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Organohalogenated contaminants in plasma and eggs of rockhopper penguins: Does vitellogenin affect maternal transfer?☆

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ABSTRACT

Although many studies have investigated organohalogenated contaminants (OHCs) in yolk, little is known about the mechanisms and timing of transfer of OHCs from the female to the egg. Vitellogenin, a yolk precursor, has been suggested to play a role in this transport.

We here report for the first time the temporal changes in OHC and an index of vitellogenin concentrations in female plasma from the pre-laying period to clutch completion in free-living birds: the southern rockhopper penguin (*Eudyptes chrysocome chrysocome*) breeding in the Falkland/Malvinas Islands. In addition, OHC concentrations in the corresponding clutches were analysed. OHC concentrations in female plasma and in the yolk of both the first (A-) and the second (B-)eggs followed a similar pattern, with hexachlorobenzene (HCB) > Σpolychlorinated biphenyls (PCBs) > Σdichlorodiphenyltrichloroethanes (DDTs) > Σmethoxylated polybrominated diphenyl ethers (MeO-PBDEs) > Σchlordanes (CHLs) > Σpolybrominated diphenyl ethers (PBDEs) ≈ Σhexachlorocyclohexanes (HCHs). The higher concentrations of MeO-PBDEs compared to PBDEs indicate a diet containing naturally-produced MeO-PBDEs. All OHC compounds except for PBDEs increased from the pre-laying period to A-egg laying and subsequently declined from A-egg laying to B-egg laying, and female plasma vitellogenin showed the same pattern. For ΣPCBs and ΣMeO-PBDEs, we found positive correlations between female plasma during A-egg laying and both eggs, and for HCB between female plasma and A-eggs only. During pre-laying, only ΣMeO-PBDEs correlated between both eggs and female plasma, and no correlations between OHC concentrations in eggs and female plasma were found during B-egg laying, highlighting that maternal transfer of OHCs is time- and compound-specific. Finally, female vitellogenin concentrations did not significantly correlate with any OHC compounds in either female plasma or eggs, and our results therefore did not confirm the suggested role of vitellogenin in the maternal transfer of OHC molecules into their eggs.

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1. Introduction

Organohalogenated contaminants (OHCs) are globally distributed due to long-distance atmospheric transfer and/or local use (Dachs et al., 2002; Semeena and Lammel, 2005; Choi et al., 2008). Due to their persistence, OHCs are especially prone to accumulate in long-lived animals (Rowe, 2008), particularly in polar regions due to the global distillation effect (Simonich and Hites, 1995). Because of their deleterious effects on health of humans (e.g. Toft et al., 2004) and wildlife (summarised in Vos

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et al., 2000), e.g. eggshell thinning in birds (Blus et al., 1972), embryotoxicity (Brunström and Reutergårdh, 1986) and effects on reproductive behaviour (Fernie et al., 2008), the production of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) has been restricted by the UN Stockholm Convention on persistent organic pollutants (POPs) (United Nations Environment Program; www.pops.int). Unlike other OHCs, methoxylated polybrominated diphenyl ethers (MeO-PBDEs) have natural sources in the oceanic food web (Teuten et al., 2005; Teuten and Reddy, 2007).

Since OHCs are generally lipophilic, they tend to accumulate through the food web and are stored in body fat reserves. Circulation of OHCs in organisms is therefore enhanced when animals are fasting and mobilise fat reserves (e.g. Bustnes et al., 2012), but OHCs are also passed on to offspring via lipophilic egg yolk (Alava et al., 2006; Eng et al., 2013) or milk (e.g. Beckmen et al., 1999). Circulating OHC concentrations in mothers and transfer rates to their offspring might then be enhanced in species that are fasting during production of eggs (captial breeders sensu Meijer and Drent, 1999) or the lactation period (Polischuk et al., 2002). Thereby, high exposure to OHCs will likely have negative implications for offspring health and survival (Brunström and Reutergårdh, 1986; Bustnes et al., 2013). For females, the transfer of OHCs into eggs or milk presents a pathway to reduce their own exposure (Tanabe et al., 1998; Donaldson and Braune, 1999). The transfer of lipophilic compounds to egg yolk can occur via passive diffusion processes during follicle recruitment and development (Russell et al., 1999; Groothuis and Schwabl, 2008). Alternatively, OHC molecules could attach in a piggy-back fashion to yolk precursors (Eng et al., 2013). Furthermore, OHCs can mimic or antagonise oestrogens (e.g. Korach et al., 1988; Jobling et al., 1995; Meerts et al., 2001), which can have disruptive effects on oestrogenic pathways leading to the production of vitellogenin (VTG), the primary yolk precursor in many animals including vertebrates. These effects are disruptive not only to breeding females, but can induce abnormal patterns of vitellogenesis in male and immature animals as well (Kime et al., 1999; Jiménez et al., 2007). OHC exposure may therefore have inter-generational consequences for population dynamics, affecting the reproductive processes of adults and potentially the development of offspring via accumulation in yolk (Kime et al., 1999; Jiménez et al., 2007).

Southern rockhopper penguins (*Eudyptes chrysocome chrysocome*) are capital breeders distributed throughout the South Atlantic Ocean, with breeding sites on the Falkland/Malvinas Islands, as well as islands in the South of Chile and Argentina (Pütz et al., 2013). Their conservation status is vulnerable following population declines across their distribution range in the 20th century (BirdLife International, 2010). In the Falkland/Malvinas Islands, the population of southern rockhopper penguins declined by more than 80% between the 1930s and 2000/01 (Pütz et al., 2003), but is currently stable with a slight recovery in numbers in the 2000s (Baylis et al., 2013).

Uptake and accumulation of OHCs in this species are predominantly through the food web, as in other seabirds from remote areas (Burger and Gochfeld, 2004). Southern rockhopper penguins feed mostly on swarming prey such as krill, (larval) fish, and squid (reviewed in Pütz et al., 2013). The species is migratory and formation of egg yolk for the first, and sometimes also the second egg, starts at sea before females arrive in their colonies for the breeding season (Poisbleau et al., 2015). Egg formation is then finalised in the colony while females are fasting. The clutch consists of two size-dimorphic eggs (Poisbleau et al., 2008; Demongin et al., 2010), with the first laid (A-)egg being smaller and hatching after the second laid (B-)egg (reversed hatching asynchrony; St. Clair, 1996). Crossin and Williams (2016) previously suggested that this size dimorphism, which is present in all crested penguin species (genus *Eudyptes*), is caused by a migratory carry-over effect. Briefly, as females are migrating back to their colonies and simultaneously start the formation of their clutches, they are constrained in the production of VTG, which especially affects the size of the A-eggs. This specific role of VTG in rockhopper penguins makes it particularly interesting to study OHC concentrations in females and their eggs in relation to VTG concentrations.

The first aim of this study was to determine OHC concentrations in female plasma during the pre-laying and egg laying periods, and in the eggs produced by the same females. Based on previous studies in the region (Van den Steen et al., 2011; Baldassin et al., 2016), we expected low to moderate concentrations of OHCs compared to seabirds in other regions like the Arctic. Secondly, we aimed to investigate potential temporal changes in OHC concentrations in plasma of females during the pre-laying and laying periods. We could not make predictions about the variation in the OHC concentrations over the laying period as females on the one hand mobilise fat tissues which releases OHCs into the plasma, but on the other hand also deposit OHCs into eggs and may therefore reduce their own OHC burdens. Thirdly, to further investigate the link between OHCs in female plasma and eggs, we specifically tested i) whether OHC concentrations in female plasma were correlated with OHC concentrations in their A- and B-eggs; ii) whether A- and B-egg OHC concentrations were correlated and whether they differ within the same clutch; iii) whether female OHC plasma concentrations at A- and B-egg laying were lowered by OHC deposition into her clutch. Finally, we predicted that VTG concentrations would correlate positively with OHC concentrations in plasma and eggs.

2. Materials and methods

2.1. Study site and birds

The study was carried out during the austral summer 2008/09 (hereafter 2008) on southern rockhopper penguins breeding at the "Settlement Colony" (51°43′S, 61°17′W) on New Island, Falkland/Malvinas Islands. The breeding biology of this population that held about 7300 pairs in 2008 has been described previously in Poisbleau et al. (2008). The birds mainly breed in open rocky areas fringed by tussac grass (*Poa flabellata*). Males arrive in the colony first (early October) and establish nest sites. Females arrive a few days later, for pairing and copulation in late October/early November. Egg laying is synchronised within this population, taking place in less than two weeks (Poisbleau et al., 2008).

Since 2006, we have marked 461 randomly-chosen adult females in the colony, equipping them with 23-mm long glass-encapsulated electronic transponders (TIRIS, Texas Instruments, USA). We determined the sex of birds through bill measurements within pairs, as males have larger bills than females (Poisbleau et al., 2010).

2.2. Adult manipulation

During the 2008 laying period, we visited the study site daily to follow egg laying. We randomly chose 17 marked females, which were homogeneously distributed within the study site and the laying period. They were captured two (8 females) or three times (9 females): the first time 5–13 days before the start of egg laying (N = all 17 females); the second and potentially third times on the day they had laid their first A-egg (N = 13 females) and/or the day they had laid their second B-egg (N = 13 females). During each capture, the birds' head was covered with a hood to minimize stress, and we then collected up to 1 mL of blood from the brachial

vein, using a 23-gauge needle and heparinized syringe. Blood samples were collected within 3 min after the first disturbance. They were stored on ice while still in the colony and centrifuged within 3 h. Red blood cells and plasma samples were stored at −20 °C in separated 1.5-mL Eppendorf tubes until analysis.

2.3. Egg collection and preparation

Once A-eggs were detected in the nests of the 17 study females, we collected and replaced them with foster eggs (*i.e.* eggs of close size found outside their own nest that we considered lost by their original parents) in order to minimize potential effects of egg removal on birds' physiology and behaviour, and on the B-egg composition. Afterwards, we checked nests daily until the laying of B-eggs. We collected B-eggs as soon as they were detected and also replaced them with foster eggs. Since incubation in rockhopper penguins typically does not start before clutch completion (Williams, 1995), A-eggs were not incubated and B-eggs were incubated less than 24 h before collection. We therefore assumed that embryo development (if any) was minimal and similar between eggs. Accordingly, no embryo development was observed during the preparation of any of the collected eggs. In total, we collected 17 entire clutches that were frozen whole at −20 °C.

We used the same method as previously (Poisbleau et al., 2009) to prepare all frozen eggs for subsequent analyses: While the egg was still frozen, we removed its shell and separated the yolk from the albumen by taking advantage that albumen thaws more quickly than yolk. A small quantity of homogenized yolk (representative of the whole yolk) was transferred to a 1.5-mL Eppendorf tube and stored at −20 °C until analysis.

2.4. OHC analyses

Egg yolk (N = 34) and plasma (N = 30) samples were analysed for OHCs at the Toxicological Centre of the University of Antwerp according to previously described methods for eggs (Van den Steen et al., 2011) and plasma (Covaci and Voorspoels, 2005; Dirtu et al., 2010), respectively. Approximately 1 g of yolk was weighed, homogenized with anhydrous Na_2SO_4 and spiked with internal standards (ε-HCH, CB 46 and CB 143, BDE 77 and BDE 128). After extraction with hexane:acetone (3:1, *v*/*v*) in an automated hot Soxhlet extractor for 2 h, the lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h). Further clean-up was performed on acid silica (Van den Steen et al., 2011).

Plasma samples were analysed according to previously described methods (Covaci and Voorspoels, 2005), using solid phase extraction (SPE) and clean-up on acid silica. Briefly, internal standards (ε-HCH, CB 46 and CB 143, BDE 77 and BDE 128) were added to a volume of plasma (typically 200–300 μL) which was further diluted with 1 mL of deionized water and 300 μL of formic acid. After sonication for 20 min, the samples were loaded onto solid phase extraction (SPE) cartridges (30 mg, 1 mL; OASIS™ HLB). The analytes were then eluted with 3 mL dichloromethane and the eluate was cleaned on acidified silica (44% sulphuric acid). The latter cartridges were eluted with 4 mL of dichloromethane. The finale cleaned eluates were concentrated under a gentle nitrogen flow until near dryness and redissolved in 100 μL of iso-octane.

Detection and quantification of compounds in egg and plasma extracts were performed by gas chromatography-mass spectrometry (GC-MS). The compounds targeted for analysis were ten PCB congeners (CB 99, CB105, CB118, CB128, CB138, CB153, CB170, CB180, CB183 and CB187), eight OCP congeners, which were treated separately in analyses as dichlorodiphenyltrichloroethane (DDTs; *p,p*'-DDE and *p,p*'-DDT), chlordanes (CHLs; oxychlordane (OxC), *trans*-nonachlor (TN) and *cis*-nonachlor (CN)), hexachlorocyclohexanes (HCHs; β-HCH and γ-HCH) and hexachlorobenzene (HCB). Furthermore, 4 PBDE congeners (BDE47, BDE99, BDE100 and BDE154) and two MeO-PBDE congeners (2'-MeO-BDE68 and 6-MeO-BDE47) were targeted. For the PCB analysis, the GC/MS was operated in electron ionisation (EI) mode and was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). For the analysis of OCPs and PBDEs, the GC/MS was operated in electron capture negative ionisation (ECNI) mode and was equipped with a similar HT-8 column. Operating details are given in (Covaci and Voorspoels, 2005).

Multi-level calibration curves were created for the quantification, and excellent correlation ($r^2 > 0.999$) was achieved. The identification of OHCs was based on the relative retention times to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. A deviation of the ion intensity ratios within 20% of the mean values obtained for calibration standards was considered acceptable. The quality control was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs, OCPs and PBDEs in whale blubber) was used to test the method accuracy indicated that the measured concentrations were within 10% of the certified values. The quality control scheme was also assessed through regular participation in inter-laboratory comparison exercises organised by the Arctic Monitoring and Assessment Programme and the National Institute of Standards and Technology. For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank and taking into account the amount of sample used for analysis. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. LOQs for the analysed compounds ranged between 0.01 and 0.1 ng/mL plasma and 0.1 and 0.5 ng/g lipid weight (lw) yolk. OHC concentrations in yolk based on lw were highly correlated with OHC concentrations based on wet weight (ww; see Table S1). We decided to use lw, following the majority of studies, and additionally present the ww data in Table S1.

2.5. VTG analyses

Plasma samples were analysed for vitellogenic zinc (VTG Zn/mL; zinc kits, Wako Chemicals) as an index for VTG, following methods described in Crossin et al. (2012). All assays were performed using a Biotek 340i microplate reader. Intra-assay coefficients of variation, using a domestic laying hen (*Gallus domesticus*) plasma pool ranged from 5.1% to 6.6%. Inter-assay coefficient of variation was 5.7%.

2.6. Statistical analyses

Samples with OHC data < LOQ were assigned a value according to f x LOQ, where f is the detection frequency; i.e. the proportion of samples ≥ LOQ (Voorspoels et al., 2002). Compounds with f < 0.50 were not taken into account for statistical analysis (i.e. CB99, CB183, *p,p*'-DDT, CN, β-HCH, BDE100 and 2'-MeO-BDE68 for female plasma, as well as BDE99 and BDE154 for eggs; see Table S2). In addition, γ-HCH was below the detection frequency in >50% of the plasma samples at A-egg laying date, and we therefore excluded HCHs from those statistical analyses that included data from A-egg laying date. We performed statistical analyses on the sum of PCBs, DDTs, CHLs, HCHs, PBDEs, MeO-PBDEs and the single congener HCB (see Table 1 for an overview of the different compounds).

Statistical analyses were conducted in R (R Core Team, 2016; version 3.1.1) for Windows. Prior to analyses, we tested for normality and homogeneity of data using Shapiro Wilk's and Levene's tests. To meet the requirement of normality, OHC

Table 1
OHC concentrations (mean ± SE) in the blood plasma (ng/mL) and yolk (ng/g lipid weight) of rockhopper penguins from the Falkland Islands. “P” stands for the pre-laying period before A-egg laying, “A” stands for the A-egg laying date and “B” stands for the B-egg laying date. Cells show a dash (–) when the compound was below the limit of quantification (LOQ) in more than 50% of the samples. “n.a.” means not analysed.

	Plasma (ng/mL)			Yolk (ng/g lw)	
Period	P	A	B	A-egg	B-egg
N	17	13	13	17	17
Lipid (%)	n.a.	n.a.	n.a.	31.3 ± 0.70	31.4 ± 0.65
ΣPCBs[a]	**0.61 ± 0.07**	**1.02 ± 0.14**	**0.55 ± 0.07**	**25.8 ± 1.12**	**27.8 ± 0.79**
p,p‘-DDE	0.62 ± 0.08	0.79 ± 0.07	0.47 ± 0.07	18.4 ± 0.67	20.4 ± 0.66
p,p‘-DDT	–	–	–	1.52 ± 0.18	1.50 ± 0.14
ΣDDTs	**0.62 ± 0.08**	**0.79 ± 0.07**	**0.47 ± 0.07**	**19.93 ± 0.70**	**21.86 ± 0.73**
OxC	0.05 ± 0.01	0.08 ± 0.02	0.04 ± 0.01	1.40 ± 0.08	1.57 ± 0.06
TN	0.04 ± 0.00	0.08 ± 0.01	0.04 ± 0.01	1.59 ± 0.08	1.73 ± 0.06
CN	–	–	–	0.35 ± 0.02	0.39 ± 0.02
ΣCHLs	**0.10 ± 0.01**	**0.16 ± 0.02**	**0.08 ± 0.02**	**3.34 ± 0.16**	**3.69 ± 0.11**
HCB	1.53 ± 0.14	2.19 ± 0.26	1.73 ± 0.24	29.71 ± 1.59	32.48 ± 1.54
β-HCH	–	–	–	0.45 ± 0.11	0.39 ± 0.05
γ-HCH	0.08 ± 0.02	–	0.10 ± 0.03	0.26 ± 0.04	0.30 ± 0.06
ΣHCHs	**0.08 ± 0.02**	–	**0.10 ± 0.03**	**0.71 ± 0.11**	**0.69 ± 0.10**
BDE47	0.05 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.14 ± 0.01	0.13 ± 0.02
BDE99	–	0.03 ± 0.02	–	–	–
BDE100	–	–	–	0.26 ± 0.03	0.31 ± 0.02
BDE154	–	0.02 ± 0.00	0.03 ± 0.01	–	–
ΣPBDEs	**0.05 ± 0.01**	**0.11 ± 0.03**	**0.07 ± 0.01**	**0.40 ± 0.03**	**0.44 ± 0.04**
2'-MeO-BDE68	–	–	–	0.56 ± 0.07	0.55 ± 0.07
6-MeO-BDE47	0.12 ± 0.02	0.17 ± 0.03	0.10 ± 0.02	6.87 ± 0.89	7.14 ± 0.95
ΣMeO-PBDEs	**0.12 ± 0.02**	**0.17 ± 0.03**	**0.10 ± 0.02**	**7.43 ± 0.94**	**7.69 ± 1.00**

[a] ΣPCBs includes CB 99, 105, 118, 128, 138, 153, 170, 180, 183 & 187.

concentrations in females were log-transformed and statistical tests were performed with log-transformed data. Following Jaspers et al. (2013), we ran Pearson correlations between MeO-PBDEs and PBDEs, but also all other OHC compounds to better understand the bioaccumulation pathways.

To analyse the changes in OHC and VTG concentrations (as dependent variables) in the females from pre-laying through to B-egg laying we ran linear mixed models in the package lme4 (Bates et al., 2011). As the number of days between first capture and A-egg laying date differed among females, we decided to run models in two steps, and tested firstly for the change in female OHC and VTG concentrations from the pre-laying period to A-egg laying date, and secondly from A-egg laying date to B-egg laying date. Models contained the number of days between capture and A-egg laying (and between A-egg laying and B-egg laying, respectively) as a covariate and female identity as random intercept. To test for the correlations of pollutant concentrations between females and their eggs, as well as between A- and B-eggs of the same clutch, we ran Pearson's correlations. As the exact process for the transfer of pollutants from female to egg (and therefore also the time period of this transfer) is unclear (Eng et al., 2013), we conducted these correlations for all sampling events of females (i.e. pre-laying period, A-egg and B-egg laying dates). Exceptionally, to enable more detailed comparisons between female and egg pollutant concentrations, these correlations were also run on the single congeners and not only on the sum of sum of PCBs, DDTs, CHLs, HCHs, HCB, PBDEs and MeO-PBDEs (see Table S3). We furthermore tested for the within-clutch difference of pollutant concentrations between A- and B-eggs using paired t-tests. To investigate whether female pollutant concentrations are influenced by the pollutant concentrations she deposited into her clutch, we ran a linear model with female pollutant concentration at A-egg laying (second capture) as dependent variable, the average pollutant concentration of the clutch, the female pollutant concentration during pre-laying (the first capture) and the number of days between these captures (to control for the fact that this period differed among females) as covariates. We repeated the same linear model with female pollutant concentration at B-egg laying (third capture) as dependent variable, and replaced the female pollutant concentration at pre-laying with that at A-egg laying (second capture) as covariate. We used the average pollutant concentration of the clutch for both of these analyses as not only A-eggs are formed completely at A-egg laying date, but also the formation of the yolk for the B-egg is finalised by then (Grau, 1982).

To investigate the role of VTG in the process of pollutant deposition from females to their eggs, we ran Pearson's correlations with female VTG (during the pre-laying period, A-egg and B-egg laying date) and simultaneous pollutant concentrations in females as well as pollutant concentrations in their eggs.

Values are presented as means ± standard errors. Sample size is consistently 17 female plasma samples for the pre-laying period, 13 female plasma samples for the A-egg laying, 13 female plasma samples for B-egg laying, and 17 entire clutches (*i.e.* 17 A-eggs and 17 B-eggs) throughout the manuscript, including all tables and figures. Significant *P*-values (<0.05) are marked in bold in tables. Although multiple statistical tests were run, we followed the recent trend in ecology and refrained from applying Bonferroni corrections, as this increases the risk of type II errors when dealing with small sample sizes (Nakagawa, 2004; Garamszegi, 2006).

3. Results

3.1. General patterns

In both female plasma and eggs, HCB had the highest concentrations among the measured OHCs (50.5% and 34.2% for plasma and eggs, respectively), followed by ΣPCBs (20.2% and 29.5%, respectively), ΣDDTs (17.6% and 23.0%, respectively), ΣMeO-PBDEs (3.8% and 8.3%, respectively) ΣCHLs (3.1% and 3.9%, respectively), ΣPBDEs (2.5% and 0.5%, respectively) and ΣHCHs (2.3% and 0.8%, respectively) (Table 1). In plasma, concentrations of CB 99, CB 183, *p,p'*-DDT, CN, β-HCH, BDE100 and 2'-MeO-BDE68 were < LOQ in more than 50% of the samples, and the same was true for γ-HCH at A-egg laying. BDE99 and BDE154 were < LOQ in more than 50% of yolk samples in both A- and B-eggs (Table 1 and Table S2).

ΣPCBs, ΣDDTs, ΣCHLs, HCB, ΣHCHs ΣPBDEs and ΣMeO-PBDEs correlated significantly with each other in plasma during the pre-laying period (except for HCB with ΣPBDEs; Table 2). Such a consistent pattern was not present any more in plasma at A- and B-egg laying or within A- and B-eggs (Table 2).

3.2. Change of female pollutants and VTG with time

ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣMeO-PBDEs in plasma increased from the pre-laying period to A-egg laying ($F_{1,16.18} = 18.55$, $P < 0.001$ for ΣPCBs; $F_{1,15.97} = 19.19$, $P < 0.001$ for ΣDDTs; $F_{1,15.77} = 19.40$, $P < 0.001$ for ΣCHLs; $F_{1,15.42} = 19.48$, $P < 0.001$ for HCB; $F_{1,14.52} = 4.83$, $P = 0.045$ for ΣMeO-PBDEs; Fig. 1). They subsequently declined from A-egg laying to B-egg laying ($F_{1,12.77} = 12.36$, $P = 0.004$ for ΣPCBs; $F_{1,12.70} = 12.86$, $P = 0.003$ for ΣDDTs; $F_{1,12.59} = 13.05$, $P = 0.003$ for ΣCHLs; $F_{1,12.36} = 13.13$, $P = 0.003$ for HCB; $F_{1,13.60} = 4.77$, $P = 0.047$ for ΣMeO-PBDEs; Fig. 1). ΣPBDEs did not change from either pre-laying to A-egg laying ($F_{1,27.92} = 0.58$, $P = 0.452$; Fig. 1) or from A-egg laying to B-egg laying ($F_{1,23.93} = 1.88$, $P = 0.183$; Fig. 1).

VTG concentrations showed a similar pattern as ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣMeO-PBDEs, with an increase from the pre-laying to A-egg laying ($F_{1,14.66} = 7.97$, $P = 0.013$; Fig. 1) and a subsequent decline to B-egg laying ($F_{1,8.53} = 118.77$, $P < 0.001$; Fig. 1).

3.3. Link between female and egg pollutants

We found several positive correlations between egg and female OHC concentrations during the pre-laying period and at A-egg laying date, but not at B-egg laying date (Table 3 and Table S3). Females with higher ΣPBDEs and ΣMeO-PBDEs during the pre-laying period, laid A-eggs with higher concentrations of these OHCs, and the same pattern was true for ΣMeO-PBDEs for B-eggs. Other pollutant concentrations were not significantly correlated between eggs and concentrations in plasma during the pre-laying period (Table 3 and Table S3). ΣPCBs, and ΣMeO-PBDEs in plasma during A-egg laying were positively correlated with those in their A- and B-eggs, while this was not the case for ΣDDTs, ΣCHLs and ΣPBDEs (Table 3). HCB concentrations in plasma during A-egg laying were positively correlated with those in A-eggs, but this relationship was only marginally significant for B-eggs. None of the analysed OHCs showed a significant correlation between plasma concentrations during B-egg laying, and either A- or B-egg

Table 2
Correlations of concentrations of different pollutant compounds within female plasma during pre-laying, A-egg laying and B-egg laying as well as within A- and B-eggs.

	ΣPCBs	ΣDDTs	ΣCHLs	HCB	ΣHCHs	ΣPBDEs
Female plasma pre-laying period						
ΣDDTs	R = 0.885, **P < 0.001**					
ΣCHLs	R = 0.851, **P < 0.001**	R = 0.863, **P < 0.001**				
HCB	R = 0.822, **P < 0.001**	R = 0.704, **P = 0.002**	R = 0.706, **P = 0.002**			
ΣHCHs	R = 0.615, **P = 0.008**	R = 0.767, **P < 0.001**	R = 0.727, **P < 0.001**	R = 0.519, **P = 0.033**		
ΣPBDEs	R = 0.682, **P = 0.003**	R = 0.693, **P = 0.002**	R = 0.657, **P = 0.004**	R = 0.429, P = 0.086	R = 0.592, **P = 0.012**	
ΣMeO-PBDEs	R = 0.889, **P < 0.001**	R = 0.793, **P < 0.001**	R = 0.778, **P < 0.001**	R = 0.799, **P < 0.001**	R = 0.517, **P = 0.033**	R = 0.521, **P = 0.032**
Female plasma A-egg laying date						
ΣDDTs	R = 0.801, **P < 0.001**					
ΣCHLs	R = 0.494, P = 0.086	R = 0.830, **P < 0.001**				
HCB	R = 0.756, **P = 0.003**	R = 0.766, **P = 0.002**	R = 0.745, **P = 0.004**			
ΣPBDEs	R = 0.523, P = 0.067	R = 0.261, P = 0.388	R = 0.163, P = 0.592	R = 0.280, P = 0.354	–	
ΣMeO-PBDEs	R = 0.541, P = 0.056	R = 0.656, **P = 0.015**	R = 0.673, **P = 0.012**	R = 0.711, **P = 0.006**	–	R = −0.058, P = 0.851
Female plasma B-egg laying date						
ΣDDTs	R = 0.630, **P = 0.021**					
ΣCHLs	R = 0.910, **P < 0.001**	R = 0.728, **P = 0.005**				
HCB	R = 0.954, **P < 0.001**	R = 0.493, P = 0.087	R = 0.849, **P < 0.001**			
ΣHCHs	R = 0.248, P = 0.414	R = −0.153, P = 0.618	R = 0.046, P = 0.882	R = 0.356, P = 0.233		
ΣPBDEs	R = 0.203, P = 0.506	R = −0.060, P = 0.845	R = 0.074, P = 0.811	R = 0.243, P = 0.424	R = 0.453, P = 0.121	
ΣMeO-PBDEs	R = 0.737, **P = 0.004**	R = 0.190, P = 0.535	R = 0.648, **P = 0.017**	R = 0.800, **P = 0.001**	R = 0.648, **P = 0.017**	R = 0.336, P = 0.262
A-eggs						
ΣDDTs	R = 0.653, **P = 0.005**					
ΣCHLs	R = 0.737, **P < 0.001**	R = 0.425, P = 0.089				
HCB	R = 0.582, **P = 0.014**	R = 0.150, P = 0.566	R = 0.684, **P = 0.003**			
ΣHCHs	R = −0.269, P = 0.297	R = 0.023, P = 0.930	R = −0.231, P = 0.373	R = −0.290, P = 0.260		
ΣPBDEs	R = 0.104, P = 0.690	R = −0.029, P = 0.913	R = 0.120, P = 0.647	R = 0.210, P = 0.426	R = −0.332, P = 0.192	
ΣMeO-PBDEs	R = 0.206, P = 0.427	R = −0.054, P = 0.838	R = 0.491, **P = 0.046**	R = 0.582, **P = 0.014**	R = −0.107, P = 0.682	R = 0.564, **R = 0.019**
B-eggs						
ΣDDTs	R = 0.419, P = 0.095					
ΣCHLs	R = 0.437, P = 0.079	R = 0.064, P = 0.807				
HCB	R = 0.388, P = 0.123	R = −0.208, P = 0.422	R = 0.640, **P = 0.006**			
ΣHCHs	R = 0.443, P = 0.075	R = 0.132, P = 0.612	R = 0.189, P = 0.468	R = 0.191, P = 0.462		
ΣPBDEs	R = 0.687, **P = 0.002**	R = 0.279, P = 0.279	R = 0.322, P = 0.208	R = 0.623, **P = 0.008**	R = 0.751, **P < 0.001**	
ΣMeO-PBDEs	R = 0.204, P = 0.432	R = −0.214, P = 0.410	R = 0.475, P = 0.054	R = 0.191, P = 0.462	R = 0.257, P = 0.320	R = 0.370, P = 0.144

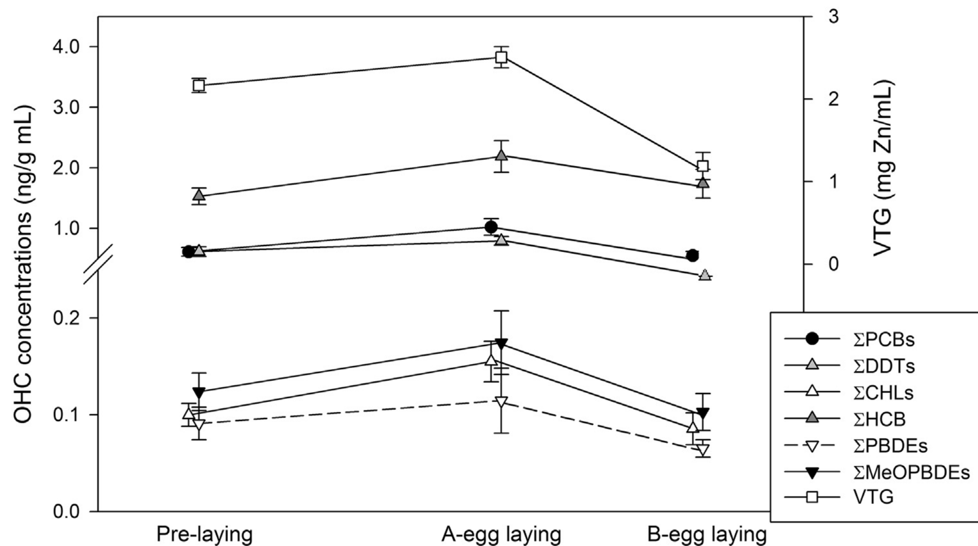

Fig. 1. Levels of OHCs (ng/mL) and VTG (mg Zn/mL) in plasma of rockhopper penguin females during the egg laying period (means ± standard error). See Table 1 for compounds included in the different groups of OHCs. ΣHCHs are not presented, due to low detection frequencies during A-egg laying. Changes that were significant are marked with solid lines, those that were not significant with dashed lines.

concentrations (Table 3 and Table S3).

Within clutches, ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣMeO-PBDEs were positively correlated between A- and B-eggs, while this was not the case for ΣHCHs and ΣPBDEs (Table 3, Fig. 2). B-eggs had higher ΣPCBs, ΣDDTs, ΣCHLs and HCB concentrations than A-eggs ($t = 2.923$, $P = 0.010$ for ΣPCBs, $t = 3.494$, $P = 0.003$ for ΣDDTs, $t = 4.268$, $P < 0.001$ for ΣCHLs, $t = 2.913$, $P = 0.010$ for HCB; Fig. 2). The other pollutants did not differ significantly between A- and B-eggs within the same clutch ($t = -0.100$, $P = 0.291$ for ΣHCHs, $t = 0.981$, $P = 0.3411$ for ΣPBDEs, $t = 1.492$, $P = 0.155$ for ΣMeO-PBDEs).

Plasma ΣPCBs, HCB and ΣMeO-PBDE concentrations at A-egg laying were positively linked to their OHC concentrations during the pre-laying period and the clutch OHC concentrations (Table 4): Females that laid clutches with high ΣPCBs, HCB and ΣMeO-PBDEs had higher plasma concentrations of these OHCs at A-egg laying date. For ΣDDTs, ΣCHLs and ΣPBDEs, this pattern was not significant. Female OHC concentrations at B-egg laying were neither linked to their OHC concentrations during A-egg laying, nor to their clutch pollutant concentrations for any of the analysed congeners (all $F \leq 5.41$, all $P \geq 0.068$).

3.4. Link between female VTG and OHC concentrations

Plasma VTG concentrations during either the pre-laying period, at A-egg laying date or B-egg laying date did not correlate with plasma OHC concentrations during the same periods (all $R \leq 0.308$, all $P \geq 0.229$). There was a weak trend, albeit not significant, for ΣPCBs in A-eggs and B-eggs to correlate with plasma VTG concentrations during the pre-laying period ($R = 0.470$, $P = 0.057$ for A-eggs; $R = 0.456$, $P = 0.066$ for B-eggs). Similarly, HCB concentration in A-eggs showed a tendency to correlate with plasma VTG concentrations during the pre-laying period, but again this was not significant ($R = 0.448$, $P = 0.071$). Plasma VTG concentrations at A- or B-egg laying date were not significantly correlated to OHC concentrations in A- or B-eggs (all $R \leq 0.329$, all $P \geq 0.197$).

4. Discussion

4.1. General OHC patterns

As expected, OHC concentrations in the plasma of female southern rockhopper penguins and in their eggs were moderate to low compared to aquatic feeding birds of similar trophic levels in more industrial areas (Watanabe et al., 2004; Zhou et al., 2016) or in the Arctic (Verreault et al., 2007; Helgason et al., 2008; Jörundsdóttir et al., 2009; see Table S4 for an overview). HCB was the dominant OHC present in southern rockhopper penguins, followed by ΣPCBs, ΣDDTs, ΣMeO-PBDEs, ΣCHLs, ΣPBDEs and ΣHCHs. This pattern, with HCB dominating over ΣPCBs and other OHC compounds has been previously observed in eggs, blood and preen gland oil of several Antarctic seabirds including penguins (van den Brink, 1997; van den Brink et al., 1998; Corsolini et al., 2006; Mello et al., 2016; Table S4). Goutte et al. (2013) found higher HCB concentrations in pelagic Antarctic organisms and pelagic feeding Antarctic seabirds compared to the benthic food chain, which coincides with southern rockhopper penguins being migratory during winter and feeding mainly on swarming pelagic prey, i.e. krill, fish and squid (Pütz et al., 2013). In contrast, ΣPCBs occurred in greater concentrations than HCB in Magellanic penguins (*Spheniscus magellanicus*) from Chile, Uruguay and Brazil (Baldassin et al., 2016; Table S4), which are known to feed particularly on anchovy (Forero et al., 2002). Another pelagic-feeding seabird, the wandering albatross (*Diomedea exulans*) breeding at the Crozet Islands, showed comparable plasma HCB concentrations (Carravieri et al., 2014) as we found in southern rockhopper penguins (see Table S4). ΣPCBs, ΣDDTs, HCB and ΣPBDEs in whole blood and whole eggs of Adélie, chinstrap and gentoo penguins (*Pygoscelis adeliae*, *P. antarctica* and *P. papua*, respectively) from King George Island (Antarctic Peninsula) were substantially greater than observed in our study in female plasma and egg yolk (Corsolini et al., 2007; Yogui and Sericano, 2009; Mello et al., 2016; see Table S4). The diet of Pygoscelid penguins along the Antarctic peninsula is dominated by euphausiids (especially for chinstrap penguins) and myctophid fishes, with a lower proportion of squid (Ratcliffe and Trathan, 2012). They therefore feed on a similar trophic level as rockhopper penguins, and although the latter take

Table 3
Correlations between pollutant concentrations in female plasma during the pre-laying period, at A-egg laying and B-egg laying and their A- and B-eggs as well as correlations between pollutant concentrations in A- and B-eggs within clutches. N = 17 for A- and B-eggs and the pre-laying period (df = 15), N = 13 for A-egg and B-egg laying dates (df = 11).

	Females	Pearson's R	P-value
Pre-laying period			
A-eggs	ΣPCBs	0.026	0.921
	ΣDDTs	0.202	0.438
	ΣCHLs	0.202	0.435
	HCB	0.289	0.256
	ΣHCHs	−0.086	0.744
	ΣPBDEs	0.498	**0.042**
	ΣMeO-PBDEs	0.785	**<0.001**
B-eggs	ΣPCBs	0.053	0.843
	ΣDDTs	0.114	0.662
	ΣCHLs	0.508	0.846
	HCB	0.170	0.514
	ΣHCHs	−0.039	0.883
	ΣPBDEs	0.017	0.947
	ΣMeO-PBDEs	0.788	**<0.001**
A-egg laying date			
A-eggs	ΣPCBs	0.579	**0.038**
	ΣDDTs	0.315	0.295
	ΣCHLs	0.385	0.194
	HCB	0.707	**0.007**
	ΣPBDEs	−0.233	0.444
	ΣMeO-PBDEs	0.867	**<0.001**
B-eggs	ΣPCBs	0.557	**0.048**
	ΣDDTs	0.133	0.663
	ΣCHLs	0.457	0.116
	HCB	0.526	0.065
	ΣPBDEs	0.438	0.134
	ΣMeO-PBDEs	0.812	**<0.001**
B-egg laying date			
A-eggs	ΣPCBs	0.257	0.397
	ΣDDTs	0.374	0.209
	ΣCHLs	0.410	0.165
	HCB	0.521	0.068
	ΣHCHs	0.500	0.082
	ΣPBDEs	0.222	0.467
	ΣMeO-PBDEs	0.443	0.129
B-eggs	ΣPCBs	0.186	0.542
	ΣDDTs	0.053	0.864
	ΣCHLs	0.243	0.423
	HCB	0.378	0.202
	ΣHCHs	−0.165	0.591
	ΣPBDEs	0.008	0.980
	ΣMeO-PBDEs	0.418	0.155
	A-eggs	**Pearson's R**	**P-value**
B-eggs	ΣPCBs	0.796	**<0.001**
	ΣDDTs	0.701	**0.002**
	ΣCHLs	0.885	**<0.001**
	HCB	0.814	**<0.001**
	ΣHCHs	−0.058	0.826
	ΣPBDEs	0.303	0.237
	ΣMeO-PBDEs	0.985	**<0.001**

proportionately more squid (Pütz et al., 2013), dietary differences seem unlikely to explain the differences in OHC concentrations. More probable, the greater pollutant concentrations in the three Pygoscelid species reflect the larger pollutant accumulation at higher latitudes (Dachs et al., 2002) compared to the sub-Antarctic Falkland Islands or – especially for PBDEs – local pollutant patterns (Choi et al., 2008; Wild et al., 2015). Indeed, at the sub-Antarctic South Shetland Islands, ΣPBDEs in whole blood of southern giant petrels (*Macronectes giganteus*), an Antarctic top predator which one might have expected to bio-accumulate this compound, were lower compared to concentrations in southern rockhopper penguins, while ΣPCBs, ΣDDTs, ΣCHLs and HCB were higher (Colabuono et al., 2016; Table S4). Similarly, at the Crozet Islands, plasma ΣPBDEs in wandering albatrosses (Carravieri et al., 2014) were lower than in southern rockhopper penguins (Table S4), presumably because this location and the albatrosses' foraging areas in the Southern Indian Ocean are less contaminated with PBDEs than the Falkland Islands and surroundings.

To date, few studies on seabirds have examined MeO-PBDEs. Arctic female glaucous gulls (*Larus hyperboreus*) had on average > 3-times greater plasma ΣMeO-PBDEs than female southern rockhopper penguins (Verreault et al., 2007, 2008), yet these studies also included several more MeO-BDEs congeners (6 and 15, respectively, while our study was based on 2; see Table S4). In contrast, ΣMeO-PBDEs in guillemot (*Uria aalge*) tissues and eggs from the North Atlantic and the Baltic sea were lower (largely below the detection limit for the analysed 7 and 3 congeners, respectively; Sinkkonen et al., 2004; Jörundsdóttir et al., 2009) compared to rockhopper penguins (Table S4). In the Southern Hemisphere, Van den Steen et al. (2011) reported lower ΣMeO-PBDE concentrations in egg yolk of imperial shags (*Phalacrocorax atriceps*) compared to southern rockhopper penguins (Table S4), which could reflect differences between the benthic feeding shags versus pelagic feeding penguins.

We found higher ΣMeO-PBDEs compared to ΣPBDEs, which seems unusual for birds in the Arctic and industrial areas (Verreault et al., 2007, 2008; Zhang et al., 2010; Jaspers et al., 2013; Zhou et al., 2016; Table S4), but has been reported before in several species of whales (overview in Alonso et al., 2014). MeO-PBDEs in whale tissues are generally assumed to be from natural origin (Teuten et al., 2005; Alonso et al., 2014). The high concentration of MeO-PBDEs in southern rockhopper penguins compared to PBDEs also suggest a natural source and reflects the low anthropogenic pollution with PBDEs around the Falkland Islands. The significant correlation between ΣMeO-PBDEs and ΣPBDEs as well as among all other OHC compounds (including ΣPCBs and ΣOCPs) in female plasma during the pre-laying period and also in A-eggs suggests similar pathways for the uptake of all OHC compounds. The fact that correlations between ΣMeO-PBDEs and ΣPBDEs (and also among the other compounds) were not consistently significant in B-eggs and in female plasma during A-egg laying and B-egg laying might be explained by differential transfer of compounds into the eggs (see below).

4.2. Female pollutants, egg pollutants and changes with time

We found an increase in plasma ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣMeO-PBDEs from the pre-laying period to A-egg laying and a subsequent decrease in the same OHCs from A-egg laying to B-egg laying, while ΣPBDEs did not change over time. Few studies have investigated OHC concentrations in female birds during the egg laying period (Bargar et al., 2001; Verreault et al., 2006; Eng et al., 2013).

The few significant correlations found between female plasma OHC concentrations at pre-laying and OHC concentrations in eggs is slightly surprising, as yolk formation for B-eggs and entire egg-formation for A-eggs is finalised on the day of A-egg laying (Grau, 1982); one would expect a better match between egg and plasma OHC concentrations during the pre-laying period than at the A-egg or B-egg laying dates. One could argue that, as females during pre-laying were captured 5–13 days before laying their first egg, this time span and thus our sampling design may have affected our results for the pre-laying period. However, plasma OHC concentrations at pre-laying were significantly correlated with plasma OHC concentrations at A-egg laying, but not with the number of days between pre-laying capture and A-egg laying date (Table 4). Alternatively, plasma OHC concentrations at B-egg laying were not

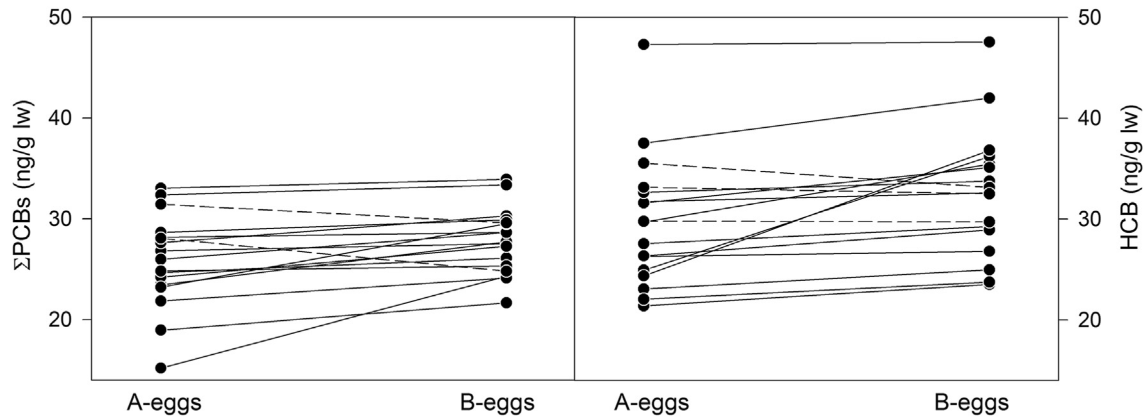

Fig. 2. Within-clutch variation of the sums of yolk ΣPCB (left side) and yolk HCB (right side) concentrations (in ng/g lw). Dashed lines represent the few clutches with decreasing concentrations between A- and B-eggs.

Table 4
Results of the linear models testing for the effects of average pollutant concentrations of the clutch (Clutch OHCs), female pollutant concentrations during pre-laying (Female pre-lay OHCs) and the number of days between pre-laying capture and A-egg laying capture (NDays) on female pollutant concentrations at A-egg laying. N = 13.

	Female pre-lay OHCs		Clutch OHCs		NDays	
	F-value	P-value	F-value	P-value	F-value	P-value
ΣPCBs	8.66	**0.016**	5.90	**0.038**	0.29	0.604
ΣDDTs	0.13	0.729	0.49	0.503	0.03	0.874
ΣCHLs	0.03	0.877	2.21	0.171	0.26	0.620
HCB	26.76	**<0.001**	9.41	**0.013**	2.01	0.190
ΣPBDEs	0.45	0.520	0.08	0.783	0.82	0.388
ΣMeO-PBDEs	24.15	**<0.001**	8.86	**0.012**	0.02	0.899

correlated with those at A-egg laying or with those in egg yolk. The latter contrasts previous findings (Verreault et al., 2006) that described a significant relation between plasma OHC concentrations at clutch completion and the eggs of these females in glaucous gulls. This suggests that the timing of maternal transfer of OHCs into eggs might be species-specific – potentially depending on whether females are fasting during egg formation (like in penguins) or not (like in gulls). Furthermore, the timing of maternal transfer may also vary among compounds within species, which would explain the strong correlations for ΣMeO-PBDEs between eggs and plasma during pre-laying, while such correlations for ΣPCBs and HCB were only present at A-egg laying date. This could be due to different transfer mechanisms from plasma to egg yolk, which likely depend on the chemical properties of the OHCs (e.g molecular structure, degree of halogenation, their affinity to fat tissues and therefore rate of bioaccumulation and release during fasting, or their affinity to tissue macromolecules such as VTG), as has been previously discussed (Bargar et al., 2001; Verreault et al., 2006). Different transfer mechanisms could also explain why the relative contribution of ΣPCBs and HCB to the entire OHC concentration in plasma compared to eggs differed by about 10% and 16%, respectively. Finally, maternal transfer of OHCs into eggs seems to be concentration-dependent (Eng et al., 2013). The latter might explain the general lack of significant results for PBDEs and HCHs for correlations among plasma and eggs as well as between A- and B-eggs in our study, while this was not the case in glaucous gulls, which showed much higher ΣPBDEs than we found (Verreault et al., 2006). Along these lines, PBDEs and HCHs not only showed low concentrations in both plasma and egg yolk, but also lower detection frequencies, and therefore higher measurement errors and associated uncertainty. This may have further affected our results and explain why we did not find correlations with PBDEs and HCHs.

Within clutches, ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣMeO-PBDEs correlated between A- and B-eggs and OHC concentrations were greater in B- than in A-eggs, refuting a previous analysis of laying-order effects in this species (Van den Steen et al., 2011). Despite the noticeable transfer of OHCs from plasma to the eggs, and the decline of plasma OHC concentrations in females from the A-egg to B-egg laying date, we could not find evidence that the OHC concentration deposited into their clutches reduced plasma post-laying pollutant concentrations. As such, females that laid eggs with higher pollutant concentrations, still had higher plasma concentrations of these OHCs at A-egg laying date (when the yolk of both eggs had been formed). Even more striking, plasma pollutant concentrations at B-egg laying date were neither related to clutch pollutant concentrations nor to plasma pollutant concentrations at A-egg laying date. As females remain fasting during the egg production and beyond B-egg laying, it remains an open question where OHCs that were circulating in female plasma at the time of A-egg laying were bound by the time B-eggs were laid. While Eng et al. (2013) also found a decline in female pollutant concentrations from laying date of the first egg to clutch completion, their results suggested an elimination pathway through egg laying, agreeing with previous studies (Tanabe et al., 1998; Donaldson and Braune, 1999).

4.3. Link between female VTG and OHC concentrations

Although female VTG concentrations showed a similar time trend as plasma ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣMeO-PBDEs, we could not – against our prediction – find a correlation between female VTG and any plasma OHCs. We only found a weak, but non-significant trend for ΣPCBs in both A- and B-eggs, and for HCB in A-eggs, respectively, to correlate with female VTG during the pre-laying period. Potentially, the lack of significant results is due to the (compared to the global scale) relatively low OHC concentrations in female southern rockhopper penguins, while documented effects of OHCs on VTG concentrations due to endocrine disruption (particularly in immature and male organisms) occurred in more polluted areas (Kime et al., 1999; Jiménez et al., 2007). We therefore do not entirely rule out the role of VTG in serving as a means of transport for OHC molecules (particularly PCBs and HCB) as hypothesized by Eng et al. (2013).

4.4. Conclusions

Southern rockhopper penguins show low to moderate exposure to OHCs, reflecting their remote and sub-Antarctic distribution range. Consistent with their highly pelagic overwinter migration behaviour, HCB was the dominant single compound, while ΣHCHs and ΣPBDEs showed the lowest concentrations, with the latter being remarkably lower than those of ΣMeO-PBDEs. As ΣMeO-PBDEs correlated with ΣPBDEs as well as all other analysed OHC compounds, they all appear to have similar pathways for uptake from the diet. We have reported for the first time the temporal pattern of OHC concentrations in female plasma from the pre-laying period to clutch completion, and of OHC concentrations within the corresponding clutches in free-living birds. Consistent with our expectations, we found significant correlations between ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣMeO-PBDEs between the two eggs of the clutch and between females and their eggs. For ΣHCHs and ΣPBDEs this was not the case, potentially due to low concentrations. The fact that egg yolk OHC concentrations (for ΣPCBs, HCB and ΣMeO-PBDEs) were only correlated with female plasma OHC concentrations at A-egg laying but not B-egg laying and only partly during the pre-laying period highlights that the timing of maternal transfer of OHCs into the eggs as well as the mechanism of maternal transfer is likely to differ among compounds. Furthermore, comparisons with existing literature suggest the timing of maternal transfer of OHCs to be species-specific. This requires attention for the design of future studies as sampling blood only once during the egg laying period may not be sufficient to link OHC concentrations in plasma and eggs. On the other hand, OHC concentrations in eggs are not suitable to assess OHC concentrations in females as some congeners and compound groups seem to accumulate relatively stronger in eggs than plasma (e.g. ΣPCBs, ΣDDTs and ΣMeO-PBDEs) while others (HCB) show the opposite pattern.

Finally, our results did not confirm the suggested role of VTG in the maternal transfer of OHC molecules into their eggs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.03.071.

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