INTERSPECIFIC PATTERNS IN LEVELS AND PROFILES OF LEGACY AND EMERGING PERSISTENT ORGANIC POLLUTANTS IN SEABIRDS FROM ANTARCTICA (ADMIRALTY BAY, KING GEORGE ISLAND)

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Introduction

Persistent Organic Pollutants (POPs) have a large utilization record around the globe, with different applications. Combined characteristics of volatility, persistence and bioaccumulative potential enable these compounds travel long distances and to reach cold places. Prohibition or restrictions of these compounds was not enough to keep them away from the Antarctic marine food web[1]. Brought to Antarctic by migratory animals, ocean currents and mainly long-range atmospheric transport[2], these compounds can be found in the local wildlife.

Seabirds can be considered good sentinels of environmental contamination, since they are able to show the pollution signal of a specific place and time, showing correspondence between the compounds concentrations observed in environmental matrixes and levels of some tissues[3]. Moreover, seabirds are conspicuous and occupy a high trophic position, enabling POPs biomagnification. However, migratory seabirds are difficult to indicate the origin of contamination. Eggs are considered a good matrix to evaluate POPs contamination in birds because they represent a noninvasive way of analysis, and reflect what was ingested by females before nesting, mainly during the fat accumulation period[4]. The eggs' contamination also can be related to stable isotope ratios or several elements to better understand relationships between exposure to pollutants and the trophic ecology of the animal. The stable-nitrogen isotope ratio ($^{15}N/^{14}N$; $\delta^{15}N$) has been widely used to delineate the trophic position of seabirds and the stable-carbon isotope ratio ($^{13}C/^{12}C$; $\delta^{13}C$) can help to identify feeding grounds, mainly migratory habits and latitudinal differences in feeding grounds[5].

Penguins are abundant birds in the Antarctic environment and the species *Pygoscelis antarctica* (Chinstrap penguin), *Pygoscelis papua* (Gentoo penguin) and *Pygoscelis adeliae* (Adelia penguin) have a circumpolar Antarctic distribution. However there are differences among each species location. Although the penguins' diet is composed mainly by krill and fishes, the proportion varies among each species. Since skuas (*Chataracta* spp.) stands as predatory species, they are territorialist and present a wider distribution. The species *C. maccormicki* (South polar skua) is a migratory seabird that breeds in Antarctic during the austral summer and migrate to the European continent in the austral winter[6].

The aim of the present work was to evaluate the presence of legacy and emerging Persistent Organic Pollutants in eggs of four seabird species (*P. antarctica, P. adeliae, P. papua and C. maccormicki*) from Admiralty's Bay, Antarctica, which show different trophic niches and migratory strategies. This effort includes the use of stable isotopic techniques in the overall ecologic characterization.

Material and methods

Sampling was carried out at Admiralty's Bay (62°10'S, 56°30'W), the largest bay of King George Island, with approximately 120 km^2 which is considered as an "Especially Managed Antarctic Area – EMAA" by the Madrid Protocol[7].

Concentrations of pesticides Hexachlorobenzene (HCB), Dichlorodiphenyltrichloroethane (DDT) and its metabolites (*o,p'*- DDD, *p,p'*-DDD, *o,p'*-DDE and *p,p'*-DDE), 22 congeners of Polychlorinated biphenyls (PCBs 28, 52, 95, 101, 105, 114, 118, 123, 132, 138, 149, 153, 156, 157, 167, 169, 170, 180, 183, 189, 194, 200), 14 congeners of Polybrominated diphenyl ethers (PBDEs 17, 28, 47,85, 99,100, 153, 154, 183, 184, 191, 196, 197 and 209) and isomers (*syn*- and *anti*-) and dechlorinated forms (-1Cl and -2Cl) of Dechlorane plus (DP) were analyzed. Each sample was spiked with labelled standards of BDE-209, DPs (syn- and anti-), DDTs (p,p'-DDE, o,p'-DDT, p,p'-DDT), PCBs (PCB-28, -52, -101, -153, -138, -180, -209), HCB and BDE-138.

A novel miniaturized analytical method optimized for the exhaustive extraction and simultaneous purification of environmentally relevant PCBs, PBDEs, DPs and organohalogenated pesticides in unhatched eggs of wild avian species was used[8].The extraction was based on a matrix solid-phase dispersion procedure. Dried samples were homogenized with acid silica. Then, they were packed on the top of columns with acid, neutral silica and sodium sulphate layers to simultaneously extract the dispersed sample and clean up the extract by column elution with hexane:dichloromethane (9:1). Final extracts were rotary evaporated until 1 mL, transferred to vials, and dried under a gentle nitrogen steam. Samples were reconstituted in a solution of TCN and $^{13}C_{12}$-BDE-139 in nonane. These compounds were used as internal standard for chromatographic analysis. The lipid content of each egg was determined gravimetrically. The quantification of DPs and related compounds as well as PBDEs was performed by high-resolution gas chromatography low-resolution mass spectrometry using a 6890N series gas chromatograph coupled with a Quadrupole Mass Spectrometer operated in selected ion monitoring mode with electron capture negative ionization (ECNI). HCB, PCBs and DDTs were determined using a 7890N gas chromatograph in selected ion monitoring (SIM) mode with electron impact (EI) ionization. DPs, HCB, PCBs and DDTs were quantified by the isotopic dilution technique. PBDEs were quantified related to $^{13}C_{12}$-BDE-139 and corrected based on labelled PBDE recoveries. Sample spike recoveries were satisfactory for all labelled compounds, ranging between 85.3-118%.

The carbon and nitrogen stable isotopes were determined at the Serveis Cientifico-Tecnics of the University of Barcelona (Barcelona, Spain). Lipid extraction of the dried powdered samples was conducted using a mixture of Chloroform/Methanol 2:1. Sample analysis was carried out following the procedure described in Roscales et al.[9]. Stable isotope ratios were determined by elemental analysis–isotope ratio mass spectrometry using a Thermo Finnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer via a CONFLOIII interface.

The statistical analyses were made using the software R version 3.1.0. Values under the quantification limit were set to zero. To test the hypothesis that species have different concentrations of HCB, PCB and DDT, we conducted generalized linear models using each pollutant as response variable. As DP and PBDE concentrations did not conform to distributions from exponential family, we used Kruskal-Wallis ANOVA. $\delta^{15}N$ and $\delta^{13}C$ show interspecies difference in their trophic niche.

Results and discussion

Total levels of all the studied families decreased according to *C. maccormicki* > *P. antarctica* > *P. adeliae* > *P. papua*, following the same order found by Cipro et al[10]. The most abundant compound found in *P. papua* and *P. adeliae* was HCB. Its persistence and long range atmospheric transport processes make it available in remote environments. ∑DDTs stood out in *P. antarctica* and ∑PCBs in *C. maccormicki* (Table 1). PBDE and DP levels were significantly higher in South polar skua than in penguins. This pattern is probably related to the migratory movements of skuas to the northern hemisphere during the non-breeding period. PBDEs and DPs are considered as emerging pollutants and higher levels have been reported in northern compared to southern hemisphere latitudes[11]. While PBDEs and DPs were largely detected above the limit of quantification (LOQ) in skuas, in the case of penguins PBDE and DP levels were mostly under the LOQ. These results suggest that skua's contamination by PBDEs and DPs mainly happens during the non-reproductive period when this species migrates to Europe. Moreover, our result suggest that the presence of DP in Antarctic food webs is negligible compared to legacy POPs.

p,p'-DDE/∑DDT ratios were 0.94, 0.93, 0.94 and 0.97 in *P. antarctica*, *P. adeliae*, *P. papua* and *C. maccormicki*, respectively. These ratios indicate a higher proportion of the metabolite DDE compared to DDT and DDD and do not suggest recent inputs of DDTs in Antarctic food webs. Generally, interspecies differences in egg pollutant levels were significant for all the contaminant families studied here ($p<0.05$). HCB levels in eggs differed significantly between skuas and *P. adeliae*, skuas and *P. papua*, *P. antarctica* and *P. adeliae* ($p<0.02$). In the case of PCBs, levels did not show significant differences, being found only between *P. antarctica* and *P. adeliae* (Figure 1). DDT concentrations were different between almost all species ($p<0.01$), with the exceptions of *P.adeliae vs. P.papua*. PBDE and DP concentrations showed significant differences only in relation to South Polar Skuas. When PBDEs are considered, only few congeneres were detected in penguins (four in *P.adeliae*, five in *P. antarctica* and two in *P.papua*) while all 14 studied congeners were identified in Skuas. Regarding PCBs, the contamination profile among the three penguin species was similar, with high contributions of less chlorinated biphenyls like the congeners 28 and 52. The presence of low-chlorinated PCBs may be related to the global fractionation that allows these congeners to reach easily polar regions compared to heavier compounds[12]. In Skuas, PCB 153 showed the highest contribution, followed by 180, which are hexa- and heptachlorinated PCBs, respectively.

Table1. Average concentrations (ng g−1 l.w.) ± confidence interval of 95 % (SD x 1.96) of the main compound groups present in the eggs of the species studied. LOQ stands for the limit of quantification.

	HCB	**∑DDTs**	**∑PCBs**	**∑PBDEs**	**∑DPs**
P. antarctica **(n=20)**	118.09±33.41 100%>LOQ	140.12±155.25 94%>LOQ	83.04±62.19 70%>LOQ	0.52±3.52 0.02%>LOQ	0.04±0.12 18%>LOQ
P. adeliae **(n=21)**	152.21±49.63 100%>LOQ	77.10±48.03 77%>LOQ	65.71±38.44 66%>LOQ	0.05±0.22 0.01%>LOQ	0.02±0.06 14%>LOQ
P. papua **(n=16)**	142.14±40.54 100%>LOQ	65.07±61.25 68%>LOQ	46.91±49.26 65%>LOQ	0.13±0.98 0.09%>LOQ	0.01±0.02 14%>LOQ
C. maccormicki **(n=16)**	133.43±113.5 100%>LOQ	1,344.75±2168.36 97%>LOQ	2,040.53±4265,97 100%>LOQ	66.33±128.43 58%>LOQ	0.34±1.13 53%>LOQ

Carbon stable isotopes ratios in Penguin eggs strongly overlapped among the studied species: *P. papua* (mean ± SD, −25.47±0.86‰), *P. antarctica* (-25.965±2.06‰) and *P. adeliae* (-24.715 ±1.38‰). Skuas had richer values of $\delta^{13}C$ (-21.60±1.45‰) than penguins (Figure 2). Differences in $\delta^{13}C$ between penguins and skuas can be related to the higher trophic position as well as larger feeding grounds of skuas and skuas' preys, which can forage on more northern grounds compared to penguins[13]. These results are in line with previous studies about South Polar Skua foraging behavior, which includes eggs and chicks of other bird species, coastal marine mollusks and penguin and seal carcasses.

$\delta^{15}N$ values also overlapped markedly among penguins' species: *P. papua* (10.4 ± 0.84‰), *P. antarctica* (12.0±3.13‰), *P. adeliae* (12.64± 3.19‰) (Figure 2). Previous studies reported an increase of about 3- 4‰ of $\delta^{15}N$ for each trophic level in marine food chains[14]. Since the main difference between penguins (*P. papua* and *P. adeliae*) was only 2.2%, it can be considered that there is no variation in their trophic level. However, *C. maccormicki* (15.9±3.97‰) had an enrichment from 3.22 to 5.5% in relation to penguins, which confirms that this species is at least one trophic level above Penguins (Figure 2).

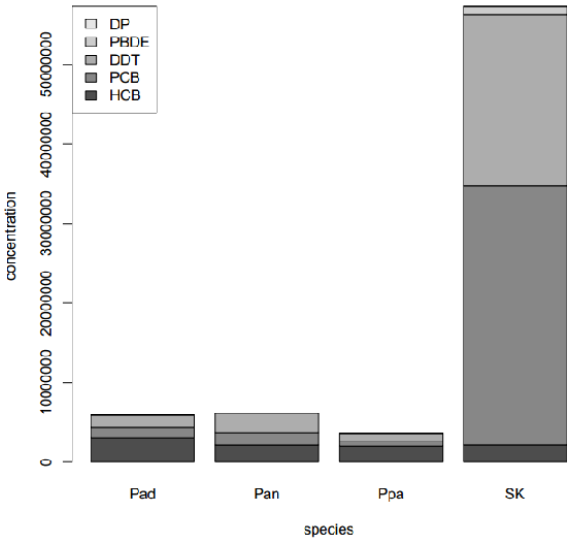

Figure 1 - Concentration of each group of compounds in relation to the total concentration of POPs (ng/g) l.w in the studied eggs. Pad, *P.adeliae*; Pan, *P.antarctica*; Ppa, *P.papua*; SK, *C.maccormicki*.

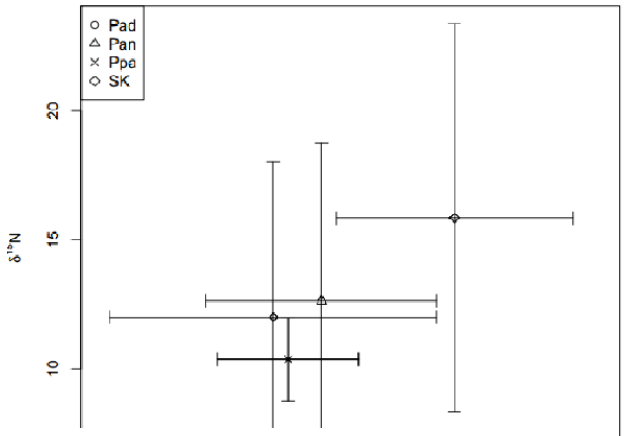

Figure 2 - $\delta^{13}C$ and $\delta^{15}N$ values (‰) in penguins and skua eggs collected at King George Island, Antarctic during the periods 2010–2011 and 2011-2012.

Acknowledgements

This study is part of the project ‘‘Estudos bioecológicos em Pingüins (***Pygoscelis antarctica***, ***P. papua*** e ***P. adeliae***) e Skuas (***Catharacta maccormicki*** e ***C. lonnbergii***): determinação de micropoluentes e níveis de estresse através de métodos de amostragem não invasivos” sponsored by CNPq/MCT 557049/2009-1. The authors are indebted to the Brazilian Navy that provided the Antarctic logistic through the ‘‘Secretaria da Comissão Interministerial para os Recursos do Mar’’ (SECIRM). Chemical and stable isotopes analysis were carried out in a partnership with researches from Dept. of Instrumental Analysis and Environmental Chemistry of the General Organic Chemical Institute of CSIC – Spain.

References

1.Corsolini S, Covaci A., Ademollo N, Focardi S, Schepens P. (2006); *Environ Poll*. 140:371-82.
2. Bargagli R. (2008); *Sci Total Environ.* 400 (1-3): 212–26.
3. Beeby A. (2001); *Environ Poll.* 112(2): 285–98.
4. Cifuentes JM, Becker PH, Sommer U, Pacheco P, Schlatter R. (2003); *Environ Poll.* 126 (1): 123-27
5. Roscales JL, Gómez-Díaz E, Verónica N, González-Sólis J. (2011); *Mar Ecol Prog Ser* 434:1–13
6. Kopp M, Peter, HU, Mustafa O, Lisovski S, Ritz MS, Phillips RA, Hahn S. (2011); *Mar Ecol-Prog Ser.* 435:263-67
7.Unep. (2002); *Fondo para el Medio Ambiente Mundial*
8. Vicente A, Roscales, JL, Jiménez B, Ramos L. (2014); *Submitted to Dioxin 2014*.
9. Roscales JL, Muñoz-Arnanz J, González-Solís JG, Jiménez B. (2010); *Environ Sci Technol*. 44: 2328–34
10. Cipro CVZ, Taniguchi S, Montone RC. (2010); *Chemosphere.* 78 (6): 767–71.
11. Amap. (2002); *Arctic Monit Assess Prog.* 112p.
12. Wania F. (2003); *Environ Sci Technol.* 37(7): 1344–51
13. Hobson KA, Piatti JF, Pitocchelli J. (1994); *J Anim Ecol.* 63: 786–98
14. Kelly JF. (2000); *Can J Zoolog* 78: 1–27