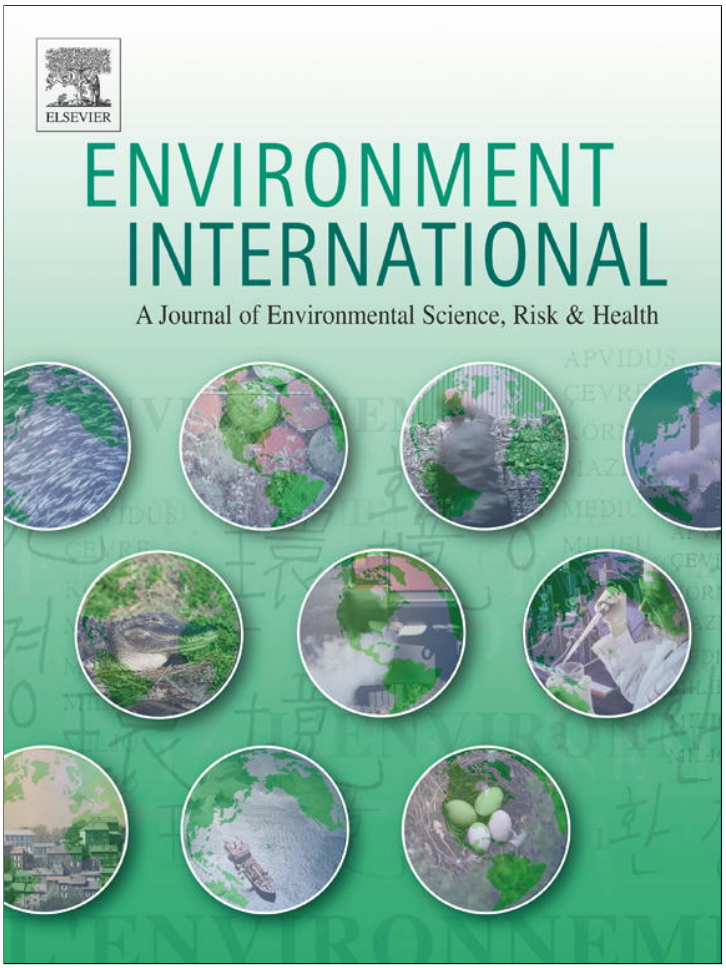

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Can starling eggs be useful as a biomonitoring tool to study organohalogenated contaminants on a worldwide scale?

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ABSTRACT

Large-scale international monitoring studies are important to assess emission patterns and environmental distributions of organohalogenated contaminants (OHCs) on a worldwide scale. In this study, the presence of OHCs was investigated on three continents (Europe, North America and Australasia), using eggs of starlings (*Sturnus vulgaris* and *Sturnus unicolor*) to assess their suitability for large-scale monitoring studies. To the best of our knowledge, this is the first study using bird eggs of the same species as a biomonitor for OHCs on an intercontinental scale. We found significant differences in OHC concentrations of the eggs among sampling locations, except for hexachlorocyclohexanes (HCHs). Mean concentrations of sum polychlorinated biphenyls (PCBs) in eggs ranged from 78 ± 26 ng/g lipid weight (lw) in Australia to 2900 ± 1300 ng/g lw in the United States. The PCB profile was dominated by CB 153 and CB 138 in all locations, except for New Zealand, where the contribution of CB 95, CB 101 and CB 149 was also high. The highest mean sum polybrominated diphenyl ether (PBDE) concentrations were found in Canada (4400 ± 830 ng/g lw), while the lowest mean PBDE concentrations were measured in Spain (3.7 ± 0.1 ng/g lw). The PBDE profile in starling eggs was dominated by BDE 47 and BDE 99 in all countries, but in Belgium, the higher brominated PBDEs had a higher contribution compared to other countries. For the organochlorine pesticides (OCPs), dichlorodiphenyltrichloroethanes (DDTs) ranged from 110 ± 16 ng/g lw in France to 17,000 ± 3400 ng/g lw in New Zealand, while HCHs and hexachlorobenzene were generally in low concentrations in all sampling locations. Chlordanes were remarkably high in eggs from the United States (2500 ± 1300 ng/g lw). The OCP profile in all countries was largely dominated by *p,p′*-DDE. In general, the worldwide trends we observed in starling eggs were in accordance with the literature on human and environmental OHC data, which suggests that there is potential for using starling eggs as a biomonitoring tool on a large geographical scale.

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1. Introduction

The presence of organohalogenated contaminants (OHCs) in the environment has been a great cause for concern, because of their persistent character, bioaccumulative potential and adverse effects on both humans and wildlife (Vos et al., 2000). For example, several OHCs have been shown to cause reproductive failure in birds through different mechanisms, such as eggshell thinning, embryotoxicity and effects on reproductive behaviours (Fernie et al., 2008; Gilbertson et al., 1991; Harris and Elliott, 2011; Vos et al., 2000). There is also evidence of long-range transport of these substances to regions where they have never been used or produced. Due to regulatory controls on the use of these compounds, there seems to be a decreasing temporal trend of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in biota (Jones and de Voogt, 1999). Nevertheless, concentrations in the environment are still high and may exert a potential health risk.

Recently, the presence of polybrominated diphenyl ethers (PBDEs) in the environment has received much attention (Law et al., 2006). PBDEs are a group of chemicals that are widely used in different materials, such as plastics, textiles and foams, because of their flame retarding properties. Large-scale production and use have led to their ubiquity in the environment and in biota, where PBDE levels have increased rapidly (Hites, 2004). Growing evidence of toxic effects on humans and wildlife has led to the ban of the three commercial formulations of PBDEs in the European Union. The Penta- and Octa-BDE mixtures have been banned in Europe since 2004, followed by banning of the Deca-BDE mixture in 2008 (European Court of Justice, 2008). Canada banned the production of all PBDE mixtures in 2006 (Canada Gazette, 2006). In the United States, manufacturers voluntarily ceased production of the Penta- and Octa-BDE mixtures by the end of 2004 and some restrictions on the use of Deca-BDE have been placed in some states. Recently, the two manufacturers of Deca-BDE in the United States have committed to phase out total production, importation, and sales of this formulation by the end of 2013 (US Environmental Protection Agency, 2009).

Monitoring studies are essential to assess the current levels and risks of different OHCs in the environment. Bird eggs have successfully been used to monitor OHCs and other chemicals (Carere et al., 2010; Donaldson et al., 1999; Elliott et al., 2005; Norstrom et al., 2002; Van den Steen et al., 2006, 2008). Eggs are easy to collect and the removal of a single egg from a large clutch is expected to have a minor effect on the population level in passerine species (Furness, 1993). Because eggs can readily be sampled from the same location each year, long-term monitoring studies using eggs are also feasible. Moreover, the study of widespread bird species enables monitoring on a larger spatial scale. Large scale geographical studies are very valuable to obtain more information about the local usage, emission patterns and spatial distribution of OHCs.

Only a few studies have monitored the concentrations of OHCs on a large geographical scale, using eggs of gulls (Larus sp.; Gebbink et al., 2011), great tits (*Parus major*; Van den Steen et al., 2009a) and blue tits (*Cyanistes caeruleus*; Van den Steen et al., 2010a). Other media that have been used to map the spatial distribution of OHCs are passive and active air samplers (Gioia et al., 2006; Jaward et al., 2004), pine needles (Jensen et al., 1992), tree bark (Simonich and Hites, 1995) and butter (Kalantzi et al., 2001). The use of birds has certain advantages over these passive media, because birds are generally exposed to OHCs via dietary intake and may be used as indicators of exposure and effects at the same time. Potential effects on reproduction and survival can be monitored and linked to the observed pollutants. Non-migratory passerine bird species are particularly useful for monitoring local contamination with OHCs. Residues in their eggs are expected to better reflect local contamination because of their relatively small home ranges, territories and foraging areas (Dauwe et al., 2006; Moore, 1966). Several previous studies used great and blue tit eggs to monitor OHCs in the European environment (Van den Steen et al., 2008, 2009a, 2010a), and in swallow species from North America (Custer, 2011).

In the present study, geographical variation in the occurrence of different OHCs was investigated in eggs of starlings collected from different locations on three continents (Europe, North America and Australasia). However, most sampling locations were situated in Europe, where eggs from 9 different countries were obtained. Concentrations and profiles of PCBs, PBDEs and OCPs were assessed and compared among the different sampling locations within Europe and among all locations together. The European starling (*Sturnus vulgaris*) and spotless starling (*Sturnus unicolor*) are passerine birds belonging to the family of Sturnidae. These closely related species are both hole nesting birds that readily nest in man-made nest boxes. Therefore, breeding populations can be rapidly established and monitored, and eggs are easily collected (Arenal et al., 2004). Another advantage of European starlings is their ubiquity, which permits biomonitoring on a large geographical scale. They are native to Europe but are an introduced species in Australia, New Zealand, North America and South Africa where they are regarded as a pest (Feare, 1984). The spotless starling is morphologically and genetically closely related to the European starling (de la Cruz-Cardiel et al., 1997; Hiraldo and Herrera, 1974), but is restricted to South West Europe (Ferrer et al., 1991). European and spotless starlings are widely used in behavioural and ecological research (Bateson and Feenders, 2010; Pinxten and Eens, 1997; Pinxten et al., 2002). Moreover, starlings have previously been shown to be good biological monitors of local contamination with PCBs and heavy metals (Arenal and Halbrook, 1997; Arenal et al., 2004; Halbrook and Arenal, 2003). Starlings mainly feed on soil invertebrates, which may be an important source of OHCs (Feare, 1984).

Based on previous studies on geographic distributions of OHCs and on their usage patterns, several predictions were made regarding the expected contamination levels in starling eggs from the different sampling locations. The highest concentrations of PCBs were expected in the United States of America (USA), the country with the highest production volume (Breivik et al., 2002). PBDE concentrations were expected to be the highest in the USA, Canada (CA) and the United Kingdom (UK). This pattern has been demonstrated previously in human samples from these countries (Hites, 2004). Several studies using environmental and biological samples showed high concentrations of DDT and other OCPs in Eastern European countries (Covaci et al., 2001; Jaward et al., 2004). Lastly, we expected the highest concentrations of PCBs and PBDEs to be in urban locations and the highest concentrations of OCPs in rural locations, as has previously been shown in great tits and common magpies (*Pica pica*) from Europe (Jaspers et al., 2009; Van den Steen et al., 2008, 2009a).

2. Materials and methods

In the breeding season of 2009 and 2010, researchers from 13 countries (on three continents: Europe, North America and Australasia) collected starling eggs in 15 existing nest box populations (Table 1). We also collected eggs from South Africa, but these could not be analysed because they had been incubated and embryo development was already advanced. Most nest box populations in this study have previously been studied in an ecological or behavioural framework, implying that there was no a priori selection of 'polluted' sites. Where necessary, the required permissions/licences were obtained from the relevant national bodies regulating the collection of wild bird eggs. We aimed to obtain one egg from 10 different nests at each sampling location. One egg was randomly collected from each nest. The eggs were collected before incubation, labelled individually and stored in a freezer (−20 °C) until transport. Eggs were sent on dry ice and stored frozen until further analysis. Seven to 8 eggs were analysed per sampling location (Table 1). In total, 106 starling eggs were analysed for PCBs, PBDEs and OCPs. A questionnaire was sent to the collectors in

Table 1
Approximate geographical coordinates of sampling locations for starling eggs, together with type of location, number of eggs analysed and information about migratory behaviour of the starling population (based on personal communications). We also collected eggs from South Africa but these could not be analysed because they had already been incubated and embryo development was advanced.

	Country	Region	Geographical coordinates	Classification	Number of eggs analysed	Resident/migratory	European starling (ES)/ spotless starling (SS)
AU	Australia	South Australia, Adelaide	34°27′ S, 138°42′ E	Rural	7	Resident	ES
BE	Belgium	North Antwerp	51°21′ N, 4°27′ E	Rural	7	Migratory (short distance)	ES
CA	Canada	Greater Vancouver	49°5′ N, 123°5′ W	Urban	7	Migratory (short distance)	ES
HR	Croatia	Hrvatsko Zagorje	46° 00′ N, 15° 55′ E	Rural	7	Migratory	ES
DE	Germany	Bavaria	47°55′ N, 11°08′ E	Rural	7	Migratory	ES
ES1	Spain	Madrid	40º44′ N, 3º49′ W	Rural	8	Resident	SS
ES2	Spain	Andalusia	37º18′ N, 3º11′ W	Rural	7	Resident	SS
FR1	France	Brittany	48°3′ N, 1°24′ W	Rural	7	Resident	ES
FR2	France	Midi-Pyrénées	43°24′ N, 1°33′ E	Rural	7	Migratory	ES
IT	Italy	Rome	41°44′ N, 12°24′ E	Urban	7	Resident/migratory	ES
NO	Norway	Nord-Trøndelag	63°29′ N, 10°53′ E	Rural	7	Migratory	ES
NZ	New Zealand	Wellington	41°10′ S, 174°54′ E	Rural	7	Resident	ES
PL	Poland	Warsaw	52°18′ N, 20°54′ E 52°16′ N, 20°53′ E	Urban	7	Migratory	ES
UK	United Kingdom	Northumberland	54°59′ N, 1°51′ W	Rural	7	Resident	ES
USA	United States of America	Illinois	37°42′ N, 89°14′ W	Rural	7	Resident	ES

order to characterise the sampling sites (rural/urban) and potential contamination sources of OHCs. Each starling population was characterised as resident or migratory based on information provided by the local researchers, although this was not based on detailed studies (Table 1). Sampling sites were located both in urban and rural areas, but most sampling locations were characterised as rural (Table 1). Urban sampling locations were closely located to a city or densely populated area. Rural sampling locations were characterised by agricultural activities (e.g. crop production, horticulture).

A homogenised sample of approximately 0.5 g whole egg (without eggshell) was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (ε-HCH, CBs 46 and 143, BDEs 77 and 128). Extraction was carried out with 100 ml hexane/acetone (3:1, *v*/*v*) in an automat Soxhlet extractor (Büchi, Flawil, Switzerland) in hot extraction mode for 2 h. The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was cleaned on a column filled with ~8 g acidified silica and eluted with 15 ml hexane and 10 ml dichloromethane. The eluate was concentrated to 100 μl under a gentle nitrogen stream and transferred to an injection vial. In all samples, concentrations of 23 PCB congeners (CBs 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194, 199, 206 and 209), 7 PBDE congeners (BDEs 28, 47, 99, 100, 153, 154 and 183), dichlorodiphenyltrichloroethane (*p*,*p*′- and *o*,*p*′-DDT) and metabolites (*o*,*p*′-DDE, *p*,*p*′-DDE, *o*,*p*′-DDD, *p*,*p*′-DDD), hexachlorocyclohexanes (HCHs; α-, β- and γ-HCHs), chlordanes (CHLs; *cis*-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor (TN) and oxychlordane (OxC)), and hexachlorobenzene (HCB) were analysed.

For the PCB analysis, an Agilent 6890 gas chromatograph (GC) connected with an Agilent 5973 mass spectrometer (MS) operated in electron ionisation (EI) mode was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The MS was used in the selected ion-monitoring (SIM) mode with two ions monitored for each PCB homologue group. One μl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 700 °C/min), pressure pulse 25 psi, pulse time 1.5 min. The splitless time was 1.5 min. Helium was used as carrier gas at constant flow (1 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.5 min, then increased to 180 °C at a rate of 15 °C/min (held for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, held for 12 min.

For the analysis of the OCPs and PBDEs, an Agilent 6890 GC connected with an Agilent 5973 MS operated in electron capture negative ionisation (ECNI) mode was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). Methane was used as moderating gas and the ion source, quadrupole and interface temperatures were set at 170, 150 and 300 °C, respectively. The MS was used in the SIM mode with two ions monitored for each pesticide in specific windows, while ions m/z = 79 and 81 were monitored for PBDEs during the entire run. One μl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 720 °C/min), pressure pulse 30 psi, pulse time 1.5 min. The splitless time was 1.5 min. Helium was used as carrier gas at constant flow (1 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.5 min, then increased to 220 °C at a rate of 15 °C/min (held for 2.0 min), further increased to 242 °C at a rate of 3 °C/min and finally raised to 300 °C at a rate of 40 °C/min, held for 15 min.

Multi-level calibration curves in the linear response interval of the detector were created for quantification, and good correlation ($r^2 > 0.999$) was achieved. Identification of each OHC 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. 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, PBDEs and OCPs in whale blubber) was used to test the accuracy of the method with values obtained here deviating less than 10% from the certified values. The recovery of the internal standards added to the samples prior to extraction was ranging between 75 and 95%, RSD < 10%. The quality control scheme was also assessed through regular participation in interlaboratory comparison exercises organized by the Arctic Monitoring and Assessment Programme (AMAP) and the National Institute of Standards and Technology (NIST). For each analyte, the mean procedural blank value was used for subtraction. BDEs 47 and 99 had blank levels which were lower than 5% of the values found in the samples. Nevertheless, the blank levels were subtracted from the sample values. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank. 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 0.2 and 1.0 ng/g lipid weight (lw).

Statistical calculations were performed using Statistica for Windows (Statsoft, 1997) and XLSTAT (Addinsoft 1995–2012). The level of significance was set at $\alpha = 0.05$ throughout this study. Data were normally distributed, thus parametric tests were used. One-way ANOVAs were used to study the differences in contamination levels among all the sampling locations. In addition, because most of the sampling locations

were situated in Europe, differences in contamination levels were also investigated among the European sampling locations. Post hoc tests (Tukey HSD) were performed if there were significant differences among the sampling locations. To compare the congener profiles among the sampling sites we conducted principal component analysis (PCA) on standardised data. Principal components (PCs) with eigenvalues above 1 were considered to account for a significant contribution to the total variance according to the latent root criterion (Hair et al., 1998). Factor loadings and factor scores were determined and used in interpreting PC patterns. Compounds with factor loadings greater than 0.65 on any PC were considered significant and were discussed. The first two PCs were used for the statistical analyses. We have included OHC data of both starling species in the same statistical analyses, as European starlings and spotless starlings are closely related species with similar ecology and behaviour (Eens and Pinxten, 1999). In addition, mean % lipids did not differ significantly between eggs of European and spotless starlings (One-way ANOVA: $F_{1,13} = 0.28$; $p = 0.61$), with a mean lipid percentage of 7.79 ± 0.71% (Table SI-1 in the Supporting information). Furthermore, excluding data of spotless starlings from the statistical analysis did not have any effect on the results. Data in the text and figures are represented as mean ± standard error.

3. Results

3.1. Egg concentrations and profiles of PCBs

Sum PCB concentrations ranged from 78 ± 26 ng/g lw in eggs from Australia to 2900 ± 1300 ng/g lw in eggs from USA (Fig. 1a). Sum PCB concentrations differed significantly among the sampling locations ($F_{14,91} = 3.44$; $p<0.001$). Sum PCB concentrations in eggs from USA were significantly higher compared to Norway, Australia, Croatia, Spain (ES1 and ES2), France (FR2) and the United Kingdom (Tukey HSD: $p<0.01$; Fig. 1a), but did not differ significantly from other sampling locations (Belgium, France (FR1), Canada, Germany, New Zealand, Poland and Italy (Tukey HSD: $p>0.06$)). When considering only the European sampling locations, PCB concentrations were significantly higher in the urban sampling location in Italy compared to rural locations from Norway, Croatia, Spain (ES1 and ES2), France (FR2) and the United Kingdom ($F_{10,67} = 4.12$; $p<0.001$; Tukey HSD: $p<0.02$ for all cases).

The PCB profile was mostly dominated by CB 153 (typically 20–30%) and CB 138 (typically 10–15%) in all sampling locations. A deviating PCB profile was found for New Zealand with a higher contribution of lower chlorinated PCBs (especially CB 95 and CB 101) and CB 149 (Fig. SI-1). A similar pattern, but less pronounced, was found in Italy and France (FR1), while in Croatia a higher contribution of CB 28 and CB 52 was observed. These differences in the PCB profile were tested by PCA (Fig. 2a). The first two PCs of the PCA accounted for 33% and 14% of the total variance (Fig. 2a). Significant differences among the sampling locations were found for both PC1 and PC2 (One-way ANOVAs: PC1: $F_{14,91} = 17.50$, $p<0.001$; PC2: $F_{14,91} = 6.05$, $p<0.001$). PC1 was positively correlated with CB 52, CB 95, CB 101, CB 110 and CB 149, and negatively correlated with CB 170, CB 180 and CB 187, while PC2 was positively correlated with CB 183. PC1 of New Zealand differed from all other sampling locations (Tukey HSD: $p<0.03$), while PC1 of France (FR1) and Italy differed significantly from all other sampling locations (Tukey HSD: $p<0.03$ for both France (FR1) and Italy), except for Australia and Germany. The PCA thus confirms the differences that were observed in the PCB profile for New Zealand, and also to some extent for Italy, France, Germany and Australia (Fig. SI-1). PC2 of Canada and USA differed significantly from Belgium, Norway, Germany, Spain (ES1 and ES2) and New Zealand (Tukey HSD: $p<0.008$ for both CA and USA). Although PC2 was positively correlated with CB 183, no clear differences could be observed from the PCB profile for CB 183, as this compound had only a minor contribution (<5%) to the total sum PCBs (Fig. SI-1).

3.2. Egg concentrations and profiles of PBDEs

Sum PBDE concentrations ranged from 3.7 ± 0.1 ng/g lw in eggs from Spain (ES2) to 4400 ± 830 ng/g lw in eggs from Canada (Fig. 1b). Sum PBDE concentrations differed significantly among the sampling locations ($F_{14,91} = 27.57$; $p<0.001$). Sum PBDE concentrations in Canadian eggs were at least 20 times higher compared to those from all other sampling locations (Tukey HSD: $p<0.001$; Fig. 1b). When considering only the European sampling locations, PBDE concentrations were significantly higher in the United Kingdom samples compared to samples from Norway, Croatia, Germany, Spain (ES1 and ES2) and France (FR1 and FR2; $F_{10,67} = 4.69$; $p<0.001$; Tukey HSD: $p<0.02$ for all cases).

The PBDE profile in starling eggs was dominated by BDE 47 (20–40%) and BDE 99 (25–55%) in all sampling locations, although the ratio of the two congeners was highly variable. In Belgium, the higher brominated PBDEs (BDE 153, BDE 154 and BDE 183) had a higher contribution in comparison to other countries (Fig. SI-2). In comparison, the first two PCs of the PCA explained 28% and 20% of the total variance (Fig. 2b) and significant differences among the sampling locations were found for both PC1 and PC2 (One-way ANOVAs: PC1: $F_{14,91} = 10.77$, $p<0.001$; PC2: $F_{14,91} = 8.69$, $p<0.001$). PC1 was positively correlated with BDE 153 and BDE 183, the higher brominated congeners. PC2 was positively correlated with BDE 47 and negatively correlated with BDE 99. PC1 of Belgium and Spain (ES1 and ES2) differed significantly from most other sampling locations. PC1 of Belgium was significantly higher than all other sampling locations (Tukey HSD: $p<0.001$), except for the locations in Spain (ES1 and ES2). PC1 of Spain (ES1) was significantly higher compared to all other sampling locations (Tukey HSD: $p<0.04$), except Belgium, Spain (ES2) and Poland. PC1 of Spain (ES2) was significantly higher than all other sampling locations (Tukey HSD: $p<0.04$), except Australia, Belgium, Spain (ES1), Poland and the United Kingdom. These differences can also be observed in the PBDE profile (Fig. SI-2). For PC2, most notable differences were observed for Canada, Germany and Italy. PC2 of Canada was significantly lower than that of Australia, Belgium, Germany, Spain (ES1), France (FR1 and FR2), Italy, New Zealand, Poland and USA (Tukey HSD: $p<0.05$). Indeed, the PBDE profile of starling eggs in Canada showed a great contribution of around 55% for BDE 99, while less than 20% was contributed by BDE 47 (Fig. SI-2). For Italy and Germany, PC2 was significantly higher than Australia, Canada, Croatia, Spain (ES2), Norway, New Zealand, Poland and the United Kingdom (Tukey HSD: I: $p<0.04$; DE: $p<0.02$). This can be related to the high contribution of BDE 47 (around 40%) in Italy and Germany (Fig. SI-2).

3.3. Egg concentrations and profiles of OCPs

Sum DDTs ranged from 110 ± 16 ng/g lw in FR1 (France) to 17,000 ± 3400 ng/g lw in New Zealand (Fig. 1c). Sum DDTs concentrations differed significantly among the sampling locations ($F_{14,91} = 11.72$; $p<0.001$). Eggs from New Zealand had significantly higher sum DDTs concentrations compared to all other sampling locations (Tukey HSD: $p<0.001$). In all samples, *p,p′*-DDE was the most abundant compound, constituting 94–99% of the sum DDTs (see Table SI-1). Sum DDTs concentrations differed significantly among the sampling locations in Europe ($F_{10,67} = 23.34$; $p<0.001$). Sum DDTs concentrations in eggs from Poland were significantly higher compared to all other sampling locations in Europe (Tukey HSD: $p<0.001$). Sum DDTs concentrations in Germany were significantly higher compared to Spain (ES2) and France (FR1 and FR2; Tukey HSD: $p<0.03$; Fig. 1c). Croatia showed significantly higher sum DDTs concentrations compared to France (FR1 and FR2; Tukey HSD: $p<0.03$; Fig. 1c).

HCB and HCHs were generally low among all sampling locations (Fig. 1d and e). HCB concentrations differed significantly among all sampling locations ($F_{14,91} = 2.43$; $p = 0.006$). Post hoc tests only revealed a non-significant tendency for HCB concentrations to be higher

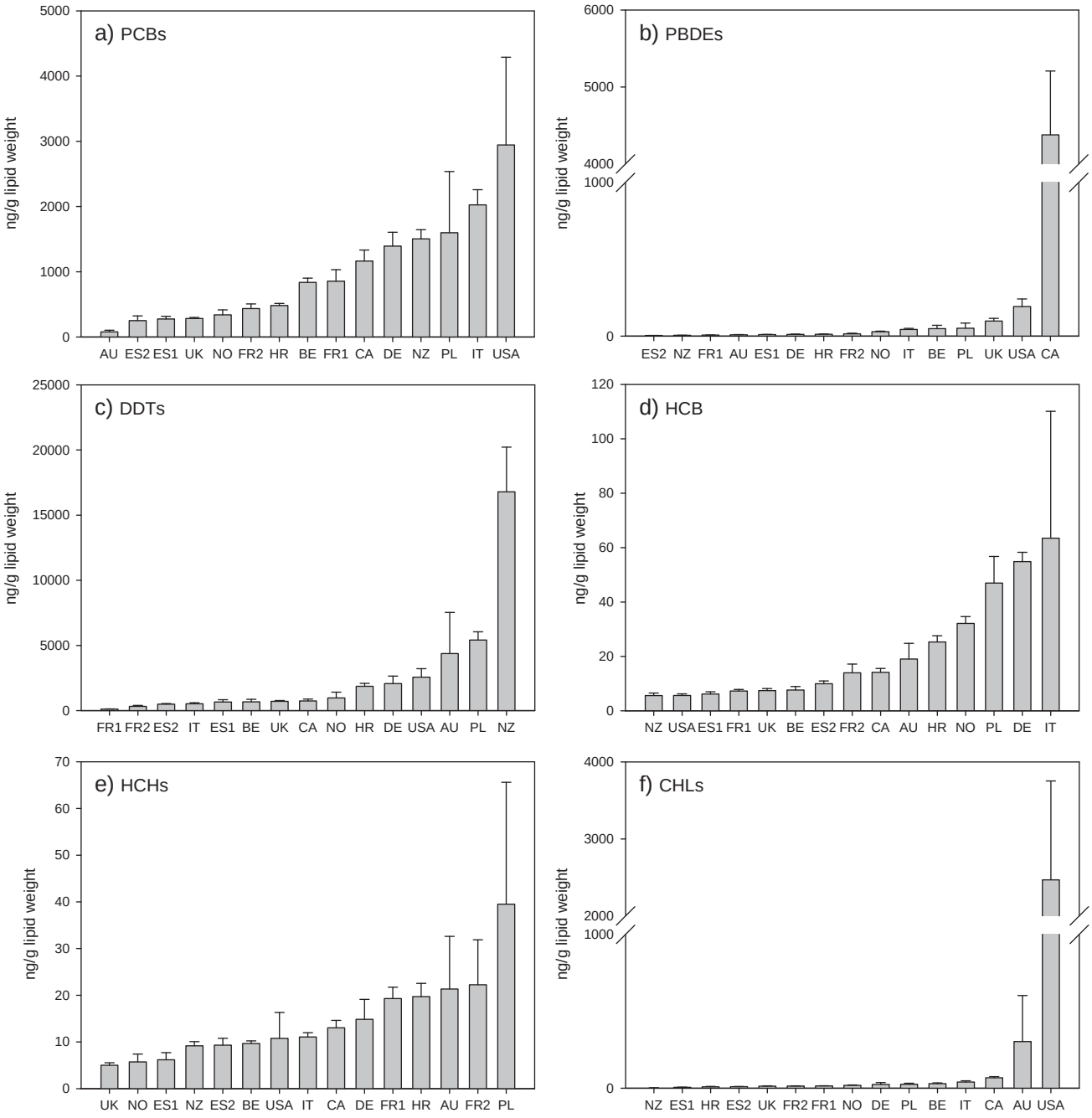

Fig. 1. Mean concentrations ± standard error of (a) sum polychlorinated biphenyls (PCBs), (b) sum polybrominated diphenyl ethers (PBDEs), (c) sum dichlorodiphenyltrichloroethanes (DDTs), (d) hexachlorobenzene (HCB), (e) sum hexachlorocyclohexanes (HCHs) and (f) sum chlordanes (CHLs): concentrations are expressed per gramme lipid weight (lw). Sampling sites were located in Australia (AU), Belgium (BE), Canada (CA), Germany (DE), Spain (ES1 and ES2), France (FR1 and FR2), Croatia (HR), Italy (IT), Norway (NO), New Zealand (NZ), Poland (PL), United Kingdom (UK) and United States of America (USA).

in Italy compared to Spain (ES1; $p = 0.07$), New Zealand ($p = 0.09$) and USA ($p = 0.09$). HCB concentrations also differed significantly among the European sampling locations ($F_{10,67} = 2.21$; $p = 0.03$). However, post hoc tests revealed no significant differences between any of the locations (Tukey HSD: $p > 0.05$). In contrast, HCH concentrations did not differ significantly among the different sampling locations (all sampling locations: $F_{14,91} = 1.26$; $p = 0.25$; European locations: $F_{10,67} = 1.43$; $p = 0.19$).

CHLs ranged from 2.8 ± 0.6 ng/g lw in New Zealand to 2500 ± 1300 ng/g lw in USA (Fig. 1f). CHL concentrations differed significantly among the sampling locations ($F_{14,91} = 3.47$; $p < 0.001$). CHLs were significantly higher in eggs from the USA compared to all other sampling locations (Tukey HSD: $p < 0.002$; Fig. 1f). For Europe, CHL concentrations were significantly higher in Italy compared to Croatia, Spain (ES1 and ES2), France (FR1 and FR2) and the United Kingdom ($F_{10,67} = 4.07$; $p < 0.001$; Tukey HSD: $p < 0.04$ for all cases).

The OCP profile of starling eggs in all sampling locations was largely dominated by *p,p′*-DDE (60–99% of total sum OCPs; Fig. SI-3). However, some differences can be observed, with a clear contribution of almost 40% for the CHLs to the OCP profile in the USA, while France and Italy had a joint contribution of about 15 to almost 30% from HCB, CHLs and HCHs (Fig. SI-3 and Fig. SI-4). To test for significant differences, we performed a PCA, which resulted in the first two PCs accounting for 38% and 22% of the total variance (Fig. 2c). Significant differences

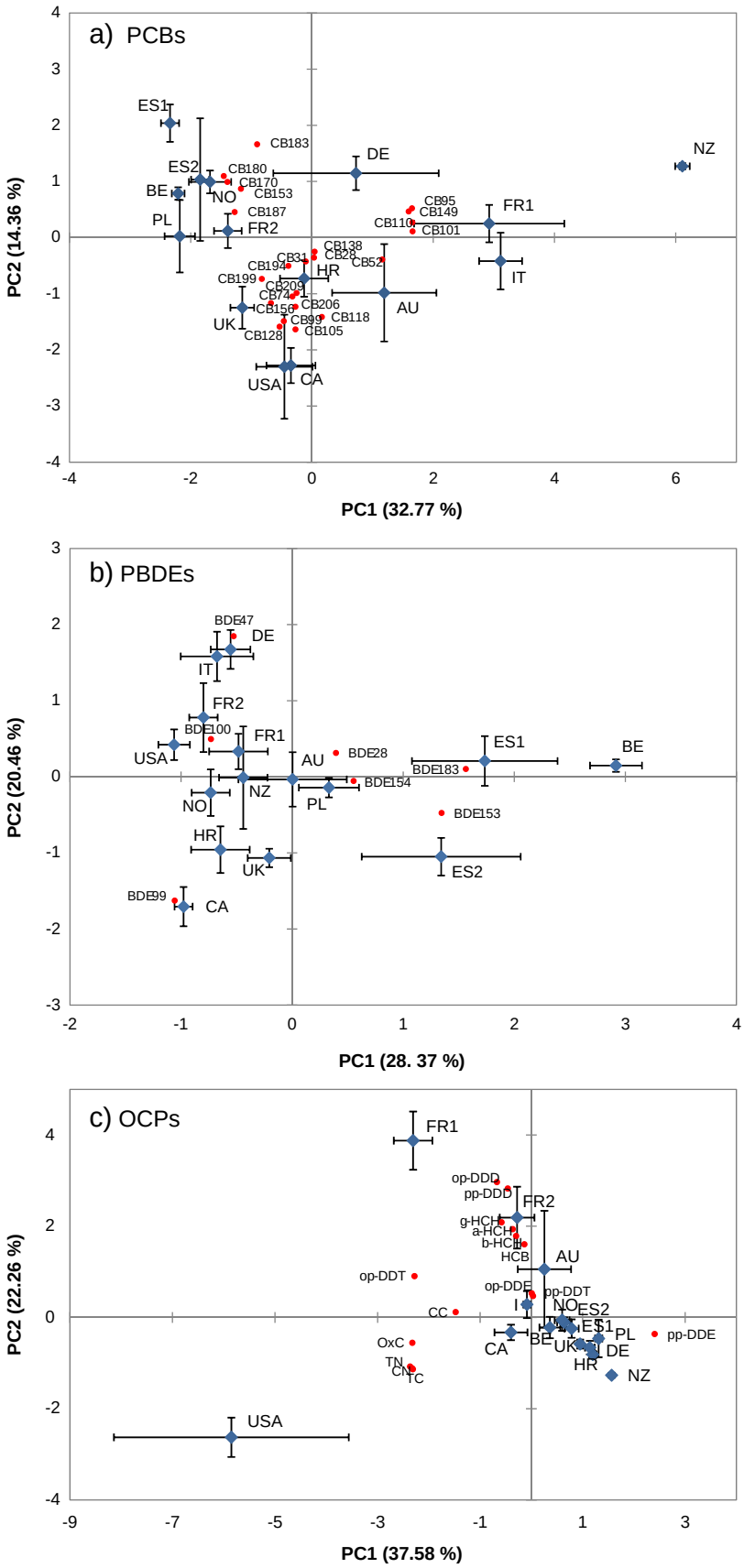

Fig. 2. Plots of mean factor scores (blue diamonds) ± standard errors and factor loadings (red circles) from the principal component analysis (PCA) for the (a) polychlorinated biphenyls (PCBs), (b) polybrominated diphenyl ethers (PBDEs) and (c) organochlorinated pesticides (OCPs). Compounds with factor loadings greater than 0.65 on any PC were considered significant. Sampling locations were located in Australia (AU), Belgium (BE), Canada (CA), Germany (DE), Spain (ES1 and ES2), France (FR1 and FR2), Croatia (HR), Italy (IT), Norway (NO), New Zealand (NZ), Poland (PL), United Kingdom (UK) and United States of America (USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

among the sampling locations were found for both PC1 and PC2 (One-way ANOVAs: PC1: $F_{14,91} = 8.89$, $p < 0.001$; PC2: $F_{14,91} = 10.67$, $p < 0.001$). PC1 was positively correlated with *p,p′*-DDE and negatively with OxC, TN, CN, TC and *o,p′*-DDT. PC2 was positively correlated with γ-HCH, *o,p′*-DDD and *p,p′*-DDD. For the USA, PC1 differed significantly from all other sampling locations (Tukey HSD: $p > 0.01$), which was in accordance with the lower contribution of *p,p′*-DDE and higher contribution of CHLs in the USA (Fig. SI-3). PC2 values for the USA were significantly lower compared to Australia, Belgium, Canada, Spain (ES1 and ES2), France (FR1 and FR2), Italy and Norway (Tukey HSD: $p < 0.03$). PC2 of one of the French populations (FR1) was significantly higher than PC2 of all other sampling locations (Tukey HSD: $p < 0.03$), except FR2 in France. PC2 of sampling location FR2 in France was also significantly higher than all other sampling locations (Tukey HSD: $p < 0.05$), except Australia, FR1 in France and Italy.

4. Discussion

4.1. Egg concentrations and profiles of PCBs

PCB concentrations differed significantly among the sampling locations. The lowest concentrations of sum PCBs were found in Australia, while the highest concentrations were found in the United States. The latter are probably due to the fact that large amounts of PCB commercial mixtures were produced and used in the USA (Breivik et al., 2002). Arenal et al. (2004) observed reduced nest provisioning behaviour and decreased chick survival in starlings from a contaminated site in southern Illinois (USA). In the present study, PCB concentrations in USA eggs (from a rural location with no known contamination) were comparable with concentrations measured at the reference sites of the latter study (Arenal et al., 2004). Therefore, starlings from the sampling location in the USA were not expected to be at risk of detrimental effects on reproduction or health. PCB concentrations in eggs from European countries in the present study were comparable with the concentrations in European great tit and blue tit eggs (Van den Steen et al., 2009a, 2010a). In Europe, the highest PCB concentrations were found in the urban locations in Italy and Poland. Van den Steen et al. (2008) also showed significantly higher PCB concentrations in great tit eggs from urban locations compared to other locations. In this study, PCB concentrations at the urban site from Italy were indeed significantly higher compared to the rural locations from Norway, Croatia, Spain (ES1 and ES2), France (FR2) and the United Kingdom. However, results from the urban sampling location in Poland did not show significantly higher PCB concentrations compared to the other locations. This might be due to the high variation in PCB concentrations within this location (Fig. 1a), which may be related to eggs being collected from two nest box colonies, 3 km apart (Table 1). Therefore, individual variation and local contamination sources (one colony was near a landfill rehabilitation area) may be of concern. Moreover, PCB concentrations at the urban site from Italy were higher than concentrations previously found in great tit and blue tit eggs collected from the same site (Van den Steen et al., 2009a, 2010a). This may be attributed to differences in diet among tits and starlings and possibly in physiological sensitivity to bioaccumulation.

Significant differences in PCB congener profiles were found among the sampling locations. Compared to the other locations, a higher contribution of lower chlorinated PCB congeners (CB 52, CB 95, CB 101, CB 110 and CB 149) was found in New Zealand, Italy and France (FR1), while a lower contribution of higher chlorinated CB congeners, CB 170, CB 180 and CB 187, was found in these locations. A slightly higher contribution of CB 183 was found in Canada and the United States. These differences in profiles are probably related to local contamination sources (Ormerod et al., 2000).

4.2. Egg concentrations and profiles of PBDEs

Sum PBDE concentrations in Canada were considerably higher compared to the other locations, followed by the USA and United Kingdom. PBDE concentrations have previously been found to be higher in human samples (such as blood and breast milk) from the USA and Canada compared to European countries (Hites, 2004). Peregrine falcon (*Falco peregrinus*) eggs from Canada showed significantly greater PBDE concentrations compared to eggs from Spain (Guerra et al., 2012). This pattern is likely related to the differential usage and (former) production of these pollutants (Birnbaum and Staskal, 2004). In addition, the extremely high concentrations in Canada might be due to the close proximity of the major urban landfill of the city of Vancouver (personal communication J. Elliott). Urbanisation and industrialisation have previously been linked to contamination with PBDEs (Harrad et al., 2008; Jaward et al., 2004; McKinney et al., 2006; Park et al., 2009; Van den Steen et al., 2008). Although the sampling location in the United Kingdom has been classified as a rural location, it is only 20 km from a large city (Newcastle upon Tyne). Therefore, local contamination sources, as well as long-range transport, may be responsible for the higher PBDE concentrations in the United Kingdom. Environmentally relevant concentrations of PBDEs have been suggested to result in adverse reproductive effects in European starlings (Van den Steen et al., 2009b), as female starlings experimentally exposed to environmentally relevant PBDE levels showed reduced egg laying (Van den Steen et al., 2009b). However, no endocrine disrupting, haematological and biochemical effects were observed in that study (Van den Steen et al., 2010b).

The PBDE profile was dominated by BDE 47 and BDE 99, but the ratio between them differed significantly among countries. The sampling location in Canada showed a high contribution of BDE 99 (55%), while BDE 47 only constituted 20% of the total PBDEs. This may be attributed to the higher usage of the Penta BDE mixture, as BDE 99 has a higher percentage than BDE 47 in that technical mixture (La Guardia et al., 2006). This pattern might also be enhanced by the close proximity to the landfill of Vancouver. Especially Belgium, but also Spain (ES1 and ES2), showed a higher contribution of BDE 153 and BDE 183, which suggests a higher contamination with Octa-BDE or debromination of the Deca-BDE mixture (Van den Steen et al., 2007). A similar profile has been found in Belgian home-produced chicken eggs (Covaci et al., 2009).

4.3. Egg concentrations and profiles of OCPs

Among the OCPs, DDTs were the most prevalent contaminants in all sampling locations, which is also in accordance with the dominance of DDTs in human adipose tissues worldwide (Li et al., 2006). The highest concentrations of sum DDTs were found in New Zealand, where DDT was used extensively to control pasture insects from about 1950 to 1968. Although its use on pasture lands was restricted in 1970, DDT was only finally banned in New Zealand in 1989 (Taylor, 1997). It has also been suggested that DDT may be responsible for the reported decline in starling numbers in New Zealand since 1945 (Flux and Flux, 1981). For Europe, significantly higher sum DDTs concentrations were found in (former) Eastern European sampling locations (Poland and Croatia), which was in line with our expectations. In comparison, high concentrations of DDT were also reported in great tit and blue tit eggs from two study sites in Poland (Van den Steen et al., 2009a).

Eggs from the USA showed a higher contribution of OxC, TN, CN, TC, *o,p′*-DDT and a lower contribution of *p,p′*-DDE. This suggests a recent exposure to DDT and other OCPs (OxC, TN, CN and TC) in that rural area. Since DDE is the major breakdown product of DDT, the overall accumulation profile of DDTs in this study suggests historical input rather than contribution from recent sources (Harris et al., 2000). DDT and its metabolites can still be found in the environment

and in biota, although it has been banned in most developed countries for more than 25 years. In general, contamination with OCPs may be related to the local historical usage of OCPs and the different usage of land (Harris et al., 2000; Van den Steen et al., 2008).

CHLs were remarkably higher in the USA compared to the other sampling locations, which is in accordance with its reported usage (Dearth and Hites, 1991). Chlordane is a pesticide that was widely used in North America on crops, lawns, gardens and forests in the 1950s, '60s and '70s. Different cases of CHL poisoning have previously been reported in birds from the USA, including starlings (Okoniewski and Novesky, 1993; Stansley and Roscoe, 1999). The OxC levels found in starling eggs (mean 990 ng/g lw, equal to 60 ng/g ww) are well below the threshold that has been reported for oxychlordane levels in brain of starlings (1.1 mg/kg), but no threshold values are available for eggs. A threshold value of 1 μg/g ww has however been suggested for 'total dieldrin equivalents' in bird eggs (Elliott and Bishop, 2011), another cyclodiene pesticide with a very similar structure to oxychlordane. Still, this threshold value is much higher than the OxC and total CHL levels found in starling eggs from the USA sampling location, again suggesting no current risk for CHL poisoning in this population.

4.4. Limitations of the study and future research directions

In general, the results were in line with our expectations. However, local contamination sources and individual variation may have an influence on OHC concentrations, especially when only one sampling location per country is considered. This could be solved in future studies by sampling more locations (especially in Australia, Canada and the United States of America) and by sampling more eggs per location. It would also be interesting to obtain samples from North and South Africa, as few pollution biomonitoring studies are available from these regions. Another issue that should be taken into account is that some starling populations in our study were partially non-resident or migratory (although this parameter has not been characterised in enough detail in all populations to include it in the statistical analyses). However, starlings are income breeders and use daily food intake to provide nutrients for eggs (Meijer and Drent, 1999). Furthermore, in non-resident European starling populations, most individuals arrive well before egg laying in the colony (Eens et al., 1991; Pinxten et al., 1990). Therefore, starling eggs are expected to largely reflect local contamination levels and migration may only have a minor influence on OHCs deposited in their eggs. Nevertheless, this is a topic that requires further study.

5. Conclusions

To the best of our knowledge, this is the first study in which bird eggs of the same species have been used as a biomonitoring tool for OHCs on an intercontinental scale. The ubiquitous European starling is highly suitable for large scale biomonitoring purposes as it makes use of nest boxes for breeding and thus the eggs can be sampled easily. In addition, starlings are relatively high on the food chain feeding mainly on soil invertebrates and therefore have the potential to accumulate high concentrations of OHCs. Furthermore, the results of this study showed that the geographical patterns of OHCs in starling eggs generally reflected the expected emission patterns and were in accordance with data from human and environmental samples. However, the influence of local contamination sources is of concern and therefore future studies should take this into account when selecting appropriate sampling locations. Overall, this study demonstrates the potential usefulness of starling eggs as a biomonitoring tool on a large geographical scale. Furthermore they may be suitable for prospective long-term studies to assess temporal trends of different OHCs. Finally, given that there is a potential to experimentally manipulate pollutant concentrations in starling eggs (see Muller and Eens, 2009 for manipulation of hormonal concentrations), it may be possible to study the (developmental) effects of ecologically relevant pollutant levels.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2012.11.003.

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