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Ingested plastics in northern fulmars (*Fulmarus glacialis*): A pathway for polybrominated diphenyl ether (PBDE) exposure?

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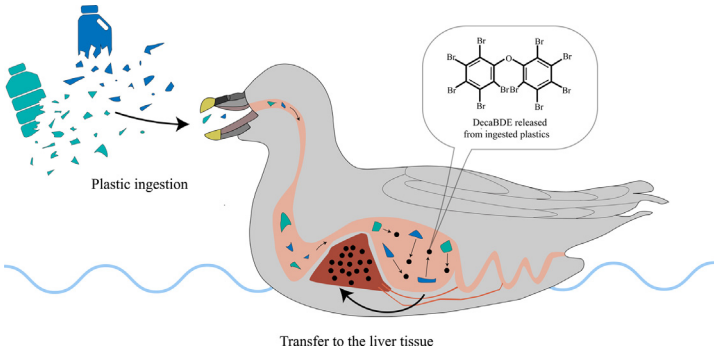
HIGHLIGHTS

- PBDEs were found in fulmar liver and plastic ingested by the same birds.
- High levels of PBDEs were detected in fulmars found dead on beaches.
- We found strong evidence for a transfer of BDE209 from plastics to the tissue of the birds.
- BDE209 may be a suitable indicator of plastic ingestion in seabirds.

GRAPHICAL ABSTRACT



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ABSTRACT

Although it has been suggested that plastic may act as a vector for pollutants into the tissue of seabirds, the bioaccumulation of harmful contaminants, such as polybrominated diphenyl ethers (PBDEs), released from ingested plastics is poorly understood. Plastic ingestion by the procellariiform species northern fulmar (*Fulmarus glacialis*) is well documented. In this study, we measured PBDEs levels in liver tissue of northern fulmars without and with (0.13–0.43 g per individual) stomach plastics. PBDE concentrations in the plastic sampled from the same birds were also quantified. Birds were either found dead on beaches in southern Norway or incidentally caught in longline fisheries in northern Norway. PBDEs were detected in all birds but high concentrations were only found in liver samples from beached birds, peaking at 2900 ng/g lipid weight. We found that body condition was a significant factor explaining the elevated concentration levels in livers of beached birds. BDE209 was found in ingested plastic particles and liver tissue of birds with ingested plastics but was absent in the livers of birds without ingested plastics. This strongly suggests a plastic-derived transfer and accumulation of BDE209 to the tissue of fulmars, levels of which might prove useful as a general indicator of plastic ingestion in seabirds.

1. Introduction

Over the past decades, global plastic production has increased dramatically and peaked at 359 million tonnes in 2018 (PlasticsEurope, 2019). Between 4.8 and 12.7 million metric tonnes of plastic litter end up in the marine environment every year (Jambeck et al., 2015). Once plastic

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litter has entered the ocean, it typically breaks down into smaller fragments due to a variety of different processes including weathering effects such as UV radiation (Andrady, 2015). The accumulation of plastic litter has been reported over a wide range of latitudes and ecosystems (Lusher et al., 2015; Cózar et al., 2017; Lacerda et al., 2019) highlighting marine plastic pollution as a global phenomenon.

Plastics have been found in a variety of different marine taxa including most trophic levels (e.g. Besseling et al., 2015; Desforges et al., 2015). Plastic ingestion by seabirds has been reported across the globe and Wilcox et al. (2015) estimated that about half of the world's seabird species contain plastics in their stomachs.

Procellariiform seabirds (albatrosses, petrels, and shearwaters) are particularly vulnerable to ingest plastics due to their unselective surface-feeding behaviour (Azzarello and Van Vleet, 1987; Moser and Lee, 1992; Tourinho et al., 2010) which makes them more likely than diving species to ingest plastics that float on the sea surface (Provencher et al., 2014; Poon et al., 2017). In addition, the structure of the digestive system of fulmarine petrels, such as the northern fulmar (*Fulmarus glacialis*, hereafter referred to as fulmar), might be another key factor affecting their levels of contained plastics (Furness, 1985; Azzarello and Van Vleet, 1987). A narrowing between the proventricular and the gizzard does not allow regurgitation of the whole stomach content (Furness, 1985; Van Franeker et al., 2011; van Franeker and Law, 2015) and hence, fulmars are likely to retain plastic items in their stomachs. They can, however, unintentionally spit out ingested plastics when for example feeding their chicks or when threatened by predators (van Franeker and Law, 2015).

In the northern hemisphere, fulmars are typically recorded with much higher amounts of ingested plastics than most other species (Van Franeker et al., 2011; Trevail et al., 2015; Acampora et al., 2016) which makes this species particularly suitable for biomonitoring trends in marine plastic pollution in these waters (Van Franeker et al., 2011; Avery-Gomm et al., 2018). In addition to their likelihood to ingest plastic items (Van Franeker and Meijboom, 2002; Van Franeker et al., 2011), fulmars are well-suited as large-scale marine biomonitors since they only obtain marine prey items and have wide migration ranges across the Barents Sea, the Greenland Sea, and the Labrador Sea (Falk and Møller, 1995; Weimerskirch et al., 2001; SEATRACK, 2020).

Therefore, the fulmar has been chosen as an indicator species for marine plastic pollution within Europe by the Oslo-Paris Convention (OSPAR). As an Ecological quality objective (EcoQO), it was set as an acceptable target that less than 10% of the fulmars found dead on beaches should have more than 0.1 g plastic particles in the stomach (OSPAR Commission, 2008). In most areas, and especially around the North Sea, this proportion is found to be much higher (van Franeker and the SNS Fulmar Study Group, 2011) and 42% of the beached fulmars found in southern Norway exceed the EcoQO threshold (van Franeker and the SNS Fulmar Study Group, 2011). Even 35% of the otherwise seemingly healthy fulmars incidentally taken as fisheries bycatch in northern Norway contained more than 0.1 g plastic in their stomachs (Herzke et al., 2016).

Ingested plastics may pose a risk to seabirds by their potential to cause physical harm, such as internal wounds or blockage of digestive organs (Gregory, 2009). In recent years, there is also a growing concern about the accumulation of toxic chemicals released from ingested plastics. A variety of organic pollutants of environmental concern have been detected in blood, feathers, and different tissues of seabirds including fulmars (e.g., Verreault et al., 2005; Helgason et al., 2008; Braune et al., 2010). Persistent organic pollutants (POPs) can sorb to plastics from the surrounding waters due to their hydrophobic character (Teuten et al., 2009; Hirai et al., 2011; Rochman, 2015; De Frond et al., 2019). Therefore, ingested plastics may increase the exposure of seabirds to POPs (Teuten et al., 2007).

An even larger concern arises from additives, including brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), that are compounded into plastic items (Schlabach et al., 2011) but can leach out of the polymer matrix and accumulate in the tissue of seabirds (e.g., Teuten et al., 2009; Tanaka et al., 2013; Herzke et al., 2016). A plastic-tissue transfer of higher-brominated PBDEs has been observed in field studies for several procellariiform species including short-tailed shearwaters (*Puffinus tenuirostris*) (e.g., Tanaka et al., 2013; Tanaka et al., 2015; Tanaka, 2017; Tanaka et al., 2020). Similarly, in vivo experiments in streaked shearwater (*Calonectris leucomelas*) chicks indicated a plastic-mediated accumulation of various additives, including BDE209, to the tissues of the birds (Tanaka et al., 2020). BDE209 has also been detected in ingested plastics samples of fulmars from the Faroe Islands (Tanaka et al., 2019). However, in fulmars from the Norwegian coast, a plastic-derived accumulation of higher-brominated PBDEs was observed more sporadically in one of 30 birds and a bioaccumulation model suggested that the PBDE exposure from ingested plastics is negligible (Herzke et al., 2016).

Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants which are added as flame retardants to a wide range of products including textiles, electronics, plastics, and polyurethane foams at concentrations ranging between 5 and 30% (Darnerud et al., 2001). PBDEs have historically been produced in three major commercially used mixtures, i.e., pentaBDE, octaBDE, and decaBDE (Chen and Hale, 2010). Over the past decades, PBDEs have become a widespread pollutant across the globe, which has led to a growing concern about their toxicological impacts on biota, including birds (e.g., McKernan et al., 2009; Verboven et al., 2009; Sullivan et al., 2013). During energy-demanding periods, such as starvation, birds are at higher risk of potential negative effects of PBDEs, because fat depots are metabolised and lipophilic compounds are relocated leading to higher contaminant concentrations (e.g., Malcolm et al., 2003; Colabuono et al., 2012). This is further corroborated by reports of high concentrations of contaminants in tissues of birds in poor physical condition (Sagerup et al., 2009; Colabuono et al., 2012).

On this background, the present study was designed to measure PBDE concentrations in the liver of fulmars with and without ingested plastics, and the corresponding levels of PBDEs in samples of the ingested plastics from the same birds. The main aim was 1) to compare PBDE patterns to confirm if ingested plastics may act as a vector for PBDE uptake in the tissue of fulmars. Based on the previous research investigating the relationship of ingested plastics and PBDE tissue concentrations in procellariiform species (Herzke et al., 2016; Tanaka et al., 2013; Tanaka et al., 2015; Tanaka et al., 2020), we expect (i) to find a plastic-mediated transfer of higher-brominated PBDEs, presumably BDE209, to the tissue of the birds. We also 2) compare the PBDE concentrations in the tissue of birds found dead (beached) and seemingly healthy (bycatch) birds to evaluate if beached birds may be suitable for identifying trends in the accumulation of additives in the tissue of fulmars. We hypothesize that (ii) fulmars in poorer body condition have higher liver PBDE concentrations compared to healthy birds (Sagerup et al., 2009; Colabuono et al., 2012; Cipro et al., 2013). Finally, we 3) compare mass, number and polymer type of ingested plastics, found in beached and bycatch birds, to identify differences in plastic accumulation levels and characteristics among the groups, which could potentially explain the occurrence of BDE209 (if observed).

2. Materials and methods

2.1. Fulmar sampling and autopsy procedures

As an à priori selection criterion, the study birds were selected from larger collections of fulmars on the basis of individual stomach plastic content, aiming to compare those exceeding the OSPAR EcoQO threshold (>0.1 g of plastics in the stomach) with those containing virtually no visible plastics (Table 1). Ten of the birds had been taken as incidental bycatch in longline fisheries in Træna, Vesterålen, and Porsangerfjorden, northern Norway, whereas five birds were found dead on beaches in Rogaland, southern Norway. Maps of the sampling areas are provided

Table 1
Method, location, date, and mass of plastic pieces in the stomachs of northern fulmars (*Fulmarus glacialis*) (IDs Fulmar 1–15). Fulmar IDs 1–5 were bycatch birds with ingested plastics, fulmar IDs 6–10 were bycatch birds without ingested plastics, and fulmar IDs 11–15 were beached birds with ingested plastics.

Sample ID	Method	Group	Location	Date	Plastic (g)
Fulmar 1	Bycatch	1	Northern Norway, Træna	November 2015	0.2596
Fulmar 2	Bycatch	1	Northern Norway, Træna	November 2015	0.2593
Fulmar 3	Bycatch	1	Northern Norway, Træna	November 2015	0.2326
Fulmar 4	Bycatch	1	Northern Norway, Porsangerfjorden	September 2016	0.1783
Fulmar 5	Bycatch	1	Northern Norway, Porsangerfjorden	September 2016	0.1367
Fulmar 6	Bycatch	2	Northern Norway, Porsangerfjorden	September 2016	0.0017
Fulmar 7	Bycatch	2	Northern Norway, Porsangerfjorden	September 2016	No
Fulmar 8	Bycatch	2	Northern Norway, Porsangerfjorden	September 2016	No
Fulmar 9	Bycatch	2	Northern Norway, Porsangerfjorden	September 2016	No
Fulmar 10	Bycatch	2	Northern Norway, Vesterålen	July 2016	No
Fulmar 11	Beached	3	Southern Norway, Rogaland	February 2016	0.4335
Fulmar 12	Beached	3	Southern Norway, Rogaland	March 2016	0.3552
Fulmar 13	Beached	3	Southern Norway, Rogaland	December 2013	0.2490
Fulmar 14	Beached	3	Southern Norway, Rogaland	April 2016	0.2317
Fulmar 15	Beached	3	Southern Norway, Rogaland	February 2016	0.1768

in the Supplementary Material (SM, Figs. S1 and S2). All carcasses were frozen and shipped to the Norwegian Institute for Nature Research (NINA) in Trondheim. The birds selected from the fisheries bycatch included five with 'high amounts' (0.13–0.25 g of plastics, 12–29 pieces of plastics, fulmar IDs 1–5) and five with 'low amounts' (0–0.001 g of plastics, 0–1 piece(s) of plastics, fulmar IDs 6–10) of ingested plastics, whereas the five found dead on beaches had all 'high amounts' of plastics (0.17–0.43 g of plastics, 3–28 pieces of plastics, fulmar IDs 11–15; Table 1).

The fulmars were examined and autopsied at NINA as part of the Norwegian seabird bycatch project and the OSPAR EcoQO monitoring, following international standardised procedures (Van Franeker, 2004). The age and sex of each bird was determined but not further investigated (for full details of age and sex, see SM, Table S1). Body condition was examined by scoring the bird's subcutaneous and intestinal fat deposits and the state of its left pectoral muscle, all three on scale 0 (none/very poor) – 3 (very good). These indices were then summed to give an overall body condition index for each bird, thus ranging from 0 to 9 (0–1 = mortally emaciated, 2–3 = critically emaciated, 4–6 = moderate body condition, and 7–9 = good body condition) (Van Franeker, 2004). For details on the individual condition of the birds, the reader is referred to the SM. Liver tissue samples and whole stomachs including the proventriculus were collected, wrapped separately in aluminium foil, placed in a zip-lock bag, and stored at −18 °C until further analyses commenced.

2.2. Plastic accumulation

Ingested plastic particles were later extracted from each stomach following the protocol for monitoring plastic ingestion by northern fulmars (Van Franeker, 2004). Briefly, both the proventriculus and gizzard of each individual were washed in a sieve (mesh size 1 mm) and stomach content was separated into different groups including plastics, other waste (non-plastic), chemical waste, and remains of natural prey items. After drying overnight at 40 °C, plastic items were counted, weighed, and stored into plastic vials.

For polymer identification of the ingested plastic samples, the Fourier Transform Infrared Spectroscopy (FTIR) Analysis was performed at the Norwegian Institute of Air Research (NILU) in Tromsø using the infrared spectrometer Cary 630 with Diamond Attenuated total reflectance (ATR) sampling accessory (Agilent Technology, Santa Clara, US). Spectra were collected between 4000 and 650 cm^{-1}, the resolution was set at 8 cm^{-1}, and 32 scans were collected when analysing the samples. Before and in between samples, the diamond crystal was cleaned with 2-propanol, and scans were collected to adjust for background noise. Plastic pieces with biofilm on the surface, which was masking the polymer spectra, were sliced, while pieces without biofilm were compressed as a whole on the crystal. Obtained sample spectra were first inspected manually and then compared to the ATR Demo reference library at NILU. Matches were ranked from zero to one (Hit Quality Index). Scores ≥0.7 were accepted if they were in accordance with the manual identification. If the automated polymer identification only scored a match below 0.7, the polymer was accepted, regardless of the quality score, if the characteristic polymer absorbance bands were identified manually (Ask, 2019). 'PE pres.' was a grouping of spectra that only produced the double peak of PE. Spectra with too much background noise and no visually identifiable signature peaks were grouped as 'unidentifiable'.

To provide standardised baseline data on plastic ingestion by fulmars, the colours of plastic pieces were also identified after performing the FTIR (Provencher et al., 2017; details are provided in the SM, Table S5).

2.3. Chemical analysis

The liver and stomach plastic samples from all birds were analysed individually for quantification of PBDEs. All samples were spiked with 20 μL of surrogate standard including ^{13}C labelled BDEs −28, −47, −99, −153, −183, −197, −206, and −209 prior to extraction. All solvents (acetone, cyclohexane, dichloromethane, isooctane, and n-hexane), solid chemicals (silica and sodium sulphate) as well as sulphuric acid applied were purchased from Merck KGaA, Darmstadt, Germany.

2.3.1. Liver samples

Between 1 and 2 g of liver tissue was homogenised with 45 g of sodium sulphate (previously burnt at 600 °C for 8 h) and extracted three times with 40 mL, 30 mL, and 30 mL cyclohexane: acetone mixture (ratio 3:1) in an ultrasonic water bath. The extracts were combined and concentrated to 1–2 mL before the samples dried overnight uncapped for lipid determination. As a next step, lipids and biological compounds were removed using 95–98% sulphuric acid. After adding approximately 6 mL of sulphuric acid, mixtures were vortexed and placed at a dark spot for 30–60 min. Samples were then centrifuged (3 rpm, 10 min) and the sulphuric acid was extracted. This step was repeated until the sulphuric acid turned colourless. An additional clean-up step was carried out applying a column chromatography (glass column dimensions: 20 mm diameter * 380 of length, equipped with a glass plug to regulate flow) packed with 5 (±0.2) g of silica gel (particle size 0.63–0.2 mm, heated for 8 h at 600 °C) and 1 cm sodium sulphate. Samples were eluted by gravity flow using 30 mL of an n-hexane and dichloromethane (DCM) mixture (5% DCM). Prior to instrumental analysis, isotopic labelled PCB159 was added to all samples as an internal injection standard and the solvent was changed to isooctane.

2.3.2. Ingested plastic samples

PBDEs were extracted from plastic particles (0.001–0.43 g) three times using 2 mL (total extraction with 6 mL) cyclohexane and acetone mixture (ratio 50:50) in an ultrasonic bath following the method described by Herzke et al. (2016). Extracts were combined, concentrated to 1 mL, and filtered before matrix removal. A gel permeation chromatography (GPC) system (Waters Cooperation, Milford, USA) with two clean-up columns (19 mm × 150 mm and 19 mm × 300 mm) containing 100-Å-pore size material with a particle size of 10 μm was applied to remove plastic polymer residues. The samples were transferred to the injection loop of the GPC system using a glass syringe with a 0.2 μm filter (Whatman Puradisc syringe filter, Sigma-Aldrich, St. Louis, USA). Columns were eluted with DCM as a mobile phase at 5 mL/min. The eluate was concentrated to 1–2 mL, followed by an acid clean-up with 95–98% sulphuric acid (performed using the same method as previously described for the liver samples). Prior to instrumental analysis, the internal injection standard was added to all samples and the solvent was changed to isooctane.

2.4. Instrumental analysis

The analytical method in this study followed the procedure described by Cooper et al. (2018) (with some modifications). The instrumental analysis was carried out at NILU, Tromsø. Liver and plastic samples were analysed for a suite of PBDEs (17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 202, 206, 207, and 209) (Wellington Laboratories, Ontario, Canada and CIL, Andover, U.S.A.). For analyte detection, gas chromatography with high-resolution accurate mass spectrometry (GC-HRAM) (TRACE 1310-Q ExactiveTM GC OrbitrapTM, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used. The GC-HRAM was equipped with a 15 m RTx 1614 MS column (0.25 μm id and 0.1 μm film thickness, Restek Corp, Bellefonte, PA, USA) and helium was used as a carrier gas at a flow rate of 1.6 mL/min.

2.5. Quality assurance

All glassware was initially burnt at 450 °C for 8 h and rinsed with n-hexane. For each sample, newly purchased equipment was used to avoid cross-contamination. Laboratory tools were rinsed in acetone and cyclohexane in an ultrasonic water bath. In addition, to avoid contamination by particles settling on surfaces, all sample handling was carried out in a clean cabinet (Bigneat Ltd. Contaminant Technology, Hampshire, UK, equipped with a chemcap filtration) or cleanroom only. Two laboratory blanks were run for a batch of ten liver samples and three procedural blanks were performed parallel with a set of plastic samples. In the blanks of the liver samples, all PBDEs were below the instrument detection limits (IDL). Most of the PBDEs in the blanks of the plastic samples were detected below IDL, except BDE47 and BDE209, which were found above IDL in two blank samples at levels of 0.05 ng/g and 0.33 ng/g, respectively. Therefore, the samples were blank corrected for these two compounds by subtracting the mass (ng) detected in the blanks. The analytical method was validated using standard reference material (SRM EDF 2524 Clean fish) purchased from Cerilliant Corporation (Analytical Reference Standards, Round Rock, USA). Two SRMs were analysed for every 10th liver sample. The relative standard deviations in the SRMs were averaging 6% for BDE47 and ranged between 4% and 16% on average for the other analysed PBDE congeners. The measured levels were within the range of the reference values. The limit of detection (LOD) was defined as the average value of the blank signal plus three times the corresponding standard deviation (SD) and the limit of quantification (LOQ) was calculated based on the average concentration of the blank signal plus ten times the SD. The LOD for PBDEs in the liver samples ranged between 0.02 and 1.55 ng/g lipid weight (lw) and 0.0001–0.015 ng/particle in the ingested plastic samples. In the liver and plastic samples, the average recoveries for the PBDE surrogate standards ranged between 36 and 80%, except BDE209 for which the average recovery was 19% in the liver and 25% in the plastic samples. Results with a recovery of <25% and <10% were removed for all PBDEs and BDE209, respectively. All concentrations are recovery corrected, compensated by the applied isotopic dilution methodology. For further details of standard deviations, LODs, and recoveries, the reader is referred to the SM.

2.6. Data analysis

PBDE concentrations are presented on lipid weight and particle number base for the liver and ingested plastic samples, respectively. However, the lipid content is provided in the SM to enable the conversion to wet weight concentrations if needed. Calculations and plots were performed using Microsoft Excel. Mean values of sumPBDE concentrations as well as mass and number of ingested plastics are given with their standard deviation (SD).

For statistical analyses, sumPBDE concentrations were log-transformed as they were not normally distributed. In the present study, the birds were separated into three different groups: bycatch birds with ingested plastics, bycatch birds without ingested plastics and beached birds with ingested plastics. For testing differences in sumPBDE among all three groups, an analysis of variance (ANOVA) was performed. To test differences in sumPBDE concentrations among two bird groups, *t*-tests for unequal variances were performed. Similarly, unequal variance *t*-tests were used to compare body condition, as well as the number and mass of plastics in beached and bycatch birds with ingested plastics.

Linear regressions were performed to determine the impact of body condition on the sumPBDE concentration in bycatch and beached birds with ingested plastics and to assess the impact of ingested plastics (yes/no) in bycatch birds with and without ingested plastics. For all statistical analyses, a statistical significance was defined as $p < 0.05$. Statistical analyses were performed using R (version 4.0.3 (2020-10-10); R Core Team, 2020).

3. Results & discussion

3.1. Polybrominated diphenyl ethers in the liver samples

Of the 25 analysed PBDEs, six lower-brominated PBDEs (BDE28, BDE47, BDE49, BDE66, BDE71, and BDE77) and eleven higher-brominated PBDEs (BDE138, BDE153, BDE154, BDE183, BDE184, BDE196, BDE197, BDE202, BDE206, BDE207, and BDE209) were detected in 7–100% of all samples (relative proportion of individual congener). The mean sumPBDE concentrations (including the above-mentioned six lower-brominated and eleven higher-brominated PBDE congeners) varied significantly between the three different groups ($F_{2,12} = 36.39$, $p < 0.0001$), i.e., bycatch birds without ingested plastics (mean sumPBDE concentration of 3.1 (±3.4) ng/g lipid weight), bycatch birds with ingested plastics (mean sumPBDE concentrations of 16.9 (±13.8) ng/g lipid weight), and beached birds with ingested plastics (mean sumPBDE concentrations of 1219.5 (±1329.5) ng/g lipid weight; Fig. 1; A and B), as explored in the next sections.

3.1.1. Beached and bycatch birds with ingested plastics

In accordance with our expectations, beached birds with ingested plastics had significant higher mean sumPBDE concentration of 1219.5 (±1329.5) ng/g lipid weight (ranging between 145 and 2913 ng/g lipid weight, Fig. 1; A) compared to bycatch birds with ingested plastics which had average sumPBDE concentrations of 16.9 (±13.8) ng/g lipid weight (ranging between 10.0 and 41.6 ng/g lipid weight; $t = -5.50$, $df = 5.45$, $p = 0.002$). Previous research suggests that the differences observed between the two groups may, among other factors, be attributed to sampling location or body condition (e.g., Colabuono et al., 2012).

3.1.1.1. Dietary exposure based on location. In the present study, fulmars were collected at different locations in northern and southern Norway

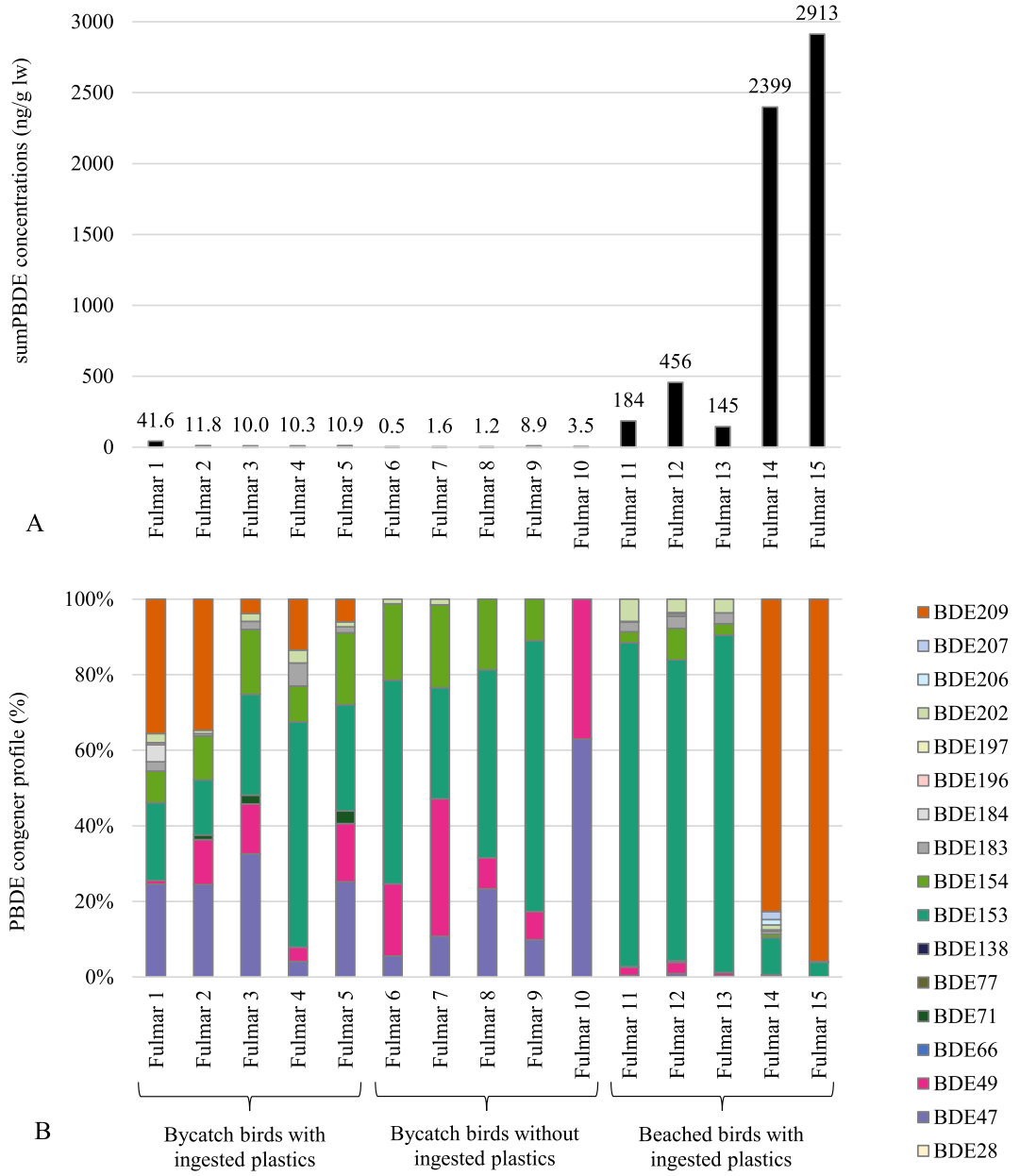

Fig. 1. PBDE concentrations and compositions in the fulmar liver samples. Bycatch birds were caught in northern Norway (Træna, Vesterålen and Porsangerfjorden), whereas the beached birds were found dead in southern Norway (Rogaland). Chart A: sumPBDE concentrations in ng/g lipid weight. Chart B: PBDE congener profile in percentages.

separated by up to 2000 km of ocean and thus, the birds likely acquired pollutants from different geographical areas. The diet of fulmars may be a major factor influencing the exposure of PBDEs (Colabuono et al., 2012) as elevated contaminant levels in prey species can, eventually, lead to higher concentrations in their tissues. As opportunistic predators, fulmars obtain different prey species from the sea surface (Phillips et al., 1999; Garthe et al., 2004). In the North Atlantic, their diet mainly consists of nereids, cephalopods, crustaceans, and fish (Mehlum and Gabrielsen, 1993; Ojowski et al., 2001) but fulmars additionally feed on discards and carrion from fishing vessels (Camphuysen and Garthe, 1997). However, fulmars are also known to vary spatially and temporally in their diet (Phillips et al., 1999).

Previous research indicated a latitudinal distribution of PBDEs with decreasing concentrations from south to north in marine fish along the Norwegian coast (Bustnes et al., 2012), which may suggest that beached birds in southern Norway might have been exposed to higher PBDE concentrations than bycatch birds collected in northern Norway. However, long-term monitoring of PBDEs in fish and mussel samples along the Norwegian coasts reveals, that significantly higher concentrations have been detected in urban areas (such as the inner Oslo fjord, but also locations in northern Norway, such as Bodø harbour) compared to more remote areas, which is most likely related to urban activities (Green et al., 2019). Still, as our selective sampling design aimed at comparing groups with either relatively equal or highly different plastic loads, we do not think sampling location played a major role in the observed differences between beached and bycatch birds with ingested plastic.

3.1.1.2. Body condition. The health status of a bird may affect the contaminant distribution (Colabuono et al., 2012). When birds suffer from starvation or sickness, body mass is reduced, and fat depots are metabolised. PBDEs, which are stored in fat tissue, will be released as birds deplete energy reserves. These compounds will then accumulate in metabolising organs resulting in higher levels of PBDEs in the liver (Malcolm et al., 2003; Colabuono et al., 2012). When comparing the two groups, beached birds were in significantly poorer physiological condition (index range 1–2, mean 1.2 (±0.45)) than bycatch fulmars (index range 3–9, mean

6.2 (±2.28); $t = 4.81$, df = 4.30, $p = 0.007$). This strongly suggests that the beached birds (dead at time of collection) have been suffering from illness or starvation, resulting in a depletion of their fat tissue reserves, mobilising an increase of PBDE concentrations in their liver tissue. In accordance with this, a linear regression revealed that the sumPBDE concentrations increased significantly with decreasing body condition (adjusted $r^2 = 0.54$, $p = 0.009$) when comparing beached and bycatch birds.

Higher tissue contaminant concentrations as a cause of poor condition have been reported in other studies (Sagerup et al., 2009; Colabuono et al., 2012; Cipro et al., 2013). In the majority of white-chinned petrels (*Procellaria aequinoctialis*), Cipro et al. (2013) detected higher sumPBDE concentrations in beached birds compared to those collected from fisheries. Similarly, Sagerup et al. (2009) reported elevated PBDE concentrations in dead glaucous gulls (*Larus hyperboreus*) from Bear Island, Svalbard compared to previous findings. Of these dead gulls, 71% were classified as emaciated. Lower levels of PBDEs have also been found in livers from healthy fulmars (Trevail et al., 2014; Herzke et al., 2016). We, therefore, find it most likely that the great difference in body condition was the key factor explaining the observed difference in PBDE concentration between bycatch and beached fulmars. The strong impact of poor body condition and the unclear underlying causes underline the concern that beached birds may not be suitable for monitoring contamination concentrations in the marine environment.

3.1.2. Bycatch birds with and without ingested plastics

Bycatch birds with ingested plastics were found with significantly higher sumPBDE concentrations ranging between from 10.0–41.6 ng/g lipid weight, averaging of 16.9 (±13.8) ng/g, compared to bycatch birds without ingested plastics with sumPBDE concentration of 3.1 (±3.4) ng/g lipid weight on average (ranging between 0.5 and 8.9 ng/g lipid weight; $t = 3.55$, df = 6.35, $p = 0.01$). A linear regression comparing bycatch birds with and without ingested plastic revealed that sumPBDE concentrations in the liver increased significantly if plastic was ingested (adjusted $r^2 = 0.56$, $p = 0.007$). The detected PBDE pattern in the liver and ingested plastics samples thus support the assumption of a plastic-derived transfer of PBDEs from plastics to accumulate in liver tissue, as explored in the next sections.

3.2. PBDE pattern in the liver and ingested plastic samples

Among all fulmars, the PBDE profile was dominated by tetra- to hexa-PBDEs namely BDE47, BDE49, BDE153, and BDE154 in 13 of the 15 liver samples (all but two of the five beached fulmars with ingested plastics). BDE153 dominated the PBDE composition in eight of these liver samples, ranging between 28%–89% (Fig. 1; B). Further details on the PBDE concentrations are provided in SM. This predominance of tetra-, penta-, and hexa-PBDEs is in accordance with previously observed PBDE patterns in fulmars (Fängström et al., 2005; Karlsson et al., 2006; Herzke et al., 2016). Lower-brominated PBDE congeners have also been detected in prey species of fulmars along the Norwegian coast, i.e., Atlantic cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*) (Fjeld et al., 2004; Bustnes et al., 2012; Boitsov et al., 2016; Boitsov, S., personal communication) suggesting a transfer to the tissue of fulmars via bioaccumulation through the food web.

In contrast, two of 15 liver samples (ID14 and ID15; both from beached birds with ingested plastics) were dominated by BDE209 contributing 83% and 96% to the sumPBDE concentrations averaging 2388 ng/g lipid weight. Notably, BDE209 was also the most abundant PBDE congener with 34% and 35% of the total PBDE concentration in two of the five bycatch birds with ingested plastics (ID1 and ID2) although decaBDE was not dominating the PBDE profile (Fig. 1; B).

BDE209 is the major compound of the commercially used decaBDE mixture and makes up to 97% of the total composition (EU Union, 2003). As pentaBDE and octaBDE have not been produced on a global scale since 2004 (Redfern et al., 2017), the use of decaBDE has increased and led to a growing emission into the environment (e.g., Abbasi et al., 2019; Kukharchyk et al., 2020). Therefore, the elevated levels of BDE209 may be a result of the restrictions of penta- and octaBDE products. In the EU, decaBDE was partially banned in 2008. In electronic applications, concentrations exceeding 0.1% (by weight) were prohibited. This initial regulation was expanded to 'any part of an article' in 2017 and entered into force in March 2019 (EU Commission, 2017). However, products, manufactured before this timeframe, will stay in use for a prolonged period in the future and will be added to the waste stream and mismanaged waste for a long time to come (Abbasi et al., 2019).

Previous studies have detected BDE209 in the liver and abdominal adipose of full-grown fulmars (Fängström et al., 2005; Jörundsdóttir et al., 2013; Herzke et al., 2016; Tanaka, 2017) but equally high or higher concentrations of BDE209 as those detected in the present study, have only been reported once in the literature. In fulmars from Bear Island, Svalbard, BDE209 was detected at a level of >5000 ng/g lipid weight (Knudsen et al., 2007). However, since this elevated concentration was found in 1 of 18 birds only, an analytical error was suggested to be the reason for the high PBDE levels. In addition, elevated BDE209 concentrations were also detected in some short-tailed shearwaters, another procellariiform species (Tanaka et al., 2013), and in fulmars with plastic ingestion (Herzke et al., 2016).

3.2.1. Transfer from ingested plastic samples

In the present study, BDE209 was detected in the livers of birds with ingested plastics (>1 mm), from both bycatch and beached birds, but not in the liver tissue of the fulmars without ingested plastics (Fig. 2; B), indicating a non-dietary exposure source of BDE209. Indeed, BDE209 was detected in seven out of ten stomach plastic samples, strongly suggesting a transfer from the ingested plastics to the liver of the fulmars (Fig. 2; B). Full details of PBDE concentrations are provided in SM.

These findings are in accordance with previous studies (Herzke et al., 2016; Tanaka et al., 2013; Tanaka, 2017; Tanaka et al., 2019; Tanaka et al., 2020). Among other compounds, Tanaka et al. (2019) detected BDE209 in ingested plastic pieces collected from 100 fulmars from the Faroe Islands. In streaked shearwater chicks fed with plastic pellets containing different additives, significantly higher additive concentrations, including BDE209, were detected in the liver, the abdominal adipose, and the preen gland oil compared to the control group (Tanaka et al., 2020). Further, a plastic-derived transfer of BDE209 and BDE183 has also been suggested for short-tailed shearwaters (Tanaka et al., 2013) as the PBDE pattern in the ingested plastics resembled the composition observed in the tissue of the birds. In addition, Tanaka et al. (2013) did not detect BDE209 and BDE183 in the prey species of the birds. These findings are confirmed by laboratory experiments demonstrating that the stomach oil of Procellariiformes may support the leaching of chemicals that are incorporated in the plastic polymer as it potentially acts as an organic solvent (Tanaka et al., 2015). Although Herzke et al. (2016) found high levels of BDE209 in the plastic sample and the corresponding muscle sample (but not in the liver tissue) of one of 30 fulmars from Norway, the authors concluded in general that the contribution of ingested plastics to the total PBDE concentration is negligible compared to their natural prey based on a bioaccumulation model (Herzke et al., 2016).

3.2.2. Dietary exposure of BDE209

Although data on PBDE concentrations in prey species of fulmars near the sampling locations are limited, PBDE209 has been detected in prey species of fulmars at all sampling locations. In two locations in northern Norway (Lofoten Islands and Varangerfjorden), PBDE209 levels in Atlantic cod ranged between <0.01–0.02 and 0.35–0.83 ng/g wet weight in the liver tissue, respectively, whereas in southern Norway, PBDE209 concentrations were ranging between <0.21–0.65 ng/g wet weight in Bømlo, and 0.21–0.27 ng/g wet weight in Lista (Fjeld et al., 2004). Thus, both bycatch and beached birds in the present study may

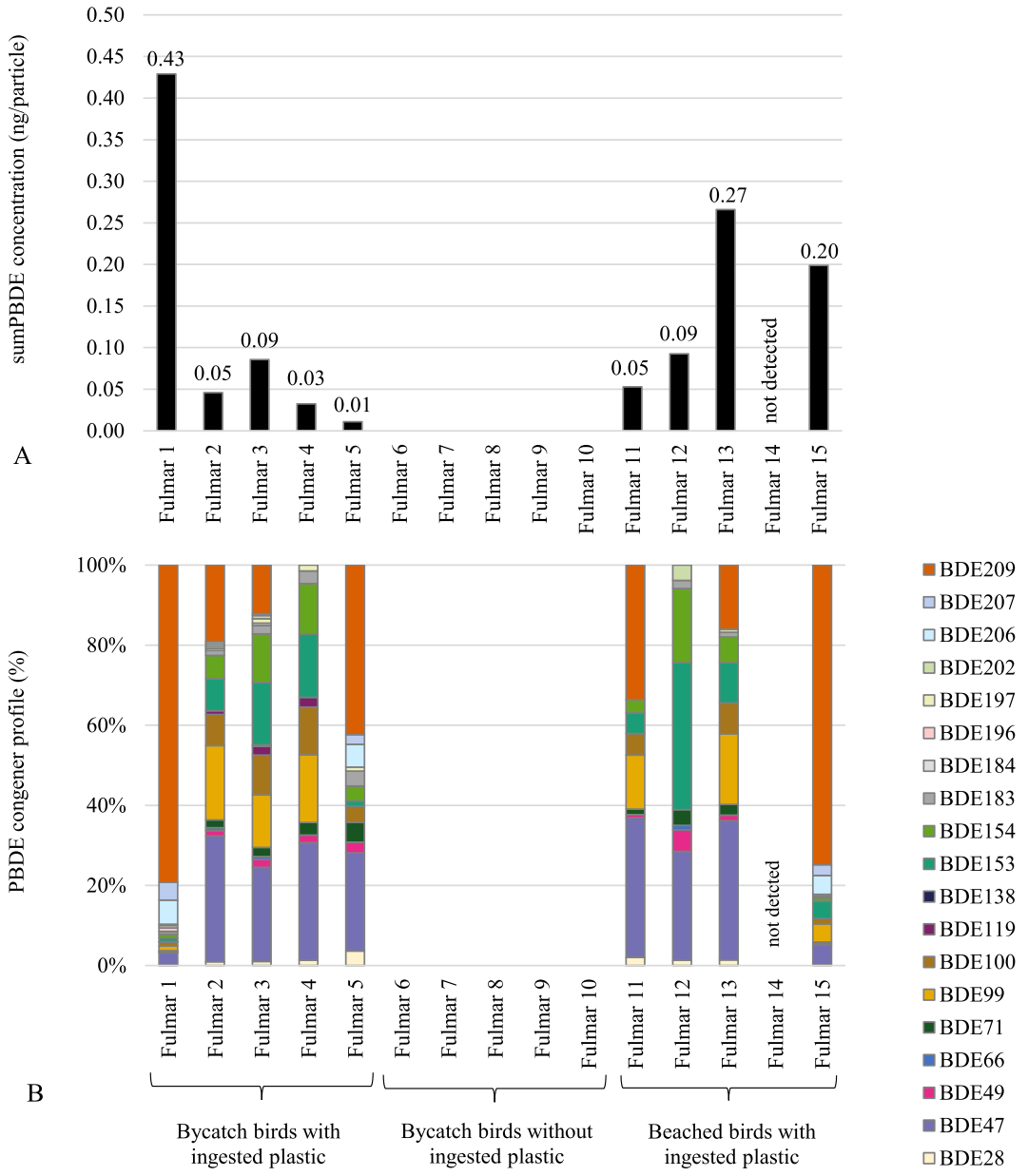

Fig. 2. PBDE concentrations and compositions in the samples of plastic ingested by the fulmars. Bycatch birds were caught in northern Norway (Træna, Vesterålen and Porsangerfjorden), whereas the beached birds were found in southern Norway (Rogaland). Chart A: sumPBDE concentrations in ng/g lipid weight. Chart B: PBDE congener profile in percentages in plastic samples.

have been exposed to PBDE209 via their diet. On the other hand, previous research also indicated the absence of PBDE209 in the fulmar prey species Atlantic cod and Atlantic herring sampled at different locations along the Norwegian coastline (Boitsov et al., 2016; Boitsov, S., personal communication). Even low PBDE209 concentrations may eventually result in elevated concentrations accumulated over the years. However, natural prey items as the only exposure source seems unlikely as PBDE209 was detected in fulmars with ingested plastics only but was absent in non-plastic exposed birds. Earlier studies also suggested a magnification of PBDE47, but assumed a potential dilution of PBDE209 through the food chain (Tomy et al., 2008; Tomy et al., 2009) supporting a plastic-derived exposure source of PBDE209. Nevertheless, the bioaccumulation and magnification of PBDE209 in fulmars and their prey species require further studies, with more species of prey organisms analysed to explore in greater detail how fulmars could accumulate PBDEs, particularly PBDE209, from natural prey. As the natural prey of fulmars mainly include juvenile fish and zooplankton (e.g., Phillips et al., 1999), this may also suggest that fisheries offal, which is known to be an important food source for fulmars (Camphuysen and Garthe, 1997), could be an important exposure source of PBDEs underlining the need for additional future studies in this field.

3.3. Debromination

The results of the present study could potentially also suggest a plastic-derived transfer of BDE183. This compound was detected in the liver (five of five bycatch and four of five beached birds) and stomach plastic samples (five of five bycatch and three of five beached birds) of fulmars with ingested plastics but not found in the livers of bycatch birds without ingested plastics. This assumption is in accordance with previous research suggesting a plastic-mediated exposure of BDE183 in procellariiformes (Tanaka et al., 2013). However, BDE209 has been shown to degrade to several lower brominated congeners via metabolic pathways (e.g., van den Steen et al., 2007) or photolytic debromination

(e.g., Eriksson et al., 2004; Söderström et al., 2004) at high rates (e.g., Sandholm et al., 2003; Thomas et al., 2005). For example, in rats that were exposed to BDE209, previous research calculated a half-life of 2.5 days (Sandholm et al., 2003). Therefore, BDE183 could be a conversion product of BDE209 (Söderström et al., 2004). The most abundant debromination products are nona-brominated congeners (BDE208, BDE207 and BDE206) (e.g., van den Steen et al., 2007; Muñoz-Arnanz et al., 2011; Letcher et al., 2014), of which BDE206 and BDE207 were detected in one liver sample of beached birds with ingested plastics (fulmar ID 14). Since BDE209 may degrade to a variety of lower PBDE congeners, some other higher-brominated PBDEs detected in our study could be debromination products. In fact, BDE202, which has also been identified as a debromination product (Stapleton et al., 2006), was detected in both liver samples of the fulmars with ingested plastics (ten of ten) and sporadically in those without ingested plastics (two of five birds), underlining the challenge of metabolite identification. This, however, also indicates that decaBDE may not be detectable in the tissue of the fulmars unless the birds have been recently exposed to BDE209.

3.4. Characteristics of ingested plastics

The study birds caught as bycatch in longline fisheries had ingested between 12 and 29 plastic pieces averaging 21.2 (±7.0) whereas the birds found dead on beaches contained between 3 and 28 plastic pieces with 15.6 (±11.6) on average in their stomachs. These differences between the bycatch and beached birds, however, were not significant ($t = -0.92$, df = 6.57, $p = 0.39$). In addition, the total mass of ingested plastic did not differ significantly between the two bird groups, ranging from 0.13–0.26 g with 0.21 (±0.05) g on average in bycatch birds and 0.17–0.43 g averaging 0.29 (±0.1) g in beached birds ($t = 1.46$, df = 6.04, $p = 0.20$).

However, among beached birds, there was a large variation in the number and mass of ingested plastics. For example, Fulmar ID 14 was found with three plastic pieces weighing 0.23 g, whereas Fulmar 15 had 28 plastic pieces weighing 0.17 g. Details of ingested plastic samples are given in the SM. Interestingly, the liver samples of these two fulmars (IDs 14 and 15), were found with the highest BDE209 concentrations, confirming previous studies (e.g., Tanaka et al., 2015; Herzke et al., 2016) that the transfer of BDE209 is more sporadic and does not correlate with mass and number of plastics as it has been suggested for other contaminants (Hardesty et al., 2015).

Although fulmars do not normally regurgitate hard indigestible items (Furness, 1985; van Franeker and Law, 2015), they can lose ingested plastic items when feeding their chicks or scaring away intruders (van Franeker and Law, 2015). The time that retained plastic items remain in the stomach mainly depends on the original type and size, including shape and thickness, of the ingested plastics, and the rate of wear in the stomach (Ryan, 2016). Eventually, ingested plastics break down into smaller fragments and are then excreted via faeces. Indeed, microplastics (<1 mm) have been found in the guano of fulmars (Provencher et al., 2018). In procellariiform species, estimates suggest that plastic pieces may be retained in the gizzard for periods ranging from only a few weeks or months (e.g. Ryan, 2015; van Franeker and Law, 2015) up to over a year (e.g., Ryan and Jackson, 1987). For example, van Franeker and Law (2015) estimated disappearance rates of ingested plastics in fulmarine petrels of approximately 75% within 4 weeks. Nevertheless, the retention time of plastics is difficult to measure. With respect to our study, this may however indicate that the detected numbers and consequently the weights of plastics may not reflect the actual amounts of plastic that contributed to the BDE209 concentrations found in the liver as the numbers may only represent a short-lived snapshot of a more dynamic turn-over process. Therefore, there might be a bias in our results as the birds of our study without ingested plastics may represent PBDE contamination from already digested or ejected plastics.

In addition, for the birds with ingested plastics, some inconsistencies may be detected when comparing the PBDE profiles of the liver and ingested plastic samples. For example, the ingested plastic sample of Fulmar 1 was found with the highest PBDE concentrations, predominated by BDE209, but the corresponding liver sample was found with relatively lower PBDE concentrations (and decaBDE did not predominate). Similar observations have been reported in short-tailed shearwaters (Tanaka et al., 2013; Tanaka et al., 2015). Previous laboratory experiments with stomach oil suggested that these irregularities may be explained by several factors, such as insufficient amount of stomach oil or digestion stage, that potentially limit the leaching of additives into the stomach (Tanaka et al., 2015). Similarly, high concentrations of PBDEs (with predominance of BDE209) were detected in one liver sample (Fulmar ID 14) but PBDEs were not found in the corresponding plastic sample. If ingested plastics do not remain long enough in the stomach, PBDEs may not be completely released into the stomach and transferred to the bird's tissue (Tanaka et al., 2015; Tanaka, 2017), which could explain the observed inconstancy.

In the present study, PE and PP made up the majority of plastic pieces ingested by bycatch and beached fulmars. In the bycatch birds, 73.6% of the ingested plastic pieces were PE, 17.9% PP, 4.7% presumably PE, and 3.8% could not be identified. The stomach plastic content for the beached birds was also predominated by PE with 74.3%, followed by PP with 22.7%, presumably PE with 1.5%, and polyamide (PA) with 1.5%. However, after the laboratory handling (extraction and various clean-up steps) at NILU, the number of plastic pieces per sample differed from the initially counted numbers. Potentially, some plastic pieces may have dissolved in the cyclohexane and acetone mixture. Therefore, the percentages of identified polymers may not be very accurate. As the polymer type of ingested plastic pieces was determined after laboratory processing, these results are presented based on the numbers of plastic pieces after extraction. Full details of plastic polymers are provided in SM. The predominance of PE and PP is in accordance with previous research, identifying these as the most frequently ingested polymers in fulmars (Ask, 2019; Tanaka et al., 2019; Kühn et al., 2021). Our findings also mirror the results of a review of 42 studies demonstrating that PE and PP are the most common polymers in marine litter (Hidalgo-Ruz et al., 2012). As PE and PP float on the ocean's surface due to their buoyancy (Yamashita et al., 2011), fulmars are likely to ingest these polymers due to their non-selective surface-feeding behaviour (Phillips et al., 1999; Garthe et al., 2004). Previous studies have indicated that concentrations of chemicals and pollutants can vary among plastic polymer types (Rochman et al., 2013; O'Connor et al., 2016). In the study of Hirai et al. (2011), BDE209 dominated the composition in PP plastic fragment samples with higher total PBDE concentrations (>100 ng/g) from different locations with over 97% in the majority of cases. A recent study by Tanaka et al. (2019) revealed that plastics ingested by different procellariiformes, among them fulmars, contained a variety of additives including various UV absorbers. In their study, polymer types of ingested plastic pieces were identified as PE or PP. Thus, the ingested plastic polymers PE and PP may pose a greater risk to seabirds regarding the transfer of other potentially harmful contaminants.

4. Conclusion

Our results confirm previous research suggesting BDE209 as an indicator of plastic contamination in biota (Tanaka et al., 2013; Rochman et al., 2014; Tanaka et al., 2020). However, to exclude bias from natural prey species as an exposure source of BDE209, more data on PBDE concentrations in natural diet items of fulmars and fisheries offal are needed. Due to the high levels of BDE209 in our study, future studies should involve the identification of toxicological risks of BDE209. The exposure to PBDEs may disrupt the endocrine hormonal system (e.g. Crisp et al., 1997; Cowens et al., 2015), which is of concern as the avian thyroid hormones are required for controlling growth as well as reproduction (e.g., Merryman and Buckles, 1998a, 1998b) and also

play an important role in feather regeneration (e.g., Pati and Pathak, 1986), and egg-laying (McNabb, 2000). Previous studies indicate decreased hatching success (McKernan et al., 2009), longer incubation periods, and lower nest temperatures (Verboven et al., 2009; Sullivan et al., 2013) caused by the exposure to PBDEs. To gain a better understanding of the behaviour and consequences of BDE209 contamination, further risk assessment of BDE209 is required.

We demonstrated that beached fulmars may not be suitable to monitor the accumulation of additives in the marine environment, as concentrations were significantly higher, likely caused by the poor body condition of the birds. However, due to the small sample size and study design in our study, we could not investigate if there is a co-variation between accumulated plastics and body condition. Future research needs to investigate if high amounts of ingested plastics lead to emaciation of the birds and as a result to higher PBDE concentrations.

Our study increases the understanding of accumulation pathways of contaminants to the tissue of biota, even though the sample size was small and subjectively selected and consequently may not allow drawing conclusions that apply to the entire population (Kitchenham and Pfleeger, 2002; Provencher et al., 2020). Yet, the differences in PBDE levels and composition observed between the different groups in our study should not be interpreted for this sole reason. Although more robust sample sizes are encouraged for future studies, similar sample sizes have been used to measure contaminants in the environment (Letcher et al., 2010) and seabirds (Hardesty et al., 2015) as previously emphasised by Provencher et al. (2020).

CRediT authorship contribution statement

Svenja Neumann: Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Mikael Harju:** Methodology, Validation, Investigation, Data curation, Writing – review & editing. **Dorte Herzke:** Conceptualization, Methodology, Validation, Writing – review & editing. **Tycho Anker-Nilssen:** Investigation, Writing – review & editing. **Signe Christensen-Dalsgaard:** Investigation, Writing – review & editing. **Magdalene Langset:** Investigation, Writing – review & editing. **Geir Wing Gabrielsen:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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