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## Persistent organic pollutants and porphyrins biomarkers in penguin faeces from Kopaitic Island and Antarctic Peninsula

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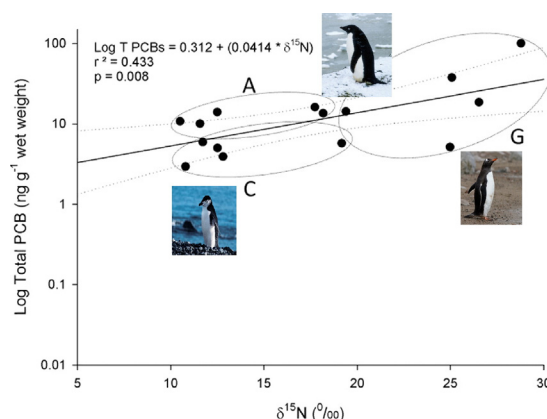
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### HIGHLIGHTS

- PCB concentrations in Antarctic penguin faeces follow trend Gentoo > Adelie > Chinstrap.
- Low chlorinated pattern dominated PCBs found in faeces samples.
- PCBs and stable isotope signal ( $\delta^{15}\text{N}$ ) relation increased with species trophic level
- Total porphyrin levels correlate well with total PCB contents in faeces.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Polychlorinated biphenyl (PCB) levels were determined in the faeces of three Antarctic Peninsula penguin species to assess viability as a non-invasive approach for sampling PCBs in Antarctic biota. These determinations were complemented with stable isotope and porphyrins assessments, and together this methodology determined the role of diet and metabolic disruption in penguins. Up to 60% of the collected faecal samples evidenced low molecular weight PCBs, of which, the more volatile compounds were predominant, in agreement with previous results. The highest PCB levels were reported in the gentoo penguin (*Pygoscelis papua*; 35.3 ng g<sup>-1</sup> wet weight average), followed by the chinstrap (*Pygoscelis antarctica*; 6.4 ng g<sup>-1</sup> wet weight average) and Adélie penguins (*Pygoscelis adeliae*; 12.9 ng g<sup>-1</sup> wet weight average). Stable isotope analyses ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) demonstrated that gentoo feeding and foraging habits differed from those of Adélie and chinstrap penguins. A strong positive correlation was found between PCB concentrations and  $\delta^{15}\text{N}$ , indicating the role of diet on the observed pollutant

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Faeces  
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levels. Porphyrins metabolite levels were also directly correlated with PCB concentrations. These results suggest that PCB levels impair the health of Antarctic penguins.

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## 1. Introduction

Persistent organic pollutants (POPs), substances of anthropogenic origin, are resistant to photolytic, chemical, and biological degradation and are thus extremely persistent in the environment. The high lipid solubility of these contaminants partially explains POP bioaccumulation in the fatty tissues of organisms (Muir et al., 1988). In a model for explaining global POP distributions, Wania and Mackay (1996) proposed a global fractionation and cold trapping hypothesis that predicts increased POP concentrations in colder environments, such as the Arctic. Supporting this, numerous studies report the occurrence of POPs in the environmental matrices of polar environments, highlighting the global distribution of these compounds in the air, seawater, and ice (Galbán-Malagón et al., 2013a, 2013b, 2013c; Cabrerizo et al., 2012).

While various reports document the wide distribution of POPs in Arctic and Antarctic biota, most are centred on the Northern hemisphere. Top predators from both hemispheres accumulate these compounds due to trophic transfer (Corsolini et al., 2002b, 2003, 2006). However, there is relatively little information available regarding contaminant distribution in Antarctic, as compared to Arctic, food webs, and available understandings on the potential risks of Antarctic contaminant distribution are limited.

Polychlorinated biphenyls (PCBs) and organochlorine pesticides, two types of POPs, have been reported in various Antarctic organisms. For example, a recent study reported increased PCB concentrations in species at lower trophic levels, in addition to commenting on the implication of this situation on the biogeochemistry of POPs (Galbán-Malagón et al., 2013c). Previous studies have also recorded POPs in upper trophic levels, such as in penguins, krill, seals, and whales (Corsolini et al., 2002a, 2002b, 2003, 2006, 2007, 2009, and 2011; Bengtson Nash et al., 2008; Taniguchi et al., 2009). Furthermore, Larsson et al. (1992) monitored atmosphere and marine organisms (i.e. fish and zooplankton) from the Ross Island for two years, finding compounds such as PCBs, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene, and lindane. Similarly, the adipose tissue of gentoo penguins displays high hexachlorobenzene levels (i.e.  $1.12 \text{ mg g}^{-1}$ ) (Inomata et al., 1996). The faecal depositions of this species, collected from the Ardley Peninsula of King George Island, also present DDT, hexachlorobenzenes, and the metabolites of these two compounds (Sun et al., 2006).

Prior analysis of the Antarctic ecosystem revealed a biomagnification and bioaccumulation of various POPs, such as PCBs, DDT, and hexachlorobenzene, in food webs that included penguins (Cipro et al., 2010). Therefore, penguins, as with other seabirds, are potentially exposed to high POP concentrations due to high trophic positioning (Hop et al., 2002). Furthermore, field studies suggest that penguin colonies are secondary POP sources and play an important role in local POP redistribution (Park et al., 2010; Cabrerizo et al., 2012; Huang et al., 2014).

Penguins are widely distributed across the Antarctic continent, making them a suitable species for studying POP bioaccumulation and trophic transfer. Of the Antarctic penguins, the *Pygoscelis* genus represents  $\approx 70\%$  of Antarctic avian biomass (Tierney et al., 2009; Biuw et al., 2010) and has varied feeding and migration patterns (Williams, 1990; Robinson and Hindell, 1996; Clarke et al., 2003; Lynnes et al., 2004; Trivelpiece et al., 2007). The Antarctic Peninsula is home to three *Pygoscelis* species, the Adélie penguin (*Pygoscelis adeliae*), chinstrap penguin (*Pygoscelis antarctica*), and gentoo penguin (*Pygoscelis papua*) (Williams, 1990; Clarke et al., 2003; Trivelpiece et al., 2007). Various halogenated contaminants have been detected in

these Antarctic *Pygoscelis* penguins (Inomata et al., 1996; Sun et al., 2006).

Stable isotopes are a useful tool for predicting and tracking anthropogenic contaminant transfer through food webs (Fry, 2006; Michener and Kaufman, 2007). In particular, the stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes have been used to establish trophic relationships in northern polar aquatic food webs (Hobson and Welch, 1992; Hobson et al., 1995), as well as to demonstrate relationships between POP concentrations and trophic position (Kidd et al., 1998; Fisk et al., 2001, 2003). The gradual enrichment of  $\delta^{15}\text{N}$  between prey and consumers is relatively predictable, and enrichment values of 2.4‰ for seabirds and 3.8‰ for an Arctic marine food web have been reported (Hobson et al., 1995; Fisk et al., 2001). Likewise, the  $\delta^{13}\text{C}$  signature only changes  $\approx 1\%$  from primary producers to primary consumers (Hobson and Welch, 1992), thus facilitating the identification of primary productivity sources (e.g. particulate organic matter vs. ice algae). Additionally, distinctions exist between onshore and offshore food webs, with the later generally presenting a more depleted  $\delta^{13}\text{C}$  signature (Hobson et al., 1995, 2002).

The exposure of Antarctic organisms to POPs can also be assessed using porphyrins, the intermediate metabolites of heme biosynthesis. Existing evidence supports that the heme biosynthesis pathway may be altered by environmental contaminants, including PCBs, hexachlorobenzene, lindane, polychlorinated dioxins, and heavy metals, leading to changes in porphyrin concentration through variations in accumulation or excretion (Marks, 1985; Casini et al., 2003; Jerez et al., 2011; Celis et al., 2012; Nicol and Foster, 2003; Vanbirgelen et al., 1996). Considering the conservation status of Antarctic biota, and of penguins in particular, excreta samples may be a valid alternative for assessing total porphyrins concentrations. This is due first to faeces sampling being a non-destructive approach that could bypass traditionally used lethal and invasive sampling procedures for assessing tissue contaminant levels. Additionally, faeces from a nest would reflect the food sources for chick(s) and of mating penguins foraging in the vicinity of breeding colonies.

The objectives of this study were to investigate the influence of trophic position and foraging ground on contaminant levels using stable isotope analyses ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) in three penguin species (gentoo, chinstrap, and Adélie); and to determine PCB exposure through porphyrins levels in faeces, a possible non-invasive sampling approach.

## 2. Methods

### 2.1. Study area

All samples were obtained from two sites, (S1) near the General Bernardo O'Higgins Chilean Military Base on the Antarctic Peninsula and (S2) near Kopaitic Island. The first site was home to a gentoo penguin colony and was located in a field adjacent to the O'Higgins Base on the Schmidt Peninsula ( $63^\circ 19'15'' \text{ S}$ ;  $57^\circ 53'59'' \text{ W}$ ). The other site was home to chinstrap and Adélie penguin nesting colonies and was located half a kilometre northeast of the O'Higgins Base on Kopaitic Island ( $63^\circ 18'51'' \text{ S}$ ;  $57^\circ 54'18'' \text{ W}$ ; see Supporting Information Fig. S1 and Table S1).

### 2.2. Collection of samples

Sampling was performed during the austral summer in January 2009 at penguin colonies near the O'Higgins Antarctic Base (see Supporting

Information Fig. S1). Samples were taken with the utmost care to avoid undo stress to the penguin colonies and nesting chicks. Fresh stool samples were collected from five nests for each penguin species (see details in Supporting Table S1). A metal blade was used to collect faeces as close to the nest as possible, choosing the freshest available faeces. Samples were individually stored in aluminium foil, labelled, placed in airtight bags, and stored at  $-20^{\circ}\text{C}$  until processing in the laboratory. A subsample was taken for porphyrin and was separately stored in another piece of aluminium foil at  $-80^{\circ}\text{C}$  until analysis.

### 2.3. Chemical extraction and clean-up

Polychlorinated biphenyls were extracted from faecal samples following methodologies developed for other tissues (Berdie and Grimalt, 1998; Vives et al., 2004; Montory et al., 2011). Briefly, the samples were thawed overnight at room temperature, and  $\approx 6\text{ g}$  was placed in a 50 mL glass centrifuge tube with a Teflon lid. As a recovery standard, 5 ng of pentachloronitrobenzene were added 30 min prior to extraction. PCBs were extracted by adding 4 mL of n-hexane:acetone (1:1), placing the samples in an ultrasound bath with water/ice for 15 min to prevent compound evaporation during extraction, and centrifuging the mixture for 5 min at 1500 rpm. The organic fraction was placed in a 100 mL flask, and the aqueous fraction was extracted in duplicate using the same process, followed by organic phase pooling. Later, the extract was treated overnight with 1 mL of concentrated  $\text{H}_2\text{SO}_4$ , and the mixture was then stirred and centrifuged for 5 min at 1500 rpm. The acid phase was removed, and this step was repeated until the organic phase was translucent. The supernatant was transferred to a new 100 mL flask. The obtained extract was passed through a packed column consisting of 1 g of  $\text{Na}_2\text{SO}_4$  and 6 g of activated florisil, which was eluted with 90 mL of n-hexane. The collected purified extract was preconcentrated through rotary evaporation and transferred to a 1.5 mL vial. This was brought almost to dryness under a nitrogen stream and was completed with isooctane to obtain a volume of 150  $\mu\text{L}$ .

### 2.4. Chemical analysis

Prior to chemical analysis, 5 ng of PCB 142 was added to samples as an internal standard to correct instrumental variability (Montory et al., 2010; Montory et al., 2011). Compound identification and quantification were performed using gas chromatography with a  $\mu$  electron capture detector (Autosystem Gas Chromatograph, 9000 series, PerkinElmer, Inc., Waltham, MA, USA) and a PTE-5 capillary column (60 m  $\times$  0.25 mm internal diameter and 0.25  $\mu\text{m}$  film thickness). The calibration curve was prepared using a CLB1 mixture containing the target compounds (see below) obtained from the National Research Council of Canada, which totaled to 51 PCB congeners. A PTE-5 capillary column (60 m  $\times$  0.25 mm internal diameter and 0.25  $\mu\text{m}$  film thickness) with helium as a carrier gas, an automatic injection mode (10  $\mu\text{L}$ ), an injector temperature of  $240^{\circ}\text{C}$ , and a detector temperature of  $360^{\circ}\text{C}$  was used. The target PCBs were classified according to the International Union of Pure and Applied Chemistry by the number and position of chlorine substitutions: Tri, 18, 31; Tetra, 44, 52, 77; Penta, 87, 101, 103, 105, 114, 118, 121; Hexa, 128, 129, 137, 138, 141, 151, 153, 154, 179, 156, 159; Hepta, 170, 171, 180, 182, 183, 185, 187, 189, 191; Octa, 195, 203, 180, 205; and Nona, 207.

### 2.5. Quality assurance and control

All glass materials used during extraction were cleaned prior to use by rinsing in triplicate with acetone, followed by an 8 h combustion process at  $450^{\circ}\text{C}$ . All metal materials were cleaned with deionized water followed by rinsing in triplicate with acetone. Then, all cleaned materials were wrapped in aluminium foil until use in sampling or laboratory processing. One blank sample was used for every five samples. Blank concentrations ranged from 9 to 33  $\text{pg g}^{-1}$  for the detected  $\Sigma\text{PCBs}$ .

Concentrations reported in the blanks were subtracted from each sample array. Average values for PCBs ranged from 4.4–10.2% for each PCB. Limits of detection and quantification were calculated from blank samples as the mean plus 3-fold and plus 10-fold the standard deviation, respectively. Obtained limits of detection and quantification ranged from 9.22 to 13.5  $\text{pg g}^{-1}$  and from 11.13 to 13.5  $\text{pg g}^{-1}$ , respectively, for individual PCBs. Extraction recoveries were evaluated as a percentage of pentachloronitrobenzene; the obtained values ranged between 56 and 84%, indicating that samples were corrected for recovery. In parallel, blank samples were run with n-hexane and 5 ng of pentachloronitrobenzene following the same extraction process, with a result of 100% extraction. More information about quality assurance and quality control protocols can be found in Berdie and Grimalt (1998); Vives et al. (2004), and Montory et al. (2010, 2011).

### 2.6. Porphyrins extraction and fluorimetric determination

Porphyrins extractions were conducted following the methodology of Lockwood et al. (1985). Briefly, a subsample of homogenized lyophilized sample (0.1 g) was placed in a graduated centrifuge tube with 1 mL of 5 N HCl and vortex-mixed. Diethyl ether (3 mL) was added; the extract was thoroughly mixed to obtain an emulsion; 3 mL of water was added, and the extract was further mixed. The mixture was then centrifuged for 10 min at 200 rpm. The lower phase containing porphyrins metabolites was used for fluorimetric determinations. Porphyrins were quantitatively established following Grandchamp et al. (1980). Briefly, this procedure is based on the different excitation/emission wavelengths for each porphyrin, where uroporphyrin: 405–595 nm; coproporphyrin: 400–595 nm; and protoporphyrin: 410–605 nm. Aqueous samples were placed in a micro-cuvette and measured using a Perkin Elmer Model LS50 spectrofluorometer. Porphyrin standards (i.e. uroporphyrin III octamethyl ester, coproporphyrin III dihydrochloride, and protoporphyrin IX dimethyl ester) were obtained from Porphyrin Products Inc. (Logan, UT, USA) and used to construct calibration curves for each porphyrin in the mixture.

### 2.7. Stable isotopes analyses

Penguin faeces samples for each species were analysed for stable nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotopes at the Environmental Isotope Laboratory, University of Waterloo, Canada. Dried samples were ground, and the aliquots (0.25–0.30 mg) were weighed in tin cups and analysed using a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (ThermoFinnigan, Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108, Milan, Italy).

### 2.8. Statistical analysis

To assess differences in concentrations between penguin species, a non-parametric Kruskal-Wallis test followed by a *post-hoc* Tukey test was performed. Linear regression analyses were used to determine relationships of porphyrins with PCB and stable isotope concentrations. All statistical analyses were performed using the STATA v11.1 statistical package (Stata Corp Ltd., College Station, TX, USA).

## 3. Results

### 3.1. PCBs occurrence

Chinstrap penguins showed the lowest PCB concentrations ( $p < 0.05$ ,  $4.7 \pm 1.2\text{ ng g}^{-1}$  wet weight [w.w.]), as compared to Adélie ( $12.93 \pm 2.5\text{ ng g}^{-1}$  w.w.) and gentoo penguins ( $35.52 \pm 38.45\text{ ng g}^{-1}$  w.w.; see concentration summaries in Supporting Information Table S2). Gentoo penguins also displayed the greatest variability of PCBs, a result obtained due to concentrations in one sample ( $5.15\text{ ng g}^{-1}$  w.w.). The



congener pattern in all three species was highly similar (Fig. 1), with a predominance of low molecular weight congeners. For example, congeners with  $\leq 5$  Cl accounted for 60% of the total quantified PCBs across species. The detailed concentrations for each PCB congener are provided in Supporting Information Table S2.

### 3.2. Porphyrins concentrations

Porphyrins concentrations were significantly different between the assessed penguin species ( $p \leq 0.05$ ). Uro, copro, proto, and total porphyrins were consistently higher in the Adélie and gentoo penguin colonies as compared to the chinstrap penguin colony (see Supporting Information Table S2). Protoporphyrins accounted for the majority of porphyrins (55–66%,  $p < 0.05$ ), followed by less abundant coproporphyrin and uroporphyrin concentrations (26–31% and 11–15%, respectively).

### 3.3. Stable isotopes

Stable isotope analyses of  $\delta^{15}\text{N}$  indicated average differences of  $>9\%$  between gentoo and chinstrap penguins, of  $\approx 8\%$  between gentoo and Adélie penguins, and of  $<2\%$  between Adélie and chinstrap penguins. From this, a significant increase in the  $\delta^{15}\text{N}$  isotopic signal was obtained in gentoo penguins ( $p < 0.05$ ). Notably, the wide range of different trophic levels consumed by each penguin species were reflected by the  $\delta^{15}\text{N}$  signatures, with  $\delta^{15}\text{N}$  signatures of 19–29, 10–18, and 10–19 respectively obtained for the gentoo, Adélie, and chinstrap penguin colonies (Fig. 2). Supporting this finding, the isotopic signal of  $\delta^{13}\text{C}$  was very narrow ( $<2\%$ ) between Adélie and chinstrap penguins, suggesting similar foraging habits (Fig. 2).

## 4. Discussion

Most of the marker PCBs established by the International Council for the Exploration of the Sea (i.e. PCBs 52, 101, 118, 138, 153, and 180) were quantified in all studied penguin populations and were present in almost all analysed samples. The only exception was PCB-138, which was only quantified in gentoo penguin faeces. Gentoo penguins also had the highest number of PCB congeners as well as the highest total PCB concentrations per sampled individual, which was roughly 3- to 5-fold higher than in other species.

Previously reported  $\Sigma$  PCB levels in Adélie ( $9.8 \pm 3.8 \text{ ng g}^{-1} \text{ w.w.}$ , ranging from 4.9 to  $17 \text{ ng g}^{-1} \text{ w.w.}$ ) and chinstrap penguins ( $4.5 \pm 2.4 \text{ ng g}^{-1} \text{ w.w.}$ , ranging from 1.7 to  $9.5 \text{ ng g}^{-1} \text{ w.w.}$ ) were similar to the currently presented values (Corsolini et al., 2007). Once again, the only exception was in gentoo penguins, where the  $\Sigma$  PCB levels of the present study were up to 10-fold higher than values ( $3.5 \pm 1.6 \text{ ng g}^{-1} \text{ w.w.}$ ) reported by Corsolini et al. (2007). However, it is worth highlighting that similar profiles were found.

Notably, the currently obtained profiles concur with previous reports on the influence of the biological pump in surface waters and on the implications of this pump in PCB biomagnification through the food web. In particular, PCB cycling studies in the Antarctic Ocean report that atmospheric PCBs reach surface waters via the biological pump (Galbán-Malagón et al., 2013a and b). This pump efficiently exports highly hydrophobic PCBs to deeper waters, and therefore, the transfer of these PCBs to higher trophic levels is likely diminished, as found in other areas (Nizzetto et al., 2012). Furthermore, the presently obtained patterns are in line with those found in Antarctic benthic and pelagic species (Corsolini et al., 2003; Goutte et al., 2013).

In turn, a positive relationship was observed between total PCB levels and the respective  $\delta^{15}\text{N}$  signals ( $r^2 = 0.43$ ,  $p < 0.05$ ), with the highest total PCB concentrations in gentoo penguins, followed by Adélie and chinstrap penguins (Fig. 3A, Supporting Information Table S2). When analysing data using the number of Cl atom substitutions, a positive relationship with  $\delta^{15}\text{N}$  was found for all PCBs ( $p < 0.05$ ) except Nona-CB (Supporting Information Fig. S2). This finding could be related

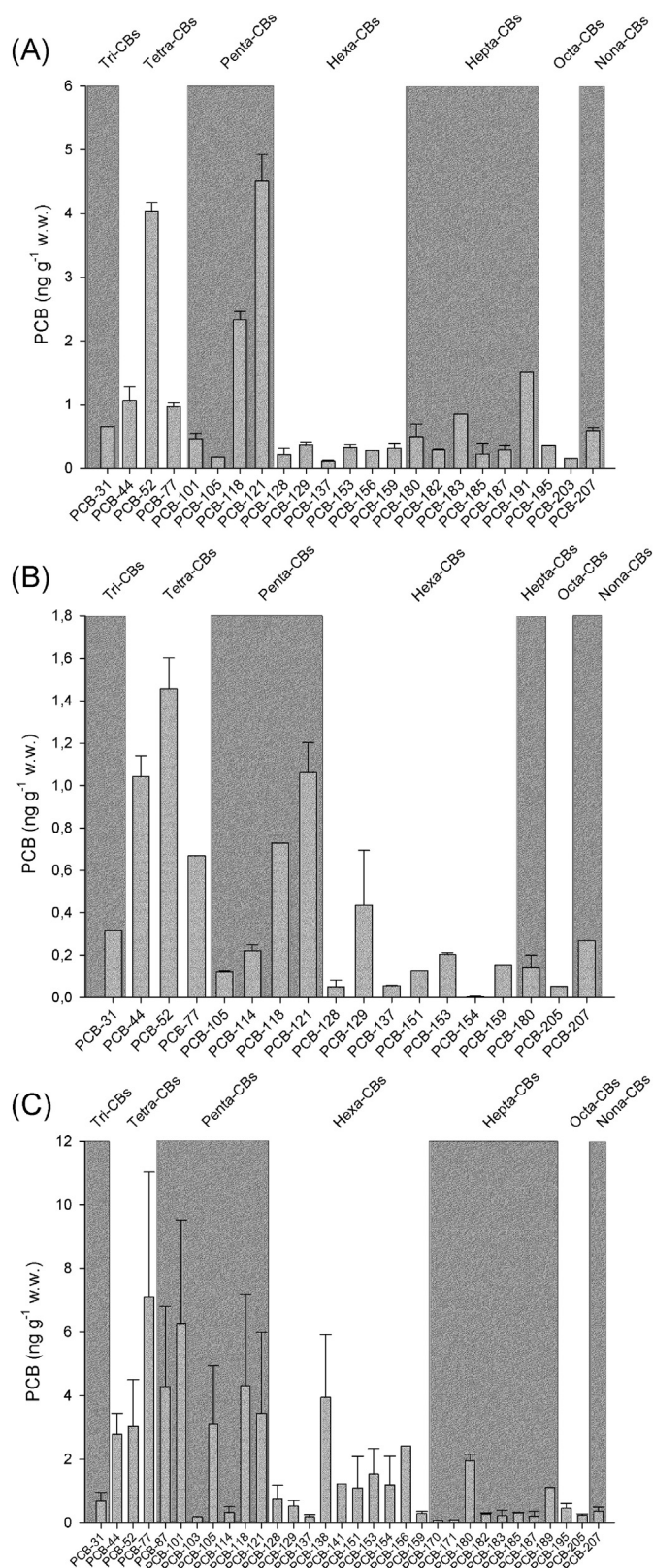
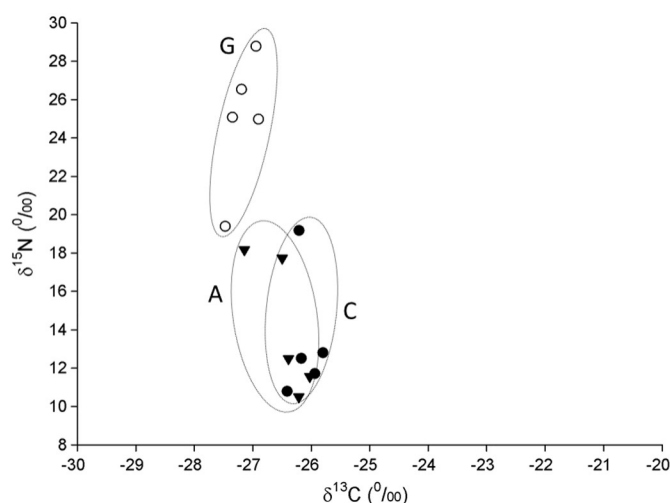


Fig. 1. PCB concentration profiles found in penguin faeces (Mean  $\pm$  Standard Error) from (A) Adélie, (B) chinstrap, and (C) gentoo penguins.

to the longer equilibration times required by the more hydrophobic PCBs, which have lower assimilation efficiencies, as demonstrated in other wildlife species (Moser and MacLachlan, 2001, Kelly et al., 2004).



**Fig. 2.** Stable isotopes ( $\delta^{13}\text{C}\text{‰}/\delta^{15}\text{N}\text{‰}$ ) in gentoo (G), Adélie (A), and chinstrap (C) penguins faeces.

The results for faecal PCB contents and stable isotopes displayed differences attributable to food web position (Fig. 2) and varied carbon sources. For example, the gentoo penguin faeces sample with the lowest

PCBs concentration (i.e.  $5.15 \text{ ng g}^{-1} \text{ w.w.}$ ) exhibited a similar trophic position to that of Adélie and chinstrap penguins. Penguin diet studies have established that the Adélie penguin diet is primarily krill-based (Lynnes et al., 2004), although occasionally fish and zooplankton may be incorporated (Tierney et al., 2009). In turn, the chinstrap penguin diet is primarily krill-based (Lynnes et al., 2004). The Adélie and chinstrap penguins chiefly forage within the upper water column, which concurs with the presently obtained findings for  $\delta^{13}\text{C}$  isotopic signals. In contrast, gentoo penguins have a more varied diet that largely consists of fish and fewer krill and squid. Therefore, gentoo penguins forage at higher trophic levels from deeper waters, which is in line with the obtained findings for the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic signals (Robinson and Hindell, 1996).

Finally, porphyrins levels were very high and, despite interspecific differences, the presently recorded faecal levels were various orders of magnitude higher than those in other seabirds along the central Chilean coast (Casini et al., 2001). There is evidence that halogenated organic compounds, such as dioxins and PCBs, cause changes in progressive uroporphyrin accumulation (Miranda et al., 1992). Furthermore, herbicide contamination promotes protoporphyrin accumulation (Leonzio et al., 1995), while heptachlor, lindane, arsenic, and mercury exposure results in coproporphyrin accumulation (Taira and San Martín De Viale, 1980; Woods et al., 1991; Bowers et al., 1992; Ng et al., 2002). Consequently, the present results suggest that penguins are, in fact, exposed to POPs. A strong positive relationship was found ( $r^2 = 0.73$ ,  $p < 0.05$ ; Fig. 3B) between total PCB concentrations and total porphyrins concentrations. This relationship was also significant when correlations were performed for each penguin species ( $p < 0.05$ ). The higher PCB levels in Adélie and gentoo penguins may be due to a higher relative trophic position than chinstrap penguins (Lynnes et al., 2004), and the carbon source (i.e.  $\delta^{13}\text{C}$  signature) suggests an offshore diet related to these contaminants, as similarly reported in other marine polar/subpolar systems (Hobson et al., 1995, 2002).

## 5. Conclusions

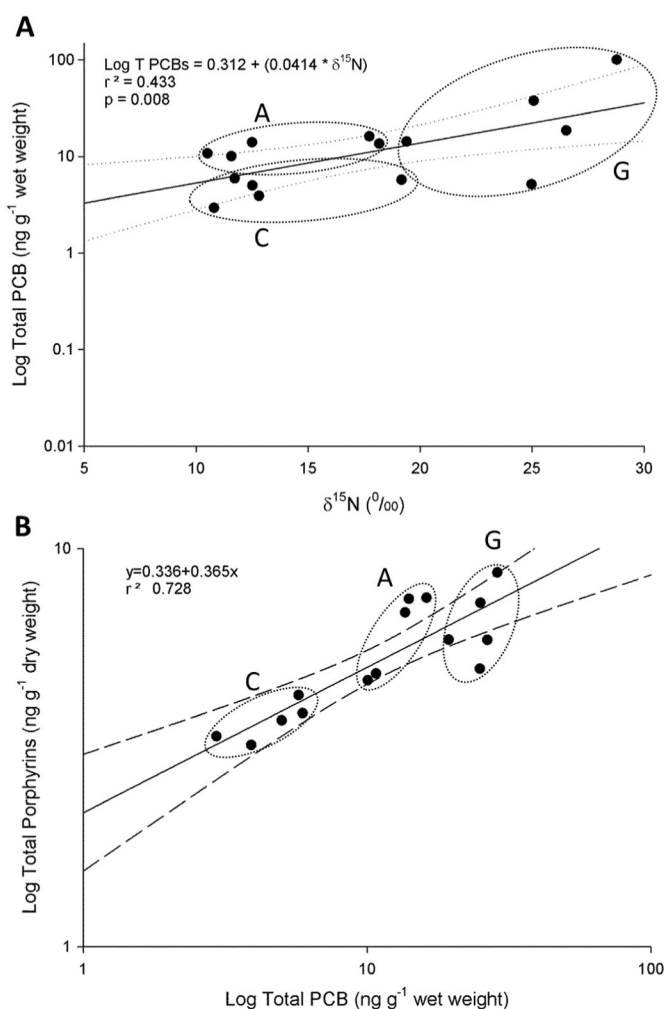
Penguin faeces sampling is a useful, non-invasive strategy for studying penguin exposure to POPs. The present results support the alignment of PCB patterns with the biological pump as a biogeochemical driver for POPs. Due to porphyrins production, PCBs could be linked to metabolic impairment in Antarctic penguins. This concurs with studies conducted on penguin blood that indicate a correlation between haematological parameters and the sum of PCBs in chinstrap penguins from the same area as that currently assessed (Jara-Carrasco et al., 2015). Taken together, these findings strongly indicate that penguins from pristine environments, such as Antarctica, are subjected to chemical exposure stress. However, better comprehensions of the toxicokinetic pathways for these pollutants are needed to propose clearer conclusions regarding potential impacts on penguin population health. Furthermore, additional studies are needed to assess the effects of exposure to other complex chemical mixtures, not only POPs.

## Abbreviations

DDT	dichlorodiphenyltrichloroethane
PCB	polychlorinated biphenyl
POPs	persistent organic pollutants
w.w.	wet weight

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**Fig. 3.** (A) Relationship between the total PCB concentrations and  $\delta^{15}\text{N}\text{‰}$  in gentoo (G), Adélie (A), and chinstrap (C) penguins faeces. (B) Correlation between total porphyrins and PCBs in gentoo (G), Adélie (A), and chinstrap (C) penguin faeces.



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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.07.091>.

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