



Individual variation in biomarkers of health: Influence of persistent organic pollutants in Great skuas (*Stercorarius skua*) breeding at different geographical locations[☆]

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ABSTRACT

Persistent organic pollutants (POPs) have been shown to cause adverse effects on a number of biomarkers of health in birds. POPs may impair immune function and alter the stress response, defined as a suite of behavioral and physiological responses to environmental perturbations. Recent studies have also proposed that POPs can induce oxidative stress. Nevertheless, there is a lack of studies simultaneously assessing the potential damaging effects of POPs on the latter biomarkers. In this study, we examined the contribution of legacy (organochlorines; (OCs)) and emerging (flame retardants; PBDEs) POPs to individual variations in stress levels (feather corticosterone), humoral immunity (plasma immunoglobulin Y levels) and oxidative stress occurring in three breeding colonies of a top predator seabird, the Great skua (*Stercorarius skua*), distributed from temperate regions to the high Arctic: Shetland (60°N), Iceland (63°N) and Bjørnøya (74°N). Our results demonstrated that plasma concentrations of OCs in Great skuas from Bjørnøya are among the highest in North Atlantic seabirds, with up to 7900 µg/kg (ww) ΣOCs. Yet, a latitudinal gradient in POP levels was observed with all compounds being significantly higher in Bjørnøya than in Iceland and Shetland (on average 4–7 fold higher for OCs and 2.5–4.5 for PBDEs, respectively). Contrary to our predictions, skuas breeding at the least contaminated site (i.e., Shetland) experienced the poorest physiological condition; i.e., the highest levels of stress hormones (25% higher) and oxidative stress (50% higher) and the lowest immunoglobulin levels (15% lower) compared to the two other colonies. Finally, our results failed to point out consistent within-colony relationships between biomarkers of health and POPs. Overall, it is suggested that other ecological factors such as food availability could constrain physiological indicators more than anthropogenic contaminants.

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1. Introduction

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCB) and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) are transported over long distances (long-range environmental transport) and have become global contaminants. Arctic ecosystems are among the areas most polluted by POPs (AMAP Assessment (2002); Wayland et al., 2010). Environmental contaminants may have strong negative effects on birds, and may cause endocrine disruption (Verreault et al., 2004), impairment of the immune system (also called immunotoxicity; Grasman et al.,

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1996) and induce oxidative stress (Hoffman, 2002; Henny et al., 2002; Fernie et al., 2005; Wayland et al., 2010). For example, pollutants have been shown to disrupt corticosterone (the major stress hormone in birds; Romero and Romero, 2002) in breeding Glaucous gulls (*Larus hyperboreus*); a rising exposure to POPs was associated with an increase in baseline plasma corticosterone levels in both sexes, but also a decrease in stress-induced plasma corticosterone levels in males (Verboven et al., 2010). Moreover, negative relationships between the contaminant burdens and diverse immune parameters have been found in Glaucous gulls (Bustnes et al., 2004) in which an experimental anti-parasite treatment alleviated the detrimental effects of POPs, suggesting interactive effects on the immune system (Bustnes et al., 2006). Finally, oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant compounds, is suggested to be the main proximate mechanism underlying degenerative processes such as aging in many species (Harman, 1956, 1972; Finkel and Holbrook, 2000). ROS are unstable and very reactive compounds that can induce damage to biomolecules such as lipids, proteins and DNA. The toxic effects of ROS can be buffered by endogenous (e.g. enzymes, glutathione) and exogenous (e.g. carotenoids, vitamin C) antioxidants (Surai, 2002). American kestrel chicks (*Falco sparverius*) dosed with a mixture of different congeners of polybrominated diphenyl ethers (PBDEs), structurally similar to PCBs, showed higher hepatic oxidative stress than control chicks (Fernie et al., 2005).

Although the latter studies have reported adverse effects of POPs on a number of biological markers, none of them has assessed these biomarkers simultaneously in the same species. Due to species-specific contamination exposure and detoxification (metabolism) pathways, POPs might not affect the species to the same extent (Fisk et al., 2001; Borgå et al., 2005). Yet, within-species studies could produce significant individual variability in biomarkers due to variations in contaminant exposure. In this context, the current study analyzed the individual variability in POPs and three important biomarkers of health (feather corticosterone levels, plasma immunoglobulin levels and oxidative stress) in a large top predator seabird, the Great skua (*Stercorarius skua*) breeding in three different colonies spread from temperate regions to the high Arctic: Shetland, UK (60°N), Iceland (63°N) and Bjørnøya (Bear Island), Svalbard (74°N), with potential different contaminant loading. More precisely, we examined the influence of both legacy (organochlorines) and emerging (PBDE) POPs and environmental factors (breeding site, body mass and red blood cell (RBC) stable isotope signature) on the latter biomarkers within each colony. Plasma concentrations of organochlorine compounds that have previously been associated with detrimental biological effects in gulls (Bustnes, 2006; Bustnes et al., 2005, 2008) as well as congeners of PBDEs (brominated flame retardants) were quantified. Moreover, we assessed avian stress by measuring feather corticosterone levels (Bortolotti et al., 2008). We used plasma immunoglobulin Y (IgY) levels as a measure of humoral immune function (Martinez et al., 2003), immunoglobulins being the most important serum proteins involved in humoral immune response in birds (Roitt et al., 1998). Finally, the balance between ROS production and antioxidant defences determines the extent of the oxidative stress, and in the current study we measured oxidative stress as the ratio between plasma total oxidant status and plasma total antioxidant status.

Based on a previous study comparing contaminant levels between Glaucous gulls and Great Black-backed gulls (*Larus marinus*) breeding in Bjørnøya and the Norwegian coast, respectively (Steffen et al., 2006), we firstly expected that there should be a latitudinal gradient in POP levels with birds from Bjørnøya exhibiting higher levels than birds from Iceland and Shetland, respectively. Based on this prediction, we hypothesized that with

increasing POP levels (between colonies): (i) feather corticosterone levels (stress levels) should be higher, (ii) IgY levels (humoral immunity) should be lower, and (iii) oxidative stress should be greater. In this context, our main objective was to examine the relative contribution of POPs and environmental factors (breeding site, body mass and stable isotopes) to individual variability in biomarkers of health within each colony. We also predicted that contaminant–biomarker relationships within each colony should be consistent between colonies.

2. Materials and methods

2.1. Study species

Great skuas (*S. skua*) are large top predators with a female-biased sexual size dimorphism that breed in the North-East Atlantic. Females lay up to two eggs and undertake most of the incubation although both sexes contribute. Chicks fledge about 40 days after hatching.

2.2. Study sites and sampling protocol

In 2009, a total of 161 incubating adult birds were caught in three different breeding colonies: in Bjørnøya, Svalbard (74°21'N, 19°05'E) ($N=51$), in south east Iceland (Öræfi, 63°57'N, 16°24'W) ($N=59$) and in Foula, Shetland (60°08'N, 2°05'W) ($N=51$) (Fig. 1). Each colony varied in size with approximately 350 breeding pairs in Bjørnøya (Strøm, 2007), 1500 in Iceland (Lund-Hansen and Lange, 1991) and 2300 in Foula (Mitchell et al., 2004). Birds were ringed with both metal and color plastic rings. Prior to catching, nests were checked on average every other day to determine the laying date and initial clutch size which were alternatively determined from hatching or egg measurements. The time into incubation (in days), at which birds were caught was subsequently calculated using both laying and hatching dates whenever known. Adult birds of both sexes were caught on their nest while incubating using remote controlled noose traps, under appropriate licences. At each capture, birds were blood sampled from the brachial or tarsal vein using heparinized syringes (under appropriate national licences in each location), body mass (± 0.1 g) and wing length were recorded and the left wing's primary feather 8 was cut and stored in individual sealed plastic bags at ambient temperature. Blood was collected in heparinized syringes within 5 min following capture and immediately transferred into Cornic tubes that were stored on ice, centrifuged within 2 h (5000 rpm), with plasma and RBCs frozen and stored at -20 °C. Plasma samples were subsequently used to assess organochlorine and brominated flame retardant concentrations, oxidative stress and immunoglobulin levels. RBCs were used to measure nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) stable isotopes. Birds were sexed from RBCs or feather pulp after DNA extraction and PCR amplification of CHD genes using primers 2550F (Fridolfsson and Ellegren, 1999) and 2757R (R. Griffiths, pers. comm.).



Fig. 1. Geographic coordinates of the three breeding colonies of Great skuas (*Stercorarius skua*): Bjørnøya, Iceland and Shetland (Foula).

2.3. Blood and feather analyses

2.3.1. Plasma organochlorines and brominated flame retardants and RBC stable isotopes

Plasma POP analyses (wet weight concentrations) and analysis of RBC stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) were performed at the Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada. See [Leat et al. \(2011\)](#) and references therein for details on the analyses. For each batch of six organochlorine samples, a reference homogenate, method blank, external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard, New Haven, CT, USA), OC standards and PCB 30 recovery standard were analyzed. Recoveries of PCB 30 in samples averaged ($\pm$ SD) $69.97 \pm 9.43\%$. Recoveries of individual PCB congeners in the inhouse reference tissue extracted with each batch of samples were within two standard deviations of the mean laboratory database value derived from laboratory control charts from GLIER accredited organic analytical laboratory (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified) established by standard cold column extraction techniques. For each batch of PBDE samples extracted, the sample injection sequences were set in the following manner: 5 external standard calibration curve for PBDEs (Wellington Laboratories certified PBDE native mixture), internal recovery standard, sample blank, internal reference homogenate (GLIER Detroit River Fish pool) and six samples. Fifty-one organochlorine compounds were measured among which 35 congeners of polychlorinated biphenyls (PCBs), *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), hexachlorobenzene (HCB) and oxychlordane while eight congeners of polybrominated diphenyl ethers (PBDEs) were measured (see detailed lists in [Tables A.1 and A.2](#) in Appendices).

2.3.2. Plasma immunoglobulin Y levels

Immunoglobulins are the most important serum proteins involved in humoral immune response in birds ([Roitt et al., 1998](#)). Plasma immunoglobulin Y (IgY) was assessed using a sensitive enzyme-linked immune absorbent assay (ELISA) method. Commercial antibodies were used as reported by [Martinez et al. \(2003\)](#). We adapted this method for Great skua adults by determining the appropriate plasma dilution (1/16,000). IgY levels were expressed in units of absorbance (Victor³ multilabel plate reader, Perkin Elmer, Turku, Finland).

2.3.3. Plasma oxidative stress

Plasma total antioxidant status (TAS) was measured using a commercial kit (TAS assay kit, RL0017, Rel Assay Diagnostics, Gaziantep, Turkey) and an automated biochemical analyzer (Cobas c 111 analyzer, Roche Diagnostics). TAS assesses the non-enzymatic antioxidants (of both dietary and endogenous sources) present in the plasma sample. The reaction rate is calibrated with Trolox, a widely used standard for TAS measurement assays and the assay results are expressed in mmol Trolox equivalent L^{-1} in reference to a standard curve.

Plasma total oxidant status (TOS) was measured using a commercial kit (TOS assay kit, RL0024, Rel Assay Diagnostics, Gaziantep, Turkey) and an automated biochemical analyzer (Cobas c 111 analyzer, Roche Diagnostics). TOS assesses both hydrogen peroxide components (that can break down to produce reactive pro-oxidants and can also be indicators of the superoxide dismutase activity) and lipid hydroperoxides (markers of damage to lipids) ([Erel, 2005](#)). The assay is calibrated with hydrogen peroxide (H_2O_2) and the results are expressed in $\mu\text{mol H}_2\text{O}_2$ equivalent L^{-1} in reference to a standard curve.

The oxidative stress index (OSI) was calculated as the ratio between total oxidant status and total antioxidant status and was expressed in arbitrary units. High ratio values reflect high plasma oxidative stress.

2.3.4. Feather corticosterone

This novel non invasive technique allows a long-term, integrated measure of stress ([Bortolotti et al., 2008](#)) as a cumulative response to both natural and anthropogenic stressors. We used the distal 15 cm of primary feather number 8 for the assay. Feather corticosterone was first extracted using a methanol-based extraction technique as reported by [Bortolotti et al. \(2008\)](#). The extracts were subsequently assayed for corticosterone using an enzyme immunoassay kit (900-097, Assay Designs Inc., USA). Intra-assay variability was 9.3% ($N=16$ samples) and inter-assay variability was 13.3% ($N=6$ samples). Feather corticosterone was expressed in pg/mm of feather.

2.4. Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics 19.0.0.1 (SPSS Inc., Chicago, IL, USA). Values are presented as means $\pm$ standard error (SE). Since not all variables were normally distributed (Kolmogorov–Smirnov test, $p < 0.05$), appropriate transformations were applied to meet parametric assumptions before parametric tests were used. Univariate linear models were first used to test for the effects of breeding site (Björnøya, Iceland or Shetland) and sex (fixed factors; full factorial model) on wing length, body mass, time into incubation, laying date, RBC stable isotopes, sum of plasma organochlorines and brominated flame retardants and biomarkers of health (dependent variables). Although time into incubation and laying date were significantly different between colonies, they were not significantly correlated to any of the POPs or biomarkers within each colony (using multiple linear regressions; $p > 0.05$ in all cases; data not shown) and therefore not included in the subsequent models.

Due to inter-correlations among the halogenated organic contaminants (HOC) and flame retardants (PBDEs), and to reduce the dimensionality of the HOC and PBDE datasets, principal components (PC) were extracted based on the $\log_{10}$ transformed concentrations of organochlorines and flame retardants, respectively within each colony. Originally 51 organochlorine compounds and 8 flame retardants were measured but only 42 and 5, respectively were used in the principal component analysis (those that were detected in more than 75% of the birds; [Tables A.1 and A.2](#)). Within each colony, we extracted two PCs to describe the organochlorine concentrations which together explained 70% of the total variance (see [Table B.1](#)) and one PC to describe the PBDE concentrations which explained at least 60% and up to 81% of the total variance (see [Table B.2](#)). These PC were referred to as “PC1-OC”, “PC2-OC” and “PC1-PBDE”. Generalized linear models (GLM) were used to test for the effects of various predictors (chosen using a stepwise model selection based on the lowest Akaike's information criterion value; see [Tables C and D](#)) on each biomarker of health (dependent variables) within each colony. Since body mass significantly differed between the sexes (see [Table 1](#); $r^2=0.47$), body mass and sex were confounded variables. As none of the biomarkers of health measured in the current study significantly differed between the sexes ([Table 2](#)), we used body mass as a predicting variable in the subsequent GLMs together with PC1-OC, PC2-OC, PC1-PBDE, RBC carbon and nitrogen stable isotopes.

Table 1

Biological profiles and measurement of red blood cell stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and plasma organochlorines (ΣOCs) and brominated flame retardants (ΣPBDEs) for Great skuas (*Stercorarius skua*) breeding in 3 different colonies: Björnøya, Iceland and Shetland. Values are means $\pm$ standard errors. The *F*- and *p*-values were calculated using univariate linear models.

Dependent variables	Björnøya	Iceland	Shetland	Location		Sex		Location $\times$ Sex	
				<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Wing length (mm)*	414.04 $\pm$ 4.34 ^a , $N=51$	418.25 $\pm$ 3.54 ^a , $N=59$	413.01 $\pm$ 3.96 ^a , $N=45$	0.55	0.58	2.14	0.14	0.63	0.54
Body mass (g)	1360.77 $\pm$ 13.91 ^a , $N=51$	1368.43 $\pm$ 11.34 ^a , $N=59$	1338.04 $\pm$ 12.70 ^a , $N=45$	1.66	0.19	128.72	< 0.0001	0.46	0.63
Time into incubation (days)	8.77 $\pm$ 1.14 ^a , $N=41$	16.66 $\pm$ 0.86 ^b , $N=55$	20.02 $\pm$ 0.93 ^c , $N=45$	29.54	< 0.0001	0.20	0.65	1.06	0.35
Laying date (julian days)*	166.58 $\pm$ 1.16 ^a , $N=41$	141.95 $\pm$ 0.87 ^b , $N=55$	148.55 $\pm$ 0.95 ^c , $N=45$	145.55	< 0.0001	0.54	0.46	0.40	0.67
	6/17/2009	5/24/2009	5/30/2009						
Red blood cell $\delta^{13}\text{C}$ (‰)	−19.07 $\pm$ 0.04 ^a , $N=51$	−18.45 $\pm$ 0.04 ^b , $N=59$	−18.48 $\pm$ 0.04 ^b , $N=51$	58.45	< 0.0001	0.12	0.72	0.19	0.82
Red blood cell $\delta^{15}\text{N}$ (‰)	14.64 $\pm$ 0.06 ^a , $N=51$	13.32 $\pm$ 0.05 ^b , $N=59$	12.69 $\pm$ 0.06 ^c , $N=51$	217.36	< 0.0001	1.25	0.26	0.21	0.81
ΣOCs^* ($\mu\text{g kg}^{-1}$ wet weight)	2651.75 $\pm$ 160.49 ^a , $N=51$	648.42 $\pm$ 130.81 ^b , $N=59$	345.14 $\pm$ 137.32 ^c , $N=51$	102.54	< 0.0001	4.79	0.03	0.31	0.73
ΣPBDEs^* ($\mu\text{g kg}^{-1}$ wet weight)	32.28 $\pm$ 4.41 ^a , $N=51$	12.89 $\pm$ 3.60 ^b , $N=59$	7.06 $\pm$ 3.78 ^c , $N=51$	14.79	< 0.0001	8.51	0.004	0.51	0.60

For each row, lowercase letters (a–c) indicate a significant difference between groups (Fisher's least significant difference post-hoc tests).

* Data were $\log_{10}$ transformed before statistical analysis.

Table 2
Statistical analysis of biomarkers of stress and health of Great skuas (*Stercorarius skua*) breeding in three different colonies: Bjørnøya, Iceland and Shetland. The *F*- and *p*-values were calculated using univariate linear models.

Biomarkers of health	Location		Sex		Location × sex	
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Feather corticosterone (pg/mm) ^a	5.51	0.005	0.37	0.54	0.71	0.49
Immunoglobulin Y levels (absorbance units)	4.04	0.02	0.29	0.59	2.33	0.10
Total antioxidant status (mM eq Trolox L ⁻¹)	19.51	< 0.0001	1.18	0.28	0.40	0.67
Total oxidant status (μM eq H ₂ O ₂ L ⁻¹) ^a	20.02	< 0.0001	0.03	0.86	0.07	0.93
Oxidative stress index (arbitrary units) ^a	19.28	< 0.0001	0.57	0.45	0.02	0.98

^a Data were log₁₀ transformed before statistical analysis.

3. Results

3.1. Variations between colonies

3.1.1. Biological profiles

There was no significant difference in wing length or body mass in incubating adults at the three different breeding sites (Table 1). As expected, we found sex differences in body mass with females being on average 11% heavier than males, independent of the colony (females' body mass: 1440 ± 8 g, *N*=114; males' body mass: 1280 ± 11 g, *N*=41). Moreover, there were significant differences between colonies in time into incubation (time elapsed since laying when the birds were caught on their nest) and laying date (Table 1). Indeed, birds started to lay eggs up to 3 weeks later in Bjørnøya than in the other two colonies, which also differed by a week from each other for laying date (Table 1). Finally, there were significant differences in both RBC carbon (δ¹³C) and nitrogen (δ¹⁵N) stable isotopes between colonies (Table 1).

3.1.2. POP concentrations

The sum of organochlorines (ΣOCs) and the sum of brominated flame retardants (ΣPBDEs) significantly differed between colonies with birds breeding in Bjørnøya showing plasma levels significantly higher than birds breeding in Iceland (by a factor of 4 × for OCs and 2.5 × for PBDEs) and Shetland (by a factor of 7 × for OCs and 4.5 × for PBDEs) (Table 1). Moreover, males had plasma POP concentrations significantly higher than females (on average 20% for OCs and 45% for PBDEs), independent of their colony of origin (Table 1).

3.1.3. Biomarkers of health

While all the biomarkers of health assessed in the current study showed no differences between males and females, there were significant differences between colonies (Table 2). Namely, feather corticosterone levels were significantly higher (by 25%) in Shetland compared to Bjørnøya and Iceland which did not differ significantly from each other (Fig. 2A). On the other hand, immunoglobulin levels were significantly lower (by 15%) in Shetland than in the other two colonies (Fig. 2B). Total antioxidant status was significantly higher in Iceland (2.30 ± 0.08 mM equivalent Trolox L⁻¹, *N*=58) than in Bjørnøya (1.54 ± 0.10, *N*=50) and Shetland (1.74 ± 0.09, *N*=44) (Table 2), whereas total oxidant status was significantly higher in Shetland (15.27 ± 1.22 μM equivalent H₂O₂ L⁻¹, *N*=44) than in Bjørnøya and Iceland (7.05 ± 1.29, *N*=50 and 8.57 ± 1.11, *N*=58, respectively) (Table 2). As a result, the oxidative stress index was significantly 50% higher in Shetland (Fig. 2C) while there were no significant relationships between TAS and TOS in any of the colonies (simple linear regressions, *p* > 0.80 in all cases) suggesting that changes in one component do not predict changes in the other. Likewise, there were no significant

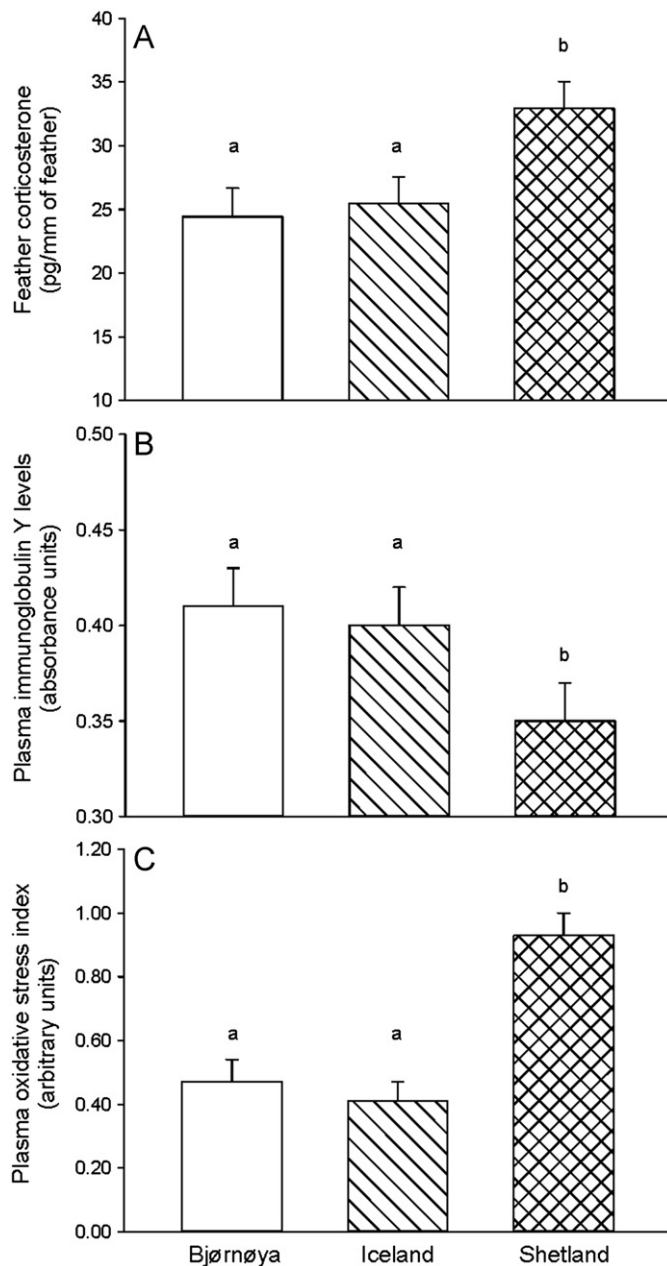


Fig. 2. Biomarkers of health: (A) feather corticosterone, (B) plasma immunoglobulin Y levels and, (C) plasma oxidative stress index in Great skuas (*Stercorarius skua*) breeding in three different colonies: Bjørnøya, Iceland and Shetland. Values are means ± standard errors. Lower case letters (a–b) indicate a significant difference between groups (Fisher's least significant difference post-hoc tests).

relationships between any of the biomarkers of health within each colony (linear regression, $p > 0.05$ in all cases; data not shown).

3.2. Factors affecting biomarkers of health within each colony

3.2.1. Feather corticosterone

Although birds breeding in Shetland had significantly higher feather corticosterone levels than birds breeding in Bjørnøya and Iceland, none of the selected predicting variables contributed to significantly explain the variations in feather corticosterone within each colony (Table 3A) except for PC1-PBDE in Iceland where increasing PC1-PBDE scores (i.e. increasing PBDE concentrations) were associated with declining feather corticosterone levels (Table 4A).

3.2.2. Immunoglobulin Y levels

Body mass was the only factor that positively explained the variations in immunoglobulin levels, but in Iceland only (Table 3B) with heavier birds showing higher IgY levels.

3.2.3. Oxidative stress

Total antioxidant status (TAS) was significantly and positively influenced by body mass in Iceland and Shetland suggesting that heavier birds showed higher plasma TAS in these colonies (Table 3C). In addition, there was a significant positive relationship between TAS and PC1-OC in Iceland and between TAS and PC2-OC in Bjørnøya (Table 3C). Since PC1-OC was loaded by higher chlorinated PCBs and persistent metabolites whereas PC2-OC was mainly loaded by lower chlorinated PCBs and organochlorine pesticides in all colonies, plasma TAS therefore increased with increasing levels of persistent and highly chlorinated PCBs, chlorinated pesticides and metabolites (i.e. increasing PC1-OC scores) in Iceland and with increasing levels of less persistent chlorinated pesticides and lower chlorinated PCBs

(i.e. increasing PC2-OC scores) in Bjørnøya. Finally, there was a positive relationship between TAS and RBC $\delta^{13}\text{C}$ values in Iceland (Table 3C; Fig. 3) suggesting that birds feeding on a diet enriched in $\delta^{13}\text{C}$ had higher plasma TAS in this colony.

Total oxidant status (TOS) was significantly and negatively correlated with PC1-OC and PC1-PBDE in Bjørnøya and Iceland suggesting that plasma TOS decreased with increasing levels of persistent PCBs, pesticides, metabolites and PBDEs (i.e. increasing PC1-OC and PC1-PBDE scores) in these colonies (Tables 3D and 4D). Likewise, oxidative stress index (OSI) decreased with increasing PC1-OC and PC1-PBDE scores in Bjørnøya and Iceland (Tables 3E and 4E). Finally, there was a negative correlation between OSI and RBC $\delta^{13}\text{C}$ values in Iceland (Tables 3E and 4E) implying that birds with higher $\delta^{13}\text{C}$ values experienced lower oxidative stress.

4. Discussion

In this study, at the interface between ecology and physiology, we examined physiological proxies that may provide information about the mechanisms of toxicity of POPs by measuring the health status of breeding birds exposed to various degrees of 'natural' contamination. To do so, we assessed markers of stress, immunity and oxidative stress within a single species, the Great skua, breeding in separate colonies along a latitudinal gradient (from temperate regions to the high Arctic) with apparent difference in exposure to POPs. In agreement with our expectations, the plasma POP concentrations (organochlorines and PBDEs) were significantly higher in Bjørnøya than in Iceland and Shetland. Yet, Shetland appeared to be the colony where the birds experienced the poorest physiological condition (see Fig. 4 for an overview of the results). Moreover, the few significant within-colony relationships observed between POP levels and biomarkers of health were not consistent between colonies.

Table 3

Factors predicting the inter-individual variations of (A) feather corticosterone, (B) plasma immunoglobulins, (C) plasma total antioxidant status, (D) plasma total oxidant status and, (E) plasma oxidative stress index within each breeding colony (Bjørnøya, Iceland and Shetland) using generalized linear models. The predicting variables presented (first and second principal component scores of organochlorines (PC1-OC and PC2-OC), red blood cell carbon and nitrogen stable isotopes (RBC $\delta^{13}\text{C}$ and RBC $\delta^{15}\text{N}$) and body mass) were chosen using a model selection based on the lowest Akaike information criterion. Numbers in bold indicate significant p -values ($p < 0.05$). SE, standard error.

Biomarkers of health (dependent variables)	Location	Predicting variables	df	Wald Chi square	p -value	Parameter estimate	SE
(A) Feather corticosterone*	Bjørnøya (N=50)	RBC $\delta^{13}\text{C}$	1	0.634	0.426	0.088	0.111
	Iceland (N=53)	PC1-OC	1	3.072	0.080	-0.04	0.023
	Shetland (N=41)	PC2-OC	1	2.599	0.107	-0.047	0.029
(B) Immunoglobulin Y levels	Bjørnøya (N=49)	RBC $\delta^{15}\text{N}$	1	2.420	0.120	-0.06	0.037
	Iceland (N=57)	Body mass	1	5.525	0.019	0.000	0.000
	Shetland (N=40)	RBC $\delta^{13}\text{C}$	1	0.739	0.390	0.049	0.057
(C) Total antioxidant status	Bjørnøya (N=50)	PC2-OC	1	6.058	0.014	0.163	0.066
		RBC $\delta^{15}\text{N}$	1	3.521	0.061	0.323	0.172
	Iceland (N=58)	Body mass	1	4.691	0.030	0.001	0.001
		PC1-OC	1	4.125	0.042	0.170	0.084
		RBC $\delta^{13}\text{C}$	1	10.088	0.001	1.071	0.337
	Shetland (N=44)	Body mass	1	6.285	0.012	0.002	0.001
(D) Total oxidant status*	Bjørnøya (N=50)	PC1-OC	1	5.325	0.021	-0.086	0.037
		RBC $\delta^{13}\text{C}$	1	2.506	0.113	0.233	0.147
	Iceland (N=58)	Body mass	1	0.315	0.574	0.000	0.000
		PC1-OC	1	4.352	0.037	-0.046	0.022
	Shetland (N=44)	PC2-OC	1	0.671	0.413	-0.034	0.012
(E) Oxidative stress index*	Bjørnøya (N=50)	PC1-OC	1	4.084	0.043	-0.076	0.038
		PC2-OC	1	2.929	0.087	-0.065	0.038
	Iceland (N=58)	PC1-OC	1	7.950	0.005	-0.078	0.028
		RBC $\delta^{13}\text{C}$	1	4.441	0.035	-0.238	0.113
	Shetland (N=44)	PC2-OC	1	1.206	0.272	-0.050	0.045

* Data were $\log_{10}$ transformed before statistical analysis.

Table 4

Factors predicting the inter-individual variations of (A) feather corticosterone, (B) plasma immunoglobulins, (C) plasma total antioxidant status, (D) plasma total oxidant status and, (E) plasma oxidative stress index within each breeding colony (Bjørnøya, Iceland and Shetland) using generalized linear models. The predicting variables presented (first principal component score of flame retardants (PC1-PBDE), red blood cell carbon and nitrogen stable isotopes (RBC $\delta^{13}\text{C}$ and RBC $\delta^{15}\text{N}$) and body mass) were chosen using a model selection based on the lowest Akaike information criterion. Numbers in bold indicate significant p-values ($p < 0.05$). SE, standard error.

Biomarkers of health (dependent variables)	Location	Predicting variables	df	Wald Chi square	p-value	Parameter estimate	SE
(A) Feather corticosterone*	Bjørnøya (N=50)	RBC $\delta^{13}\text{C}$	1	0.634	0.426	0.088	0.111
	Iceland (N=53)	PC1-PBDE	1	7.387	0.007	-0.060	0.022
	Shetland (N=41)	RBC $\delta^{15}\text{N}$	1	0.456	0.500	-0.041	0.061
(B) Immunoglobulin Y levels	Bjørnøya (N=49)	RBC $\delta^{15}\text{N}$	1	2.420	0.120	-0.06	0.037
	Iceland (N=57)	Body mass	1	5.525	0.019	0.000	0.000
	Shetland (N=40)	PC1-PBDE	1	2.252	0.133	0.027	0.018
(C) Total antioxidant status	Bjørnøya (N=50)	Body mass	1	1.091	0.296	0.001	0.000
	Iceland (N=58)	Body mass	1	3.314	0.069	0.001	0.001
		RBC $\delta^{13}\text{C}$	1	6.986	0.008	0.890	0.337
	Shetland (N=44)	Body mass	1	6.285	0.012	0.002	0.001
(D) Total oxidant status*	Bjørnøya (N=50)	PC1-PBDE	1	5.213	0.022	-0.081	0.036
	Iceland (N=58)	PC1-PBDE	1	7.977	0.005	-0.061	0.022
	Shetland (N=44)	RBC $\delta^{15}\text{N}$	1	0.695	0.405	-0.068	0.082
(E) Oxidative stress index*	Bjørnøya (N=50)	PC1-PBDE	1	7.167	0.007	-0.101	0.038
	Iceland (N=58)	PC1-PBDE	1	6.338	0.012	-0.072	0.029
		RBC $\delta^{13}\text{C}$	1	4.263	0.039	-0.238	0.115
	Shetland (N=44)	Body mass	1	0.599	0.439	-0.000	0.000

* Data were $\log_{10}$ transformed before statistical analysis.

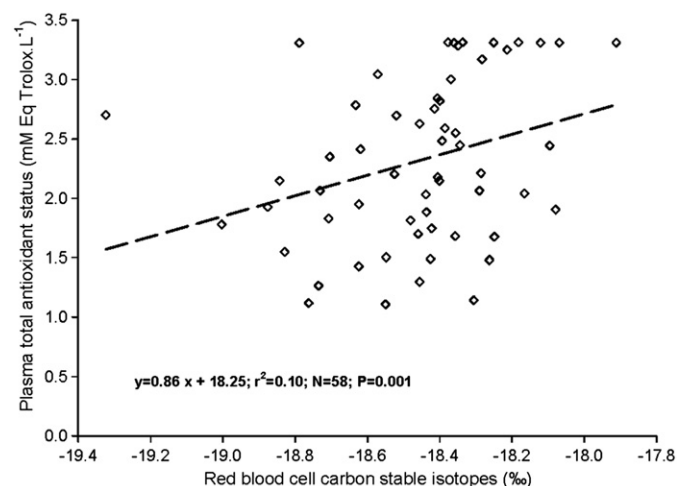


Fig. 3. Relationship between plasma total antioxidant status and red blood cell carbon stable isotope values in Great skuas (*Stercorarius skua*) breeding in Iceland.

4.1. Variations in POPs, stable isotopes and biomarkers of health between colonies

Firstly, we observed that the organochlorine plasma levels found in the Great skuas breeding in Bjørnøya were up to three times higher compared to other high trophic level birds like the Glaucous gulls from Bjørnøya (Verreault et al., 2010). Yet, there were large significant differences in pollutant levels between colonies with Bjørnøya exhibiting the highest organochlorine and PBDE plasma concentrations compared to Iceland and Shetland. Similarly to what was observed in other seabird species such as Glaucous gull, Great Black-backed gull and South polar skua (*Catharacta maccormicki*; Bustnes et al., 2003, 2007, 2008), Great skuas also showed significant sex differences in OC and PBDE levels with males having significantly higher levels than females. Regardless of sex, all contaminants measured were significantly higher in Bjørnøya (on average 4–7 fold higher for OCs and 2.5–4.5 for PBDEs) than in Iceland and Shetland, respectively. This latitudinal gradient in POP levels might be

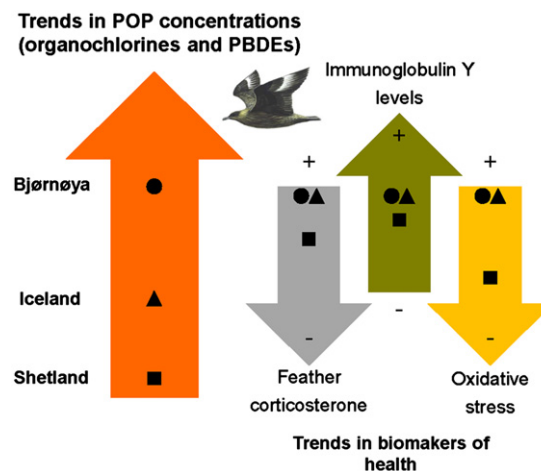


Fig. 4. Schematic trends in persistent organic pollutants (organochlorines and PBDEs) and biomarkers of health (feather corticosterone, plasma immunoglobulin Y levels and plasma oxidative stress index) in Great skuas (*Stercorarius skua*) breeding in three different colonies: Bjørnøya (filled circle), Iceland (filled triangle) and Shetland (filled square). The plus signs (+) indicate the most favorable values for biomarkers while the minus signs (–) indicate the least favorable values.

imputable to differences in diet i.e. trophic levels between colonies on their breeding grounds. Accordingly, the analysis of regurgitated pellets provided evidence supporting that the diet during the breeding season of Great skuas in Bjørnøya was mainly composed of birds (80% of pellets; Knutsen, 2010), whereas fish accounted for 70% and 60% of the pellets in Iceland and Shetland, respectively (Eliza H.K. Leat, unpublished data). Moreover, although the comparison of stable isotopes between colonies alone is not sufficient to interpret potential trophic differences between individuals breeding at different sites since the baseline isotopic values were not investigated in the current study (Cherel and Hobson, 2007), the significant differences observed between colonies tend to corroborate the assumption that the Bjørnøya skuas might feed at a higher trophic level than the skuas further south. Nonetheless, the wintering areas of these birds, determined using geolocation data loggers could also influence plasma POP levels measured during summer as the birds of the current

study were shown to winter in distinct and non-overlapping areas depending on their breeding colony (Magnusdottir et al., 2012).

Similarly to the contaminant levels, all the biomarkers of health varied significantly among colonies (see below) although no sex differences were observed for any of the biomarkers further implying that they might not be related to sex and/or body mass (which differs between the sexes) in Great skuas.

4.2. Within-colony variations in biomarkers of health in relation to organochlorines, flame retardants, body mass and stable isotopes

In order to highlight potential causal relationships, we statistically investigated what environmental variables predicted best the inter-individual variations of each biomarker of health within each colony.

4.2.1. Feather corticosterone

Feather corticosterone was only affected (negatively) by plasma PBDE in Iceland while it was not affected by any of the other parameters tested (organochlorines, body mass and stable isotopes) in any of the colonies. The assessment of feather corticosterone represents a long-term, integrated measure of avian stress physiology (Bortolotti et al., 2008); i.e. corticosterone deposited into feathers as they grow is assumed to mirror both baseline and stress-induced levels (Bortolotti et al., 2008; Lattin et al., 2011). Therefore, one cannot predict to what extent baseline and stress-induced levels can be mutually affected. Either way, because the primary flight feathers used in the current study molt during winter (Thompson et al., 1998) and because there is no evidence for the presence of corticosterone in preen oil further limiting an external transfer on this hormone to feathers (Lattin et al., 2011), our measure of feather corticosterone may therefore reflect the stress levels experienced by birds during winter. The different time scales at which feather corticosterone and plasma POP concentrations were collected might therefore explain the lack of consistent relationships between the latter two parameters among colonies. Yet, feather corticosterone levels were significantly higher by 25% in Shetland compared to the two other colonies. This might indicate that birds breeding in Shetland suffered higher stress levels than birds breeding in Bjørnøya and Iceland during winter. Accordingly, geolocation loggers deployed on some of the birds included in this study showed that Bjørnøya and Iceland birds have somewhat overlapping wintering areas while the birds breeding in Shetland have more distinct wintering areas (Magnusdottir et al., 2012). Even if winter conditions were to be more stressful (for many potential reasons) for the Shetland birds, it does not necessarily imply that these birds would suffer high stress levels during the following breeding season although one can only assume that poor winter conditions could predispose birds to poor breeding conditions.

4.2.2. Plasma immunoglobulin levels

Plasma immunoglobulin levels (IgY) were significantly lower in Shetland compared to the two other colonies suggesting a lower immune capacity in the least polluted area. More importantly, we did not find any significant relationship between IgY and organochlorines or PBDEs in any of the colonies. These results are not in line with the immuno-suppressive effect of POPs proposed in many species (Vos and Luster, 1989) although other studies have provided contrasting results regarding the immunotoxicity of various environmental stressors. For example, female American kestrels experimentally exposed to PCBs exhibited a higher humoral immune response to a non-pathogenic antigen while males showed a decrease in their response (Smits and Bortolotti, 2001). The opposite pattern was observed in free-living

Pied flycatchers exposed to heavy metal pollution with males showing a stronger humoral response to tetanus toxoid while females' response was not altered (Eeva et al., 2005).

4.2.3. Oxidative stress

We only observed significant relationships between oxidative stress parameters and POPs (organochlorines and PBDEs) in two out of three colonies (Bjørnøya and Iceland). However, contrary to our predictions, our results indicated that plasma TAS increased with increasing organochlorine concentrations whereas plasma TOS decreased with increasing organochlorine and PBDE concentrations. These unforeseen results could reflect the activation of the endogenous antioxidant machinery to reduce oxidative damage induced by increasing plasma POP levels. For example, in two seabird species the expression of cytochrome P450 1A (enzyme involved in detoxification reactions) was significantly higher in birds from a formerly oil spilled area compared to birds from uniled areas (Trust et al., 2000). Nevertheless, in the current study supporting evidence for such a scenario (activation of the antioxidant machinery) could be a positive relationship between TAS and TOS (reflecting the increased production of antioxidants in response to higher oxidative damage) that was not reported in any of the colonies. Alternatively, the oxidative balance has been hypothesized to be influenced by the quality of birds' diet which is closely related to their habitat quality and foraging capacity (Costantini, 2010; van de Crommenacker et al., 2011). For example, van de Crommenacker et al. (2011) demonstrated in the Seychelles warblers (*Acrocephalus sechellensis*) that oxidative status can be influenced by variations in territory quality (assessed through spatio-temporal variations in food availability) with oxidative stress being greater in birds occupying low quality territories. In Iceland, where TAS was significantly higher compared to the two other colonies, RBC $\delta^{13}\text{C}$ values were positively correlated to TAS (Fig. 2) and negatively to oxidative stress index (OSI). Accordingly, Shchepinov (2007) suggested that a higher $\delta^{13}\text{C}$ intake would make biomolecules incorporating higher isotopes less susceptible to oxidative damage. On the contrary, a significant positive relationship was evidenced between $\delta^{13}\text{C}$ values and oxidative damage in Adelie penguins (*Pygoscelis adeliae*) with higher $\delta^{13}\text{C}$ values being associated to greater oxidative damage (Beaulieu et al., 2010). In the current study, TOS was not affected by RBC $\delta^{13}\text{C}$ values in Iceland nor in any of the colonies. Moreover, while TOS was significantly higher in birds breeding in Shetland resulting in significantly higher oxidative stress index in this colony compared to the other two, RBC $\delta^{13}\text{C}$ values did not significantly explain the variations in any of the oxidative stress parameters in this colony.

4.3. Alternative scenarios

There are alternative scenarios that may account for both the unexpected relationships between POP levels and biomarkers of health and the lack of consistent relationships between colonies. First, the biomarkers of health that were measured in the current study might not be influenced by organic pollutants. For example, heavy metals (mainly mercury) can also explain the variations of oxidative stress among colonies (reviewed in Koivula and Eeva, 2010). For example, heavy metal exposure was responsible for increased lipid peroxidation and higher hepatic enzyme levels in nestling Pied flycatchers (*Ficedula hypoleuca*; Berglund et al., 2007). Future studies should therefore investigate the spatial variations in mercury in relation to inter-colony variations in biomarkers of health. Secondly, regardless of pollutant levels, corticosterone was shown to be a modulator of both immunity and oxidative stress. Indeed, high corticosterone levels were

shown to suppress immunity (Råberg et al., 1998) and to increase oxidative stress (Costantini et al., 2008). Our results tend to support this physiological hypothesis with the Shetland birds showing the highest corticosterone levels, the highest oxidative stress index but the lowest immunoglobulin levels although there were no significant relationships between any of these parameters.

Finally, the differences in biomarkers among colonies might be more influenced by numerous ecological factors such as local climatic conditions, food resources (abundance and quality), predation pressure and/or population densities that can differ between colonies and act as confounding factors. Although ecological data on the Bjørnøya and Iceland populations are quite scarce, the population appears to have remained quite steady over the past decades in Iceland (Mitchell et al., 2004) and even increasing in Bjørnøya (Strøm, 2007). On the other hand, ecological factors have been quite extensively documented for the Shetland population. Long-term data collected on this Great skua population revealed declining numbers at the study colony in Foula since the late 1970s mainly attributed to a decrease in sandeel (*Ammodytes marinus*) availability (Hamer et al., 1991). Changes in food supply have been linked to a decrease in egg size (Hamer et al., 1991), a lower breeding success (Ratcliffe et al., 1998) and reduced adult survival (Hamer et al., 1991; Ratcliffe et al., 2002) in the Shetland population over the past 30 years. In addition to environmental pollutants, inter-colony variations in ecological factors such as qualitative change in the diet between populations might therefore play a non-negligible part in explaining variations in biomarkers of health and the lack of consistent relationships between biomarkers and POPs between colonies. For example, herring gull chicks (*Larus argentus*) experimentally exposed to PCBs and thereafter fasted showed a significant decrease in their catalase activity compared to control chicks (Hegseth et al., 2011). Future studies should assess the impact of food supplementation on biomarkers of health and contaminants in chicks whose pollutant levels are strictly limited to the sampling area (i.e. breeding site). Shetland appears to be the ideal colony to perform such an experimental manipulation since ecological factors seem stronger than in the other colonies.

5. Conclusions

In conclusion, Shetland, the least polluted colony of the three, appeared to be the colony where the birds experienced the least favorable physiological condition. Indeed, they suffered the highest feather corticosterone levels, the lowest immunoglobulin levels and the highest oxidative stress index of the three colonies. The latter results contradict our predictions that higher POP levels would translate into lower physiological status. Accordingly, our results did not allow highlighting any repeatable relationships between persistent organic pollutants and biomarkers of health between colonies which suggests that the influence of organic contaminants on the biomarkers of health could be context-dependent and that ecological factors such as food availability and/or climatic conditions (i.e. the ecological context) should be taken into account whenever inferring about the effects of contaminants.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at: <http://dx.doi.org/10.1016/j.freeradbiomed.2012.07.016>.

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