Persistent Organic Pollutants in two species of migratory birds from Rothera Point, Adelaide Island, Antarctica.

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Carcasses of South Polar Skuas (*Catharacta maccormicki*) and Kelp gulls (*Larus dominicanus*) were opportunistically collected around of Rothera Research station (67°35'8"S and 68°7'59"W) during the 2016/2017 austral summer. Samples of their tissues (muscle, liver and subcutaneous fat) were analysed for persistent organic pollutants (POPs). Organochlorine pesticides (OCPs) showed the highest concentrations, notably for pp’-DDE and HCB. The Polychlorinated biphenyls (PCBs)-profiles demonstrated a clear dominance of hexa- and hepta-CBs, while concentrations of polybrominated diphenyl ethers (PBDEs) remained low. The concentrations of some POPs (e.g. HCB) were lower than in past studies on similar species, however others were within the previous range (PCBs) or even higher than previous reported values (DDE). Although no major interspecific differences in the absolute concentrations of POPs were detected, their profiles varied, being likely related to feeding and migration patterns of each species. The current study provides important baseline data for future monitoring of POPs in Antarctica.

Keywords:  
  
POPs; Antarctica; birds; PBDEs; PCBs; OCPs;

1. Introduction

Persistent Organic Pollutants (POPs) encompass a group of contaminants including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) (Vallack et al., 1998). POPs have been demonstrated to potentially affect reproductive, developmental, neurologic, endocrine, and immunologic systems of animals and humans (Noyes et al., 2009). Despite these risks being known since the 1960s, only in 2001 international agreement was achieved on the ban, restricted use or reduction of hazardous POPs by the Stockholm convention (Gavrilescu, 2005; “Listing of POPs in the Stockholm Convention,” n.d.). POPs are known to accumulate in multiple compartments of ecosystems all around the world (Gavrilescu, 2005; Vallack et al., 1998). Even the remote continent of Antarctica has been established as an environmental sink for POPs (Bargagli, 2008; Wania, 2003). Because of a unique combination of relative volatility and environmental persistence, some POPs are able to reach the Antarctic continent via long-range atmospheric transport process and the cold condenser effect (Schenker et al., 2014). Therefore more volatile POPs can reach high concentrations in Antarctic apex predators, including marine birds (Van den Brink, 1997). In addition, the birds also migrating North of the Antarctic convergence are exposed to relatively higher amounts of other POPs in their wintering grounds (Bourgeon et al., 2012; Martínez-López et al., 2015; Pozo et al., 2014). In this manner birds that migrate into and out of Antarctica may be exposed to a wide spectrum of POPs, with both Antarctic as well as more Northerly signatures (Battaglia et al., 1997). This has been shown for South Polar Skua *(Stercorarius maccormicki)* (Carravieri et al., 2017; Corsolini et al., 2011; Focardi et al., 1992; Mello et al., 2016; Roscales et al., 2016) However, little information is available on the exposure of related migrating species, one of them being the Kelp gull (*Larus dominicanus*).

South Polar Skuas (*C. maccormicki*) and kelp gulls (*L. dominicanus*) are two species of migratory seabirds that nest around the whole Antarctic continent, including the Western Antarctic Peninsula during austral summer. The South Polar Skua (widely hybridised with the brown Skua (*Stercorarius antarcticus)* (Ritz et al., 2006)) may be found almost everywhere around Antarctica, but in the same time can commence a trans-equatorial migration when foraging (Hahn and Bauer, 2008; Trillmich, 1978). The exact patterns of its migration have been investigated in detail (Kopp et al., 2011). Kelp gulls are less inclined towards very long haul migration than Skuas, but can move all around the Sub-Antarctic islands and South America (Bertellotti and Yorio, 1999; Harrison, 1991). These reports indicate that Skuas and Kelp gulls demonstrate consistent migration patterns on both populational and individual scales (Krietsch et al., 2017). They have both been shown to be highly affected by global climate change (Micol and Jouventin, 2001), and established migration routes are therefore likely to change, or even cease to exist (Constable et al., 2014). Skuas and Kelp gulls are predominantly opportunistic omnivore avian predators and scavengers in the marine food chain of the Southern Ocean. Their diet composition may shift dramatically when travelling from Antarctica to South America: as they move north the amount of krill and marine fish and invertebrates gradually decreases, being substituted with terrestrial insects and even garbage (Reinhardt et al., 1988),(Bertellotti and Yorio, 1999). Skuas prey on other bird species (Furness and Hislop, 2009; Reinhardt et al., 1988), while Kelp gulls may forage on live whales (Rowntree et al., 1998) and seal pups (Seguel et al., 2017) in Antarctica and on rodents in South America (Ruiz and Simeone, 2001). Both species may also exhibit cannibalistic behaviour towards both chicks and adults (Coulson and Coulson, 1993; Reinhardt et al., 1988).

The first research on POPs concentrations in migratory birds in the Antarctic dates back to the 1960s (Risebrough et al., 1968), however the data is often incomplete or sporadic (see Appendix Table 1 for details). For permanent Antarctic species, concentrations of POPs have been shown to be declining over the last decade, but it remains unknown whether this also applies to migratory species reaching Antarctica (van den Brink et al., 2011). Since the 1990s explicit studies were performed to compare POPs in the migratory Antarctic birds to the native Antarctic non-flying birds (mainly Adélie penguins) (Court et al., 1997; Kim et al., 2015; Wolschke et al., 2015). These studies reported that concentrations of POPs in the former are almost 2 times higher than in the latter. However, direct comparisons between birds of different migratory routes are lacking. Skuas and Giant Petrels continue to be the most well-researched species among the Antarctic marine predators on the topic of POPs contamination (Colabuono et al., 2016; Corsolini et al., 2011; Court et al., 1997; Kim et al., 2015; Mello et al., 2016; Norheim et al., 1982; Roscales et al., 2016; Yogui and Sericano, 2009), while information on others is more limited (Cipro et al., 2013; Fromant et al., 2016; Van den Brink, 1997).

In the current study we report a wide range of POPs in different tissues of opportunistically obtained Skua and Kelp gull specimen. This is the first article to do so in various tissues of Kelp gulls. The data will provide important insights to the task of monitoring of POPs in Antarctic birds.

1. Material and methods

2. 1 Sample collection and preparation.

Three corpses of South Polar Skua (*C. maccormicki*) and Kelp gulls (*L. dominicanus*) were opportunistically collected on Rothera Point (67°35'8"S, 68°7'59"W, Fig. 1) and nearby islands in Ryder Bay during the austral summer of 2016-2017. Only adult birds found dead were collected, with the main cause of death determined to be collisions with masts or buildings. The corpses were stored and transported at -20°C until further analysis in the laboratory in the Netherlands. During necropsies, samples of liver, muscle and subcutaneous fat were collected where possible, and stored in hexane pre-cleaned 60ml amber glass vials. Due to scavengers, birds were found in different physical states, and therefore, it was not possible to sample all the targeted organs in each specimen.

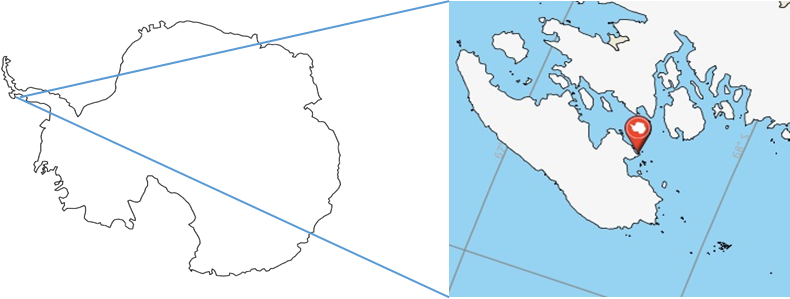


Figure1. Location of Rothera research station on the Antarctic continent (left) and on Adelaide Island (right).

2. 2 Chemical analysis.

The whole procedure is based on the EN 15741:2009 (European Committee for Standardization CEN/TC 327, 2012) method with some adjustments during the extraction step (details in Appendix 2A). In short, the procedure was as follows: After homogenization, 2 to 6g of sample was transferred into hexane pre-cleaned 60ml amber glass tubes, and spiked with 13C12 standards. MiliQ water was added until the total sample volume was 13ml. After this 10ml of ethyl acetate was added, and the mixture was vigorously shaken in an overhead shaker for at least 10 minutes. This was followed by the addition of a mix of 2g of sodium chloride and 4g of magnesium sulphate. The samples were then centrifuged for at least 10 minutes at 350G. The ethyl acetate supernatant was then transferred into a Turbovap® tube, and the procedure was repeated 2 times, starting from the addition of 10ml of ethyl acetate, so that the total extract volume at the end of the process was 30ml. The samples were then concentrated to 1ml, and carefully transferred to new pre-cleaned 60ml amber glass tubes. 27ml of hexane and 10g of 40% acidic silica were added to each sample and left overnight. The hexane fraction was then transferred to a hexane pre-cleaned Turbovap® tube, and evaporated to 1ml, after which the sample was passed through a clean-up column which consisted of 1g of activated silica and 8g of 40% acidic silica. The sample was eluted with 25ml of hexane and subsequently by a mixture of 18ml hexane and 12ml dichloromethane. The resulting solvent mixture was evaporated in a Turbovap® to 1ml, and a solvent exchange to iso-octane was performed. The resulting 1ml of extract in iso-octane was then stored at -20°C until measurements of PCBs, PBDEs and OCPs were made.

Table 1 presents the full list of POPs analysed in this study.

Table 1.Chemical compounds analysed in the current study.

|  |  |  |
| --- | --- | --- |
| **PCBs** | **PBDEs** | **OCPs** |
| PCB 28 | BDE-47 | HCB |
| PCB 52 | BDE-66 | HCH-α |
| PCB 101 | BDE-85 | HCH-γ |
| PCB 105 | BDE-99 | HCH-β |
| PCB 114 | BDE-100 | HCH-δ |
| PCB 118 | BDE-138 | Heptachlor |
| PCB 123 | BDE-153 | op'-DDE |
| PCB 138 | BDE-154 | trans-Chlordane |
| PCB 153 | BDE-183 | cis-Chlordane |
| PCB 156 |  | pp'-DDE |
| PCB 157 |  | Endosulfan-α |
| PCB 167 |  | op'-DDD |
| PCB 180 |  | op'-DDT |
| PCB 189 |  | pp'-TDE(DDD) |
|  |  | pp'-DDT |
|  |  |  |

PCBs and PBDES were quantified by a Magnetic Sector Autospec GC-HRMS from Waters (Manchester, UK) equipped with an Agilent 6890 GC (Santa Clara, USA). Because of practical constraints OCPs were measured by an Agilent 7010B Triple Quadrupole coupled with an Agilent 7890 GC (Santa Clara, USA). A DB-5MS 60m × 0.25mm × 0.25μm fused silica capillary column (Agilent J&W, Folson, USA) was used for PCB analysis, while a CL-Pesticide 30m x 0.25mm x 0.25μm column (Restek, Bellefonte, USA) was fitted for the analysis of PBDEs and OCPs. The measurements were conducted at RIKILT laboratories in Wageningen, the Netherlands. For further details on the GC-methods see Appendix 2B. Limits of quantification (LOQ) were set for each individual compound as the lowest quantifiable standard, while the limit of detection (LOD) was calculated as 3 times the concentration of the compound in the extract of the corresponding blank sample. Concentrations in samples were not adjusted for blanks nor corrected for recovery rates, however all values are reported in Appendix 2C. The lipid content was determined gravimetrically on a fraction of each sample after extraction.

2. 3 QA/QC.

Each measurement batch contained 7 tissue samples, a procedural blank and sample of a certified reference material (SRM 1947, National Institute of Standards and Technology). The samples were considered valid if the recoveries of all internal standards were 50%-150% and the difference between measured and estimated SRM concentrations was <50%. The detailed QA data is shown in Appendix 3.

2. 4 Data treatment.

The low number (1-3 per specific tissues per specimen) of samples did not allow for a comprehensive interpretation of results based on statistical tests. Therefore further analysis and discussion chiefly refer to the obtained values mostly in a descriptive manner. The only exception are the samples of muscles, where 3 samples were collected for both Skuas and Kelp gulls each, and therefore they could be analysed statistically. Mann–Whitney–Wilcoxon (MWW) tests were conducted on them using the Real Statistics© package in Microsoft Excel with the null hypothesis being that the sums of concentrations of PCBs, DDTs and PBDEs in did not different between Skuas and Kelp gulls. The results of the test are presented in Appendix 4.

The mean values were calculated in cases when more than one sample was available per specific tissue in a species. The standard deviation (SD) values were calculated only for instances with 3 samples present.

Concentrations of POPs across different tissues in a single organism may be similar due to lipid-mediated exchange of contaminants between tissues. In the current study there were higher concentrations of PCBs and OCPs in muscles and livers of Skuas than in fat (Haddad et al., 2000). Although similar patterns were reported for non-Antarctic birds (Jaspers et al., 2006; Zheng et al., 2018), the most probable explanations are that the bird specimens were found in different stages of decomposition and natural variability (Carravieri et al., 2017).

3. Results and discussion

PCBs

Figure 2. Concentrations (mean and standard deviations back-transformed from log-transformed data) of PCBs in muscles, livers and subcutaneous fat of Skuas (blue bars) and Kelp gulls (orange bars and dots (in cases where only 1 sample was available)). Note that log scale is used ubiquitously.

Concentrations of PCBs (Fig.2) are generally higher in subcutaneous fat and livers than in muscles for both species. The patterns of individual congeners, nevertheless, appear quite similar with a predominance of hexa- and hepta-CBs, i.e. PCB 153, PCB 180 and PCB 138. Among tri-, tetra- and hepta-CBs the highest concentrations are attributed to PCB 118. The predominance of hexa- and hepta-CBs in both Skuas and Kelp gulls agrees with many preceding studies for various Antarctic marine birds (including Skuas) (Corsolini et al., 2017, 2011; Mello et al., 2016; Roscales et al., 2016), although this may not observed for penguins(Van den Brink, 1997). This outcome may arise from characteristics of the metabolism of PCBs by avian species, which allows them to eliminate lower chlorinated PCBs faster than the higher chlorinated ones (Court et al., 1997; Maervoet et al., 2004). No statistically significant differences were found among concentrations of PCBs in the muscles of the two species (Appendix 4).

Although PCB concentrations in this study are higher than those reported recently for Antarctic seabirds(Cipro et al., 2013; Corsolini et al., 2011; Fromant et al., 2016; Kim et al., 2015; Mello et al., 2016; Roscales et al., 2016; Yogui and Sericano, 2009) and are comparable to the ones from 15-20 years ago (Court et al., 1997; Van den Brink, 1997), Antarctic birds are known to demonstrate high individual variability in their PCB contents (Mello et al., 2016), which also varies with their seasonal breeding activities (van den Brink et al., 1998). From the current samples, no indications of their reproductive condition could be acquired, so it was not feasible to account for such seasonal variability. Neither was it possible to find significant differences between concentrations of PCBs in Skuas and Kelp gulls. Therefore although PCBs concentrations in the current study may indicate that concentrations may be stable over time, more detailed information is needed to account for different factors affecting the uncertainty when comparing between studies.

OCPs

Figure 3. Concentrations (mean and standard deviations back-transformed from log-transformed data) of OCPs in muscles, livers and subcutaneous fat of Skuas (blue bars) and Kelp gulls (orange bars and dots (in cases where only 1 sample was available)). Note that log scale is used ubiquitously.

OCPs are the most abundant group of POPs among the individual compounds and congeners analysed (Fig. 3), possibly due to their application method which involves their dispersion over large areas and therefore an increased tendency for the long-range atmospheric transfer (Fromant et al., 2016). In addition, recent reports (Brisbois, 2014; Carvalho, 2017; Hjorth et al., 2011; Joyce, 1997) indicate that despite multiple legislative restrictions, several OCPs (e.g. HCB) are still being produced and/or sold (sometimes as by-products) in South America, which facilitates their continuous intake into environments which the collected birds were very likely to have visited (Martínez-López et al., 2015).

The profile of OCPs is quite similar among the species (the MWW test (Appendix 4) has not revealed any significant differences among their concentrations in muscles of Skuas and Kelp gulls): the highest concentrations measured were for p,p’-DDE, followed by HCB, p,p’-DDT, p,p’-DDD, oxychlordane and HCB-β. Concentrations of other pesticides are low.

The overall burden of total DDTs (DDTs, DDEs and DDDs) is higher than reported in recent studies for Skua eggs, but is similar to an earlier report for livers of adult Skuas (Court et al., 1997). Furthermore, the DDT and especially the DDE concentrations in the both species of the current study are higher than found in penguins (Kumar et al., 2002; Montone et al., 2016; Weichbrodt et al., 1999). This may demonstrate that exposure to DDT is a continuing issue for the former during their winter migrations. Blood samples of giant petrels and Skuas were yet reported to have lower DDT concentrations, and this may result from their different migration patterns and/or site-specific variations (Colabuono et al., 2015; Kim et al., 2015; Roscales et al., 2016).

HCB is the second most abundant OCP. The concentrations of HCB were higher in this study than for eggs of Antarctic flying birds (including Skuas) recently reported (possibly due to the absence of potential for HCB to accumulate in eggs) (Mello et al., 2016), but are yet consistent with reported values for blood of giant petrels (Colabuono et al., 2016). Compared to Antarctic non-flying birds, HCB concentrations reported here were similar for Kelp gulls, and were higher for Skuas (Roscales et al., 2016; van den Brink et al., 1998).

The chlordane group (Oxychlordane, trans-Chlordane, cis-Chlordane, Heptachlor and Heptachlor epoxide) is mainly represented by Oxychlordane, a metabolite of other chlordanes (Eisler, 1990), while their concentrations are higher than those found in other studies (Colabuono et al., 2016; Fromant et al., 2016; Kim et al., 2015). The HCH group is chiefly represented by β- and γ- stereoisomers, which are the mostly used isomers (Kristine L. Willett et al., 1998).

There were no significant statistical differences among concentrations of OCPs in muscles of Skuas and Kelp gulls (Appendix 4).

PBDEs

Figure 4. Concentrations (mean and standard deviations back-transformed from log-transformed data) of PBDEs in muscles, livers and subcutaneous fat of Skuas (blue bars) and Kelp gulls (orange bars and dots (in cases where only 1 sample was available)). Note that log scale is used ubiquitously.

The concentrations of PBDEs (Fig. 4) are low compared to other POPs. They are comparable, however, to previous studies for eggs of Skuas (Mello et al., 2016; Yogui and Sericano, 2009), but lower than the ones reported in blood of giant petrels and prions. This can be explained by the different migration behaviour (Mello et al., 2016) – petrels and prions tend to travel less further North and therefore are less directly exposed to industrial chemicals (Fromant et al., 2016; Roscales et al., 2016). Composition of congeners is similar in all tissues of both species: the highest values are attributed to BDE-47 followed by BDE-153, BDE-154, BDE-99, and BDE-100. While concentrations of other congeners are minimal, they exhibit high variability as seen in their large standard deviations. The presence of PBDE-183 in quantifiable concentrations has been suggested to occur due to direct exposure to decaBDEs in Antarctica, and may be an indicator of emerging pollution of local seas by microplastics (Fromant et al., 2016).

The MWW test (Appendix 4) demonstrated significant differences between PBDE concentrations in muscles of Skuas and Kelp gulls, which was the only instance when significant differences were found in this study. Because Skuas tend to travel longer distances than Kelp gulls (Bertellotti and Yorio, 1999; Kopp et al., 2011; Krietsch et al., 2017), concentrations of PBDEs in the former can be expected to be higher.

The second explanation may arise from different feeding habits of the two birds species during their stay in South America. Skuas are more likely to forage on landfills and therefore to be exposed to industrial chemicals (such as PBDEs), while Kelp gulls prefer to forage in agricultural areas (Bertellotti and Yorio, 1999; Hahn and Peter, 2003; Reinhardt et al., 1988; Votier et al., n.d.).

4. Conclusions

The present study provides an important baseline for any future evaluation of trends of POPs in Antarctic marine avian predators, near the Antarctic Peninsula and the Southern regions of South America. The concentrations of PCBs, OCPs and PBDEs were generally in good agreement with similar earlier reports. This study confirms that Skuas and Kelp gulls are still subjected to direct exposure to POPs, whose concentration patterns are linked not only to environmental levels in the areas the birds are visiting, but also to the characteristic ecological traits of each species.

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