, common gulls, great black-backed gulls, herring gulls and lesser black-backed gulls) breeding within 2 km of a municipal solid waste landfill facility in western Scotland and reference conspecifics breeding 50–110 km distant. The eggs of landfill breeders of all species exhibited generally and sometimes significantly higher BFR burdens, particularly in the case of PBDEs, the most frequently-detected compounds of interest. The focal taxon in Chapter III was herring gulls. This was due to their numerical superiority in terms of i. individuals observed foraging on the study landfill (comprising over 90 % of total gull observations) and ii. the eggs of this species forming the largest individual proportion of total collected eggs across all species (36 %). For these reasons, herring gulls were identified as a putative sentinel avian taxon for the evaluation of BFR emissions from municipal solid waste landfill in a north-west European context.

Dietary intake can be an important pathway of avian contaminant exposure (e.g., Hebert et al., 2000; Ma et al., 2018; Manosa et al., 2003; Newsome et al., 2010; Santos et al., 2017) (but see Brown et al., 1997, on the importance of inhalation as an exposure pathway). The diet of free-living birds can be determined via various methods, often used in concert (Duffy and Jackson, 1986). These include the direct observation of food consumption (e.g., Bielefeldt et al., 1992; Götmark, 1984b; Götmark et al., 1986), the analysis of pellets, faeces and regurgitant (e.g., Ewins et al., 1994; Lenzi et al., 2016; Votier et al., 2003), collection of prey remains in the vicinity of roosting and nest sites (e.g., Graham et al., 1995; Redpath et al., 2001) and analysis of stomach contents via necropsy (e.g., Bernhardt et al., 2010; Seif, 2017). In recent decades however, SIA has come to prominence as a key analytical tool in the study of avian trophic ecology (Bearhop et al., 2002; Fox and Bearhop, 2008; Hebert et al., 1999; Hobson, 1987; Hobson et al., 2015; Hobson and Clark, 1992a, 1992b; Hobson et al., 1994; Inger and Bearhop, 2008). There are several advantages of SIA over other methods of avian dietary evaluation. Isotopes are based on time-integrated information relating to assimilated, rather

than ingested, food and can therefore reveal diet over longer timescales, as well as eliminating the potential for under or overestimation of the total dietary proportions of soft and hard foods, respectively (Inger and Bearhop, 2008; Hobson and Clark 1992a,b). The use of the carbon stable isotope ratio of ^{13}C to ^{12}C, expressed as $\delta^{13}C$ to elucidate the proportion of marine *vs*. terrestrial / freshwater dietary components is well established (e.g., Hebert et al., 1999; Hobson, 1987; Hobson and Sealy, 1991; Inger and Bearhop, 2008), with ^{13}C in marine biomes being enriched compared to terrestrial C-3 systems (Hobson et al., 1997). Use of the nitrogen stable isotope ratio of ^{15}N to ^{14}N, expressed as $\delta^{15}N$, is equally well established as a means of understanding the trophic level at which an organism forages (Bearhop et al., 2002; Hebert et al., 2006; Hobson et al., 1994; Inger and Bearhop, 2008; Kelly, 2000): elevated trophic levels correspond with enriched $\delta^{15}N$ values. A third stable isotope ratio, that of sulphur ^{34}S to ^{32}S ($\delta^{34}S$), has been demonstrated to be effective in characterising nutrient sources in biota that utilise both marine and terrestrial environments, such as gulls. For example, Hobson et al. (1997) reported that American herring gulls breeding on the Canadian Atlantic coast (Newfoundland) laid eggs with significantly enriched $\delta^{34}S$ signatures compared with conspecifics breeding inland in the Laurentian Great Lakes. The authors concluded that the substantial difference in $\delta^{34}S$ signatures of sulphates obtained from terrestrial *vs*. marine food webs indicated that it was more effective than carbon as a measure of the nutrients derived by birds that inhabit both marine and terrestrial ecosystems. Similarly, Roscales et al. (2016) found that mean $\delta^{34}S$ signatures, as well as those of $\delta^{13}C$ and $\delta^{15}N$, were significantly higher in the eggs of the marine piscivore, Audouin's gull, compared with the more dietary generalist yellow-legged gull in the case of birds breeding sympatrically in the southern Mediterranean Sea. Nevertheless, the analysis of $\delta^{34}S$ has featured considerably less frequently in the avian SIA literature compared with that of $\delta^{13}C$ and $\delta^{15}N$ to date (Blight et al., 2015; Hobson et al., 1997; Moreno et al., 2010; Peterson et al., 2017; Ramos et al., 2013; Roscales et al., 2016). Research on $\delta^{34}S$ values in avian tissues is therefore of particular interest in advancing our understanding of how it can be used in such dietary studies. It

should be noted that Fox and Bearhop (2008) advised caution in the use of SIA to elucidate the diets of birds that consume anthropogenic refuse given that they likely ingest items originating from various isotopic sources.

Different tissues provide dietary isotopic information over different timescales, ranging from liver (a period of hours) to bone collagen (potentially the duration of an individual's lifetime) (Hobson and Clark 1992a). The fractionation rate of stable isotopes from the diet into consumer tissue is expressed by the following equation (reproduced from Hobson et al., 1994):

$$D_t = D_d + \Delta_{dt} \qquad \text{(Eqn 4.1)}$$

where D_t is the abundance of the isotope in consumer tissue, D_d is the isotopic abundance in the consumer's diet and Δ_{dt} is the isotopic fractionation factor between diet and tissue.

Hobson (1995) reported that the egg yolks of Japanese quails (*Coturnix japonica*) that were switched from a grain-based to a ^{13}C-enriched diet reached a new isotopic equlibrium value in approximately eight days. In larger birds such as the large white-headed gull taxa, isotopic fractionation may take somewhat longer given that Roudybush et al. (1979) observed the period of yolk formation in great black-backed gulls to be 12 days and in American herring gulls 11–13 days. Eggs are convenient for avian dietary analysis because the nutrients required for their production derive from the diet of the laying female (Hobson et al., 1997; Klaassen et al., 2004). This is particularly important in avian taxa such as larids, that can generally be categorised as 'income breeders' that are more reliant on exogenous nutrient reserves, as opposed to 'capital breeders' that use endogenous (stored) nutrient reserves for reproduction (Drent and Daan, 1980).

Stable isotope analysis, predominantly utilising δ^{15}N and or δ^{13}C signatures, is routinely used in avian ecotoxicological studies, facilitating the statistical analysis of contaminant burdens in relation to trophodynamics (e.g., Burgess et al., 2013; Chen et al., 2012; Davis et al., 2017; Gebbink et al., 2011; Hebert and Weseloh, 2006; Jardine et al., 2006; Peterson et al., 2017; Roscales et al., 2016;

Vicente et al., 2015). In this chapter, relationships between $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ in the eggs of great black-backed gulls, lesser black-backed gulls and herring gulls (i.e., those species known to forage at the study landfill), comprised of samples from landfill-breeding and reference birds, were analysed alongside egg concentrations PBDEs, HBCDD and six novel brominated flame retardants (NBFRs). Table 2.9 lists the number of eggs analysed by species, divided between landfill and reference breeding birds.

The aim of this chapter was to use stable isotopes as dietary tracers to elucidate BFR trophodynamics in both landfill and reference-breeding great black-backed gulls, herring gulls and lesser black-backed gulls. The working hypothesis was that landfill-breeding gulls would exhibit depleted $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ values as a result of having diets containing a relatively lower marine component and being potentially at a lower trophic level compared to reference conspecifics. Such information was anticipated to strengthen our understanding of the potential threats facing a species group that are deemed to be of conservation concern in a UK (and in the case of herring gull, European) context.

4.3 Materials and methods

Site selection, field sampling, determination of egg stable isotope signatures and statistical analyses were all undertaken as described in Chapter II.

4.4 Results

4.4.1 Herring gulls

4.4.1.1 Stable isotope ratios in herring gull eggs

Table 4.1 shows the arithmetic mean (± SE), median and range for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ ratios in the eggs of landfill-breeding vs. reference herring gulls collected during 2017 and 2018 (i.e., years in which both landfill and reference eggs were obtained).

Table 4.1 The mean (± SE), median and range for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (‰) in the eggs of landfill-breeding (n = 26) and reference (n = 23) herring gulls collected in western Scotland in 2017 and 2018.

Stable isotope ratio	Landfill mean ± SE	Reference mean ± SE	Landfill median (range)	Reference median (range)	$P^{\dagger}$
$\delta^{13}C$	-23.9 ± 0.2	-19.0 ± 0.2	-23.9 (-25.7– -21.6)	-19.5 (-21.0– -17.5)	<0.001‡
$\delta^{15}N$	10.8 ± 0.4	11.0 ± 0.3	10.3 (4.8–15.8)	10.6 (8.6–14.1)	0.93
$\delta^{34}S$	10.7 ± 0.5	12.5 ± 0.7	9.9 (7.1–16.5)	13.7 (7.1–17.6)	0.06

† Significance test for difference between landfill and reference egg stable isotope ratios derived using Wilcoxon Mann-Whitney U test.
‡ Isotopic ratios significantly higher in reference eggs.

Eggs collected in 2017 and 2018 were pooled for the purposes of statistical analysis given that there was no significant difference between years in terms of $\delta^{13}C$ (Mann Whitney / Wilcoxon U = 279.5, P = 0.68,) $\delta^{15}N$ (U = 343.5, P = 0.38) or $\delta^{34}S$ (U = 292.5, P = 0.88). The eggs of reference birds contained significantly enriched $\delta^{13}C$ values (P < 0.001; Figure 4.1). There was no significant difference between colony types in terms of $\delta^{15}N$ values (P = 0.93). Reference eggs contained enriched $\delta^{34}S$ values which approached significance (P = 0.06). In the case of all herring gull eggs combined (2016–18; n = 63), $\delta^{13}C$ was also significantly enriched in reference eggs (n =23) (P < 0.001), whilst there were no significant differences between landfill (n = 40) and reference eggs in terms of $\delta^{15}N$ (P = 0.78) or $\delta^{34}S$ (P = 0.11). Variance in $\delta^{13}C$ values for the eggs of landfill-breeding gulls (2.33) was significantly higher than for reference birds (1.00) (F = 2.32, d.f. = 39, P = 0.03). Conversely, variance was equal for landfill and reference breeders in terms of $\delta^{15}N$ (F = 1.60, d.f. = 39, P = 0.44) and $\delta^{34}S$ (F = 0.77, d.f. = 39, P = 0.51).

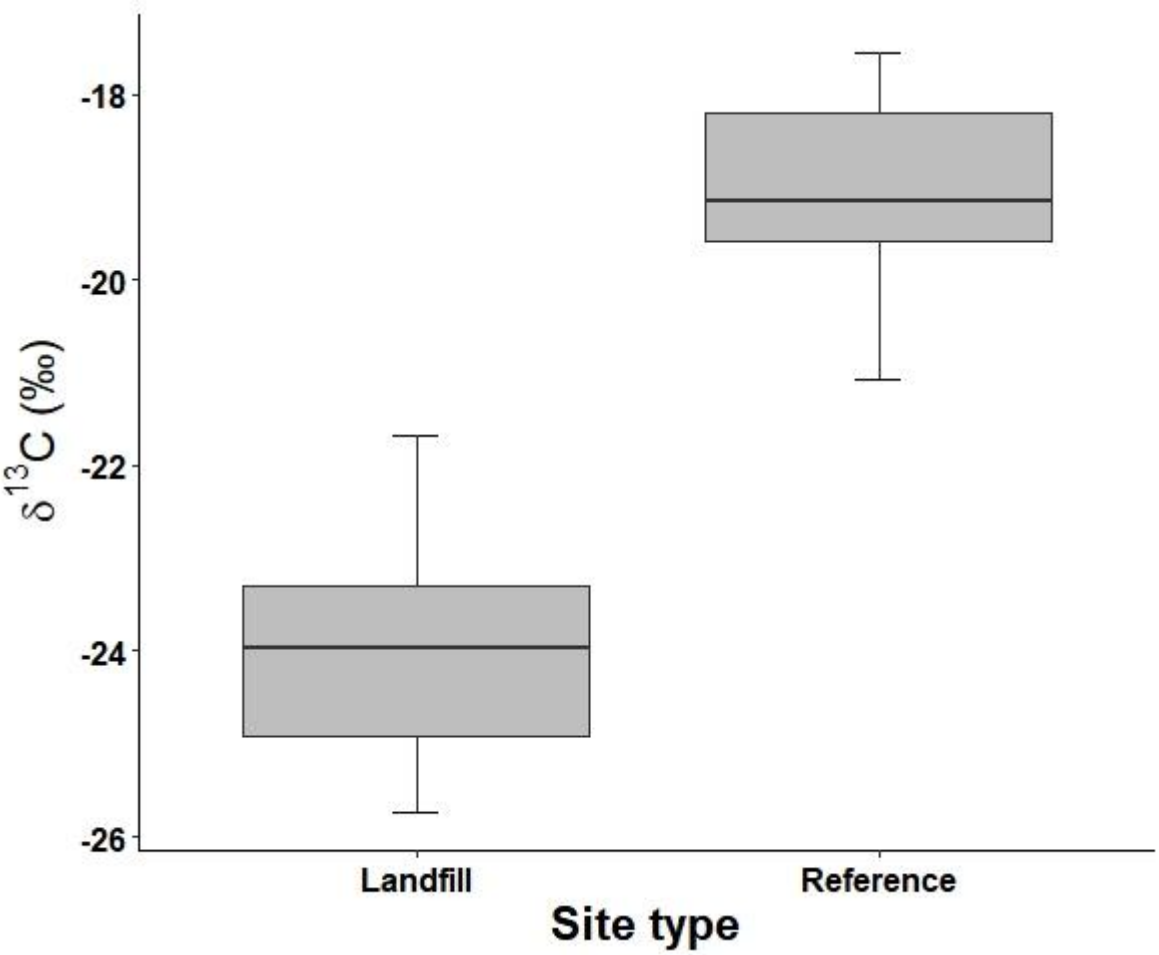

Figure 4.1 Box and whisker plot showing significantly depleted $\delta^{13}C$ values in the eggs of landfill-breeding (n = 26) *vs*. reference (n = 23) herring gulls collected in western Scotland in 2017 and 2018. Lines in boxes indicate medians, boxes indicate the 25th and 75th percentiles, whiskers indicate the 10th and 90th percentiles.

Figure 4.2 depicts bivariate plots of $\delta^{13}C$ against $\delta^{15}N$, $\delta^{34}S$ against $\delta^{15}N$ and $\delta^{34}S$ against $\delta^{13}C$, including arithmetic mean centroids, for the eggs of landfill-breeding and reference herring gulls pooled for 2017 and 2018. Of particular note is the significant positive correlation between $\delta^{15}N$ and $\delta^{34}S$ values (adjusted r-squared = 0.58, d.f. = 47, $P < 0.001$; Figure 4.2 B).

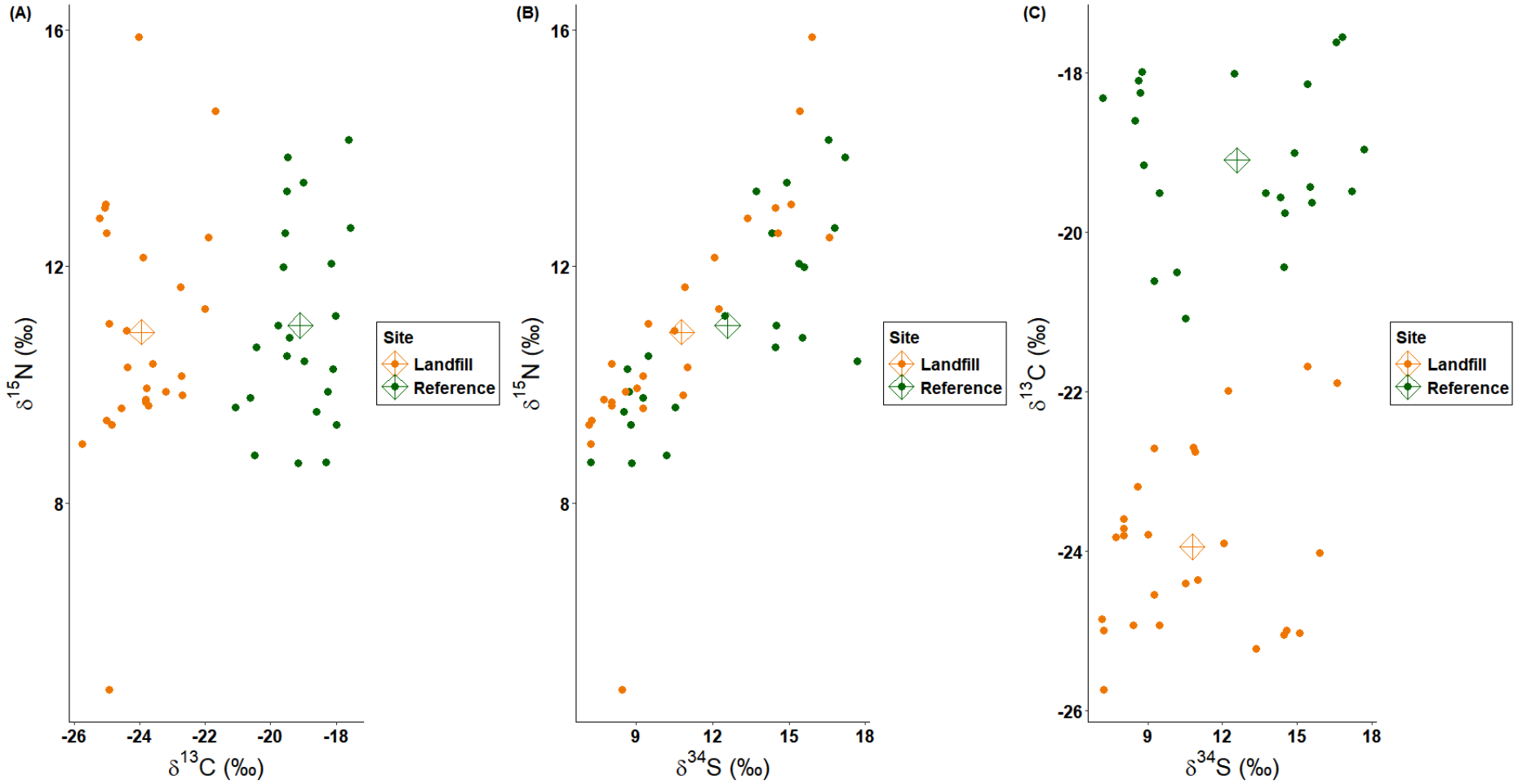

Figure 4.2 Bivariate plots of isotope ratios of (A) $\delta^{13}C$ against $\delta^{15}N$, (B) $\delta^{34}S$ against $\delta^{15}N$ and (C) $\delta^{34}S$ against $\delta^{13}C$ in eggs of landfill (n = 26) and reference (n = 23) breeding herring gulls collected in western Scotland during 2017–18. Diamonds are arithmetic means shown as centroids.

4.4.1.2 BFR concentrations in relation to stable isotope ratios in herring gulls

In the case of herring gull eggs collected in 2017 (n = 25), a significant negative relationship existed between $\delta^{13}C$ values and concentrations of BDE-209 (adjusted r-squared = 0.44, d.f. = 23, P <0.001). Furthermore there was a clear $\delta^{13}C$ enrichment in the eggs laid by reference individuals (Figure 4.3). Analyses of herring gull egg data involving single and multiple years of landfill and reference eggs

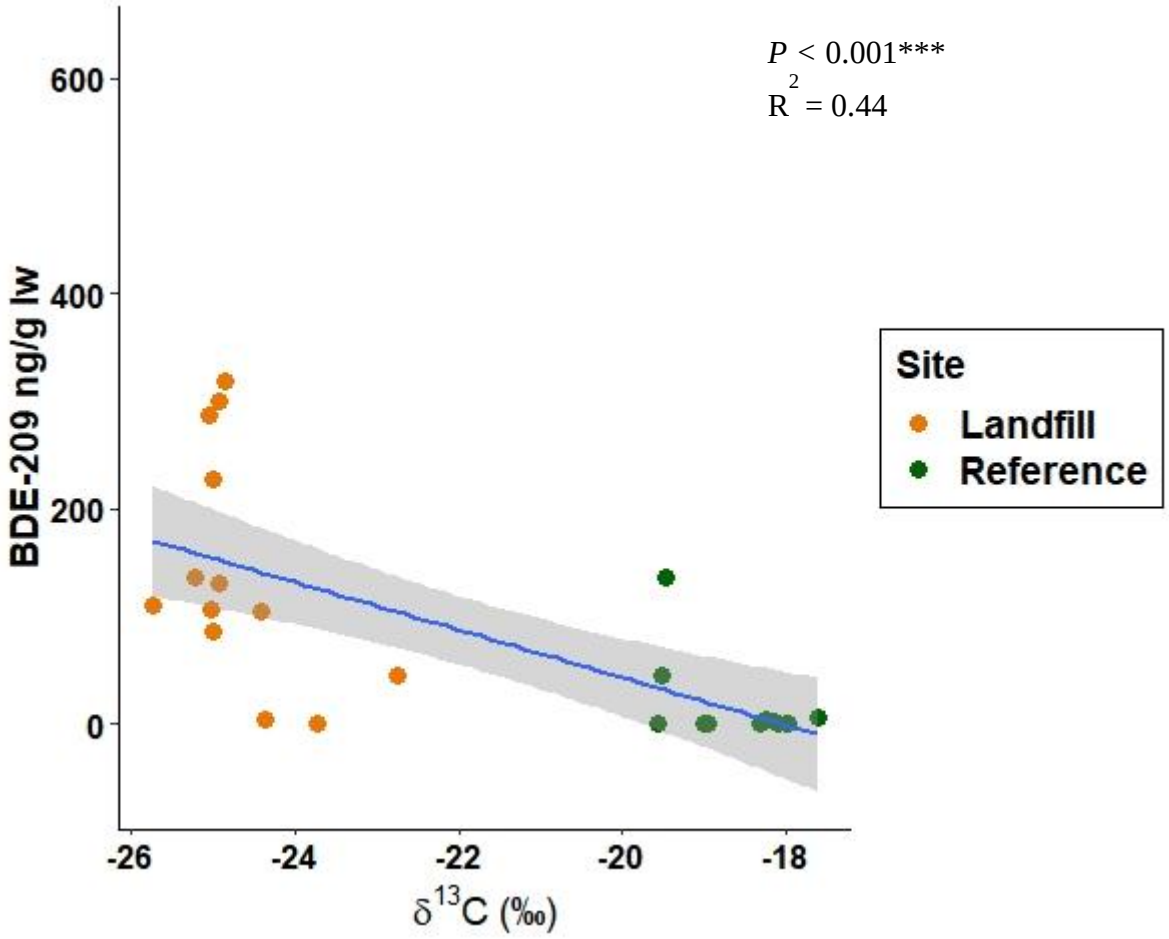

Figure 4.3 Simple linear regression plot displaying the relationship between concentrations of BDE-209 and $\delta^{13}C$ enrichment in eggs of landfill-breeding (n = 13) and reference (n = 12) herring gulls collected in western Scotland in 2017.

(using data both combined and separated via site type) identified no further significant relationships between $\delta^{15}N$, $\delta^{13}C$ or $\delta^{34}S$ values and concentrations of the BFR compounds of interest.

4.4.1.3 Egg traits in relation to SIA ratios in herring gulls

Figure 4.4 shows a significant, albeit weak, relationship between egg volume and $\delta^{13}C$ values in all herring gull eggs combined (2016–18, adjusted r-squared = 0.10, d.f. = 61, P <0.001).

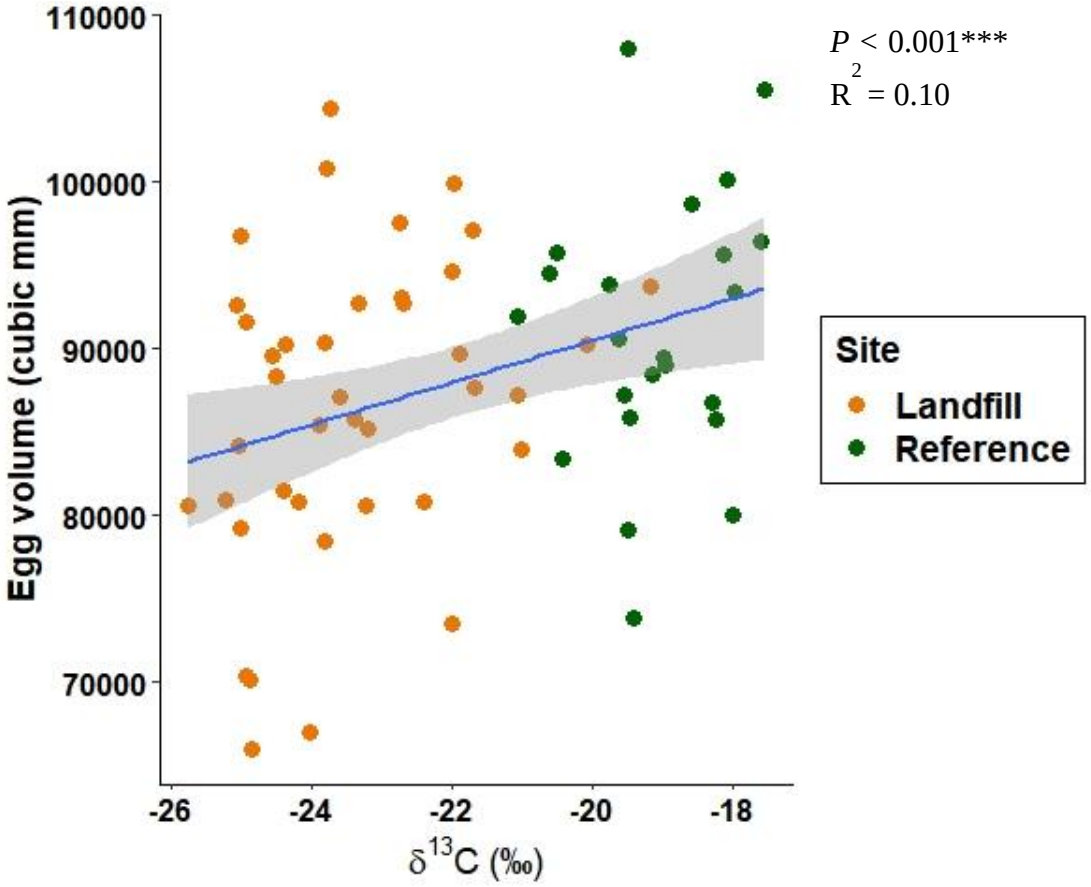

Figure 4.4 Simple linear regression plot displaying the relationship between egg volume (mm^3) and $\delta^{13}C$ enrichment in eggs (n = 63) laid by landfill-breeding and reference herring gulls collected in western Scotland during 2016–18.

Conversely, no relationships were observed between egg volume and $\delta^{15}N$ (adjusted r-squared = 0.01, d.f. = 61, P = 0.13) or $\delta^{34}S$ (adjusted r-squared = -0.07, d.f. = 61, P = 0.40) enrichment. A significant positive relationship between egg volume and $\delta^{13}C$ values was found for 2017 and 2018 combined (adjusted r-squared = 0.08, d.f. = 47, P = 0.02). There was also a significant, although again weak, positive relationship between eggshell thickness and $\delta^{13}C$ values for herring gull eggs collected in 2018 (adjusted r-squared = 0.15, d.f. = 22, P = 0.03; Figure 4.5).

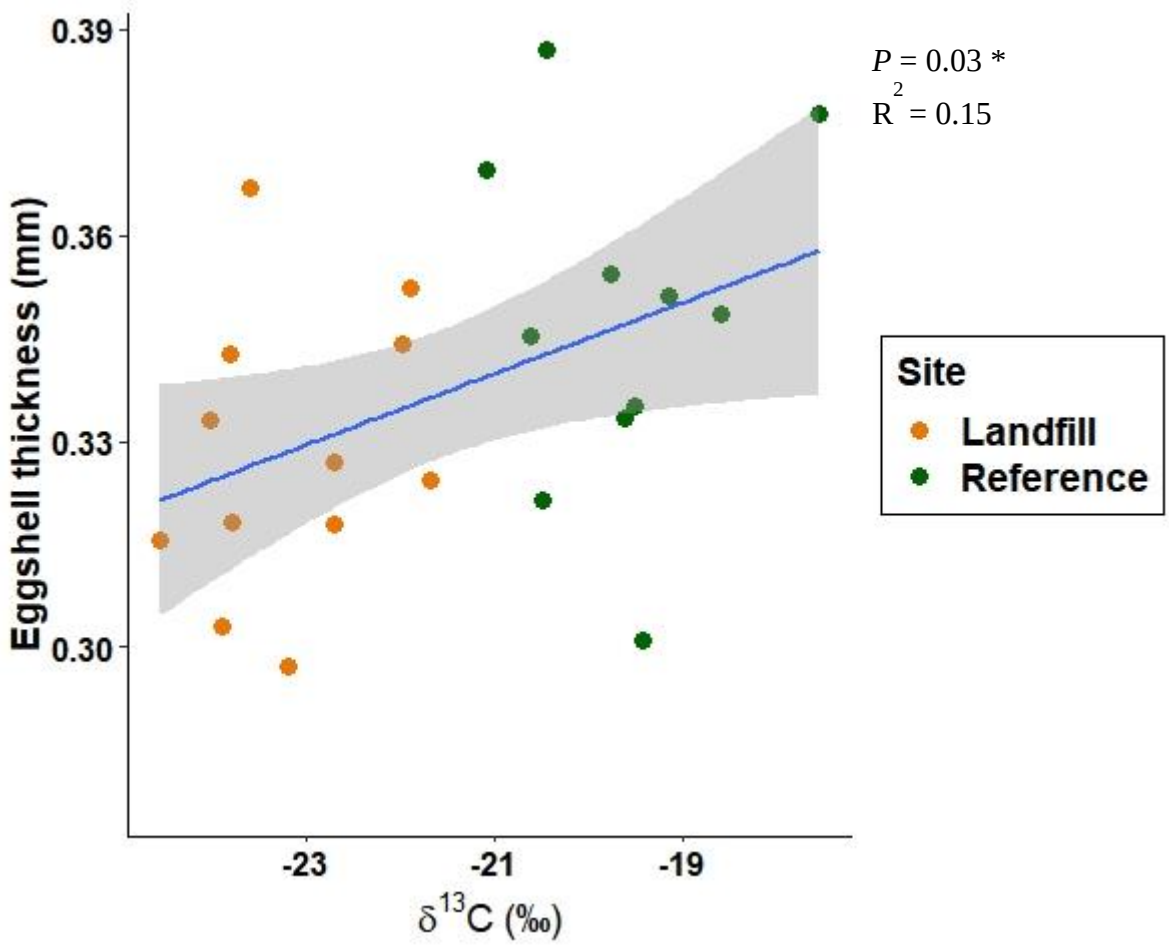

Figure 4.5 Simple linear regression plot displaying the relationship between shell thickness (mm) and $\delta^{13}C$ enrichment in eggs (*n* = 24) laid by landfill-breeding and reference herring gulls collected in western Scotland in 2018.

A similar relationship existed for all herring gull eggs combined (2016 –18): adjusted r-squared = 0.05, d.f. = 61, *P* = 0.04), but not in the case of eggs obtained in 2017 (adjusted r-squared = 0.007, d.f. = 23, *P* = 0.28), landfill-only (adjusted r-squared = -0.01, d.f. = 38, *P* = 0.58) or reference-only eggs (adjusted r-squared = -0.04, d.f. = 21, *P* = 0.84).

4.4.2 Stable isotope ratios in great black-backed gull eggs

Table 4.2 shows the mean, median and range for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in the eggs of landfill-breeding *vs.* reference great black-backed gulls for which both SIA and FR data were obtained. Given disparities between years in terms of the SIA data available, coupled with small sample sizes, statistical analysis was not possible. Data are therefore illustrative only.

Table 4.2 The mean, median and range for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (‰) isotope ratios in the eggs of landfill-breeding (*n* = 5; 2016–18) and reference (*n* = 6; 2017 & 2018) great black-backed gulls collected in western Scotland during 2016–18.

Stable isotope ratio	Landfill mean† (2016–18; *n* = 5)	Reference mean† (2017 & 2018; *n* = 6)	Landfill median (range; *n* = 5)	Reference median (range; *n* = 6)
$\delta^{13}C$	-21.32	-20.91	-21.69 (-23.19– -19.62)	-20.80 (-24.94– -17.61)
$\delta^{15}N$	12.17	10.20	12.58 (10.00–13.31)	9.55 (8.75–14.36)
$\delta^{34}S$	14.45	11.39	14.2 (12.62–17.23)	9.89 (7.37–16.80)

†Arithmetic mean.

4.4.3 Stable isotope ratios in lesser black-backed gull eggs

Table 4.3 shows the mean, median and range for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in the eggs of landfill-breeding *vs.* reference lesser black-backed gulls collected during 2016–18 for which both SIA and FR data were obtained.

Table 4.3 The mean, median and range for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (‰) in the eggs of landfill-breeding (*n* = 7; 2016–18) and reference (*n* = 2; 2018) lesser black-backed gulls collected in western Scotland during 2016–18.

Stable isotope ratio	Landfill mean† (2016; *n* = 7)	Reference mean† (2018 & 2018; *n* = 2)	Landfill median (range; *n* = 7)	Reference median (range; *n* = 2)
$\delta^{13}C$	-24.61	-25.80	-25.06	-25.80
			(-26.11– -22.92)	(-26.02– -25.58)
$\delta^{15}N$	11.21	8.73	10.5	8.73
			(8.48–13.67)	(7.59–9.87)
$\delta^{34}S$	12.19	11.50	12.68	11.50
			(8.40–15.47)	(10.28–12.73)

† Arithmetic mean.

As for great black-backed gulls, small sample sizes and different years to which landfill *vs*. reference samples relate preclude statistical analysis.

4.4.4 SIA ratios in all three species combined

Figure 4.6 shows bivariate plots of $\delta^{13}C$ against $\delta^{15}N$, $\delta^{34}S$ against $\delta^{15}N$ and $\delta^{34}S$ agaibst $\delta^{13}C$ in the eggs of landfill and reference-breeding great black-backed gulls, herring gulls and lesser black-backed gulls. The eggs of great black-backed gulls exhibited the highest mean values for all three isotope ratios in the case of landfill-breeding gulls. This is in contrast to data for reference birds, for which the highest mean isotopic values were in herring gull eggs. The eggs of reference lesser black-backed gulls (*n* = 2) exhibited especially low mean isotopic values relative to the other two species.

4.5 Discussion

This chapter has produced five key findings concerning stable isotope values in herring gull eggs. First, $\delta^{13}C$ values were significantly depleted in the eggs of landfill breeders. Secondly, there was a significant positive relationship between $\delta^{34}S$ and $\delta^{15}N$ values. Thirdly, for 2017 eggs there was a

significant negative relationship between BDE-209 and $\delta^{13}C$ enrichment. Fourthly, a significant but weak positive relationship existed between $\delta^{13}C$ and egg volume. Lastly, eggshell thickness was weakly but significantly positively correlated with $\delta^{13}C$ values for eggs collected in 2018.

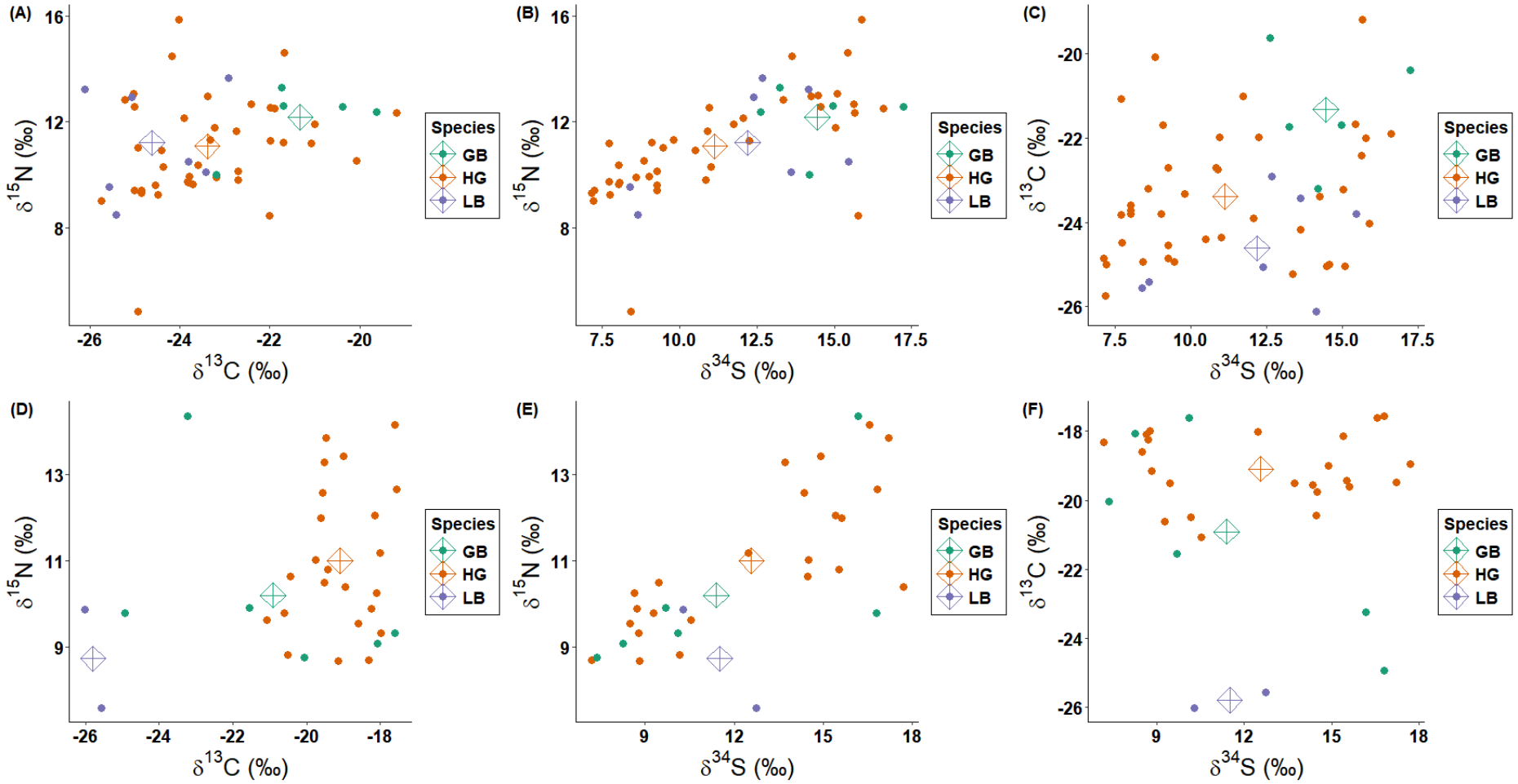

Figure 4.6 Bivariate plots of $\delta^{13}C$ against $\delta^{15}N$, $\delta^{34}S$ against $\delta^{15}N$ and $\delta^{34}S$ against $\delta^{13}C$ in the eggs of landfill and reference breeding great black-backed gulls (GB; *n* = 5 and 6 respectively), herring gulls (HG; *n* = 40 and 23) and lesser black-backed gulls (LB, *n* = 7 and 2) (A–C: landfill breeding birds; D–F: reference birds) collected in western Scotland during 2016–18. Diamonds are arithmetic means shown as centroids.

The significantly depleted $\delta^{13}C$ values for the eggs of landfill-breeding herring gulls (Figure 4.1) suggests a greater proportion of marine-derived items in the diets of reference individuals and some degree of colony-specific dietary homogeneity. These results also indicate the utility of SIA for landfill-associated species, i.e., countering the concerns raised by Fox and Bearhop (2008) that such birds could exhibit a wide variation in isotopic signatures due to the different foods available in waste streams. Similar significant between-colony differences have been reported elsewhere, for example, in the case of Laurentian Great Lakes-breeding American herring gulls (Hebert et al., 1999). The mean $\delta^{13}C$ signature of the eggs of landfill breeding herring gulls in this chapter (i.e., -24.69 ± 0.20 ‰ in 2017 and -23.20 ± 0.23 ‰ in 2018) was commensurate with the mean $\delta^{13}C$ value for protein in the typical human diet in western Europe (-23.6 ‰; Nakamura et al., 1982). Notwithstanding any potential differences between humans and gulls in terms of isotopic fractionation, this indicates a substantial element of landfill-derived items in the diet of such birds. The $\delta^{13}C$ value of bone collagen for purely marine consumers is approximately -13 ‰ (Schoeninger and DeNiro, 1984), which is considerably more enriched than the egg values in this chapter, indicating that none of the herring gulls in the present study foraged on an entirely marine diet. However, the mean $\delta^{13}C$ value in the eggs of reference herring gulls in 2017 (-18.75 ± 0.92 ‰) is comparable to that of the marine specialist Audouin's gull breeding in the southern Mediterranean (-18.60 ‰; Roscales et al, 2016). The $\delta^{13}C$ and $\delta^{15}N$ isotopic data for herring gulls in the present study is comparable with dietary information as incorporated into Bayesian mixing models in a previous study for this species in the same geographical area (i.e., western Scotland and Northern Ireland; O'Hanlon et al., 2017), although their study did not examine $\delta^{34}S$ values.

The significant positive relationship between $\delta^{34}S$ and $\delta^{15}N$ values in herring gull eggs (Figure 4.2 B) (mirrored in the case of great black backed and lesser black-backed gulls despite very small sample sizes; Figures 4.6 B and E), has also been reported in the eggs of both Audouin's gulls and yellow-legged gulls (Roscales et al., 2016), as well as chick feathers in yellow-legged gulls (Morera-

Pujol et al., 2018) in Spain. Sulphur values in biota are known to increase with proximity to coastal environments (Michener and Lajtha, 2007) and these data may indicate that dietary items obtained in such habitats (for example, predatory fish and other birds) occupy a relatively elevated trophic level (as demonstrated via elevated $\delta^{15}N$ values). The absence of such a correlation with respect to $\delta^{13}C$ and $\delta^{15}N$ may result from the association of the former isotopic value with benthic (i.e., potentially lower trophic level) environments (France, 1995). However, such conclusions can only be tentative in the absence of dietary samples for the colonies concerned.

The more terrestrial diets of landfill breeding herring gulls in the present chapter appear to result in them being more exposed to BDE-209 (i.e., the major PBDE congener used in the UK; Drage et al., 2016; Ganci et al., 2019; Harrad et al., 2008) (Figures 3.2 and 4.3). This is also to be expected given the known association of this extremely hydrophobic, high molecular weight congener with anthropogenic and terrestrial matrices (Tongue et al., 2019). Chen et al. (2012) undertook a pan-Canadian study of PBDE, HBCDD and NBFR (including BTBPE, DBDPE and PBB) concentrations in the eggs of four species (i.e., ring-billed gulls, California gulls, glaucous-winged gulls and American herring gulls) in relation to their $\delta^{13}C$ and $\delta^{15}N$ values. In keeping with the present chapter, the authors observed that elevated BDE-209 concentrations were associated with the eggs of terrestrial and freshwater breeders (as opposed to marine-breeding individuals), which in turn exhibited relatively lower $\delta^{13}C$ egg signatures. Chen et al. (2012) also reported a significant negative relationship between $\delta^{15}N$ values and α-HBCDD in a freshwater colony of California gulls, suggesting that their foraging at lower trophic levels resulted in reduced egg concentrations of this BFR. The fact that no relationships were observed between HBCDD concentrations and stable isotope ratios in the present chapter may be explained by the lack of significant differences in HBCDD concentrations in the eggs of reference *vs*. landfill herring gulls. This may result from reference gulls visiting a different landfill (Gartbrek, Islay) with a greater proportion of building (i.e., potentially-HBCDD containing) waste (Tables 2.1 and 2.2).

The bioaccumulation and biomagnification potential of different PBDE congeners is influenced by their octanol-water partition coefficient (K_{OW}) and the nature of the food web concerned (i.e., aquatic *vs.* terrestrial; Chen and Hale, 2010; Kelly et al., 2007). Predatory birds associated with terrestrial ecosystems that predominantly consume terrestrial invertebrates, mammals and other birds tend to exhibit a relatively greater percentage of higher brominated congeners, particularly in Europe, in keeping with their greater historic use in that region (Table 1.1; Voorspoels et al., 2006). Conversely, PBDE profiles of birds at the apices of aquatic food chains are often characterised by a greater relative percentage of more bioavailable, lower brominated congeners, especially in North America, where such congeners were subject to greater use (Table 1.1; Chen and Hale, 2010; Elliott et al., 2005; Law et al., 2003; Henny et al., 2009, 2011). In the present chapter, no relationships were identified between egg concentrations of lower brominated congeners and stable isotope values (applying to birds of either colony site type), which may be expected in a European context given the relatively low use of such congeners (Harrad et al., 2008). Roscales et al. (2016) studied $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ as dietary tracers in relation to concentrations and profiles of PBDEs and other POPs (polychlorinated dibenzo-p-dioxins and furans [PCDD/Fs] and non-*ortho* polychlorinated biphenyls [no-PCBs]) in eggs of sympatrically-breeding Audouins gulls and yellow-legged gulls in Spain. There was a significant negative relationship between BDE-209 concentrations and stable isotope values as well as a greater proportion of more bioavailable, lower brominated and chlorinated PBDE congeners in the eggs of the marine specialist Audouin's gull compared to the generalist and more terrestrially-associated yellow-legged gull. This difference was attributed to the greater proportion of fish in the diet of Audouin's gulls, as demonstrated by significantly enriched $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in this species. Roscales et al. (2016) also reported greater variance in terms of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in eggs of yellow-legged gulls compared to Audouin's gulls, as may be expected given the generalist foraging ecology of this species (Duhem et al., 2005; Ramos et al., 2013; Ramos et al., 2008; Steigerwald et al., 2015). In the present chapter, variance in $\delta^{13}C$ values for the eggs of

landfill breeding herring gulls (2.33) was significantly higher than for reference conspecifics, suggesting that landfill-breeding individuals may have relied upon a wider range of resources during the egg formation period.

Various studies have utilised SIA to elucidate BFR burdens in the eggs of waterbirds. For example, no significant relationship was found between ∑PBDEs and $\delta^{15}N$ values in eggs of ancient murrelets (*Synthliboramphus antiquus*), rhinoceros auklets (*Cerorhinca monocerata*) and Leach's storm petrels (*Oceanodroma leucorhoa*) breeding in the Canadian Pacific, although a significant positive relationship between ∑PBDEs and $\delta^{13}C$ was found when eggs of all species were combined (Miller et al., 2014). Canadian-breeding great blue herons (*Ardea herodias*) specialising in intertidal zone prey showed no significant correlations between egg BFR (PBDE and HBCDD) concentrations and $\delta^{13}C$ or $\delta^{15}N$ (Champoux and Boily, 2017). In keeping with the present study, contaminant profiles (particular PBDEs, mercury and DDT) correlated with $\delta^{13}C$, but not with $\delta^{15}N$ in eggs of American dippers (*Cinclus mexicanus*) breeding in Canada (Morrissey et al., 2010). In eggs of Eurasian dippers (*C. cinclus*) $\delta^{34}S$ values were strongly depleted in urban rivers which was suggested to indicate a strong anthropogenic signal (Morrissey et al., 2013).

The significant albeit weak relationship between egg volume and $\delta^{13}C$ values for herring gulls (Figure 4.4) suggests that birds with a more marine diet, which is probably more nutrient-rich (O'Hanlon et al., 2017) lay larger eggs. Similarly, those American herring gull populations in the Laurentian Great Lakes with a more terrestrial diet lay smaller eggs and have reduced reproductive success compared to populations which consume a greater proportion of fish (Hebert, 1999). The significant, although weak correlation between shell thickness and $\delta^{13}C$ in herring gull eggs collected in 2018 (Figure 4.5) indicates that those birds consuming a greater proportion of marine-origin prey laid eggs with thicker shells. Eggshell thickness is a measure of egg viability and therefore reproductive success (Zapata et al., 2018). This may result from greater calcium consumption in such birds, with important sources of dietary calcium in gulls including mussels (Mytilidae) and fish

(Pierotti and Annett, 1990). However, Chapter III also observed that herring gull eggshells were significantly thinner in those eggs containing >50 ng/g ww $\sum_8$PBDEs including BDE-209 (Figure 3.5). It therefore appears possible that both BFR exposure, as well as the extent of marine food in the diet of herring gulls may influence eggshell thickness. Corman et al. (2018) found no correlation between shell thickness and $\delta^{13}C$ values in the eggs of European herring gulls breeding at three colonies along the German North Sea and Baltic Sea coastlines during 1998–2015. However, they did not discuss whether landfill sites were used by birds breeding at these colonies.

Interspecific comparisons of egg isotope values (Figure 4.6) are illustrative only, given the very small sample sizes of eggs collected from great black-backed gulls and lesser black-backed gulls across different years. However, the elevated isotopic values of eggs of landfill-breeding great black-backed gulls suggests that they have a broad foraging niche at an elevated trophic level, in keeping with their foraging (predatory) ecology (Cramp and Simmons, 1983). The relatively depleted $\delta^{13}C$ values in the eggs of reference lesser black-backed gulls (n = 2) indicates that their diet may be predominantly terrestrial. This is unexpected considering that they breed on Colonsay (an island), i.e., where marine prey might be regarded as being readily available. There has been a significant decline in lesser black-backed gull populations across the Hebrides, with a concomittant increase in birds breeding in inland urban centres inland (Bowler, 2014). A relatively large maximum foraging range of 71.9 km has been recorded during the breeding season for this species (compared with 10.5 km for breeding herring gulls) (Thaxter et al., 2012). Despite the very small sample sizes, isotopic egg data from the present chapter indicates that lesser black-backed gulls breeding on Colonsay may be commuting to urban areas to forage. This is further supported by the substantial mean contribution of BDE-209 (of $\sum_8$ PBDEs) in their eggs compared to landfill-breeding conspecifics (60.78 % and 23.21 %, respectively; Figure 3.6). Further study of the foraging movements of Hebridean breeding lesser black-backed gulls may help explain this species' apparent inland shift.

The fact that there was no significant difference in $\delta^{15}N$ values between the eggs of landfill and reference breeding herring gulls indicates that individuals from both colonies were foraging at broadly comparable trophic levels. It has been postulated that enriched $\delta^{15}N$ may also be a marker of anthropogenic activity in Norwegian-breeding white-tailed eagles, given its association with contaminant burdens, potentially reducing its potential as a proxy for trophic level in that species (Eulaers et al., 2014). There is also some evidence that gulls shift their diets prior to egg-laying and thereby influencing stable isotope values in their tissues. For example, the plasma of pre-laying adult glaucous-winged gulls contained significantly lower $\delta^{15}N$ values compared to incubating birds, which Davis et al. (2017) suggested may have reflected a possible requirement of pre-laying females to consume marine invertebrates at a lower trophic level than fish in order to derive nutrients for egg production. Somatic nitrogen values for incubating gulls (incubation being apparently relatively synchronous for all species and colonies) in the present chapter may therefore have been depleted compared to other periods in their annual cycles.

In the present chapter, enriched $\delta^{34}S$ values of eggs laid by reference herring gull eggs in comparison to those of landfill-breeding individuals approached statistical significance. It has been demonstrated that refuse in the diet of yellow-legged gulls results in depleted $\delta^{13}C$ and $\delta^{34}S$ tissue values (Ramos et al., 2009). Roscales et al. (2016) determined that out of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ ratios they analysed in gull eggs, the latter was the best predictor of POP concentrations in gull eggs, although in the present study $\delta^{13}C$ was of the greatest utility in this respect.

Earlier SIA studies (e.g., Hobson et al., 1997; Hobson and Clark 1992b) asserted that in order to make robust connections between the isotopic signatures of egg contents and the likely diet of laying females, diet-tissue isotopic fractionation factors should be calculated using isotopic data for prey items, if available. Hobson (1995) calculated $\delta^{15}N$ and $\delta^{13}C$ fractionation factors for egg yolks of carnivorous bird species (gyrfalcons *Falco rusticolus*, prairie falcons *F. mexicanus* and peregrine falcons), reporting a + 3.4 ‰ fractionation factor for nitrogen isotopes and a fractionation factor of

close to 0 ‰ in the case of carbon. The study taxa (gulls) in the present thesis are dietary generalists, although a proportion of their diets will be carnivorous (Cramp and Simmons, 1983). The above fractionation factors suggest a mean $\delta^{15}N$ value in the diet of landfill-breeding herring gulls of 10.71 ‰, with the equivalent figure for reference individuals being 10.63 ‰. Bond and Jones (2009) in a review of the literature suggested that fractionation factors for seabirds fell between 2 ‰–5 ‰ for $\delta^{15}N$ and 0 ‰–2 ‰ for $\delta^{13}C$, but stressed that fractionation factors are unique to each tissue–consumer species–prey species situation, indicating that the calculation of mean isotopic values for subjects in the present study may be less straightforward than suggested in earlier literature.

Future research into contaminant exposure across avian species assemblages associated with landfill would likely benefit from ongoing use and development of SIA. The present study, as well as others (e.g., Arizaga et al., 2013; Caron-Beaudoin et al., 2013; Roscales et al., 2016) have successfully used such methods to elucidate the diets of birds that use landfill. The potential issue of bird exposure to a range of isotopic values at landfill sites might be addressed by combining isotopic data with other dietary data such as regurgitant, pellets, as well as the use of GPS telemetry to obtain spatiotemporal data in relation to diet. In this thesis, SIA data supports the proposed use of European herring gulls as a bioindicator species of BFR emissions from municpal solid waste landfill in north-west Europe, revealing important findings in terms of differences in the diet and BFR exposure of landfill-associated subjects and reference conspecifics.

4.6 Conclusions

This chapter has demonstrated that herring gulls breeding in proximity to a municipal solid waste landfill exhibit significantly depleted $\delta^{13}C$ egg values and has identified a negative relationship between egg concentrations of BDE-209 and $\delta^{13}C$ enrichment. This suggests that the more terrestrial diet of landfill breeding individuals appears to result in them being exposed to this congener, formerly widely used in the UK and typically associated with landfill abiotic matrices. Reference eggs contained enriched $\delta^{34}S$ values that approached significance. Egg size and volume were both

positively, though weakly, correlated with a greater marine component in the diet of herring gulls. Despite very limited sample sizes, insights have been obtained into BFR trophodynamics in great black-backed and lesser black-backed gulls. These include evidence to suggest that lesser black-backed gulls breeding in Scotland's Inner Hebrides forage in predominantly terrestrial environments, where, as demonstrated in Chapter III, they appear to be exposed to higher brominated PBDE congeners.

The present chapter documents the first use of dietary tracers to investigate the role of landfill as a source of BFR exposure in UK-breeding gulls. To the author's knowledge, a total of two published studies to date (Chen et al. 2012; Roscales et al., 2016) have employed SIA to elucidate BFR contamination in the eggs of generalist larids. Both of these studies also reported that gulls exhibiting a greater proportion of terrestrial items in their diets (i.e., as evidenced by depleted $\delta^{13}C$ *in ovo* values) have elevated BDE-209 egg burdens. However, unlike the present chapter, both studies also observed positive relationships between stable isotopic signatures and the relative proportion of lower brominated BDEs, suggesting that marine-foraging gulls were at greater risk of exposure to these potentially more bioavailable congeners. Differences in terms of local ecological conditions, species' ecology and use of different BFR formulations in different jurisdictions may account for such disparity.

Studies using isotopic signatures in isolation are not able to resolve the extent to which the inhalation of aerolised particulates, dermal exposure or exposure via feather maintenance using preen oil containing deposited particulates play a part in BFR toxicokinetics. Work on flame retardant contamination in gulls has yet to utilise direct behavioural observations to assess how foraging and loafing behaviours of birds may influence BFR burdens. Chapter V examines the behaviour of three species of gulls that used the study landfill in relation to their flame retardant egg burdens.

CHAPTER V

BEHAVIOURAL ECOLOGY OF LANDFILL-FORAGING GULLS IN THE CONTEXT OF POTENTIAL FLAME RETARDANT EXPOSURE PATHWAYS

5.1 Synopsis

The exposure routes of BFR contamination in free-living avifauna are poorly characterised. Landfill-associated birds exhibit elevated BFR burdens compared to reference conspecifics. Approximately 250 bird species are associated with landfill globally, several of which are of conservation concern. Exposure to BFRs has been demonstrated to exert various deleterious effects on birds, including behavioural change, immunosuppression and oxidative stress. These ubiquitous chemicals, several of which are classified as POPs, are under increasing scrutiny as a challenge for avian conservation. This chapter addresses the potential exposure routes of FRs in three landfill-associated gull species (great black-backed gulls, European herring gulls and lesser black-backed gulls), based on field observations at a study landfill in western Scotland (UK). Behaviour is analysed in the context of BFR and SIA data for the eggs of gulls breeding in proximity to the landfill and reference conspecifics. The results suggest that direct ingestion of anthropogenic items is likely to be less important than dermal exposure, inhalation of aerosolised particulates and exposure via preening.

5.2 Introduction

Chapter III discussed BFR profiles and concentrations in the eggs of landfill-breeding and reference populations of gulls breeding in western Scotland. Chapter IV used trophic ecology (via stable isotope ratios in eggs) to elucidate relationships between trophodynamics and BFR burdens. The eggs of landfill breeders contained higher concentrations of PBDEs and the NBFR, DBDPE. Stable isotope analysis established that landfill-breeding herring gulls had significantly depleted $\delta^{13}C$ ratios compared to reference individuals, indicating a relatively greater component of terrestrial dietary

items in the case of the former. Carbon stable isotope ratios were significantly positively correlated with egg concentrations of BDE-209 in landfill breeders. However, given that no relationships were observed in terms of d $\delta^{15}N$ and s $\delta\ ^{34}S$ ratios and BFRs, diet alone may be insufficient to explain BFR burdens. Another method of examining BFR exposure risk in landfill-associated birds is the analysis of their behaviour at such sites.

Behavioural studies play an increasingly important role in ecotoxicological research, particularly in terms of elucidating the patho-physiologic effects of toxicants at the level of the whole organism. A recent review of the effects of FRs on birds noted that all studies examining FR exposure in relation to bird behaviour (i.e., frequency of vocalisation, courtship behaviour and parental behaviour) published up to 2015 (n = 7) reported significant negative effects (Guigueno and Fernie, 2017). Exposure to other contaminants such as metals and trace elements has also been shown to affect bird behaviour (Burger and Gochfeld, 2000; Whitney and Cristol, 2018). However, studies of the behavioural repertoire of free-living organisms are also important in terms of identifying the various potential pathways by which contaminant burdens may be accrued. Research on human FR uptake has estalished that exposure is primarily via dietary sources (Schecter et al., 2008), the incidental ingestion / inhalation of contamined dust (Roosens et al., 2009) / air (Allen et al., 2007) and via dermal contact (Abdallah and Harrad, 2018). Such exposure routes may also be important in the case of free-living birds, although limited research has been undertaken in this area to date. A review of the 18 studies published to 2018 reporting FR concentrations in landfill-associated avifauna (Tongue et al., 2019) observed that few studies undertook detailed analyses of the potential pathways by which such birds accrued their pollutant burdens. Direct ingestion of FR-treated items or their abraded particles, either deliberately or inadvertently, as a result of foraging, ‘gritting’ (the intake of grit to augment gizzard function in grinding ingested food material), preening, bathing or drinking, may be an important FR exposure route for birds associated with landfill (Seif, 2017), and diet has been shown to be an important pathway of contaminant exposure in birds (e.g., Hebert et al., 2000;

Ma et al., 2018; Manosa et al., 2003; Newsome et al., 2010; Santos et al., 2017). Another possible exposure route may be the inhalation of airborne gaseous and particulate-phase FRs, especially since the substrate of municipal solid waste landfill facilities is subject to regular perturbation via the operation of on-site machinery and large aggregations of foraging birds themselves (Coulson, 2015; Fernie et al., 2018a; Gentes et al., 2015; Sorais et al., 2017). In comparison to other vertebrates such as mammals, birds appear to be particularly susceptible to airborne environmental insults: Given the demands of flight, the multiple air sacs of the avian respiratory system require a larger gas uptake relative to mammals, placing birds at potentially greater risk of exposure to airborne contaminants, *ceteris paribus* (Brown et al., 1997; Sanderfoot and Holloway, 2017). Furthermore, compared to mammals, bird species possess fewer airway macrophages, phagocytic cells important in the clearance of particulates from the lower airways (Brown et al., 1997; Hussell et al., 2014; Sanderfoot and Holloway, 2017). As a consequence, little is known about the mechanisms responsible for the clearance of particulates from the avian respiratory system. Landfill-foraging birds may also be at risk of dermal contact with BFRs via their skin or their bare parts, such as the feet, which in the case of gulls, possess a relatively large surface area since they are fully webbed. This may place gulls at higher risk of dermal BFR exposure compared to other landfill-associated birds, such as vultures (Cathartidae, Gypaetinae and Aegypiinae), crows (Corvidae) and starlings (Sturnidae), which do not have webbed feet, as well as storks (Ciconiidae) and ibises (Threskiornithidae), which have partially-webbed feet (Miller and Fowler, 2015). Dermal exposure via the metatarsal pad has been shown to constitute an important exposure pathway for insecticide contamination in various avian taxa, including Canada geese (*Branta canadensis*), feral pigeons (*Columba livia*), Neotropical warblers (Setophaga spp.) and brown-headed cowbirds (*Molothrus ater*) (Alharbi et al., 2016; Henderson et al., 1994; Vyas et al., 2003, 2004, 2006). Dermal exposure remains an under-explored area of avian toxicology (Mineau, 2011).

Gull use of landfill has been widely studied, and includes research in terms of the numbers and species composition of gull assemblages associated with such sites (Belant et al., 1995; Bosch, 1994; Duhem et al., 2003a; Götmark, 1984a; Kihlman and Larsson, 1974; Patton, 1988), the effects of changes in the availability of landfilled refuse on gull populations (Duhem et al., 2003b; Kilpi and Ost, 1998; Pons, 1992; Weiser and Powell, 2011), the relative contribution of landfilled waste to gulls diets (Abdennadher et al., 2014; Duhem et al., 2005; Ramos et al., 2008) and differences in gull use of landfill between sexes and temporally (Coulson et al., 1987; Monaghan and Metcalfe, 1986; Sibly and McCleery, 1983). However, few studies have been published which examine in any detail the behavioural repertoire of gulls when foraging on landfill and, aside from work using GPS telemetry to examine landscape-scale habitat utilisation (Blanco et al., 2018; Gentes et al., 2015), none has undertaken detailed ethological research of gull behaviour on landfill in order to identify potential BFR exposure pathways. Of particular relevance to this thesis was the study by Greig et al. (1986) comparing and analysing the foraging strategies of four gull taxa on landfill sites in north-eastern England. The authors obtained video footage of black-headed gulls, great black-backed gulls and herring gulls (among the latter, differentiating between the smaller, resident *L.a. argenteus* and the larger winter-visiting nominate *L.a. argentatus*, breeding from Scandinavia eastwards to the Kola Peninsula, with individuals of either subspecies trapped and colour-ringed). Greig et al. (1986) classified the incidence of different foraging behaviours for individual taxa under different feeding conditions via the use of an ethogram (i.e., a list of behavioural categories). This was adapted for use in the present study to identify potential routes of BFR exposure in gulls frequenting landfill (Table 5.1). The foraging ecology of the three focal species in the present chapter is well doccumented (e.g., Verbeek 1977 a,b,c; Verbeek, 1979). When foraging in intertidal habitats, herring gulls methodically dig and pull with the bill at items such as marine invertebrates. This led Verbeek (1977a) to assert that such behaviour had preadapted this species to exploit novel environments such as landfill, where it behaves similarly: digging and pulling at the substrate in order to isolate and obtain human food

Table 5.1 Ethogram of those behaviours of great black-backed gulls, herring gulls and lesser black-backed gulls observed on the study landfill recorded as potential routes of BFR exposure.

Individual behaviour	Description
Pecking at substrate / food item	Downward movement of the head so that one or both mandibles comes into contact with substrate / food item.
Swallowing an item	Obvious ingestion of item (food item seen to enter mouth and, with larger items, apparently entering oesophagus and / or crop).
Standing stationary on the landfill substrate	Feet still whilst bird stood on substrate surface.
Walking across landfill surface	Moving over the landfill surface on foot.
Preening	Tending to feathers with the bill (e.g., for feather maintenance).

refuse which may be admixed with other anthropogenic waste and soil due to the activities of on-site machinery. The resourcefulness of herring gulls foraging in anthropogenic environments has been documented elsewhere (e.g., Henry and Aznar, 2006; Holman et al., 2019). Verbeek (1977b) observed that adult herring gulls are more successful at uncovering (and hence obtaining) food items on landfill (3.6 objects moved per minute, compared to immature birds, particularly individuals in their first calendar year, which moved 0.2 objects per minute). This might explain why adults are more numerous than first-year birds on landfill (Monaghan, 1980). The relatively greater use of landfills by adult herring gulls may also place them at a higher risk of contaminant exposure compared to immatures. This would be exacerbated by the bioaccumulative nature of POPs (i.e., as an organism ages, body burdens increase; Shaw and Chadwick, 1998). Verbeek (1977a) found that landfill-foraging herring gulls independently obtained more food items than did lesser black-backed gulls (2.43 *vs*. 1.67 pecks that resulted in food items being swallowed, per minute). Verbeek (1979) also hypothesised that landfill-foraging great black-backed gulls obtained the majority of their food by

stealing on the ground from the other two species, especially herring gulls; predominantly via attacks on the ground.

Landfill sites are also routinely used by gulls for the purposes of loafing: Horton et al. (1983) attributed the widespread loafing behaviour of landfill-foraging gulls in the south of England to the 'superabundance' of food at such sites, which may have offered birds increased opportunities to conserve energy by resting. Belant et al. (1993) asserted that in the northern US, landfill was equally important for American herring gulls for loafing purposes as it was in terms of foraging, presumably by facilitating social interaction and providing an information centre (Ward and Zahavi, 1973). Coulson (2015) hypothesised that widespread loafing by European herring gulls at landfill facilities resulted from a hesitancy on the part of individuals to be the first to alight at the active tip face and forage, since large gulls can be particularly wary when in close proximity to humans (Burger and Gochfeld, 1983a). Preening is a typical behaviour performed by gulls loafing on landfill (Coulson, 2015; Curtis, 1993). As with foraging, preening may be a potential BFR exposure route for landfill-associated gulls. The toxicological implications of aerosolised pesticides on the plumage of queleas *Quelea* spp. in sub-Saharan Africa exposed to such chemicals as part of bird control operations were highlighted by Mineau (2011). External anthropogenic chemical contamination of plumage has been demonstrated to occur in the case of mallards *Anas platyrhynchos* (Espin et al., 2010), clapper rails *Rallus crepitans* (Summers et al., 2010) and white-tailed eagles (Jaspers et al., 2011). Preening may lead to the ingestion of FR-adhered particulates which have undergone aerial deposition onto avian plumage (Garcia-Fernandez et al., 2013). Furthermore, the waxy nature of preen oil, derived from the uropygial gland and applied to feathers with the bill for purposes such as feather maintenance may inadvertently facilitate the adherence of particulates onto the plumage (Jaspers et al., 2007b). In the case of herring gulls, preen oil contains monoester waxes composed of approximately 30 saturated C_7–C_{16} fatty acids and 50 saturated C_{11}–C_{20} alcohols (Fischer et al., 2017). However, endogenous organic pollutant contamination of plumage via preen oil has been found to be substantially more

important than external contamination, such as in common buzzards and Eurasian magpies (Jaspers et al., 2007a; Jaspers et al., 2008). This has implications when assessing whether feather contamination may result from atmospheric deposition onto the plumage or whether it may arise due to internal contaminant burdens, derived from the application of preen oil (Jaspers et al., 2011). In gulls, preen oil is known to contain FRs (e.g., $\sum_4$PBDEs 1.6±0.4 [SE] ng/g lw in yellow-legged gulls in Turkey; Kocagöz et al., 2014). Preening may also lead to direct dermal exposure to FRs as such molecules could potentially make contact with the skin when feathers are parted at the calamus (Rajchard, 2010). Furthermore, feathers have been demonstrated to provide an insufficient barrier to protect the skin of birds from chemical exposure (Pope and Ward, 1972). The aerial photograph in Figure 2.8 depicts the respective parts of the study landfill where birds foraged and loafed.

The aim of this chapter was to analyse and compare the foraging and preening behaviours of the three species (great black-backed gulls, herring gulls and lesser black-backed gulls) frequenting the study landfill in order to determine species-specific routes of potential exposure in such heavily BFR-contaminated environments. The key objective was, via field observations and consultation of the literature, to produce a behavioural ethogram to establish and compare the frequency with which each species performed behaviours considered likely to result in BFR exposure. The working hypothesis of this study was that, as a result of their behavioural ecology, herring gulls spent proportionately greater amounts of time pecking and walking in comparison to other species, thus potentially putting them at greater risk of BFR contamination via contaminated substrate, notwithstanding the possibility of intra- and interspecific differences in terms of organohalogen toxicokinetics.

5.3 Materials and Methods

Site selection, field sampling and the production and analyses of video recordings, including statistical analyses, were all undertaken as described in Chapter II.

5.4 Results

5.4.1 Numbers of foraging birds

Herring gulls were the most numerous gull species foraging at the landfill by several orders of magnitude, comprising on average 91 % of all foraging gulls (range: 90–97 %). Great black-backed gulls comprised on average 6 % (range: 2–7 %) and lesser black-backed 2.5 % (range: 0–3 %). The arithmetic mean total number of foraging gulls over the period during which behavioural observations took place was 440.80 ± 3.26, with the maximum number of foraging birds being estimated at approximately 800. Given their numerical dominance, herring gulls were the primary focus of foraging analyses, in keeping with the flame retardant (Chapter III) and stable isotope (Chapter IV) data. These were the only gull species present on the landfill, except for one each of glaucous gull *L. hyperboreus* and Iceland gull *L. glaucoides*, both second calendar-year individuals that were observed on one occasion each. In total, 2,329 foraging bird-observations were made, comprising 1,961 of herring gulls, 255 of great black-backed gulls and 113 of lesser black-backed gulls. Birds were not individually marked and it was therefore not possible to obtain insight into the turnover of individuals on the landfill. The total number of foraging birds increased during the course of the operational day (slope = 0.13, $t = 9.89$, d.f. = 2,327, $P < 0.001$; adjusted r-squared = 0.03; $n = 2{,}329$; Figure 5.1).

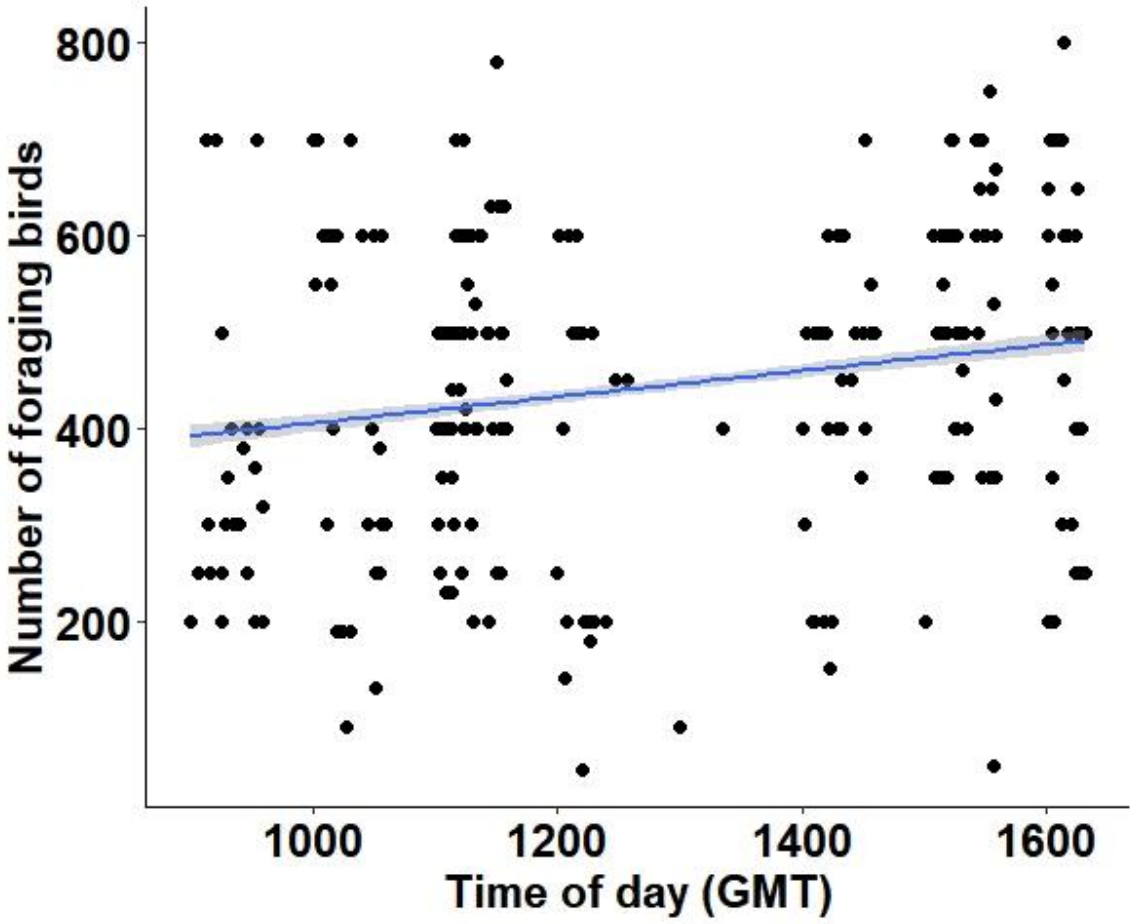

Figure 5.1 The relationship between the number of foraging gulls at the study landfill in western Scotland and time of day (data obtained during April 2018). The limited observations during approximately 13.00–14.00 hrs reflect a comfort break taken by the observer at this time.

5.4.2 Foraging behaviour

5.4.2.1 Number of pecks at the substrate

The number of pecks at the substrate made by birds was zero-inflated (Figure 5.2A). Median peck rates were 0 (arithmetic mean: 2.15 ± 0.21 SE); range: 0–20 for great black-backed gulls, 2 (2.98 ± 0.06; 0–21) for herring gulls and 2 (1.88 ± 0.16; 0–7) for lesser black-backed gulls. Herring gulls exhibited the lowest proportion of zero pecks (23.3 %), followed by lesser black-backed gulls (30.08 %) and great black-backed gulls (50.5 %). The frequency of pecks at the substrate differed significantly between species (Kruskal-Wallis: $\chi^2 = 58.25$, d.f. = 2, $P < 0.001$; Figure 5.3A).

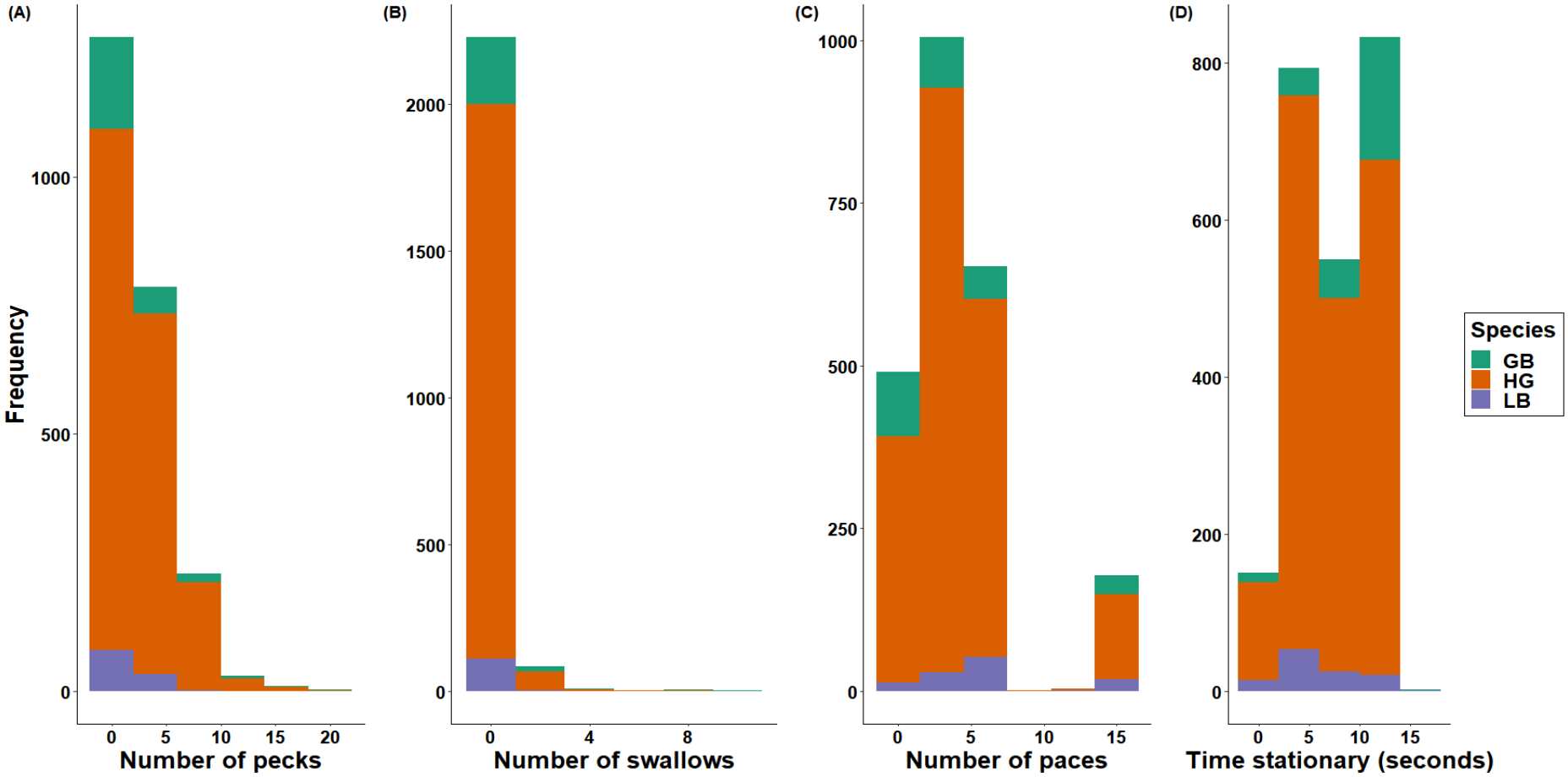

Figure 5.2 Frequency distribution of pecks at the substrate (A), swallowing events (B), paces across the substrate and (C) time spent stationary on the substrate (D) per 15 second observation of great black-backed gulls (GB; *n* = 255), herring gulls (HG; *n* = 1,961) and lesser black-backed gulls (LB; *n* = 113) foraging at the study landfill in western Scotland during April 2018.

Post-hoc comparisons (Wilcoxon Rank Sum test, Holm-adjusted) revealed that there were significant differences between great black-backed gulls and herring gulls (P <0.001) and between lesser black-backed gulls and herring gulls (P <0.001).

5.4.2.2 Number of swallowing events

The number of occasions that birds were observed to swallow items was highly zero-inflated: out of a total of 2,329 bird-observations, 2,023 (86.8 %) resulted in no items being swallowed (Figure 5.2 B). Median swallowing rates were 0 (arithmetic mean: 0.42 ± 0.08; range: 0–10) for great black-backed gulls, 0 (mean: 0.19 ± 0.01; range: 0–8) for herring gulls and 0 (mean: 0.07 ± 0.03; range: 0–8) for lesser black-backed gulls. The difference between species in terms of the number of swallowing events was close to being significant (χ^2 = 8.34, d.f. = 2, P = 0.015; Figure 5.3B) (α = 0.01; Chapter II).

5.4.2.3 Number of paces

The median number of paces made by each species whilst foraging was 2.5 (arithmetic mean: 3.94 ± 0.30; range: 0–15) for great black-backed gulls, 2.5 (mean: 4.26 ± 0.08; range: 0–15) for herring gulls and 7.5 (mean: 6.59 ± 0.43; range: 0–15) for lesser black-backed gulls (respective frequencies displayed in Figure 5.2C). Interspecies differences were significant (χ^2 = 46.31, d.f. = 2, P <0.001; Figure 5.3C). Post-hoc tests showed that significant differences existed between lesser black-backed gulls and the other two species (both P values < .0001).

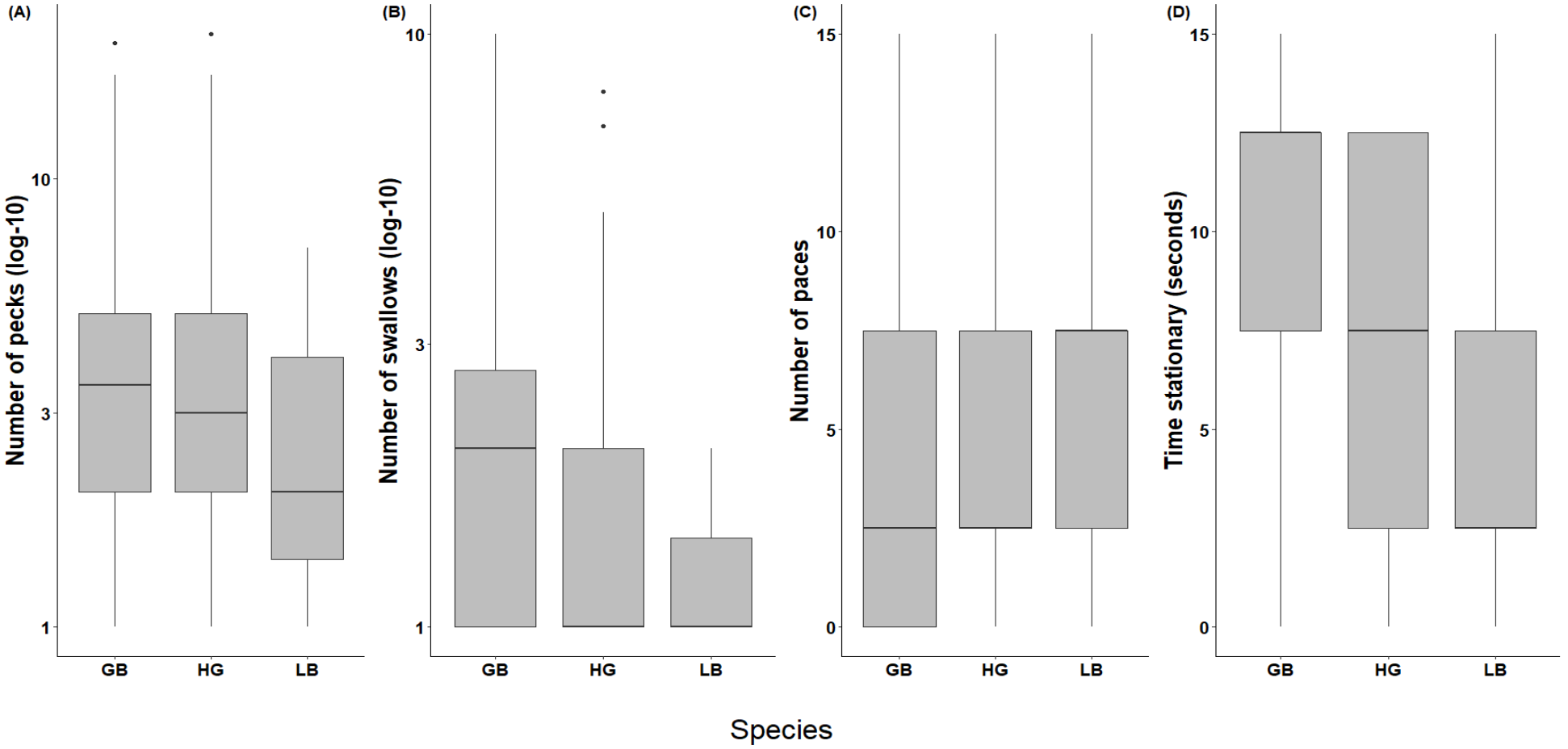

Figure 5.3 Box and whisker plot showing rates of pecking at the substrate (A), number of swallowing events (B), number of paces across the substrate (C) and time spent stationary on the substrate (D) per 15-second observations of great black-backed gulls (GB; *n* = 255), herring gulls (HG; *n* = 1,961) and lesser black-backed gulls (LB; *n* = 113) foraging at the study landfill in western Scotland in April 2018. Black lines within boxes indicate medians, boxes indicate the 25th and 75th percentiles, whiskers indicate the 10th and 90th percentiles. Dots denote outliers.

5.4.2.4 Time spent stationary

The median amount of time spent stationary during foraging, per species, was 12.5 (arithmetic mean: 9.53 ± 0.26; range: 0–15) for great black-backed gulls, 7.5 (mean: 6.90 ± 0.10; range: 0–12.5) for herring gulls and 2.5 (mean: 5.17 ± 0.40; range: 0–15) for lesser black-backed gulls (respective frequencies shown in Figure 5.2 D). Interspecies differences in time spent stationary during foraging were significant (χ^2 = 93.50, d.f. = 2, P <.0001; Figure 5.3 D). Post-hoc tests identified significant differences between all species (for all pairwise comparisons, P < .0001).

5.4.3 Preening behaviour

5.4.3.1 Numbers of loafing birds

The area of the landfill where birds loafed (a plastic-covered embankment of landfilled waste located approximately 30 m. immediately south-west of the active tip face; Figure 2.8) was occupied by gulls during the course of the operational day. Gulls of all three species gathered on the embankment early in the morning (from at least 07.00 hrs GMT) prior to the start of tip operational activities at approximately 09.00 GMT. The total number of loafing birds decreased during the day (slope = -0.1, t = -3.20, d.f. = 127, adjusted r-squared = 0.06, P = 0.001, n = 650; Figure 5.4). During loafing, birds would engage in any of the following activities (in approximate descending order of frequency): a. standing, b. sitting

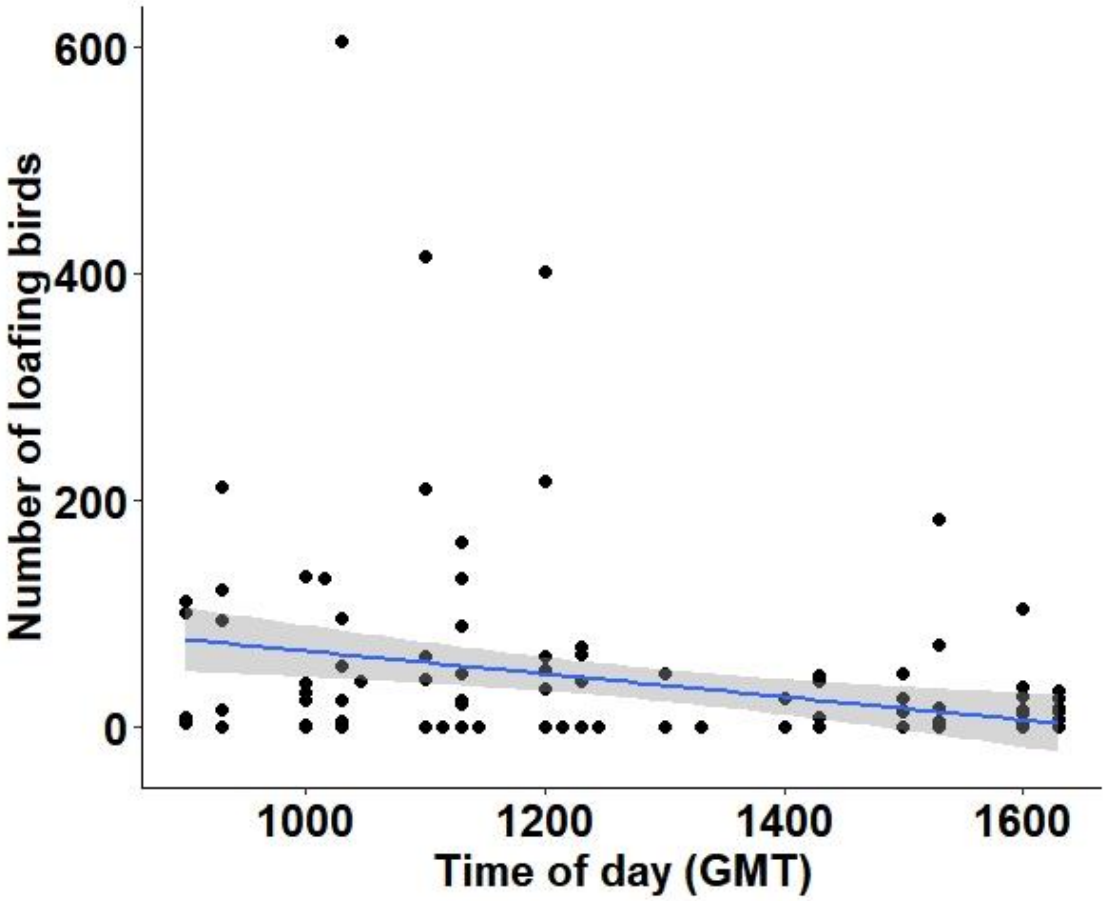

Figure 5.4 The relationship between the number of loafing gulls at the study landfill in western Scotland and time of day (data obtained during April 2018). The limited observations during approximately 13.00–14.00 hrs reflect a comfort break taken by the observer at this time.

on their bellies, c. preening, and, d., social behaviour, including long-calling, pair bonding and direct copulation. During the occurrence of heterosexual behaviour, allopreening was not observed. The arithmetic mean number of loafing gulls was 37.7 ± 3.28 (range: 0–605), constituting 16.2 ± 7.62 % of total gulls on site. As with foraging behaviour, the most numerous species at the loafing area was herring gulls, comprising on average 86.3 % (range: 0–100 %) of all loafing birds. The remainder were lesser black-backed gulls (10.2 %; range: 0–100 %) and great black-backed gulls (3.5 2 %; range: 0–26 %).

5.4.3.2 Preening observations

Preening episodes were infrequently observed: of a total of 129 observations of loafing birds, preening was recorded for 20, or 15.5 % of such observations. Preening occurred in discrete episodes numbering 3 (14 %) in great black-backed gulls, 13 (12 %) in herring gulls and 4 (14 %) in lesser black-backed gulls. The average duration of time spent preening by individual species was similar, being 116, 100 and 111 seconds for great black-backed gulls, herring gulls and lesser black-backed gulls, respectively. Sample sizes were insufficient to permit statistical tests of preening data.

5.4 Discussion

The study landfill appears to have been an important foraging and loafing site, particularly for herring gulls (comprising over 90 % of all gulls present), but also for great black-backed gulls and lesser black-backed gulls. In light of the number of foraging observations (2,329) relative to the total numbers of foraging birds present (maximum of 800 at any one time), it is possible that individual birds were observed on more than one occasion and future studies would benefit from working with individually-marked birds. There were significant interspecies differences in terms of foraging strategy, although pecking at the substrate and the swallowing of items were infrequently observed. This may suggest that ingestion of food items is unlikely to be the main source of BFR contamination in gulls frequenting landfill, with dermal contact via the feet / skin or via inhalation of airborne particulates potentially of importance as exposure routes. Exposure to BFRs via consumption of human food refuse was considered unlikely to be the primary source of BFR burdens in landfill-foraging ring-billed gulls in Canada due to the trace amounts of these compounds in human foodstuffs (Desjardin et al., 2019). Chapter III also observed that the eggs of black-headed gulls and common gulls breeding in proximity to the landfill contained elevated BFR concentrations in comparison with reference conspecifics, despite neither species being observed to use the landfill, potentially implicating airborne contamination. Nevertheless, the fact that the terrestrial diets of landfill-breeding

herring gulls (in 2017 only) appear to result in them being exposed to BDE-209 (Figure 4.4) indicates the need for further work to understand the relative importance of respective exposure pathways.

5.4.1 Foraging

Despite data being zero inflated, the fact that herring gulls showed the lowest proportion of zero pecks is in accordance with the ethological literature (eg., Greig et al., 1986; Verbeek, 1977a). For this species, pecking in the substrate may represent a relatively important route of BFR exposure, (as evidenced by the negative relationship observed between $\delta^{13}C$ values and BDE-209 egg concentrations). The fact that there was no significant difference in peck rates between great black-backed gulls and lesser black-backed gulls may be explained by the former species spending a greater proportion of time on the landfill observing herring gulls (in anticipation of stealing their food) and the latter being easily displaced by herring gulls as the latter worked over the surface, thereby being competitively excluded. Food appears to have been scarce at the study landfill. Captive herring gulls have been shown to tolerate starvation for up to eight days, leading (Spaans, 1971) to conclude that this species is adapted to exploit a sporadic food supply. The intermittent availability of food on landfill may to some extent be compensated by the relatively high energetic value of human refuse to gulls when such items are obtained (van Donk et al., 2017, 2019). As discussed in Chapter IV, $\delta^{13}C$ values in the eggs of landfill-breeding herring gulls (significantly depleted compared to reference birds) are commensurate with those for protein in the typical human diet in western Europe (Nakamura et al., 1982), suggesting that birds must obtain a certain quantity of food from the landfill.

Dermal exposure via the relatively large, webbed feet of gulls may constitute an important potential BFR exposure route for individuals foraging on landfill. The significantly higher number of paces across the substrate made by foraging lesser black-backed gulls (Figure 5.3 C) suggest that this species may be at comparatively greater risk of landfill dermal BFR exposure via the feet, *ceteris paribus*. Table 3.7 showed that mean $\sum_8$PBDE inc. BDE-209 concentrations in the eggs of lesser black-backed gulls were the highest of all five species from which eggs were collected from landfill

colonies in 2016. Such data may relate to greater dermal exposure in this species, although it could equally mean that lesser black-backed gulls metabolise PBDEs less effectively. In contrast, foraging great black-backed gulls spent significantly more time stationary compared to the other two species and this may potentially expose the skin of great black-backed gulls to BFRs for longer periods (Figure 5.3 D). The behaviours of both lesser black-backed gulls (moving quickly across the substrate) and great black-backed gulls (standing still for longer periods) both require further investigation as to their relative implications for BFR exposure in landfill-associated individuals in either species. Dermal exposure via the skin and feet of birds to organophosphate insecticides has been identified as a contamination pathway (Alharbi et al., 2016; Henderson et al., 1994; Mineau, 2011; Vyas et al., 2003, 2004, 2007). The toxicity of the organophosphate insecticide diazinon in Canada geese has been found to be greater in birds exposed dermally (via the feet) in comparison to orally-dosed individuals (Vyas et al., 2006). In feral pigeons, plasma cholinesterase activity recovered more slowly following foot exposure to diazinon and another organophosphate insecticide, parathion compared to birds exposed orally. It was hypothesised that pesticide residues accumulated under the scutes of the feet of feral pigeons could enter the bloodstream (Henderson et al., 1994). Dermal contact with the organophosphate insecticide chlorpyrifos applied to foliage in agricultural plantations in their central American wintering grounds is the likely explanation for such chemicals being detected in the feet of six Neotropical warblers (*Setopahaga* spp.) in Canada, with a maximum concentration of 17.9 ± 29 pg/mg reported in the feet of an individual black-throated blue warbler (*Setophaga caerulescens*) (Alharbi et al., 2016). The feet of sacrificed brown-headed cowbirds following organophsphate exposure and weathered for up to 28 days retained 37 % and 43 % of their chlorpyrifos and diazinon concentrations, respectively (Vyas et al., 2003). Dermal contact is increasingly recognised as an important pathway of FR exposure in humans (Abdallah et al., 2015; Abdallah and Harrad, 2018; Abdallah et al., 2016) and further research is required to establish its importance in terms of the exposure of birds and other wildife to BFRs.

5.4.2 Preening

Preening has been identified as a contaminant exposure pathway in an increasing number of avian toxicological studies. For example, ingestion via the preening of contaminated feathers, preen oil and inhalation of aerosolised particulates are considered the most important *deca*-BDE exposure pathways for landfill-associated ring-billed gulls (*Larus delawarensis*) in Canada (Desjardins et al., 2018; Gentes et al., 2015), notwithstanding the possibility that BFR molecules may adsorb to human food refuse on landfill prior to ingestion. In northern bobwhites (*Colinus virgianus*) dermal uptake, preening and inhalation were all found to be more important than oral exposure in terms of parathion contamination (Driver et al., 1991). The preening observations in the present chapter suggest that there was little difference between the three study taxa in terms of the proportion of loafing individuals engaging in preening. Likewise, the average duration of preening was similar for all three species, hinting at the potential importance of preening as an exposure route for all three species. However, sample sizes were small and did not permit statistical testing. Preening is recognised as a 'comfort behaviour' in birds (Delius, 1988; Van Rhijn, 1977). Given the infrequency of preening episodes observed at the study landfill, it is possible that the presence of a novel structure, i.e., the portable chair hide, positioned approximately 50 m from the loafing area, may have led to loafing birds being in an enhanced state of alertness. Loafing birds were frequently observed to look in the direction of the chair hide until the end of the final week of observations, indicating that habituation to the hide did not occur. The incidence of preening in Sandwich terns (*Thalasseus sandvicensis*) and common terns (*Sterna hirundo*) was negatively correlated with the frequency that an observer placed their hand outside of a temporary hide and with which humans passed within 50 m. of breeding colonies of both species in the Netherlands (Van Iersel and Bol, 1958). Vehicles provide suitable vantage points from which to observe the behaviour of bald eagles (*Haliaeetus leucocephalus*) on landfill, and they may disturb birds less than temporary hides (Elliott et al., 2006a). However, a vehicle would have been unsuitable at the study landfill in this thesis given that the full extent of the

loafing area was not completely visible from the site's vehicular track. Where suitable, further work, perhaps involving vehicles, rather than small hides, may be required to obtain a more accurate understanding of the incidence of preening among gulls of different species loafing at municipal solid waste landfill.

5.4.3 Inhalation as a potential source of BFR contamination

The avian respiratory system is known to differ markedly from that of mammals in several important respects. Many such differences (for example, typically a reduced number of macrophages, different enzymatic processes and a near-constant airflow in the lung) mean that birds may be more susceptible to airborne contaminants relative to other groups (Brown et al., 1997; Hussell et al., 2014; Sanderfoot and Holloway, 2017). Preening and inhalation are considered to be more important sources of exposure compared to their invertebrate diet in terms of polycyclic aromatic compound (PAC) contamination in tree swallows (*Tachycineta bicolor*) breeding in Canada's Athabasca oil sands region (Fernie et al., 2018a,b) and respiration has been proposed as a pathway for FR exposure in landfill-associated avifauna (Gentes et al., 2015; Sorais et al., 2017). Similarly, elevated lead concentrations in nestling blood and addled (failed) eggs of black kites (*Milvus migrans*) breeding in proximity to a solid waste incinerator were postulated to result from exposure to airborne lead particulates (Blanco et al., 2003), whilst Peach et al. (2018) reported a significant positive correlation between the abundance of territorial male house sparrows and reduced levels of atmospheric nitrogen dioxide across London (UK), leading the authors to suggest that further work is required to understand the impacts of airborne pollutants on the viability of urban bird populations. However, in what may be an evolutionary response, some bird populations regularly exposed to anthropogenic pollution appear to be better adapted to survive in contaminated environments. For example, airway macrophages are more commonly found in urban-living feral pigeons, laughing doves (*Spilopelia senegalensis*), cape starlings (*Lamprotornis nitens*) and house sparrows compared to rural conspecifics (Lorz and Lopez, 1997; Steyn and Maina, 2015). Similarly, hepatic mixed-function

oxidase in black-headed gulls associated with landfill has been shown to perform greater detoxification activity compared to conspecifics frequenting other habitats (Fossi et al., 1991).

5.6 Conclusions

This chapter is the first study globally to characterise likely BFR exposure routes in landfill-associated gulls, providing behavioural data for a landfill assemblage of three species. An ethogram identified those behaviours that were most likely to be pathways of BFR exposure. Significant interspecies differences were identified in terms of foraging strategy. Given the extent of zero inflation regarding pecking and swallowing data for all species, ingestion of food items is unlikely to be the only source of BFR contamination in landfill-breeding gulls, despite herring gulls (in 2017 only) exhibiting elevated BFR burdens in relation to depleted $\delta^{13}C$ values in comparison with reference conspecifics. Further research is required to understand the roles played by dermal exposure, inhalation of aerosolised particulates and exposure via preening as BFR exposure pathways for landfill-associated gulls. Dermal exposure could be examined via anlyses of BFR concentrations and profiles on the feet of gulls, especially at landfills where culling is practiced. Work on inhalation exposure risk is already underway, including the use of passive air samplers affixed to ring-billed gulls (Sorais et al., 2020). Further work on preening could be undertaken on captive subjects.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Brominated flame retardants, a large group of synthetic organohalogenated compounds with superior fire retardant properties, have been used extensively in product manufacture worldwide, in line with the exponential growth in the output of polymeric items and in compliance with improvements in fire safety standards and regulations (Alaee et al., 2003; de Boer and Stapleton, 2019). However, intensive research efforts over the past four decades have conclusively demonstrated that two of the most widely-used BFRs, PBDEs and HBCDD, are toxic to many organisms and are environmentally persistent. They are also bioaccumulative, biomagnify at elevated trophic levels and are capable of long-range atmospheric transport (Andersson and Blomkvist, 1981; Darnerud, 2003; de Wit et al., 2010; Jansson et al., 1987; Law et al., 2003). These findings culminated in commercial PBDE formulations and HBCDD progressively being listed as Persistent Organic Pollutants (POPs) and subject to regulation.

The sensitivity of birds to organohalogens was first identified following the widespread deployment of organochlorine pesticides in the second half of the 20th Century which, in raptors, led to eggshell thinning and direct mortality (Hickey and Anderson, 1968; Newton and Bogan, 1974; Ratcliffe, 1967, 1970). The effects of BFRs on avian receptors was reviewed by Guigueno and Fernie (2017), with impacts reported for various endpoints, including reproduction, behaviour and endocrine function, among others. PBDEs and HBCDD are regularly detected in waste streams such as landfill, as the products to which they were formerly applied enter obsolescence. The substantial quantities of human food refuse often deposited in landfill facilities implies that the various avian taxa which utilise this predictable anthropogenic subsidy worldwide are at risk of PBDE (Appendix 2) and HBCDD (Appendix 3) exposure. Such birds, several of which are of global conservation concern (Appendix 1), are also increasingly likely to be exposed to novel BFRs, those brominated compounds that have

replaced PBDEs and HBCDD despite knowledge gaps in terms of their effects and environmental fate (Appendix IV; Covaci et al., 2011).

Given this situation, the primary aim of this thesis was to assess whether five species of gull (black-headed gulls, common gulls, great black-backed gulls, herring gulls and lesser black-backed gulls) breeding in proximity to a UK landfill constitute effective bioindicators of legacy BFR and NBFR emissions from such facilities. The two primary working hypotheses were that: i. gulls breeding close to landfill exhibit elevated BFR egg concentrations compared to reference conspecifics, and ii., that European herring gulls, by virtue of their abundance and foraging ecology on landfill, represent the most effective bioindicator species for future work on contaminants in landfill-associated gulls in north-west Europe. In order to test these hypotheses, work was undertaken to meet the following two objectives: i., to compare BFR burdens in the eggs of five different gull species breeding in proximity to a landfill and ii., to assess the BFR increment in such eggs by comparing their concentrations and profiles with the eggs of reference conspecifics breeding away from the study landfill (Chapter III). In addition, stable isotopes of carbon, nitrogen and sulphur ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) were measured in the same eggs to test an additional hypothesis, i.e., that the trophic level at which gulls forage differs between landfill and reference conspecifics and will influence the concentrations and relative abundance of BFRs in their eggs (Chapter IV). Furthermore, observations of gulls frequenting the study landfill were undertaken, with a focus on behaviour likely to influence BFR exposure in order to test a fourth hypothesis, i.e., that the behaviour of birds on landfill will influence BFR egg concentrations and profiles (Chapter V).

The primary outcomes of this thesis are summarised below:

- The strongest evidence for a BFR increment in gull populations breeding in proximity to landfill is that the eggs of landfill-breeding herring gulls contained significantly higher $\sum_8$PBDE concentrations compared to those of reference conspecifics (Table 3.1), likely aided by comparatively robust sample sizes for this species. This, coupled with this species' known

association with landfill and widespread distribution reinforces the general view that herring gulls are a valuable biomonitoring taxon for BFR emissions from landfill in north-west Europe.

- In terms of individual PBDE congeners, the eggs of landfill-breeding herring gulls contained significantly elevated concentrations of BDE-100 and BDE-209. In the case of the latter, these data provides further evidence of it's the dominance of BDE-209 in landfill, especially in Europe, as previously demonstrated in research investigating both abiotic (Morin et al., 2017) and avian matrices (Morales et al., 2012; Roscales et al., 2016). This congener has a history of substantially greater use as a FR in Europe (and especially in the UK) relative to North America in particular (BSEF, 2003; Harrad et al., 2008; Söderström et al., 2004). BDE-209 is known to undergo sequential metabolic debromination and photocatalytic degradation to lower brominated and more bioavailable congeners in birds (Francois et al., 2016; Letcher et al., 2014; Van den Steen et al., 2007) and abiotic matrices (Gerecke et al., 2006; Robrock et al., 2008), respectively, which may explain elevated BDE-100 concentrations in landfill breeding herring gulls. Alternatively it may reflect herring gull / congener-specific toxicokinetics.

- In contrast to the significant differences in PBDE egg concentrations between landfill and reference herring gulls, there was no such difference between sites in terms of HBCDD levels. HBCDD was used primarily in extruded polystyrene insulation foam in the construction industry (Alaee, 2003). Waste data for a small landfill, located approximately 30 km south-west of the reference herring gull colony on the island of Islay (Table 2.2) showed that this site received proportionally more construction waste compared to the study landfill. It may be the case that reference herring gulls were foraging at this landfill to some extent, potentially accounting for their relatively high HBCDD burdens compared to PBDEs. Local observers

report that herring gulls forage at the Islay landfill (J. Dickson 2018, personal communication).

- Eggshell thickness for landfill-breeding herring gulls was significantly (on average 5 %) thinner than for reference conspecifics (Figure 3.4), comparable to reductions in eggshell thickness reported in experimentally PBDE-dosed captive American kestrels. Eggs containing in excess of 50 ng/g ww $\sum_8$PBDEs had significantly thinner shells (Figure 3.5).Whether this is causative or merely correlative is unknown. Work is required to understand the influence of BDE-153 on eggshell thinning. Furthemore, the reproductive implications of a 5 % reduction in eggshell thickness for this species are unknown.

- Great black-backed gulls, herring gulls and lesser black-backed gulls (i.e., those gull species recorded frequenting the study landfill) exhibited generally elevated egg concentrations of BDE-99, BDE-100, BDE-154 and BDE-153 compared to black-headed gulls and common gulls, which were not recorded at the landfill. This may suggest that association with landfill, as well as general foraging ecology and trophic level plays a role in interspecific differences in PBDE burdens, notwithstanding small sample sizes in the case of great black-backed gulls. In terms of individual HBCDD diastereomers, significant differences existed predominantly between the three large *vs*. two small species, although there were significant differences as regards β-HBCDD concentrations between common gulls and black-headed gulls and between lesser black-backed gulls and great black-backed gulls in the case of γ-HBCDD, indicating species-specific differences in HBCDD metabolism or diet.

- The only NBFR detected to any substantial degree was DBDPE. It was detected in the eggs of landfill breeding gulls only, at concentrations of up to 7724.20 ng/g lw in a single great black-backed gull egg, understood to be the highest concentration of this NBFR reported in biota to date, globally (Table 3.4). Relatively high DBDPE concentrations in the eggs of

black-headed and common gulls (i.e., species not observed to frequent the landfill; Tables 3.2 and 3.3, respectively) are harder to explain. However, the extent to which these individuals may have used landfill in the non-breeding season is unknown, as is the half-life of DBDPE in birds. Given the proximity (approximately 400 m and 900 m, respectively) of these colonies to the landfill (Figure 2.1), this may represent DBDPE contamination from the landfill via particulate-phase air concentrations. DBDPE has been recorded in outdoor air at concentrations up to 307 pg/gm^3 in China during 2012–19 (Li et al., 2017). This chemical, which is structurally similar to BDE-209, appears to be increasingly employed as a *deca*-BDE replacement (Wemken et al., 2019) and its occurrence in landfill-associated avifauna is perhaps to be expected (Betts, 2009; de Wit et al., 2019; Guo et al., 2019; Stubbings et al., 2019).

- Stable isotope analysis demonstrated that the eggs of reference herring gulls were significantly δ^{13}C enriched (Figure 4.1), suggesting a greater proportion of marine-derived items in their diet compared to landfill-breeding conspecifics. The more terrestrial diets of landfill breeding herring gulls appeared to result in them being more exposed to BDE-209 (Figure 4.3), in keeping with studies elsewhere (Chen et al., 2012; Roscales et al., 2016).

- The significant positive, though weak, relationship between egg volume and δ^{13}C values for herring gulls (Figure 4.4) suggests that a diet comprised of a greater relative proportion of marine items (i.e., a likely more nutritious diet; O'Hanlon et al., 2017) equates with a small increase in egg size. Similarly, in the case of Great Lakes (i.e., freshwater)-breeding American herring gulls, a reduced proportion of fish in the diet has been equated with smaller egg volume, as well as reduced reproductive success (Hebert, 1999). The correlation between shell thickness and δ^{13}C for herring gull eggs collected in 2018 (Figure 4.5) also indicates that those birds consuming a greater proportion of marine prey laid eggs with relatively thicker shells. It therefore appears possible that both flame retardants, and/or the extent of marine material

in the diet of herring gulls may influence eggshell thickness to some extent, although these data are correlative only and provide no indication as to the cause.

- Despite very small sample sizes for reference lesser black-backed gulls (n = 2), the relatively depleted $\delta^{13}C$ values in their eggs indicates that their diet is predominantly terrestrial. This is unexpected considering that they breed on an Atlantic island (Colonsay) where marine prey might be regarded as relatively easily available. There has been a significant decline in lesser black-backed gull populations across the Hebridean archipelago, with a concomitant increase in birds breeding in urban centres inland (Bowler, 2014). A relatively large breeding season maximum foraging range of 71.9 km has been recorded in this species (compared with an equivalent figure for herring gull of 10.5 km) (Thaxter et al., 2012). Despite the very small sample sizes, isotopic data from the present study indicates that the sampled Colonsay-breeding lesser black-backed gulls from this study may be commuting to urban areas to forage. This is reinforced by the substantial mean contribution of BDE-209 in the eggs of Colonsay breeders compared to landfill-breeding conspecifics (60.78 % and 23.21 %, respectively; Figure 3.6).

- Reference herring gull eggs contained enriched $\delta^{34}S$ values compared to landfill breeders. This approached statistical significance. Similarly, it has been demonstrated that refuse in the diet of yellow-legged gulls results in depleted $\delta^{13}C$ and $\delta^{34}S$ tissue values (Ramos et al., 2009). Roscales et al. (2016) determined that, of the three stable isotope ratios they analysed, $\delta^{34}S$ values were the best predictor of POP concentrations in gull eggs. In the present study, however, $\delta^{13}C$ values were of the greatest utility in this regard.

- Behavioural observations confirmed the importance of the study landfill as a foraging and loafing site for great black-backed gulls, herring gulls and lesser black-backed gulls, with a maximum of approximately 800 individuals present at any one time. Of this total, herring

gulls comprised over 90 % of all gulls present. There were significant interspecies differences in terms of foraging strategy, although pecking at the substrate and the swallowing of items were infrequently observed (Figures 5.2 A and B). The intermittent availability of food on landfill may to some extent be mitigated by the relatively high energetic value of human refuse to gulls when such items are obtained (van Donk et al., 2017, 2019). These data may suggest that ingestion of food items may not be the only pathway of BFR contamination in gulls frequenting landfill despite the evidence in this study linking diet to elevated BDE-209 in herring gulls. Other exposure routes, for example, dermal contact via the feet / skin or via inhalation of airborne particulates may therefore also be of importance in determining BFR burdens in birds frequenting landfill. There were limited data in terms of preening activity, although all three species undertook preening, with interspecific similarities in terms of regularity and individual bout lengths, indicating that it may be an equally important exposure route for all three species, although more research is required.

6.2 Research gaps and future perspectives

Heightened research interest in the presence and deleterious effects of BFRs in birds in recent years has resulted in significant advances in our knowledge of this area. Nevertheless, in terms of BFR exposure in landfill-associated avifauna, substantial research gaps remain and further research is necessary in order to:

i. Clarify the extent to which the apparently widely-used BDE-209 replacement, DBDPE is elevated in the tissues (e.g., eggs) of landfill-associated birds across various jurisdictions and the potential effects of DBDPE exposure at different endpoints in avian receptors.
ii. Establish whether eggshell thinning is a phenomenon in other landfill-associated species aside from European herring gulls and attempt to evaluate its reproductive implications with captive subjects experimentally dosed with FRs.

iii. Elucidate spatiotemporal data in terms of BFR profiles and concentrations in birds associated with landfill via the use of GPS telemetry combined with tissue and stable isotope analyses.

iv. Via experimentation with captive / necropsied subjects and / or modelling, attempt to understand better the roles played by dermal contact, inhalation and preening as BFR exposure pathways for landfill-associated birds. More research and modelling are required to determine the relative importance of different exposure routes.

v. Understand the role of BFR contamination in driving the population declines reported in certain vultures and gull species that routinely associate with landfill, through studies with captive birds or *in vivo* experiments. Given the ubiquity of BDE-209 in landfill, it will also be important to develop our understanding of toxicokinetics as a result of BDE-209 debromination in such species. Similarly, work should be undertaken to obtain insight into the potential range of different anthropogenic chemicals of increasing environmental concern in birds frequenting landfill, including flame retardants, perfluoroalkyl acids and volatile methylsiloxanes, although such work may admittedly be expensive.

vi. Elucidate BFR concentrations and profiles in free-living birds across South America, much of Africa and most Asian countries. Such countries are where human populations and development, and therefore quantities of anthropogenic waste are increasing most rapidly. In countries where the funds exist to undertake analysis of FRs in birds utilising landfill, research should be undertaken using internationally-established sampling protocols and eggs as the sampling tissue of choice given their proven utility in environmental monitoring studies. Ideally, future research would involve the same avian group (i.e., corvids), which may be a more suitable group in those tropical regions where gulls may use landfill less regularly. Such studies would benefit from the reporting of a minimum common suite of key chemicals. In terms of PBDEs, a minimum of the

following congeners should be measured: BDEs -28, -47, -99, -100, -153, -154, -183 and -209. Among HBCDDs, α-, β- and γ-diastereomers should also be quantified. It will be increasingly important to monitor other FRs given the restrictions applying to legacy BFRs, the selection of which may depend on local legislation and use.

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Appendix 1 Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Lesser whistling duck (*Dendrocygna javanica*)	'Least concern'	(Mehra et al.,2017)
Canada goose (*Branta canadensis*)	'Least concern'	(Seamans, 2009)
Knob-billed duck (*Sarkidiornis melanotos*)	'Least concern'	(Mehra et al.,2017)
Ruddy shelduck (*Tadorna ferruiginea*)	'Least concern'	(Mehra et al.,2017)
Paradise shelduck (*Tadorna variegata*)	'Least concern'	(Plaza and Lambertucci, 2017)
Garganey (*Spatula querquedula*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Northern shoveler (*Spatula clypeata*)	'Least concern'	(Mehra et al., 2017)
Northern pintail (*Anas acuta*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Eurasian teal (*Anas crecca*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Painted francolin (*Francolinus pictus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Grey francolin (*Francolinus pondicerianus*)	'Least concern'	(Mehra et al., 2017)
Rain quail (*Coturnix coromandelica*)	'Least concern'	(Mehra et al., 2017)
Jungle bush-quail (*Perdicula asiatica*)	'Least concern'	(Mehra et al., 2017)
Rock bush-quail (*Perdicula agroondah*)	'Least concern'	(Mehra et al., 2017)
Grey junglefowl (*Gallus sonneratii*)	'Least concern'	(Mehra et al., 2017)
Indian peafowl (*Pavo cristatus*)	'Least concern'	(Mehra et al., 2017)
Little grebe (*Tachybaptus ruficollis*)	'Least concern'	(Mehra et al., 2017)
Wood stork (*Mycteria americana*)	'Least concern'	(Rumbold et al., 2009)
Painted stork (*Mycteria leucocephala*)	'Near-threatened'	(Mehra et al., 2017)
Asian openbill (*Anastomus oscitans*)	'Least concern'	(Mehra et al., 2017)
White stork (*Ciconia ciconia*)	'Least concern'	(Plaza and Lambertucci, 2017)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Greater adjutant (*Leptopilos dubius*)	'Endangered'	(Choudhury, 2009)
Marabou stork (*Leptopilos crumenifer*)	'Least concern'	(Plaza and Lambertucci, 2017)
African sacred ibis (*Threskiornis aethiopicus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Black-headed ibis (*Threskiornis melanocephalus*)	'Near-threatened'	(Mehra et al., 2017)
Australian white ibis (*Threskiornis molucca*)	'Least concern'	(Plaza and Lambertucci, 2017)
Red-naped ibis (*Pseudibis papillosa*)	'Least concern'	(Mehra et al., 2017)
American white ibis (*Eudocimus albus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Glossy ibis (*Plegadis falcinellus*)	'Least concern'	(Mehra et al., 2017)
Black-crowned night heron (*Nycticorax nycticorax*	'Least concern'	(Mehra et al., 2017)
Striated heron (*Butorides striata*)	'Least concern'	(Mehra et al., 2017)
Indian pond heron (*Ardeola grayii*)	'Least concern'	(Mehra et al., 2017)
Western cattle egret (*Bubulcus ibis*)	'Least concern'	(Mehra et al., 2017)
Grey heron (*Ardea cinerea*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Great egret (*Ardea alba*)	'Least concern'	(Mehra et al., 2017)
Intermediate egret (*Ardea intermedia*)	'Least concern'	(Mehra et al., 2017)
Little egret (*Egretta garzetta*)	'Least concern'	(Mehra et al., 2017)
Little cormorant (*Microcarbo niger*)	'Least concern'	(Mehra et al., 2017)
Turkey vulture (*Cathartes aura*)	'Least concern'	(Plaza and Lambertucci, 2017)
Black vulture (*Coragyps atratus*)	'Least concern'	(Plaza and Lambertucci, 2017)
California condor (*Gymnogyps californianus*)	'Critically Endangered'	(Plaza and Lambertucci, 2017)
Andean condor (*Vultur gryphus*)	'Near-threatened'	(Plaza and Lambertucci, 2017)
Western osprey (*Pandion haliaetus*)	'Least concern'	(Mehra et al., 2017)
Black-winged kite (*Elanus caeruleus*)	'Least concern'	(Mehra et al., 2017)
Egyptian vulture (*Neophron percnopterus*)	'Endangered'	(Mehra et al., 2017)
Hooded vulture (*Necrosyrtes monachus*)	'Critically Endangered'	(Plaza and Lambertucci, 2017)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
White-rumped vulture (*Gyps bengalensis*)	Critcally 'Endangered'	(Mehra et al., 2017)
Indian vulture (*Gyps indicus*)	'Critically Endangered'	(Mehra et al., 2017)
Griffon vulture (*Gyps fulvus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Red-headed vulture (*Sarcogyps calvus*)	'Critically Endangered'	(Mehra et al., 2017)
Tawny eagle (*Aquila rapax*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Steppe eagle *(Aquila nipalensis*)	'Endangered'	(Mehra et al., 2017)
Eastern imperial eagle (*Aquila heliaca*)	''Vulnerable'	(Mehra et al., 2017)
Shikra (*Accipiter badius*)	'Least concern'	(Mehra et al., 2017)
Western marsh harrier (*Circus aeruginosus*)	'Least concern'	(Mehra et al., 2017)
Red kite (*Milvus milvus*)	'Least concern'	(Pain et al., 2007)
Black kite (*Milvus migrans*)	'Least concern'	(Mehra et al., 2017)
Brahminy kite (*Haliastur indus*)	'Least concern'	(Mehra et al., 2017)
White-tailed eagle (*Haliaeetus albicilla*)	'Least concern'	(Bekmansurov et al., 2017)
Bald eagle (*Haliaeetus leucocephalus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Black-chested buzzard *Geranoaetus melanoleucus*	'Least concern'	(Lobos et al. 2011)
Purple swamphen (*Porphyrio porphyrio*)	'Least concern'	(Mehra et al., 2017)
Common moorhen (*Gallinula chloropus*)	'Least concern'	(Mehra et al., 2017)
White-breasted waterhen *Amaurornis phoenicurus*	'Least concern'	(Mehra et al., 2017)
Eurasian coot (*Fulica atra*)	'Least concern'	(Mehra et al., 2017)
Grey-crowned crane (*Balearica regulorum*)	'Endangered'	(Plaza and Lambertucci, 2017)
Eurasian stone-curlew (*Burhinus oedicnimus*)	'Least concern'	(Mehra et al., 2017)
Black-winged stilt (*Himantopus himantopus*)	'Least concern'	(Mehra et al., 2017)
American avocet (*Recurvirostra americana*)	'Least concern'	(Weir, 1997)
Yellow-wattled lapwing (*Vanellus malabaricus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Red-wattled lapwing (*Vanellus indicus*)	'Least concern'	(Mehra et al., 2017)
Little ringed plover (*Charadrius dubius*)	'Least concern'	(Mehra et al., 2017)
Kentish plover (*Charadrius alexandrinus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Greater painted-snipe (*Rostratula benghalensis*)	'Least concern'	(Mehra et al., 2017)
Pheasant-tailed jacana (*Hydrophasianus chirurgus*)	'Least concern'	(Mehra et al., 2017)
Bronze-winged jacana (*Metopidius indicus*)	'Least concern'	(Mehra et al., 2017)
Black-tailed godwit (*Limosa limosa*)	'Near-threatened'	(Mehra et al., 2017)
Ruff (*Calidris pugnax*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Little stint (*Calidris minuta*)	'Least concern'	(Mehra et al., 2017)
Common snipe (*Gallinago gallinago*)	'Least concern'	(Mehra et al., 2017)
Common sandpiper (*Actitis hypoleucos*)	'Least concern'	(Mehra et al., 2017)
Green sandpiper (*Tringa ochropus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Common redshank (*Tringa totanus*)	'Least concern'	(Mehra et al., 2017)
Marsh sandpiper (*Tringa stagnatilis*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Wood sandpiper (*Tringa glareola*)	'Least concern'	(Mehra et al., 2017)
Common greenshank (*Tringa nebularia*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Bonaparte's gull (*Chroicocephalus philadelphia*)	'Least concern'	(Plaza and Lambertucci, 2017)
Silver gull (*Chroicocephalus novaehollandiae*)	'Least concern'	(Smith and Carlisle, 1993)
Black-headed gull (*Chroicocephalus ridibundus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Laughing gull (*Leucophaeus atricapilla*)	'Least concern'	(Plaza and Lambertucci, 2017)
Audouin's gull (*Ichthyaetus audouinii*)	'Least concern'	(Blanco and Marchamalo, 1999)
Mediterranean gull (*Ichthyaetus melanocephalus*)	'Least concern'	(Jurinović, 2012)
Mew gull (*Larus canus*)	'Least concern'	(Wallace et al., 1997)
Ring-billed gull (*Larus delawarensis*)	'Least concern'	(Plaza and Lambertucci, 2017)
California gull (*Larus californicus*)	'Least concern'	(Ackerman et al., 2006)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Great black-backed gull (*Larus marinus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Kelp gull (*Larus atlanticus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Glaucous-winged gull (*Larus glaucescens*)	'Least concern'	(Elliott et al., 2006)
Western gull (*Larus occidentalis*)	'Least concern'	(Plaza and Lambertucci, 2017)
Glaucous gull (*Larus hyperboreus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Iceland gull (*Larus glaucoides*)	'Least concern'	(Seif et al., 2017)
American herring gull (*Larus smithsonianus*)	'Least concern'	(Belant et al., 1993)
European herring gull (*Larus argentatus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Caspian gull (*Larus cachinnans*)	'Least concern'	(Skórka and Wójcik, 2008)
Yellow-legged gull (*Larus michahellis*)	'Least concern'	(Plaza and Lambertucci, 2017)
Slaty-backed gull (*Larus schistisagus*)	'Least concern'	(Zelenskaya, 2014)
Lesser black-backed gull (*Larus fuscus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Least tern (*Sternula antillarum*)	'Least concern'	(DeVault et al., 2005)
River tern (*Sterna aurantia*)	'Near-threatened'	(Mehra et al., 2017)
South polar skua (*Stercorarius maccormicki*)	'Least concern'	(Plaza and Lambertucci, 2017)
Brown skua (*Stercorarius antarcticus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Rock dove (*Columba livia*)	'Least concern'	(Mehra et al., 2017)
Eurasian collared dove (*Streptopelia decaocto*)	'Least concern'	(Mehra et al., 2017)
Red turtle dove (*Streptopelia tranquebarica*)	'Least concern'	(Mehra et al., 2017)
Laughing dove (*Spilopelia senegalensis*)	'Least concern'	(Mehra et al., 2017)
Mourning dove (*Zenaida macroura*)	'Least concern'	(Plaza and Lambertucci, 2017)
Greater coucal (*Centropus sinensis*)	'Least concern'	(Mehra et al., 2017)
Jacobin cuckoo (*Clamator jacobinus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Eurasian eagle-owl (*Bubo bubo*)	'Least concern'	(Saurola, 2009)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Spotted owlet (*Athene brama*)	'Least concern'	(Mehra et al., 2017)
Indian nightjar (*Caprimulgus asiaticus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Pallid swift (*Apus pallidus*)	'Least concern'	(Moulaï, 2007)
House swift (*Apus nipalensis*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Indian roller (*Coracias benghalensis*)	'Least concern'	(Mehra et al., 2017)
White-throated kingfisher (*Halcyon smyrnensis*)	'Least concern'	(Mehra et al., 2017)
Common kingfisher (*Alcedo atthis*)	'Least concern'	(Mehra et al., 2017)
Pied kingfisher (*Ceryle rudis*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Oriental bee-eater (*Merops orientalis*)	'Least concern'	(Mehra et al., 2017)
Eurasian hoopoe (*Upupa epops*)	'Least concern'	(Mehra et al., 2017)
Indian grey hornbill (*Ocyceros birostris*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Eurasian wryneck (*Jynx torquilla*)	'Least concern'	(Moulaï, 2007)
Southern crested caracara (*Caracara plancus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Chimango caracara (*Milvago chimango*)	'Least concern'	(Plaza and Lambertucci, 2017)
Lesser kestrel (*Falco naumanni*)	'Least concern'	(Mehra et al., 2017)
Common kestrel (*Falco tinnunculus*)	'Least concern'	(Mehra et al., 2017)
Red-necked falcon (*Falco chicquera*)	'Near-threatened'	(Mehra et al., 2017)
Laggar falcon (*Falco jugger*)	'Near-threatened'	(Mehra et al., 2017)
Peregrine falcon (*Falco peregrinus*)	'Least concern'	(Mehra et al., 2017)
Rose-ringed parakeet (*Psittacula krameri*)	'Least concern'	(Mehra et al., 2017)
Common iora (*Aegithina tiphia*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Brown shrike (*Lanius cristatus*)	'Least concern'	(Mehra et al., 2017)
Isabelline shrike (*Lanius isabellinus*)	'Least concern'	(Mehra et al., 2017)
Bay-backed shrike (*Lanius vittatus*)	'Least concern'	(Mehra et al., 2017)
Long-tailed shrike (*Lanius schach*)	'Least concern'	(Mehra et al., 2017)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Southern grey shrike (*Lanius meridionalis*)	'Vulnerable'	(Mehra et al., 2017)
Eurasian golden oriole (*Oriolus oriolus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Black drongo (*Dicrurus macrocercus*)	'Least concern'	(Mehra et al., 2017)
White-throated fantail (*Rhipidura albicollis*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Indian Paradise Flycatcher (*Terpsiphone paradisi*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Steller's jay (*Cyanocitta stelleri*)	'Least concern'	(Plaza and Lambertucci, 2017)
Eurasian magpie (*Pica pica*)	'Least concern'	(Plaza and Lambertucci, 2017)
Black-billed magpie (*Pica hudsonia*)	'Least concern'	(Elder, 2006)
Alpine chough (*Pyrrhocorax graculus*)	'Least concern'	(Delestrade, 1994)
Western jackdaw (*Coloeus monedula*)	'Least concern'	(Plaza and Lambertucci, 2017)
House crow (*Corvus splendens*)	'Least concern'	(Mehra et al., 2017)
Rook (*Corvus frugilegus*)	'Least concern'	(Plaza and Lambertucci, 2017)
American crow (*Corvus brachyrhynchos*)	'Least concern'	(Plaza and Lambertucci, 2017)
Fish crow (*Corvus ossifragus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Carrion crow (*Corvus corone*)	'Least concern'	(Plaza and Lambertucci, 2017)
Hooded crow (*Corvus cornix*)	'Least concern'	(Vuorisalo et al., 2003)
Large-billed crow (*Corvus macrorhynchos*)	'Least concern'	(Mehra et al., 2017)
Indian jungle crow (*Corvus culminatus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Pied crow (*Corvus albus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Northern raven (*Corvus corax*)	'Least concern'	(Plaza and Lambertucci, 2017)
Chihuahuan Raven (*Corvus cryptoleucus*)	'Least concern'	(Restani, 2008)
Brown-necked raven (*Corvus ruficollis*)	'Least concern'	(Belkacem et al.,2017)
Eurasian blue tit (*Cyanistes caruleus*)	'Least concern'	(Moulaï, 2007)
Great tit (*Parus major*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Rufous-tailed lark (*Ammomanes phoenicura*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Ashy-crowned sparrow-lark (*Eremopterix griseus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Woodlark (*Lullula arborea*)	'Least concern'	(Moulaï, 2007)
White-eared bulbul (*Pycnonotus leucotis*)	'Least concern'	(Mehra et al., 2017)
Red-vented bulbul (*Pycnonotus cafer*)	'Least concern'	(Mehra et al., 2017)
Common bulbul (*Pycnonotus barbatus*)	'Least concern'	(Moulaï, 2007)
Barn swallow (*Hirundo rustica*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Wire-tailed swallow (*Hirundo smithii*)	'Least concern'	(Mehra et al., 2017)
Eurasian crag martin (*Ptyonoprogne* rupestris)	'Least concern'	(Moulaï, 2007)
Dusky crag martin (*Ptyonoprogne concolor*)	'Least concern'	(Mehra et al., 2017)
Common house martin (*Delichon urbicum*)	'Least concern'	(Moulaï, 2007)
Red-rumped swallow (*Cecropis daurica*)	'Least concern'	(Mehra et al., 2017)
Common chiffchaff (*Phylloscopus collybita*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Greenish warbler (*Phylloscopus trochiloides*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Clamorous reed warbler (*Acrocephalus stentoreus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Sedge warbler (*Acrocephalus schoenobaenus*)	'Least concern'	(Alker and Redfern, 1996)
Eastern olivaceous warbler (*Iduna pallida*)	'Least concern'	(Moulaï, 2007)
Zitting cisticola (*Zisticola juncidis*)	'Least concern'	(Moulaï, 2007)
Ashy prinia (*Prinia socialis*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Tawny-bellied babbler (*Dumetia hyperythra*)	'Least concern'	(Mehra et al., 2017)
Common babbler (*Turdoides caudata*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Large grey babbler (*Turdoides malcolmi*)	'Least concern'	(Mehra et al., 2017)
Jungle babbler (*Turdoides striata*)	'Least concern'	(Mehra et al., 2017)
Blackcap (*Sylvia atricapilla*)	'Least concern'	(Moulaï, 2007)
Lesser whitethroat (*Sylvia curruca*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Eastern orphean warbler (*Sylvia crassirostris*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Sardinian warbler (*Sylvia melanocephala*)	'Least concern'	(Moulaï, 2007)
Yellow-eyed babbler (*Chrysomma sinense*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Eurasian wren (*Troglodytes troglodytes*)	'Least concern'	(Moulaï, 2007)
Short-toed treecreeper (*Certhia brachydactyla*)	'Least concern'	(Moulaï, 2007)
Bank myna (*Acridotheres ginginianus*)	'Least concern'	(Mehra et al., 2017)
Common myna (*Acridotheres tristis*)	'Least concern'	(Mehra et al., 2017)
Asian pied starling (*Gracupica contra*)	'Least concern'	(Mehra et al., 2017)
Brahminy starling (*Sturnia pagodarum*)	'Least concern'	(Mehra et al., 2017)
Common starling (*Sturnus vulgaris*)	'Least concern'	(Plaza and Lambertucci, 2017)
Spotless starling (*Sturnus unicolor*)	'Least concern'	(Baglione and Canestrari, 2009)
Common blackbird (*Turdus merula*)	'Least concern'	(Moulaï, 2007)
Song thrush (*Turdus philomelos*)	'Least concern'	(Moulaï, 2007)
Indian robin (*Copsychus fulicatus*)	'Least concern'	(Mehra et al., 2017)
Oriental magpie-robin (*Copsychus saularis*)	'Least concern'	(Mehra et al., 2017)
Spotted flycatcher (*Muscicapa striata*)	'Least concern'	(Moulaï, 2007)
Asian brown flycatcher (*Muscicapa dauurica*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Rufous-tailed Jungle Flycatcher *Cyornis ruficauda*	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
European robin (*Erithacus rubecula*)	'Least concern'	(Moulaï, 2007)
Common nightingale (*Luscinia megarynchos*)	'Least concern'	(Moulaï, 2007)
Red-breasted flycatcher (*Ficedula parva*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Black redstart (*Phoenicurus ochruros*)	'Least concern'	(Mehra et al., 2017)
Blue rock thrush (*Monticola solitarius*)	'Least concern'	(Moulaï, 2007)
Siberian stonechat (*Saxicola maurus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Pied Bush Chat (*Saxicola caprata*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Brown rock chat (*Oenanthe fusca*)	'Least concern'	(Mehra et al., 2017)
Purple-rumped sunbird (*Leptocoma zeylonica*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Purple sunbird (*Cinnyris asiaticus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
House sparrow (*Passer domesticus*)	'Least concern'	(Mehra et al., 2017)
Eurasian tree sparrow (*Passer montanus*)	'Least concern'	(Tang et al., 2015)
Yellow-throated sparrow (*Gymnoris xanthocollis*)	'Least concern'	(Mehra et al., 2017)
Baya weaver (*Ploceus philippinus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Red avadavat (*Amandava amandava*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Indian silverbill (*Euodice malabarica*)	'Least concern'	(Mehra et al., 2017)
Scaly-breasted munia (*Lonchura punctulata*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Chestnut munia (*Lonchura atricapila*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Yellow wagtail (*Motacilla flava*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Grey wagtail (*Motacilla cinerea*)	'Least concern'	(Mehra et al., 2017)
White wagtail (*Motacilla alba*)	'Least concern'	(Mehra et al., 2017)
White-browed wagtail (*Motacilla maderaspatensis*)	'Least concern'	(Mehra et al., 2017)
Paddyfield pipit (*Anthus rufus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
Common chaffinch (*Fringilla coelebs*)	'Least concern'	(Moulaï, 2007)
Common rosefinch (*Carpodacus erythrinus*)	'Least concern'	(Tuljapurkar and Bhagwat, 2007)
European greenfinch (*Chloris chloris*)	'Least concern'	(Moulaï, 2007)
Common linnet (*Linaria cannabina*)	'Least concern'	(Moulaï, 2007)
European serin (*Serinus serinus*)	'Least concern'	(Moulaï, 2007)
Red-winged blackbird (*Agelaius phoeniceus*)	'Least concern'	(Plaza and Lambertucci, 2017)
Shiny cowbird (*Molothrus bonariensis*)	'Least concern'	(Plaza and Lambertucci, 2017)

Appendix 1 (continued). Bird species recorded at municipal solid waste landfill sites and surroundings, including sewage treatment facilities (in taxonomic order). Systematics and nomenclature follow Gill and Donsker (2018). Taken from Tongue et al. (2019).

Species	IUCN conservation status	Reference
Brown-headed cowbird (*Molothrus ater*)	'Least concern'	(Plaza and Lambertucci, 2017)
Common grackle (*Quiscalus quiscula*)	'Least concern'	(Plaza and Lambertucci, 2017)
Boat-tailed grackle (*Quiscalus major*)	'Least concern'	(Plaza and Lambertucci, 2017)

Appendix 2 Bird species associated with landfill in various countries where tissue compartments were investigated for polybrominated diphenyl ethers (PBDEs). Entries are listed in chronological order of publication of the study. Concentrations are shown in ng/g wet weight unless otherwise stated.

Species	Tissue (*n* = sample size)	Median ∑PBDEs	Range ∑PBDEs	Country	Reference
Glaucous gull (*Larus hyperboreus*)[a]	Egg yolk (*n* = 31)	ND	81.1–321	Norwegian Arctic	(Verreault et al., 2007)
African sacred ibis (*Threskiornis* aethiopicus)[b]	Egg (*n* = 2)	ND	3.53–22.9	South Africa	(Polder et al., 2008)
White stork (*Ciconia* ciconia)[c]	Egg (*n* = 33)	6.59	2.79–20.5	Spain	(Muñoz-Arnanz et al., 2011a)
Ring-billed gull (*Larus* delawarensis)[d]	Egg (*n* = 10)	144	ND	Canada	(Chen et al., 2012a)
California gull (*Larus* californicus)[d]	Egg (*n* = 10)	166	ND	Canada	(Chen et al., 2012a)
Glaucous-winged gull (*Larus glaucescens*)[d]	Egg (*n* = 10)	79.1	ND	Canada	(Chen et al., 2012a)
Herring gull (*Larus* argentatus)[d]	Egg (*n* = 305)	ND	62–781	Canada	(Chen et al., 2012a)
Yellow-legged gull (*Larus* michahellis)[e]	Egg (*n* = 36)	38.5	35.9–40.4	Spain	(Morales et al., 2012)
Common starling (*Sturnus* vulgaris)[f]	Egg (*n* = 259)	280	25–533	Canada	(Chen et al., 2013)
Eurasian tree sparrow (*Passer* montanus)[g]	Pectoral muscle (*n* = 37)	78.7	33.1–375.6	China	(Tang et al., 2015) §

Species	Tissue (*n* = sample size)	Median ∑PBDEs	Range ∑PBDEs	Country	Reference
Black kite (*Milvus* migrans)[h]	Tail feather (*n* = 5)	2.4	0.70–7.5	Pakistan	(Abbasi et al., 2016a) §

§ Abbasi et al. 2016a provide feather concentrations in ng/g dry weight. Tang et al., 2015 report concentrations in ng/g lipid weight.

For all studies, the detection method was gas chromatography-mass spectrometry (GC-MS).

ND = not detected.

The BDE congeners screened for in each study were as follows:

[a] -17, -25, -28, -47, -49, -54, -66, -75, -77, -85, -99, -100, -116, -119, -138, -139, -140, -153, -154/BB153, -155, -156, -171, -180, -181, --183, -184, -190, -191, -196, -197, -201, -202, -203, -205, -206, -207, -208, -209.

[b] -28, -47, -99, -100, -153, -154, -183, -209.

[c] -17, -28, -47, -66, -85, -99, -100, -153, -154, -183, -184, -191, -194, -195, -196, -197 + -204, -198+-199+200+203, -201, -202, -205, -206, -207, -208, -209.

[d] -17, -25, -28, -47, -49, -54, -66, -75, -77, -85, -99, -100, -116, -119, -138, -139, -140, -153, -154/BB153, -155, -156, -171, -180, -181, --183, -184, -190, -191, -196, -197, -201, -202, -203, -205, -206, -207, -208, -209.

[e] -28, -47, -99, -100, -153, -154, -183, -209

[f] -28, - 47, -49, -66, -85, -99, -100, -138, -153, -154, -183, -209

[g] -28, -47, -99, -100, -153, -154, 183, -209

[h] -28, -47, -99, -100, -153, -154, -183.

Appendix 3 Bird species associated with landfill in various countries where tissue compartments were investigated for hexabromocyclododecane (HBCDD). Entries are listed in chronological order of publication of the study. Concentrations are shown in ng/g wet weight unless otherwise stated.

Species	Tissue (*n* = sample size)	Median Total HBCDD	Range Total HBCDD[a]	Country	Reference
African sacred ibis (*Threskiornis aethiopicus*)	Egg (*n* = 2)	n.d.	3.53–22.9	South Africa	(Polder et al., 2008)
Glaucous gull (*Larus hyperboreus*)	Egg (*n* = 31)	n.d.	13–23	Norwegian Arctic	(Verboven et al., 2008)
Ring-billed gull (*Larus delawarensis*)	Liver (*n* = 28)	n.d.	n.d.–19.8	Canada	((Gentes et al., 2012))
California gull (*Larus californicus*)	Egg (*n* = 10)	8.3	n.d.	Canada	(Chen et al., 2012a)
Glaucous-winged gull	Egg (*n* = 10)	4.5	n.d.	Canada	(Chen et al., 2012a)
Herring gull *Larus argentatus*	Egg (*n* = 305)	16.6†	n.d.	Canada	(Chen et al., 2012a)
Black kite (*Milvus migrans*)	Tail feather (*n* = 5)	1.5	0.5–8.1	Pakistan	(Abbasi et al., 2016a) §

§ Abbasi et al. 2016aprovide feather concentrations in ng/g dry weight.

n.d.: No data provided.

†: Mean concentration.

[a]Sum of α–, β-, and γ-HBCDD

Appendix 4 Bird species associated with landfill in various countries where tissue compartments were investigated for novel brominated flame retardants (NBFRs). Entries are listed in chronological order of publication of the study. Concentrations are shown in ng/g wet weight unless otherwise stated.

NBFR	Species	Tissue (*n* = sample size)	Median NBFRs	Range NBFRs	Country	Reference
BEHTBP						
	Ring-billed gull (*Larus delawarensis*)	Liver *n* = 28	n.d.	ND– 17.6	Canada	(Gentes et al., 2012))
	Glaucous gull (*Larus hyperboreus*)	Liver *n* = 31	n.d.	ND–2.05	Canadian Arctic	(Verreault et al., 2018)
BTBPE						
	Glaucous gull	Liver *n* = 4	n.d.	ND–0.96	Norwegian Arctic	(Vorkamp et al., 2015)
	Herring gull	Egg *n* = 91	0.10	0.05–0.20	Laurentian Great Lakes (Canada / USA)	(Gauthier et al., 2009)
DBE-DBCH						
	Herring gull	Egg *n* = 78	0.16	0.11–0.54	Laurentian Great Lakes	(Gauthier et al., 2007)
	Ring-billed gull	Liver *n* = 28	n.d.	ND–0.30	Canada	(Gentes et al., 2012)
DPDBE						
	Herring gull	Egg *n* = 91	n.d.	n.d.–44	Canada	(Gauthier et al., 2009)
EHTBB						
	Ring-billed gull	Liver *n* = 28	n.d.	ND–1.55	Canada	(Gentes et al., 2012)
	Glaucous gull	Liver *n* = 31	n.d.	ND–0.92	Canadian Arctic	(Verreault et al., 2018)
HBB						
	Glaucous gull	Liver *n* = 31	n.d.	ND–0.53	Canadian Arctic	(Verreault et al., 2018)

NBFR	Species	Tissue (*n* = sample size)	Median NBFRs	Range NBFRs	Country	Reference
	Herring gull	Egg *n* = 78	0.45	0.27–0.66	Canada	(Gauthier et al., 2007)
HCDBCO						
	Ring-billed gull	Liver *n* = 28	n.d.	ND–0.02	Canada	(Gentes et al., 2012)
OBIND						
	Ring-billed gull	Liver *n* = 28	n.d.	ND–0.37	Canada	(Gentes et al., 2012)
PBEB						
	Herring gull	Egg *n* = 78	0.05	0.03–1.4	Canada	(Gauthier et al., 2007)
	White stork (*Ciconia ciconia*)	Egg *n* = 33	n.d.	0.01–9.79	Spain	(Muñoz-Arnanz et al., 2010)
	Glaucous gull	Liver *n* = 31	n.d.	0.03–0.23	Norwegian Arctic	(Verreault et al., 2007b)
PBT						
	Glaucous gull	Egg yolk *n* = 31	n.d.	ND–0.12	Norwegian Arctic	(Verreault et al., 2007)
	Herring gull	Egg *n* = 78	0.01	0.004–0.02	Canada	(Gauthier et al., 2007)
TBBP-A-dbpe						
	Herring gull	Egg *n* = n.d.	n.d.	ND–0.36	Laurentian Great Lakes	(Letcher and Chu, 2010)

Abbreviations
BEHTEBP: bis(2 ethylhexyl) tetrabromophthalate
BTBPE: 1,2-bis(2,4,6-tribromophenoxy) ethane
DBE-DBCH: 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane
DBDPE: Decabromodiphenylethane or 1,2-bis(pentabromodiphenyl)ethane
EHTBB: 2-ethylhexyl-2,3,4,5-tetrabromobenzoate
HBB: Hexabromobenzene
HCDBCO: Hexachlorocyclopentadienyldibromocyclooctane
OBIND: Octabromo-1,3,3-trimethyl-1-phenylindane
PBEB: Pentabromoethylbenzene
PBT: Pentabromotoluene
TBBP-A-dbpe: Tetrabromobisphenol-A-bis(2,3-dibromopylether)

n.d.: No data provided.
ND: Below detection / quantification limit

Appendix 5 Research Group QA/QC Protocol

SUMMARY OF ANALYTICAL METHODS AND ASSOCIATED QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) PROCEDURES FOR SEMI-VOLATILE ORGANIC COMPOUNDS

Prepared by: Prof. Stuart Harrad, Persistent Organic Pollutants Research Group, Public Health Building, School of Geography, Earth & Environmental Sciences, University of Birmingham

1. Overview

This document describes the generically applicable methods and procedures that all researchers within the group must follow to ensure the reliability of their analytical data. Methods that apply only to a specific group of pollutants are not covered here. If you have any questions about anything relating to analysis, please ask your supervisor or an experienced member of the Research Group for advice.

2. Instrument Calibration

A full 5-point calibration must be conducted at the beginning of any measurement campaign. The exact concentrations and content of the calibration standard mixes will vary according to the pollutant class being measured but as a guide, the table below gives a typical example.

Compound	*Standard A Concn (pg µl^{-1})*	*Standard B Concn (pg µl^{-1})*	*Standard C Concn (pg µl^{-1})*	*Standard D Concn (pg µl^{-1})*	*Standard E Concn (pg µl^{-1})*
All "native"[1] standards	20	50	200	500	1000
Internal standards, Sampling Evaluation standards, recovery determination standards	200	200	200	200	200

These standards are used to calculate relative response factors (RRFs) for each of the "target" compounds. The RRF is defined as the instrument response for a unit amount of target pollutant relative to the instrument response obtained for the same amount of the internal standard (IS). For example, if the response of a unit amount of the target compound is 1.5 times that for the same amount of the internal standard, the RRF =1.5. It is calculated as in equation 1.

$$RRF = \frac{A_{NAT}}{A_{IS}} \times \frac{C_{IS}}{C_{NAT}} \quad \text{(Eqn 1)}$$

[1] "native" refers to the ^{12}C or ^{1}H isotope of the target compound. The term is used to distinguish it from the ^{13}C or ^{2}D (deuterated) isotope used as the internal standard.

where A_{NAT} is the peak area for the "native" compound in the standard; A_{IS} is the peak area of the internal standard in the standard; C_{NAT} is the concentration of the "native" compound in the standard; and C_{IS} is the concentration of the internal standard in the standard.

Calculation of RRFs for each of the standards A–E should reveal them to be essentially identical in each standard. Ideally, the relative standard deviation, i.e., average × 100 % of RRFs for a given target compound, should not exceed 10 %. If they do, consult your supervisor before proceeding.

A full 5 point calibration typically only needs to be conducted infrequently, when the instrument (e.g. GC/MS) has been shut down for a long period, undergone a general maintenance or when an on-going accuracy check proves unsatisfactory. The average RRF for any subsequent full calibration should be within 10 % of the average RRF obtained for the 1st 5-point calibration. If they do not, then you must consult your supervisor immediately.

Before each batch of samples is analysed on the GC/MS, one of the calibration standards (usually Standard C, but others are fine) must be run (this is called a continuing calibration). The RRFs obtained from this analysis must be within 25% of the RRFs obtained for that standard in the initial 5-point calibration. If they do not, please consult your supervisor before proceeding. At the end of each batch of samples, the same calibration standard must be run. The RRFs obtained from this analysis must be within 25% of the RRFs obtained for that standard in the initial 5-point calibration. The RRFs that must be used for calculating concentrations in samples in that batch will be an average of those obtained for the 2 standards run for that batch. A minimum of two continuing calibrations MUST be conducted EVERY 24 hours that samples are run.

Generally, the following calibration issues would be considered significant QC violations that require immediate consultation of your supervisor prior to recalibration:

- Instrument not calibrated by a continuing calibration (i.e. the continuing calibration yields RRFs that are outside the ±25% criterion outlined above).
- GC/MS tune criteria significantly out of tune (> 20 % for any atomic mass unit).
- A response (i.e. peak area) for the target analytes less than 5% of its original value (with no technical justification for such a low response).

3. GC/MS tuning tips

At the start of each session, an autotune should be run. The results should be printed out and a record kept. Following the autotune (which should detect any major problems with the GC/MS), a manual tune must be conducted. Routinely, a manual tune is necessary once a week. The purpose of the manual tune is to maximise sensitivity and instrument performance for the particular group of compounds you are targeting. As a general rule, while during tuning the detector voltage should be set at 200V, you should set the detector voltage to 450V (*i.e.* that necessary to detect compounds in the concentration range 10- 1000 pg/component) in your acquisition file used when running standards and samples. You should also tune with the oven temperature at a temperature similar to that at which your target compounds will elute form the GC column. Typically for a DB-5 type column it will be 250°C, but may be lower for other columns (DO NOT EXCEED THE MAXIMUM ALLOWABLE ISOTHERMAL OPERATING TEMPERATURE FOR THE COLUMN). You should choose the m/z values most appropriate to the mass range of the pollutants which you are targeting – your supervisor will be able to advise you on the best choice. The autotune uses m/z 69, 219, and 502 – this is not appropriate for PCBs for example, which lie in the mass range 256 to 394, and the manual tune should be based on tuning masses 219, 264,

and 414.

4. LC/MS/MS tuning tips

At the start of each session, an auto tune should be run. The results should be printed out and a record kept. If the instrument fails to pass through the autotune phase then a manual tune is necessary using the provided poly propylene glycol standards (PPGs). Make sure to use the correct standard for each type of source/ionisation. Please consult your supervisor or an experienced member of the research group before performing the manual tune operation. After performing the manual tune, it's advisable to run some pure solvent through the instrument at an ion source temperature of 300-350 °C for 1 hour prior to injecting your samples/calibration standards in order to remove any traces of PPGs in the ion source.

5. Determination of Internal Standard Recoveries

It is important to note that use of the internal standard quantification method means that NO correction of concentrations for recovery is required. However, it is important that recoveries of internal standards are calculated for each sample as a QA/QC measure. Typically, such recoveries should be around 70%, but they may routinely fall in the range 30%-150%. If values exceed 150%, the sample extract should be re-analysed and the recovery recalculated. If recoveries are below 30%, then the signal to noise (S:N) ratio of the internal standard must be calculated. The data are acceptable provided that the S:N ratio exceeds 20:1. If it is less than 20:1 the sample extract should be re-analysed and the recovery recalculated. If the recovery percentage and S:N ratio is still unacceptable then data for that sample must be considered invalid.

Internal standard (IS) recoveries are calculated thus:

$$\%\ \text{IS Recovery} = \left[\left(\frac{A_{IS}}{A_{RDS}}\right)_S \times \left(\frac{A_{RDS}}{A_{IS}}\right)_{STD} \times \left(\frac{C_{IS}}{C_{RDS}}\right)_{STD} \times \left(\frac{C_{RDS}}{C_{IS}}\right)_S \times 100\right] \quad \text{(Eqn 2)}$$

where $(A_{IS}/A_{RDS})_S$ = ratio of internal standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{IS})_{STD}$ = ratio of recovery determination standard peak area to internal standard peak area in the calibration standard (the average of values obtained for both calibration standards run for a batch of samples is used); $(C_{IS}/C_{RDS})_{STD}$ = ratio of concentration of internal standard to concentration of recovery determination standard in the calibration standard; and $(C_{RDS}/C_{IS})_S$ = ratio of concentration of recovery determination standard to concentration of internal standard in the sample (assuming 100% recovery). Note that this can be calculated as the amount of internal or recovery determination standard added to the sample divided by the volume of the sample extract used for GC/MS analysis (typically 25-50 µ1).

6. Determination of Sampling Evaluation Standard Recoveries

Recoveries of sampling evaluation standards (*i.e.* those added to the PUF plug in air or aqueous sample analysis) are calculated for each sample as QA/QC measure. Note that SESs are NOT added to solid samples like soil or grass. Typically such recoveries should be around 70%, but they may routinely fall in the range 30%-150%. Note that although SES recoveries should be

recorded for every sample, they are a QA/QC check only, and are NOT used to correct concentrations for sampling losses. If values exceed 150%, the sample extract should be re-analysed and the recovery recalculated. If it still exceeds 150%, then data for that sample must be considered invalid. If recoveries are below 30%, then the signal to noise (S:N) ratio of the sampling evaluation standard must be calculated. The data are acceptable provided that the S:N ratio exceeds 20:1. If it is less than 20:1 the sample extract should be re-analysed and the recovery recalculated. If the recovery percentage and S:N ratio is still unacceptable then data for that sample must be considered invalid.

Sampling evaluation standard (SES) recoveries are calculated thus:

$$\%\ \text{SES Recovery} = \left[\left(\frac{A_{SES}}{A_{RDS}}\right)_S \times \left(\frac{A_{RDS}}{A_{SES}}\right)_{STD} \times \left(\frac{C_{SES}}{C_{RDS}}\right)_{STD} \times \left(\frac{C_{RDS}}{C_{SES}}\right)_S \times 100\right] \quad \text{(Eqn 3)}$$

where $(A_{SES}/A_{RDS})_S$ = ratio of sampling evaluation standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{SES})_{STD}$ = ratio of recovery determination standard peak area to sampling evaluation standard peak area in the calibration standard (the average of values obtained for both calibration standards run for a batch of samples is used); $(C_{SES}/C_{RDS})_{STD}$ = ratio of concentration of sampling evaluation standard to concentration of recovery determination standard in the calibration standard and $(C_{RDS}/C_{SES})_S$ = ratio of concentration of recovery determination standard to concentration of sampling evaluation standard in the sample (assuming 100% recovery). Note that this can be calculated as the amount of sampling evaluation or recovery determination standard added to the sample divided by the volume of the sample extract used for GC/MS analysis (typically 25-50 µ1).

7. Determination and On-Going Monitoring of Accuracy

The principal means of determining method accuracy is *via* analysis of one or more certified or standard reference materials (CRMs or SRMs). Your supervisor will recommend a suitable CRM/SRM. Before you commence analysis of any samples as part of your research, you must conduct 6 replicate analyses of a suitable CRM or SRM, and obtain satisfactory data for these analyses. Essentially a CRM or SRM is a sample that has been analysed a large number of expert laboratories worldwide and that has had agreed concentrations of target pollutants assigned to it. These values are usually cited as an average ± a standard deviation. The values you obtain will be compared with these. You must discuss your data with your supervisor and will only be allowed to proceed with analysis of your samples once acceptable accuracy of data are obtained.

As an ongoing measure of accuracy, you must analyse 1 aliquot of the same CRM/SRM for every 20 samples – *i.e.* every 21st sample you analyse must be a CRM/SRM. If satisfactory data are not obtained, then you must consult your supervisor immediately.

Additional means of evaluating accuracy include participation in interlaboratory comparisons. Your supervisor will advise you as and when such comparisons are to take place.

8. Determination of Precision

This is defined as the relative standard deviation (*i.e.* $100 \times {}_{n-1}$/average) of concentrations obtained from 6 replicate analyses of the same sample. Usually, this is a CRM/SRM. Typically, precision should be no more than 30%, but you must discuss your data with your supervisor.

9. Determination of Blank Concentrations

This is defined as the concentration of a target pollutant present in an analysis where the sample is omitted, but internal standards etc. are added. Note that for air analyses, a method blank should consist of analysis of a clean PUF plug and filter paper. For calculation of blank sample concentrations, you should assume the sample mass or volume to be that typically used – *e.g.* 1,000 m^3 for air samples, 50 g for soil or grass samples and 0.2 g for indoor dust samples. A field blank is analyte-free media exposed to sampling location conditions, storage, preservation, and all analytical procedures. For a dust sample, a field blank is 0.2 g of pre-cleaned anhydrous sodium sulfate vacuumed through the vacuum cleaner, collected in a nylon sock, wrapped in aluminium foil and stored with dust samples until analysis. Field blanks are used to assess any contamination contributed from sampling location conditions and the transport, handling, and storage of the samples. One blank analysis must be conducted for every 5 samples – *i.e.* every 6^{th} analysis you perform must be a blank. Where the concentration of a target pollutant in a blank for a given batch of samples is 5-20% of the concentration in a sample from that batch, the blank concentration must be subtracted from that in the sample. Where the concentration in the blank exceeds 20% of that in a sample from that batch, data for that target pollutant in that sample must not be reported.

10. Determination of Detection limits

Two categories of detection limits exist.

1. instrument detection limit (IDL)
2. sample detection limit (SDL)

The IDL is defined as that amount of pollutant that gives a signal to noise ratio of 3:1. It is best determined by calculating the signal to noise ratio for the pollutant in your calibration standard A. To illustrate, if the concentration of a target pollutant in that standard = 20 pg/µ1 and 1 µ1 is injected, then if a signal to noise ratio of 50:1 is obtained, then the IDL
= 20 × (3/50)= 1.2 pg/injection.

The SDL can then be calculated as in equation 4:

$$\text{SDL} = \frac{IDL \times FEV}{VFEI \times SS} \times \frac{100}{\%IS\,\text{Recovery}} \quad \text{(Eqn 4)}$$

Where *FEV* = final extract volume (µ1), *VFEI* = volume of final extract injected (µ1); *SS* = sample size (m^3 or g); and *%ISRecovery* = percentage recovery of internal standard used to quantify the target pollutant in a particular sample.

To illustrate, if the IDL = 1.2 pg/injection, the final extract volume for a sample is 50µl, 1 µ1 is injected; the sample size is 1,000 m^3, and the percentage internal standard recovery in that sample = 70%, then the SDL = ((1.2 × 50)/ (1 × 1000)) × 100/70 = 0.086 pg m^{-3}.

Where the concentration in the sample blank exceeds the SDL calculated as above, an appropriate number (ideally 7-10) of blanks should be prepared and analysed, the effective SDL (also called the Minimum reported value (MRV)) is 3 times the standard deviation of the blank analyses. This is the lowest value you are allowed to report. This is not an unusual occurrence.

11. Calculation of concentrations in samples

Concentrations in samples may be calculated *via* the equation below:

$$\text{Concentration} = \frac{A_{NAT}}{A_{IS}} \times \frac{1}{RRF} \times \frac{M_{IS}}{SS} \quad \text{(Eqn 5)}$$

Where A_{IS} = peak area of internal standard in sample; A_{NAT} = peak area of target pollutant in sample; *RRF* = relative response factor for the target pollutant (see equation 1); M_{IS} = mass of internal standard added to sample (pg) and *SS* = sample size (m^3 or g).

To illustrate, where A_{NAT} = 10,000 units; A_{IS} = 20,000 units; *RRF* = 1.5; M_{IS} = 20,000 pg; and *SS* = 50 g, the concentration of the target pollutant will be (10,000/20,000) × (1/1.5) × (20,000/50) = 133.33 pg g^{-1}.

12. Correct Storage of Calibration and internal standards

Once prepared in CERTAN vials, all standards should be stored in a freezer unless required for analysis. You should record the weight of the CERTAN vial and contents before and after each use. Before weighing, allow the vial and contents to reach room temperature, and wipe off any condensation before weighing. If at any time, the weight before use deviates from the weight recorded after the previous use by more than 5 %, you must consult your supervisor immediately.

13. Criteria for Quantification of a Peak as a Target pollutant

For a given peak to be identified as a target pollutant in a sample, various criteria must be met. These are:

1. the signal to noise ratio of the peak must exceed 3:1
2. the relative retention time (RRT) of the peak in the sample must be within 0.2% of the average value determined for the 2 calibration standards run for the sample batch. Note RRT = retention time of target pollutant/retention time of internal standard used to quantify target pollutant.

The above criteria apply to all target pollutants. For organochlorine and organobromine pollutants, the following criterion also applies.

- the isotope ratio of the peak in the sample must be within 15% of the average value determined for the 2 calibration standards run for that sample batch. If it falls outside this range, then you must consult your supervisor, but it is likely that the peak cannot be quantified due to a co-eluting interference. For example, for trichlorinated PCBs, where 2 m/z values are monitored (*i.e.* 255.95 and 257.95) the isotope ratio = area of peak for 255.95 trace/area of peak for 257.95 trace. Note that for calculating RRFs and concentrations, the m/z value providing the largest peak must be used.

Appendix 6 Detection frequencies (%) for PBDE congeners and HBCDD diastereomers in the eggs of landfill and reference breeding black-headed gulls, common gulls, great black-backed gulls, herring gulls and lesser black-backed gulls in 2017 and 2018.

Species	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	α-HBCDD	β-HBCDD	γ-HBCDD
Black-headed gull											
2016 landfill	33	66	66	25	66	16	40	75	75	0	60
2017 reference	0	80	20	0	0	0	40	20	3	0	0
Common gull											
2016 landfill	28	85	71	50	64	71	0	64	92	21	35
2017 reference	0	33	0	0	0	0	0	0	0	0	0
Great black-backed gull											
2016 & 17 landfill	42	100	100	85	100	100	100	85	100	0	100
2017 & 18 reference	0	100	62	100	50	62	12	75	100	0	0

Herring gull											
2017 landfill	94	50	50	55	50	22	16	100	100	0	61
2017 reference	21	92	78	92	85	85	92	57	50	35	50
2018 landfill	86	93	53	0	33	20	40	46	80	6	46
2018 reference	0	63	36	0	45	27	18	54	54	9	54
Lesser black-backed gull											
2016 landfill	45	100	81	72	90	72	45	90	100	0	27
2017 reference	0	50	0	0	0	0	0	50	100	50	100

Appendix 7 The mean (± standard error), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 32) and reference (*n* = 25) herring gulls collected in western Scotland in the 2017 and 2018 breeding seasons (ng/g wet weight).

Compound	Landfill mean	Reference mean	Landfill median (range)	Reference median (range)	$P^{\dagger}$
$\sum_8$PBDEs inc. BDE-209	34.7 ± 9.9	19.5 ± 9.6	11.9 (0.3–247.8)	4.2 (0.4– 237.8)	**0.03*** ‡
$\sum_7$PBDEs exc. BDE-209	23.0 ± 9.3	16.4 ± 9.6	2.1 (0.1–230.0)	3.8 (0.02–187.0)	0.58
BDE-28§	<0.1	<0.1	<0.1 (<0.1– 5.5)	<0.1 (<0.1– 1.6)	-
BDE-47	2.05 ± 1.1	2.3 ± 1.0	<0.05 (<0.05–31.4)	<0.05 (<0.05–16.1)	0.96
BDE-99	<0.05	<0.05	<0.05 (<0.05–2.6)	<0.05 (<0.05–2.9)	-
BDE-100§	<0.05	<0.05	<0.05 (<0.05–7.2)	<0.05 (<0.05– <0.7)	**<0.001*** ‡
BDE-153	12.9 ± 6.3	7.0 ± 4.5	<0.05 (<0.05–160.0)	<0.05 (<0.05–112.9)	0.14
BDE-154§	2.5 ± 1.5	3.5 ± 3.2	<0.05 (<0.05–37.7)	<0.05 (<0.05–81.0)	-
BDE-183	4.3 ± 1.7	3.0 ± 2.7	<0.05 (<0.05–37.0)	<0.05 (<0.05–67.5)	0.20
BDE-209	11.7 ± 4.3	<0.05	4.6 (<0.05–109.4)	<0.05 (<0.05–50.8)	**0.002** ‡
Total-HBCDD	8.8 ± 2.6	11.0 ± 2.7	1.6 (0–61.2)	4.8 (0–41.0)	0.65
α-HBCDD	5.1 ± 1.4	6.1 ± 1.6	<0.06 (<0.06–32.3)	<0.06 (<0.06–30.2)	0.99
β-HBCDD§	<0.06	<0.06	<0.06 (<0.06–7.7)	<0.06 (<0.06–4.8)	-
γ-HBCDD	3.4 ± 1.0	4.6 ± 1.2	<0.08 (<0.08–23.4)	2.9 (<0.08–24.1)	0.56
BTBPE§	<0.04	<0.04	<0.04 (<0.04– <0.04)	<0.04 (<0.04–0.20)	-
DBDPE§	<3.6	<3.6	<3.6 (<3.6–182.6)	<3.6 (<3.6 –<3.6)	-
EH-TBB§	<0.06	<0.06	<0.06 (<0.06– <0.06)	<0.06 (<0.06– <0.06)	-
PBB§	<0.1	<0.1	<0.1 (<0.1– <0.1)	<0.1 (<0.1– <0.1)	-
PBEB§	<0.01	<0.01	<0.01 (<0.01– <0.01)	<0.01 (<0.01– <0.01)	-

Means include ± standard error. † Significance test for difference between landfill and reference egg concentrations derived using Wilcoxon Mann-Whitney U test.

§ Section 3.4.1 provides details of those compounds of interest which were excluded from analyses.

‡ Significantly higher for the eggs of landfill-breeding birds.

<LOD: Below limit of detection.

Average wet weight limits of detection (ng/g lw): BDE-28: 0.1; BDE-47: 0.05; BDE-99: 0.05; BDE-100: 0.05; BDE-153: 0.05; BDE-154: 0.05; BDE-183: 0.05; BDE-209: 0.05; α-HBCDD: 0.06; β-HBCDD: 0.06; γ-HBCDD: 0.08; BTBPE 0.04; DBDPE: 3.6; EH-TBB 0.06; PBB 0.1; PBEB: 0.01.

Appendix 8 Lipid weight FR concentrations in the eggs of ten three-egg (i.e., full) herring gull clutches obtained during 2016–18. Egg size measured via volume (mm^3)

	$\sum_8$PBDEs inc. BDE-209	**$\sum_7$PBDEs exc. BDE-209**	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-183**	**BDE-209**	**Total-HBCDD**	**α-HBCDD**	**γ-HBCDD**	**DBDPE**
Clutch 1 (landfill; 2016a)													
Largest ('a') egg	132.46	131.29	24.48	9.75	81.77	9.21	1.70	0.11	1.17	19.29	16.32	2.96	0.82
Median ('b') egg	733.11	411.03	113.18	41.56	195.84	6.48	6.81	0.08	322.08	79.45	67.59	11.86	0.61
Smallest ('c') egg	26.30	25.49	4.12	1.98	15.69	2.52	0.55	0.08	0.81	1.23	0.40	0.82	26.97
Clutch 2 (landfill, 2016b)													
Largest ('a') egg	90.75	47.48	9.15	3.03	30.00	0.24	3.66	0.64	43.27	5.89	4.37	1.51	0.86
Median ('b') egg	307.71	134.24	0.42	9.74	101.35	11.08	8.52	1.95	173.47	181.97	176.81	5.16	1.19
Smallest ('c') egg	59.18	36.05	6.87	2.52	20.02	3.10	2.73	0.39	23.13	1.19	0.50	0.69	0.70
Clutch 3 (reference, 2017a)													
Largest ('a') egg	292.64	288.74	N/A	274.52	0.42	0.33	0.42	0.16	3.90	513.37	511.03	2.17	4.41
Median ('b') egg	350.89	212.46	N/A	98.46	112.13	0.37	0.46	0.18	138.42	40.61	0.93	39.67	1.31
Smallest ('c') egg	621.06	149.52	N/A	64.09	27.06	20.61	24.37	9.56	471.54	412.52	252.95	159.33	28.36

	$\sum_8$PBDEs inc. BDE-209	**$\sum_7$PBDEs exc. BDE-209**	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-183**	**BDE-209**	**Total-HBCDD**	**α-HBCDD**	**γ-HBCDD**	**DBDPE**
Clutch 4 (reference; 2017b)													
Largest ('a') egg	40.63	14.86	N/A	9.94	0.45	3.01	0.45	0.18	25.77	183.90	15.48	168.23	21.77
Median ('b') egg	79.39	76.83	N/A	59.63	13.85	0.26	1.84	0.13	2.55	394.77	394.46	0.31	0.91
Smallest ('c') egg	345.28	23.46	N/A	13.95	0.52	4.30	1.22	0.20	321.82	1390.39	144.05	820.45	355.97
Clutch 5 (landfill; 2017a)													
Largest ('a') egg	651.38	19.66	0.74	1.82	0.74	14.71	0.74	0.29	631.72	2.5	1.13	1.13	27.27
Median ('b') egg	76.52	29.74	0.39	0.30	15.60	12.09	0.88	0.15	46.77	122.02	120.23	1.48	35.71
Smallest ('c') egg	87.65	46.32	0.56	1.25	5.21	34.38	4.22	0.22	41.32	183.70	91.45	70.25	88.89
Clutch 6 (landfill, 2017b)													
Largest ('a') egg	244.24	30.91	0.60	8.24	5.73	13.73	1.86	0.24	213.32	89.58	88.13	1.21	29.05
Median ('b') egg	256.82	130.13	0.41	8.52	0.41	119.87	0.41	0.16	126.68	38.00	3.51	34.32	19.90
Smallest ('c') egg	187.35	46.11	0.58	7.82	0.58	34.71	1.71	0.23	141.23	118.79	71.69	46.86	27.84

	$\sum_8$PBDEs inc. BDE-209	$\sum_7$PBDEs exc. BDE-209	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-183	BDE-209	Total-HBCDD	α-HBCDD	γ-HBCDD	DBDPE
Clutch 7 (reference, 2018a)													
Largest ('a') egg	29.60	21.89	N/A	0.73	0.52	N/A	17.51	0.70	7.70	2.29	1.04	1.04	4995.89
Median ('b') egg	60.29	50.01	N/A	3.34	0.69	N/A	0.69	0.27	10.27	165.55	163.88	1.38	33.33
Smallest ('c') egg	384.53	375.42	N/A	3.19	0.48	N/A	253.67	0.24	9.11	2.70	1.23	1.23	29.55
Clutch 8 (reference; 2018b)													
Largest ('a') egg	316.86	306.13	N/A	5.96	0.72	N/A	297.27	0.28	10.72	3.18	1.44	1.44	34.78
Median ('b') egg	98.67	93.14	N/A	0.60	0.60	N/A	0.60	0.24	5.52	347.59	346.15	1.20	6.25
Smallest ('c') egg	179.69	170.77	N/A	1.67	0.60	N/A	158.88	0.24	8.91	2.65	1.20	1.20	28.91
Clutch 9 (landfill; 2018a)													
Largest ('a') egg	429.25	426.16	2.60	422.21	0.33	N/A	0.33	0.13	3.09	110.21	109.40	0.67	3.49
Median ('b') egg	3219.80	2490.31	89.55	81.64	2163.57	N/A	47.01	6.38	729.49	69.52	0.86	68.66	1.20
Smallest ('c') egg	956.64	951.94	31.69	918.20	0.51	N/A	0.51	0.20	4.70	88.58	81.39	6.99	5.31
Clutch 10 (landfill, 2018b)													
Largest ('a') egg	4721.25	4382.29	0.47	4.79	18.01	N/A	3047.61	590.94	338.96	83.23	0.95	82.09	2342.85
Median ('b') egg	2199.07	1541.14	0.32	0.32	33.89	N/A	1156.39	84.59	657.93	100.78	0.65	100.00	3.41

Smallest ('c') egg	530.79	89.08	0.31	87.52	0.31	N/A	0.31	0.12	441.71	146.81	0.62	146.06	3.24

N/A: Exlcuded from statistical analyses due to detection frequencies being <30 % for that site-type/year.

Appendix 9 Mean (± standard error for landfill), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 12; 2016) and reference (*n* = 5; 2017) black-headed gulls collected in western Scotland (ng/g wet weight)

Compound	Landfill mean (2016) †	Reference mean (2017)	Landfill median (range) (2016)	Reference median (range) (2017)
$\sum_8$PBDEs inc. BDE-209	9.0 ± 2.4	0.8	7.1 (0.04–26.1)	0.1 (0.08–4.0)
$\sum_7$PBDEs exc. BDE-209	6.1 ± 2.1	0.3	4.5 (0.01–26.1)	0.1 (0.07–1.2)
BDE-28	0.9 ± 0.6	<0.1	<0.1 (<0.1–8.2)	<0.1 (<0.1– <0.1)
BDE-47	0.7 ± 0.4	0.1	0.1 (<0.05–3.9)	0.07 (<0.05–0.3)
BDE-99	1.2 ± 0.4	0.1	0.6 (<0.05–5.1)	<0.05 (<0.05–0.8)
BDE-100§	0.1 ± 0.09	<0.05	<0.05 (<0.05–1.1)	<0.05 (<0.05– <0.05)
BDE-153	2.7 ± 2.1	<0.05	<0.05 (<0.05–1.1)	<0.05 (<0.05– <0.05)
BDE-154§	<0.05	<0.05	<0.05 (<0.05–0.3)	<0.05 (<0.05– <0.05)
BDE-183	0.2 ± 0.2	<0.05	<0.05 (<0.05–3.1)	<0.05 (<0.05–0.09)
BDE-209	2.9 ± 1.0	0.5	1.8 (<0.05–11.3)	<0.05 (<0.05–2.7)
Total-HBCDD	0.9 ± 0.3	1.5	0.4 (0–4.0)	0.1 (0–7.1)
α-HBCDD	0.8 ± 0.3	1.5	0.4 (<0.06–3.9)	0.1 (<0.06–7.1)
β-HBCDD§	0.8 ± 0	1.5	0.4 (<0.06–3.9)	0.1 (<0.06–7.1)
γ-HBCDD	<0.08	<0.08	<0.08 (<0.08–0.5)	<0.08 (<0.08– <0.08)
BTBPE§	<0.04	<0.04	<0.04 (<0.04– <0.04)	<0.04 (<0.04– <0.04)
DBDPE§	<3.6	<3.6	<3.6 (<3.6– 41.5)	<3.6 (<3.6– <3.6)
EH-TBB§	<0.06	<0.06	<0.06 (<0.06– <0.06)	<0.06 (<0.06– <0.06)
PBB§	<0.1	<0.1	<0.1 (<0.1– <0.1)	<0.1 (<0.1– <0.1)
PBEB§	<0.01	<0.01	<0.01 (<0.01–<0.01)	<0.01 (<0.01–<0.01)

† Arithmetic mean ± standard error (the latter omitted for reference eggs due to sample size)
n.d.: not detected
§ Table 3.2 lists those compounds of interest which were excluded from analyses.
Average wet weight limits of detection (ng/g ww): BDE-28: 0.1; BDE-47: 0.05; BDE-99: 0.05; BDE-100: 0.05; BDE-153: 0.05; BDE-154: 0.05; BDE-183: 0.05; BDE-209: 0.05; α-HBCDD: 0.06; β-HBCDD: 0.06; γ-HBCDD: 0.08; BTBPE 0.04; DBDPE: 3.6; EH-TBB 0.06; PBB 0.1; PBEB: 0.01.

Appendix 10 The mean (± standard error for landfill), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 14) and reference (*n* = 6) common gulls collected in western Scotland during 2016–17 (ng/g wet weight).

Compound	Landfill mean[§]	Reference mean[§]	Landfill median (range)	Reference median (range)
$\sum_8$PBDEs inc. BDE-209	34.5 ± 0.2	0.07	22.4 (1.8–105.9)	0.06 (0.04–0.1)
$\sum_7$PBDEs exc. BDE-209	27.2 ± 6.5	0.06	20.6 (1.7–97.4)	0.05 (0.03–0.1)
BDE-28[§]	0.4 ± 0.3	<0.1	<0.1 (<0.1–4.9)	<0.1 (<0.1– <0.1)
BDE-47[§]	1.1 ± 0.2	<0.05	1.0 (<0.05–2.5)	<0.05 (<0.05–0.1)
BDE-99	2.0 ± 0.9	<0.05	0.1 (<0.05–12.2)	<0.05 (<0.05– <0.05)
BDE-100	0.6 ± 0.3	<0.05	<0.05 (<0.05–3.9)	<0.05 (<0.05– <0.05)
BDE-153	14.9 ± 4.9	<0.05	9.1 (<0.05– 66.7)	<0.05 (<0.05– <0.05)
BDE-154	7.9 ± 2.1	<0.05	5.5 (<0.05–28.6)	<0.05 (<0.05– <0.05)
BDE-183[§]	<0.05	<0.05	<0.05 (<0.05–0.05)	<0.05 (<0.05– <0.05)
BDE-209	7.3 ± 3.2	<0.05	1.6 (<0.05–42.2)	<0.05 (<0.05– <0.05)
Total-HBCDD	3.6 ± 1.1	<0.01	1.5 (0.01–18.0)	0 (0–0)
α-HBCDD	2.5 ± 0.6	<0.06	1.5 (<0.06–10.4)	<0.06 (<0.06– <0.06)
β-HBCDD[§]	0.3 ± 1.1	<0.06	<0.06 (<0.06–18.0)	<0.06 (<0.06–<0.06)
γ-HBCDD	0.7 ± 0.3	<0.08	<0.08 (<0.08–4.1)	<0.08 (<0.08–<0.08)
BTBPE	<0.04	<0.04	<0.04 (<0.04–<0.04)	<0.04 (<0.04–<0.04)
DBDPE	<3.6	<3.6	<3.6 (<3.6–227.00)	<3.6 (<3.6–<3.6)
EH-TBB	<0.06	<0.06	<0.06 (<0.06–<0.06)	<0.06 (<0.06–<0.06)
PBB	<0.1	<0.1	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)
PBEB	<0.01	<0.01	<0.01 (<0.01–<0.01)	<0.01 (<0.01–<0.01)

[§] Arithmetic mean ± standard error (the latter omitted for reference eggs due to sample size)
n.d.: not detected
[§] Table 3.2 lists those compounds of interest which were excluded from analyses.
[†] Average wet weight limits of detection (ng/g ww): BDE-28: 0.1; BDE-47: 0.05; BDE-99: 0.05; BDE-100: 0.05; BDE-153: 0.05; BDE-154: 0.05; BDE-183: 0.05; BDE-209: 0.05; α-HBCDD: 0.06; β-HBCDD: 0.06; γ-HBCDD: 0.08; BTBPE 0.04; DBDPE: 3.6; EH-TBB 0.06; PBB 0.1; PBEB: 0.01.

Appendix 11 The mean, median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 7; 2016–18) and reference (*n* = 8; 2017 and 2018) great black-backed gulls collected in western Scotland (ng/g wet weight)

Compound	Landfill mean†	Reference mean†	Landfill median (range)	Reference median (range)
$\sum_8$PBDEs inc. BDE-209	35.2	9.4	19.2 (3.2–108.6)	4.9 (0.7–41.0)
$\sum_7$PBDEs exc. BDE-209	27.8	8.1	14.2 (2.4–92.4)	4.6 (0.6–33.4)
BDE-28	1.5	<0.1	<0.1 (<0.1–5.6)	<0.1 (<0.1– <0.1)
BDE-47	3.1	5.2	2.7 (0.5–6.6)	2.6 (0.3–21.1)
BDE-99	16.6	0.5	4.3 (0.1–62.0)	0.09 (<0.05–2.0)
BDE-100	3.9	1.0	1.3 (<0.05–13.0)	0.8 (<0.05–3.5)
BDE-153	1.6	0.5	1.7 (0.2–3.0)	0.1 (<0.05–2.7)
BDE-154	0.3	0.6	0.4 (0.05–0.8)	0.1 (<0.05–3.3)
BDE-183	0.5	0.1	0.4 (0.1–1.2)	<0.05 (<0.05–0.8)
BDE-209	7.4	1.2	5.0 (<0.05–16.2)	0.2 (<0.05–7.6)
Total-HBCDD‡	9.3	0.9	9.9 (4.0–13.5)	0.7 (0.1–1.9)
α-HBCDD‡	4.8	0.9	5.2 (1.5–7.5)	0.7 (0.1–1.9)
β-HBCDD‡	<0.06	<0.06	<0.06 (<0.06–<0.06)	<0.06 (<0.06–<0.06)
γ-HBCDD‡	4.5	<0.08	4.4 (2.5–6.60)	<0.08 (<0.08 – <0.08)
BTBPE	<0.04	<0.04	<0.04 (<0.04–<0.04)	<0.04 (<0.04–<0.04)
DBDPE	<3.6	<3.6	<3.6 (<3.6–484.0)	<3.6 (<3.6– <3.6)
EH-TBB	<0.06	<0.06	<0.06 (<0.06– <0.06)	<0.06 (<0.06– <0.06)
PBB	<0.1	<0.1	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)
PBEB	<0.01	<0.01	<0.01 (<0.01– <0.01)	<0.01 (<0.01– <0.01)

† Arithmetic mean ± standard error (the latter omitted for landfill eggs due to sample size)
<LOD: Below limit of detection.
§ Table 3.2 lists those compounds of interest which were excluded from analyses.
Average wet weight limits of detection (ng/g ww): BDE-28: 0.1; BDE-47: 0.05; BDE-99: 0.05; BDE-100: 0.05; BDE-153: 0.05; BDE-154: 0.05; BDE-183: 0.05; BDE-209: 0.05; α-HBCDD: 0.06; β-HBCDD: 0.06; γ-HBCDD: 0.08; BTBPE 0.04; DBDPE: 3.6; EH-TBB 0.06; PBB 0.1; PBEB: 0.01.
‡ HBCDD data obtained for 7 eggs, comprised of 4 landfill (from 2016) and 3 reference eggs (from 2017).

Appendix 12 The mean (± standard error for landfill), median and range of concentrations of the compounds of interest in the eggs of landfill-breeding (*n* = 11; 2016) and reference (*n* = 2; 2018) lesser black-backed gulls collected in western Scotland (ng/g wet weight).

Compound	Landfill mean[†]	Reference mean[†]	Landfill median (range)	Reference median (range)
$\sum_8$PBDEs inc. BDE-209	60.7 ± 26.8	7.2	34.8 (6.5–315.0)	7.2 (0.06–14.4)
$\sum_7$PBDEs exc. BDE-209	51.3 ± 26.7	0.5	17.9 (2.9–304.3)	0.5 (0.04–1.1)
BDE-28	0.2 ± 0.1	<0.1	<0.1 (<0.1–1.2)	<0.1 (<0.1–<0.1)
BDE-47[§]	2.1 ± 0.8	0.5	0.9 (0.1–12.8)	0.5 (<0.05–1.1)
BDE-99	6.8 ± 2.3	<0.05	1.7 (<0.05–28.0)	<0.05 (<0.05–<0.05)
BDE-100	1.5 ± 0.5	<0.05	0.5 (<0.05–7.2)	<0.05 (<0.05–<0.05)
BDE-153	23.4 ± 13.5	<0.05	0.6 (<0.05–184.1)	<0.05 (<0.05–<0.05)
BDE-154	19.3 ± 12.6	<0.05	0.2 (<0.05–170.9)	<0.05 (<0.05–<0.05)
BDE-183	3.1 ± 1.6	<0.05	0.1 (<0.05–19.9)	<0.05 (<0.05–<0.05)
BDE-209	8.0 ± 2.8	6.6	2.1 (<0.05–34.0)	6.6 (<0.05–13.3)
Total-HBCDD	2.6 ± 0.7	11.3	1.3 (0.1–9.5)	11.3 (7.5–15.2)
α-HBCDD	1.5 ± 0.4	4.8	1 (<0.06–5.7)	4.8 (4.0–5.6)
β-HBCDD[§]	<0.06	0.9	<0.06 (<0.06–<0.06)	0.9 (<0.06–1.8)
γ-HBCDD[§]	1.0 ± 0.6	5.6	<0.08 (<0.08–9.5)	5.61 (3.5–7.6)
BTBPE	<0.04	<0.04	<0.04 (<0.04–<0.04)	<0.04 (<0.04–<0.04)
DBDPE	<3.6	<3.6	<3.6 (<3.6–68.4)	<3.6 (<3.6–<3.6)
EH-TBB	<0.06	<0.06	<0.06 (<3.6–<3.6)	<0.06 (<3.6–<3.6)
PBB	<0.1	<0.1	<0.1 (<0.1–<0.1)	<0.1 (<0.1–<0.1)
PBEB	<0.01	<0.01	<0.01 (<0.01–<0.01)	<0.01 (<0.01–<0.01)

[†]Arithmetic mean ± standard error (the latter omitted for reference eggs due to sample size)
<LOD: Below limit of detection
[§] Table 3.2 lists those compounds of interest which were excluded from analyses.
Average wet weight limits of detection (ng/g ww): BDE-28: 0.1; BDE-47: 0.05; BDE-99: 0.05; BDE-100: 0.05; BDE-153: 0.05; BDE-154: 0.05; BDE-183: 0.05; BDE-209: 0.05; α-HBCDD: 0.06; β-HBCDD: 0.06; γ-HBCDD: 0.08; BTBPE 0.04; DBDPE: 3.6; EH-TBB 0.06; PBB 0.1; PBEB: 0.01.

Appendix 13 Toxicological impacts of FRs on birds in the literature with the percentage of samples from the present study that meet / exceed lowest observed effect levels.

Toxicological impact; minimum concentration at which impacts occurred	Black-headed gull	Common gull	Great black-backed gull	Herring gull	Lesser black-backed gull
Plasma TT4 concentration in nestlings negatively correlated with PBDE burdens. Egg concentrations of 198 ng/g ww ∑PBDEs.[a]	0 %	0 %	0 %	2 %	11 %
Reduced TT4 released by thyroid gland; baseline TT3 lower. Egg concentrations of 288 ng/g ww ∑PBDEs.[b]	0 %	0 %	0 %	0 %	5 %
Negative association with plasma retinol. Egg concentrations of 198 ng/g ww ∑PBDEs.[c]	0 %	0 %	0 %	2 %	11 %
Reduced copulation, pair bonding and nest attendance. Egg concentrations of 198 ng/g ww ∑PBDEs.[d]	0 %	0 %	0 %	0 %	5 %

Toxicological impact; minimum concentration at which impacts occurred	Black-headed gull	Common gull	Great black-backed gull	Herring gull	Lesser black-backed gull
Reduced nestling plasma TT4 and FT4. Higher T in breeding males. Egg concentrations of 22 ng/g lw Total-HBCDD.[e]	21 %	33 %	60 %	56 %	52 %
Reduced courtship behaviour, reduced nest temperature, reduced parental care in males. Egg concentrations of 22 ng/g lw Total-HBCDD.[e]	21 %	37 %	60 %	57 %	52 %
Exposed chicks consumed more food. Egg concentrations of 198 ng/g ww ∑PBDEs.[f]	0 %	0 %	0 %	1 %	11 %
Delayed hatching, shorter humerus length and reduced total thyroid weight. Egg concentrations of 2000 ng/g lw ∑PBDEs.[g]	0 %	4 %	1 %	5 %	17 %

Toxicological impact; minimum concentration at which impacts occurred	Black-headed gull	Common gull	Great black-backed gull	Herring gull	Lesser black-backed gull
Reduced courtship vocalisations, bonding behaviour in females, reduced male parental behaviours, compensatory increase in females. Egg concentrations of 283 ng/g ww ∑PBDEs.[h]	0 %	0 %	0 %	0 %	5 %
In exposed birds: PHA response negatively associated with BDE-47; structural changes in spleen, bursa and thymus; negative association between spleen somatic index and ∑PBDEs. Egg concentrations of 100 ng/g ww ∑PBDEs.[i]	0 %	4 %	1 %	10 %	17 %
Exposed chicks consumed more food. Egg concentrations of 198 ng/g ww ∑PBDEs.[j]	0 %	0 %	0 %	1 %	11 %

Toxicological impact; minimum concentration at which impacts occurred	Black-headed gull	Common gull	Great black-backed gull	Herring gull	Lesser black-backed gull
Female chicks generally smaller. Egg concentrations of 288 ng/g ww ∑PBDEs.[b]	0 %	0 %	0 %	0 %	5 %
Thinner eggshells, reduced fertility, hatching and fledging success. Egg concentrations of 188 ng/g ww.[k]	0 %	0 %	0 %	3 %	11 %
Fewer eggs and smaller clutches. Egg concentrations of 288 ng/g ww ∑PBDEs.[l]	0 %	0 %	0 %	0 %	5 %

References:

[a] Fernie et al. (2005b)
[b] Fernie and Marteinson (2016)
[c] Sullivan et al. (2010)
[d] Marteinson et al. (2010)
[e] Marteinson et al. (2011b)
[f] Fernie et al. (2006)
[g] Rattner et al. (2013)
[h] Marteinson et al. (2012a)
[i] (Fernie et al., 2005a)
[j] Fernie et al. (2006)
[k] Fernie et al. (2009)

Abbreviations:
FT4: Free thyroxine
PHA: Phytohemagglutinin
T: Testosterone
TT3: Total triiodothyrine
TT4: Total thyroxine