

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/305522640>

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

Article in Science of The Total Environment · July 2016

DOI: 10.1016/j.scitotenv.2016.07.091

CITATIONS

0

READS

231

10 authors, including:



Ignacio Rudolph

6 PUBLICATIONS 85 CITATIONS

[SEE PROFILE](#)



Gustavo Chiang

Fundacion MERI (Melimoyu Ecosystem Resea...

23 PUBLICATIONS 136 CITATIONS

[SEE PROFILE](#)



Cristóbal Galbán-Malagón

Universidad Andrés Bello

25 PUBLICATIONS 269 CITATIONS

[SEE PROFILE](#)



Miguel Martinez

Universidad de La Salle

4 PUBLICATIONS 2 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



The first GEF/UNEP international water and science conference [View project](#)



Exposición a contaminantes y su relación con biomarcadores de salud en dos núcleos de alta densidad de cóndor andino (*Vultur gryphus*) en Chile. (PhD thesis: Melanie Duclos) [View project](#)



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

Ignacio Rudolph^a, Gustavo Chiang^{b,c}, Cristóbal Galbán-Malagón^d, Rafael Mendoza^e, Miguel Martínez^f, Carlos Gonzalez^f, José Becerra^g, Mark R. Servos^h, Kelly R. Munkittrick^b, Ricardo Barra^{a,*}

^a Aquatic Systems Department, Faculty of Environmental Sciences and EULA-Chile Centre, Universidad de Concepción, Concepción 4070386, Chile

^b Canadian Rivers Institute, University of New Brunswick, St. John, New Brunswick E2L 4L5, Canada

^c Fundación MERI, Santiago 7650720, Chile

^d Department of Ecology and Biodiversity, Faculty of Ecology and Natural Resources, Universidad Andres Bello, Santiago 8370371, Chile

^e Instituto de Investigación Pesquera, Dpto. de Estudios Ambientales, Talcahuano 4260000, Chile

^f Departamento de Microbiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción 4070386, Chile

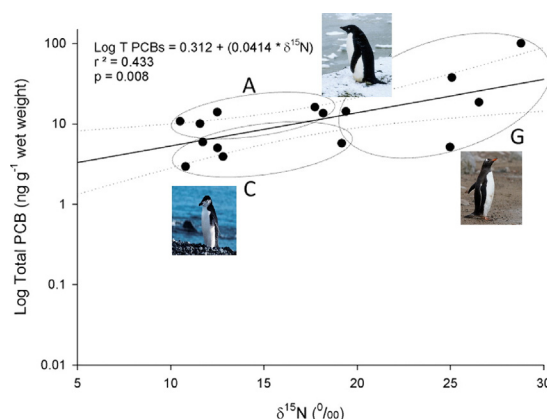
^g Departamento de Botánica, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción 4070386, Chile

^h Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

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



ARTICLE INFO

Article history:

Received 8 April 2016

Received in revised form 13 July 2016

Accepted 13 July 2016

Available online xxxx

Editor: D. Barcelo

Keywords:

PCB

Penguin

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⁻¹ wet weight average), followed by the chinstrap (*Pygoscelis antarctica*; 6.4 ng g⁻¹ wet weight average) and Adélie penguins (*Pygoscelis adeliae*; 12.9 ng g⁻¹ 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

* Corresponding author.

E-mail address: ricbarra@udec.cl (R. Barra).

Faeces
Stable isotope
Porphyrin
Antarctica

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

© 2016 Elsevier B.V. All rights reserved.

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 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°C until processing in the laboratory. A subsample was taken for porphyrin and was separately stored in another piece of aluminium foil at -80°C until analysis.

2.3. Chemical extraction and clean-up

Polychlorinated biphenyls were extracted from faecal samples following methodologies developed for other tissues (Berdié 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 H_2SO_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 Na_2SO_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 μL .

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 μ 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 μm 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 μm film thickness) with helium as a carrier gas, an automatic injection mode (10 μL), an injector temperature of 240°C , and a detector temperature of 360°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°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 pg g^{-1} for the detected ΣPCBs .

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 pg g^{-1} and from 11.13 to 13.5 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 Berdié 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 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 ≤ 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 Σ 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 Σ 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

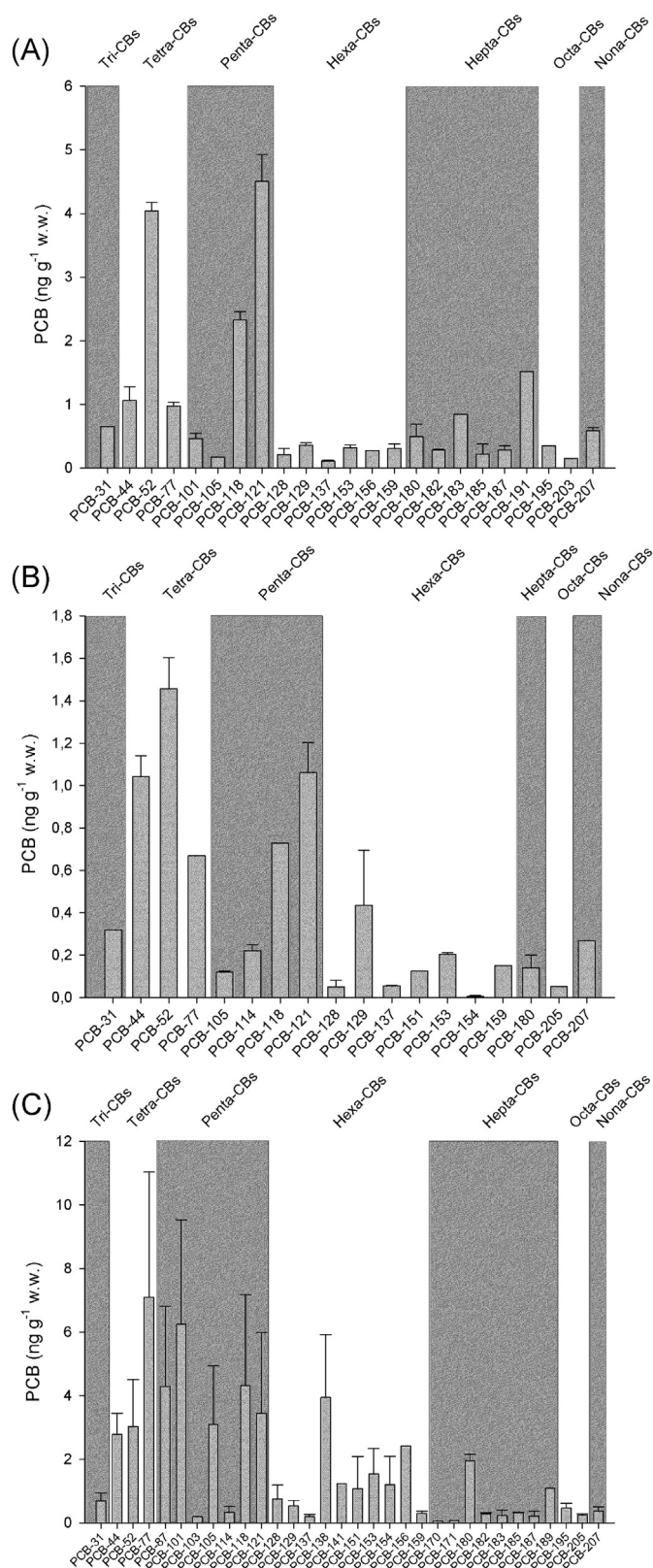


Fig. 1. PCB concentration profiles found in penguin faeces (Mean $\pm$ Standard Error) from (A) Adélie, (B) chinstrap, and (C) gentoo penguins.

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).

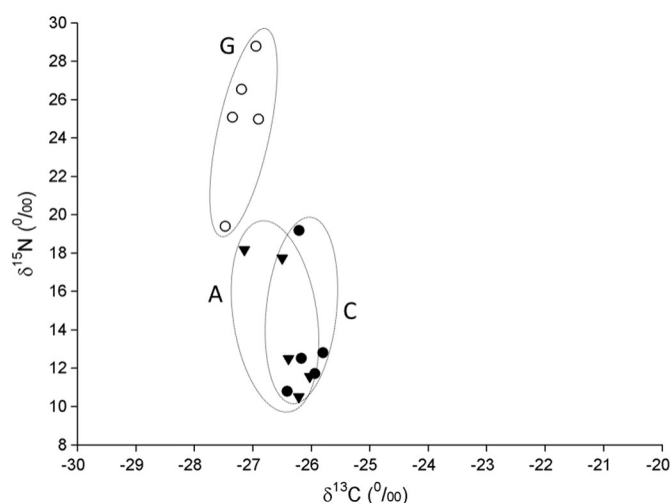


Fig. 2. Stable isotopes ($\delta^{13}\text{C}\text{‰}/\delta^{15}\text{N}\text{‰}$) in gentoo (G), Adélie (A), and chinstrap (C) penguins faeces.

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

Funding

This work was funded by INACH [grant number T18-09, to R. Barra]; the Research Exchange Scholarship from the Embassy of Chile in Italy, to G. Chiang; and FONDECYT [grant number 1140466, to R. Barra]. R Barra thanks also the support of CONICYT FONDAP CRHIAM 15130015.

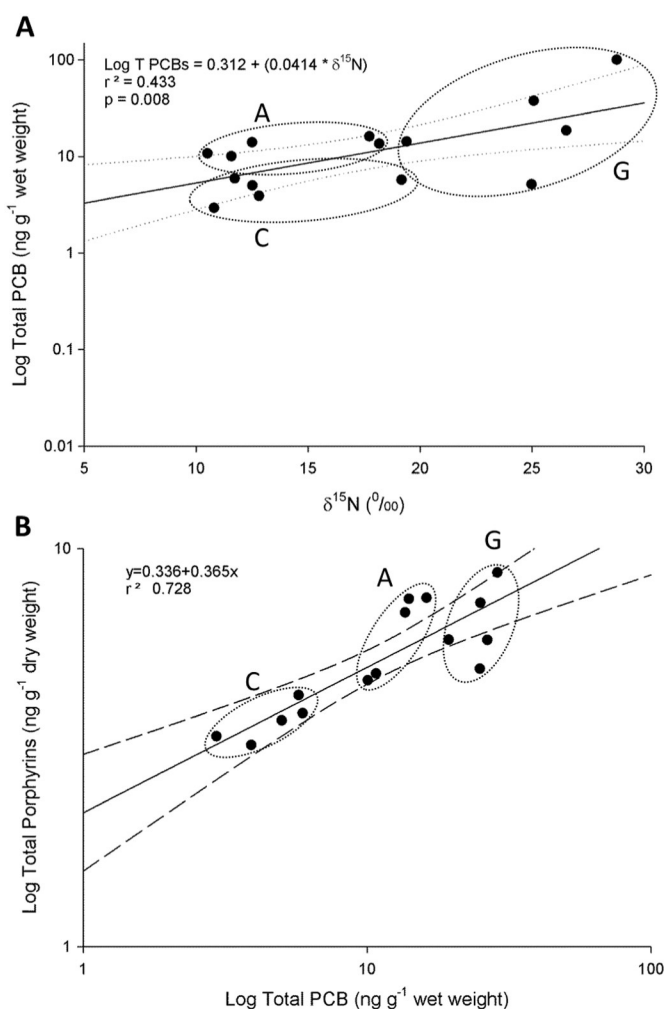


Fig. 3. (A) Relationship between the total PCB concentrations and $\delta^{15}\text{N}\text{‰}$ in gentoo (G), Adélie (A), and chinstrap (C) penguins faeces. (B) Correlation between total porphyrins and PCBs in gentoo (G), Adélie (A), and chinstrap (C) penguin faeces.

Acknowledgements

The authors thank the support provided by the Antarctic Division of the Chilean Army and by Dr. Silvia Casini.

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>.

References

- Bengtson Nash, S.M., Poulsen, A.H., Kawaguchi, S., Vetter, W., Schlabach, M., 2008. Persistent organohalogen contaminant burdens in antarctic krill (*Euphausia superba*) from the eastern antarctic sector: a baseline study. *Sci. Total Environ.* 407 (1), 304–314.
- Berdie, L., Grimalt, J.O., 1998. Assessment of the sample handling procedures in a labor-saving method for the analysis of organochlorine compounds in a large number of fish samples. *J. Chromatogr. A* 823 (1–2), 373–380.
- Biuw, M., Lydersen, C., De Bruyn, P.J.N., Arriola, A., Hofmeyr, G.G.J., Kritzing, P., Kovacs, K.M., 2010. Long-range migration of a Chinstrap penguin from Bouvetoya to Montagu Island, South Sandwich Islands. *Antarct. Sci.* 22 (2), 157–162.
- Bowers, M.A., Aicher, L.D., Davis, H.A., Woods, J.S., 1992. Quantitative-determination of porphyrins in rat and human urine and evaluation of urinary porphyrin profiles during mercury and lead exposures. *J. Lab. Clin. Med.* 120 (2), 272–281.
- Cabrero, A., Dachs, J., Barceló, D., Jones, K.C., 2012. Climatic and biogeochemical controls on the remobilization and reservoirs of persistent organic pollutants in Antarctica. *Environ. Sci. Technol.* 47 (9), 4299–4306.
- Casini, S., Fossi, M.C., Gavilan, J.F., Barra, R., Parra, O., Focardi, S., 2001. Porphyrin levels in excreta of sea birds of the Chilean coasts as non destructive biomarker of exposure to environmental pollutants. *Arch. Environ. Contam. Toxicol.* 41, 65–72.
- Casini, S., Fossi, M.A., Leonzio, C., Renzoni, A., 2003. Review: porphyrins as biomarkers for hazard assessment of bird populations: destructive and non destructive use. *Ecotoxicology* 12, 297–305.
- Celis, J., Jara, S., González-Acuña, D., Barra, R., Espejo, W., 2012. A preliminary study of trace metals and porphyrins in excreta of Gentoo penguins (*Pygoscelis papua*) at two locations of the Antarctic Peninsula. *Archivos de Medicina Veterinaria* 44 (3), 311–316.
- Cipro, C.V.Z., Taniguchi, S., Montone, R.C., 2010. Occurrence of organochlorine compounds in *Euphausia superba* and unhatched eggs of *Pygoscelis* genus penguins from Admiralty Bay (King George Island, Antarctica) and estimation of biomagnification factors. *Chemosphere* 78 (6), 767–771.
- Clarke, J., Kerry, K., Fowler, C., Lawless, R., Eberhard, S., Murphy, R., 2003. Post-fledging and winter migration of Adélie penguins *Pygoscelis adeliae* in the Mawson region of East Antarctica. *Mar. Ecol. Prog. Ser.* 248, 267–278.
- Corsolini, S., 2009. Industrial contaminants in Antarctic biota. *J. Chromatogr. A* 1216 (3), 598–612.
- Corsolini, S., Kannan, K., Imagawa, T., Focardi, S., Giesy, J.P., 2002a. Polychloronaphthalenes and other dioxin-like compounds in arctic and antarctic marine food webs. *Environ. Sci. Technol.* 36 (16), 3490–3496.
- Corsolini, S., Romeo, T., Ademollo, N., Greco, S., Focardi, S., 2002b. POPs in key species of marine antarctic ecosystem. *Microchem. J.* 73 (1–2), 187–193.
- Corsolini, S., Ademollo, N., Romeo, T., Olmastroni, S., Focardi, S., 2003. Persistent organic pollutants in some species of a Ross sea pelagic trophic web. *Antarct. Sci.* 15 (1), 95–104.
- Corsolini, S., Borghesi, N., Ademollo, N., Focardi, S., 2011. Chlorinated biphenyls and pesticides in migrating and resident seabirds from east and west Antarctica. *Environ. Int.* 37 (8), 1329–1335.
- Corsolini, S., Borghesi, N., Schiamone, A., Focardi, S., 2007. Polybrominated diphenyl ethers, polychlorinated dibenzo-dioxins, -furans, and -biphenyls in three species of antarctic penguins. *Environ. Sci. Pollut. Res.* 14 (6), 421–429.
- Corsolini, S., Covaci, A., Ademollo, N., Focardi, S., Schepens, P., 2006. Occurrence of organochlorine pesticides (OCPs) and their enantiomeric signatures, and concentrations of polybrominated diphenyl ethers (PBDEs) in the Adélie penguin food web, Antarctica. *Environ. Pollut.* 140, 371–382.
- Fisk, A.T., Hobson, K.A., Norstrom, R.J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater polynya marine food web. *Environ. Sci. Technol.* 35 (4), 732–738.
- Fisk, A.T., Hoekstra, P.F., Gagnon, J.M., Duffe, J., Norstrom, R.J., Hobson, K.A., Kwan, M., Muir, D.C.G., 2003. Influence of habitat, trophic ecology and lipids on, and spatial trends of, organochlorine contaminants in Arctic marine invertebrates. *Mar. Ecol. Prog. Ser.* 262, 201–214.
- Fry, B., 2006. *Stable Isotope in Ecology*. Springer, New York (308 p. ISBN 978-0-387-30513-4).
- Galbán-Malagón, C., Cabrero, A., Caballero, G., Dachs, J., 2013c. Atmospheric occurrence and deposition of hexachlorobenzene and hexachlorocyclohexanes in the southern ocean and Antarctic Peninsula. *Atmos. Environ.* 80, 41–49.
- Galbán-Malagón, C.J., Del Vento, S., Berrojalbiz, N., Ojeda, M., Dachs, J., 2013a. Polychlorinated biphenyls, hexachlorocyclohexanes and hexachlorobenzene in seawater and phytoplankton from the southern ocean (weddel, south scotia, and bellingshausen seas). *Environ. Sci. Technol.* 47 (11), 5578–5587.
- Galbán-Malagón, C.J., Del Vento, S., Cabrero, A., Dachs, J., 2013b. Factors affecting the atmospheric occurrence and deposition of polychlorinated biphenyls in the southern ocean. *Atmos. Chem. Phys.* 13 (23), 12029–12041.
- Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G., 2013. Persistent organic pollutants in benthic and pelagic organisms off Adélie land, Antarctica. *Mar. Pollut. Bull.* 77, 82–89.
- Grandchamp, B., Deybach, J.C., Grelier, M., De Verneuil, H., Nordmann, Y., 1980. Studies of porphyrin synthesis in fibroblasts of patients with congenital erythropoietic porphyria and one patient with homozygous coproporphyrin. *Biochim. Biophys. Acta* 629 (3), 577–586.
- Hobson, K.A., Welch, H.E., 1992. Determination of trophic relationships within a high arctic marine food web using delta-C-13 and delta-N-15 analysis. *Mar. Ecol. Prog. Ser.* 84 (1), 9–18.
- Hobson, K.A., Ambrose, W.G., Renaud, P.E., 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the northeast water polynya: insights from delta C-13 and delta N-15 analysis. *Mar. Ecol. Prog. Ser.* 128 (1–3), 1–10.
- Hobson, K.A., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J.M., Fortier, M., 2002. A stable isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) model for the north water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. *Deep-Sea Res.* II 49, 5131–5150.
- Hop, H., Borga, K., Gabrielsen, G.W., Kleivane, L., Skaare, J.U., 2002. Food web magnification of persistent organic pollutants in poikilotherms and homeotherms from the Barents Sea. *Environ. Sci. Technol.* 36 (12), 2589–2597.
- Huang, T., Sun, L., Wang, Y., Chu, Z., Qin, X., Yang, L., 2014. Transport of nutrients and contaminants from ocean to island by emperor penguins from Amanda Bay, East Antarctica. *Sci. Total Environ.* 468, 578–583.
- Inomata, O.N.K., Montone, R.C., Lara, W.H., Weber, R.R., Toledo, H.H.B., 1996. Tissue distribution of organochlorine residues - PCBs and pesticides - in Antarctic penguins. *Antarct. Sci.* 8 (3), 253–255.
- Jara-Carrasco, S., González-Acuña, D., Chiang, G., Celis, J., Espejo, W., Mattatall, P., Barra, R., 2015. Immunohematological alterations attributed to persistent organic pollutants on three populations of chinstrap penguins (*Pygoscelis antarctica*) in the south Shetland Islands and Antarctica peninsula. *Antarct. Sci.* 27, 373–381.
- Jerez, S., Motas, M., Palacios, M.J., Valera, F., Cuervo, J.J., Barbosa, A., 2011. Concentration of trace elements in feathers of three Antarctic penguins: geographical and interspecific differences. *Environ. Pollut.* 159, 2412–2419.
- Kelly, B.C., Gobas, F.A.P.C., MacLachlan, M.S., 2004. Intestinal absorption and biomagnification of organic contaminants in fish, wildlife, and humans. *Environ. Toxicol. Chem.* 23 (10), 2324–2336.
- Kidd, K.A., Hesslein, R.H., Ross, B.J., Koczenski, K., Stephens, G.R., Muir, D.C.G., 1998. Bioaccumulation of organochlorines through a remote freshwater food web in the Canadian Arctic. *Environ. Pollut.* 102 (1), 91–103.
- Larsson, P., Jarnmark, C., Sodergren, A., 1992. PCBs and chlorinated pesticides in the atmosphere and aquatic organisms of Ross Island, Antarctica. *Mar. Pollut. Bull.* 25 (9–12), 281–287.
- Leonzio, C., Fossi, M.C., Casini, S., 1995. Porphyrins as biomarkers of methylmercury and PCB exposure in experimental quail. *Bull. Environ. Contam. Toxicol.* 56 (2), 244–250.
- Lockwood, W.H., Poulus, V., Rossi, E., Curnow, D.H., 1985. Rapid procedure for fecal porphyrin assay. *Clin. Chem.* 31 (7), 1163–1167.
- Lynnes, A.S., Reid, K., Croxall, J.P., 2004. Diet and reproductive success of Adélie and Chinstrap penguins: linking response of predators to prey population dynamics. *Polar Biol.* 27 (9), 544–554.
- Marks, G.S., 1985. Exposure to toxic agents: the heme biosynthetic pathway and hemo-proteins as indicator. *CRC Crit. Rev. Toxicol.* 15 (2), 151–179.
- Michener, R.H., Kaufman, L., 2007. Stable Isotopes ratios as tracers in marine food webs: an update. In: R.H., M., K., L. (Eds.), *Stable Isotopes in Ecology and Environmental Science*, second ed. BlackWell, Oxford, UK, pp. 238–282 (ISBN-13: 978-1-4051-2680-9).
- Miranda, C.L., Henderson, M.C., Wang, J.L., Nakaue, H.S., Buhler, D.R., 1992. Comparative effects of the polychlorinated biphenyl mixture, Aroclor-1242, on porphyrin and xenobiotic metabolism in kidney of japanese-quail and rat. *Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol.* 103 (1), 149–152.
- Montory, M., Habit, E., Bahamonde, P., Fernandez, P., Grimalt, J.O., Saez, K., Rudolph, I., Barra, R., 2011. Polychlorinated biphenyls in farmed and wild *Onchorhynchus kisutch* and *Onchorhynchus mykiss* from the Chilean Patagonia. *Environ. Sci. Pollut. Res.* 18 (4), 629–637.
- Montory, M., Habit, E., Fernandez, P., Grimalt, J.O., Barra, R., 2010. PCBs and PBDEs in wild Chinook salmon (*Onchorhynchus tshawytscha*) in the northern Patagonia, Chile. *Chemosphere* 78 (10), 1193–1199.
- Moser, G.A., MacLachlan, M.S., 2001. The influence of dietary concentration on the absorption and excretion of persistent lipophilic organic pollutants in the human intestinal tract. *Chemosphere* 45, 201–211.
- Muir, D.C.G., Norstrom, R.J., Simon, M., 1988. Organochlorine contaminants in Arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordan-related compounds. *Environ. Sci. Technol.* 22, 1071–1079.
- Ng, J.C., Qi, L.X., Moore, M.R., 2002. Porphyrin profiles in blood and urine as a biomarker for exposure to various arsenic species. *Cell. Mol. Biol.* 48 (1), 111–123.
- Nicol, S., Foster, J., 2003. Recent trends in the fishery for Antarctic krill. *Aquat. Living Resour.* 16 (1), 42–45.
- Nizzetto, L., Gioia, R., Li, J., Pomati, F., Bettinetti, R., Dachs, J., Jones, K.C., 2012. The phytoplankton biological pump for airborne hydrophobic chemical pollutants. *Environ. Sci. Technol.* 46 (6), 3204–3211.
- Park, H., Lee, S.-H., Kim, M., Kim, J.H., Lim, H.S., 2010. Polychlorinated biphenyl congeners in soils and lichens from King George Island, South Shetland Islands, Antarctica. *Antarct. Sci.* 22 (01), 31–38.
- Robinson, S.A., Hindell, M.A., 1996. Foraging ecology of Gentoo penguins *Pygoscelis papua* at Macquarie Island during the period of chick care. *Ibis* 138 (4), 722–731.

- Sun, L.G., Yin, X.B., Liu, X.D., Zhu, R.B., Pan, C.P., Zhao, Y.Z., Liu, F.M., Jiang, S.R., Wang, Y.H., 2006. Levels of hexachlorocyclohexanes and dichloro-diphenyl-trichloroethanes in penguin droppings collected from Ardley Island, the maritime Antarctic. *Hum. Ecol. Risk Assess.* 12 (2), 328–338.
- Taira, M.C., San Martín De Viale, L.C., 1980. Porphyrinogen carboxylase from chick embryo liver-in vivo effect of heptachlor and lindane. *Int. J. Biochem.* 5–6, 1033–1038.
- Taniguchi, S., Montone, R.C., Bicego, M.C., Colabuono, F.I., Weber, R.R., Sericano, J.L., 2009. Chlorinated pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in the fat tissue of seabirds from King George Island, Antarctica. *Mar. Pollut. Bull.* 58 (1), 129–133.
- Tierney, M., Emmerson, L., Hindell, M., 2009. Temporal variation in Adélie penguin diet at Bechervaise Island, east Antarctica and its relationship to reproductive performance. *Mar. Biol.* 156 (8), 1633–1645.
- Trivelpiece, W.Z., Buckelew, S., Reiss, C., Trivelpiece, S.G., 2007. The winter distribution of Chinstrap penguins from two breeding sites in the South Shetland Islands of Antarctica. *Polar Biol.* 30 (10), 1231–1237.
- Vanbirgelen, A., Devito, M.J., Akins, J.M., Ross, D.G., Diliberto, J.J., Birnbaum, L.S., 1996. Relative potencies of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls derived from hepatic porphyrin accumulation in mice. *Toxicol. Appl. Pharmacol.* 138 (1), 98–109.
- Vives, I., Grimalt, J.O., Catalan, J., Rosseland, B.O., Battarbee, R.W., 2004. Influence of altitude and age in the accumulation of organochlorine compounds in fish from high mountain lakes. *Environ. Sci. Technol.* 38 (3), 690–698.
- Wania, F., Mackay, D., 1996. Tracking the distribution of persistent organic pollutants. *Environ. Sci. Technol.* 30 (9), A390–A396.
- Williams, T.D., 1990. Annual variation in breeding biology of Gentoo penguins, *Pygoscelis papua*, at bird island, south Georgia. *J. Zool.* 222, 247–258.
- Woods, J.S., Bowers, M.A., Davis, H.A., 1991. Urinary porphyrin profiles as biomarkers of trace-metal exposure and toxicity-studies on urinary porphyrine excretion patterns in rats during prolonged exposure to methyl mercury. *Toxicol. Appl. Pharmacol.* 110 (3), 464–476.