Assessment of contaminant levels and trophic relations at a World Heritage Site by measurements in a characteristic shorebird species

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A B S T R A C T

The River Elbe is responsible for influxes of contaminants into the Wadden Sea World Heritage Site. We investigated levels of polychlorinated biphenyls (PCBs), oxychlorodane (OxC), hexachlorobenzene (HCB), hexachlorocyclohexanes (α-, β-, γ-HCHs), dichlorodiphenyltrichloroethane (DDT) and its metabolites, and polybrominated diphenyl ethers (PBDEs) in blood and feathers from Eurasian oystercatchers (Haematopus ostralegus; n = 28) at the Elbe and compared it with a non-riverine site about 90 km further north. (1) Mean levels of all contaminants in feathers and serum were significantly higher at the river (∑PCBs: 27.6 ng/g feather, 37.0 ng/ml serum; ∑DDTs: 5.3 ng/g feather, 4.4 ng/ml serum) compared with the non-riverine site (∑PCBs: 6.5 ng/g feather, 12 ng/ml serum; ∑DDTs: 1.4 ng/g feather, 0.5 ng/ml serum). Mean ∑HCH and HCB levels were < 1.8 ng/g in feather and < 1.8 ng/ml in serum at both sites. (2) Levels of most detectable compounds in serum and feathers were significantly related, but levels were not consistently higher in either tissue. (3) There was no significant relationship between trophic level in individual oystercatchers (expressed as δ15N) or the degree of terrestrial feeding (expressed as δ13C) and contaminant loads. (4) PBDEs were not detected in significant amounts at either site. The results of this study indicate that the outflow from one of Europe’s largest river systems is associated with significant historical contamination, reflected by the accumulation of contaminants in body tissues in a coastal benthivore predator.

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1. Introduction

The North Sea is one of the most intensively used marine regions in the world (Halpern et al., 2008). It receives major influxes of nutrients and contaminants from several large river systems, including the Elbe (Bakker et al., 2009). Moreover, the Wadden Sea, located in the south-eastern North Sea, was declared a World Heritage Site in 2009 (CWSS, 2008) and provides food and space for millions of breeding and resting birds. Monitoring of population trends and numbers to assess changes in the quality of this important wetland site have identified alarming negative trends (Laursen et al., 2010; Blew et al., 2013; Kofijberg et al., 2013). Birds are widely used as indicators of health of the marine environment. They are found in the upper levels of the food web and therefore provide useful evidence for the accumulation of persistent contaminants (e.g., Furness, 1993; Thompson and Hamer, 2000; Dittmann et al., 2011), as well as for important changes in trophic relations (e.g., Montevecchi, 1993; Montevecchi and Myers, 1996; Schwemmer et al., 2013).

The contaminant loads of birds inhabiting this internationally important ecosystem have been monitored in terms of persistent organic substances (i.e., polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), chlor dane) and heavy metals (i.e., mercury) since the 1980s, and regularly since the 1990s (Becker and Muñoz Cifuentes, 2004). The results of this monitoring have provided insights into long-term changes in contaminant loads in the eggs of piscivorous (i.e., terns) and benthivorous (i.e., oystercatchers Haematopus ostralegus) marine birds.
High breeding and resting numbers (Laursen et al., 2010; Kofijberg et al., 2013) mean that oystercatchers are one of the most characteristic species in the Wadden Sea. Contaminants in egg shells of oystercatchers have been shown to differ between different sites on the Wadden Sea, with the outflow of the River Elbe as an important hotspot (Becker et al., 1992; Beyerbach et al., 1993; Becker and Muñoz Cifuentes, 2004; Becker and Dittmann, 2009). Although overall levels of all contaminants in most parts of the Wadden Sea (including the Elbe river area) have declined during recent years, levels remain highest in the vicinity of the Elbe outflow (Becker and Dittmann, 2009). Intertidal mudflats and salt marshes in the Elbe estuary provide important feeding and resting areas for various waterbird species, including oystercatchers (Kofijberg et al., 2003).

We investigated the relevance of different types of organic contaminants in the south-eastern Wadden Sea, with a focus on major river influxes. This study had four major objectives:

(1) We compared contaminant levels and compositions of PCBs, HCB, oxychlordane (OXC), α-, β-, γ-HCHs, DDT and its metabolites, and polybrominated diphenyl ethers (PBDEs) between the River Elbe area and Hallig Oland, another important breeding site located about 90 km further north, with no riverine influence. Oystercatchers are very local during the breeding period (Schwemmer and Garthe, 2011), and individual oystercatchers thus reflect contaminant loads from their respective site.

(2) We compared contaminant patterns in blood and feathers from oystercatchers at the two sites to detect differences between different tissues (e.g., Jaspers et al., 2006; Voorspoels et al., 2006; Ahrens et al., 2009). Levels in feathers might integrate contaminant loads over a relatively long period of time and thus be influenced by contaminant levels in areas used outside the breeding period (Jaspers et al., 2007). Blood levels, however, indicate contaminant levels during the breeding period, and thus reflect contaminant loads at distinct breeding sites. We also examined the relationship between contaminant levels in feathers and those in blood, as suggested by Jaspers et al. (2007, 2008) for other bird species.

(3) Trophic position of consumers such as birds and the origin of prey can be investigated by analysis of stable isotopes (Inger and Bearhop, 2008). We therefore measured carbon (δ13C) and nitrogen (δ15N) in red blood cells, serum and feathers to assess potential differences in feeding behaviours between individuals breeding at the two sites. δ13C in body tissues of consumers provides information on prey origins (i.e., low δ13C values indicate terrestrial origin, whereas high values indicate marine origin); δ15N levels provide information on the trophic level of the prey (i.e., high δ15N indicates prey from higher trophic levels; Fry, 2006; Inger and Bearhop, 2008; Ceia et al., 2014). We therefore aimed to relate levels of different types of pollutants to the feeding habits of the birds (Jaspers et al., 2007). We hypothesised that higher contaminant loads would be accompanied by higher δ15N levels as a consequence of birds foraging at higher trophic levels, and by higher δ13C levels as a result of foraging in more contaminated marine environments (when foraging terrestrially, oystercatchers commonly use pastures which in our study area are not treated with agricultural contaminants).

(4) Finally, we investigated the importance of PBDE contamination in birds in the Wadden Sea, particularly at the River Elbe site. PBDEs have been less-well investigated than many other substances, but have been suggested to influence hormone levels in different organisms (WWF, 2000; Jaspers et al., 2006; Voorspoels et al., 2006).

2. Methods

2.1. Study area

Incubating adult oystercatchers (n=28) were caught in the saltmarsh of Kaiser Wilhelm Koog (53° 57’ 55 N, 8° 54’ 14E; n=11) and on pastures on Hallig Oland (54° 41’ 52N, 8° 42’ 18E; n=17) in summer 2008 using walk-in nest traps (Fig. 1). The first site was located in the immediate vicinity of the River Elbe outflow, which is a significant source of contaminants (Bakker et al., 2009), while the latter site was located on an island in the north-eastern Wadden Sea, with no major river inflows. Both study areas are about 90 km apart. As oystercatchers show very local foraging flights (maximum up to 4 km distance) during the breeding period (Schwemmer and Garthe 2011) we can exclude that oystercatchers switched between the two study sites.

2.2. Sampling

Caught birds were ringed and about 1 ml of blood was taken from the brachial vein and preserved in serum tubes. Bird-catching and blood-sampling procedures complied with EC Directive 86/609/EEC for animal experiments and current German laws. Permits were obtained from the Ministerium für Landwirtschaft Umwelt und ländliche Räume (file numbers V 312-72241.121-37 (69-6/07) and V 312-72241.121-37 (27-3/08)). Samples were...
and spiked with internal standards (10 ng CB 143, 1 ng BDE 77, and 2 ng BDE 154, and 183) and 2-MeO-BDE68 and 6-MeO-BDE47 were analysed in both tissues.

The concentrations of contaminants in body feathers and serum were analysed at the Toxicological Centre (University of Antwerp, Belgium), as described previously (Eulaers et al., 2011). Briefly, individual feathers were rinsed thoroughly with distilled water and dried overnight at ambient temperature. After cutting into approximately 1 mm pieces, feather samples were weighed into approximately 1 mm pieces, feather samples were weighed and dried overnight at ambient temperature. After cutting into approximately 1 mm pieces, feather samples were weighed and spiked with internal standards (10 ng CB 143, 1 ng BDE 77, and 2 ng 5-α-HCH). Analytes were incubated overnight at 45 °C in 8 mL of HCI (4 M) and 8 mL of hexane:dichloromethane (4:1, v:v). The extracts were cleaned on 500 mg acidi fication material (Cat. No. 89962). After elution with 5 mL dichloromethane, the extracts were evaporated to dryness and reconstituted in 100 μL iso-octane.

Serum samples were spiked with internal standards (10 ng CB 143, 1 ng BDE 77, and 2 ng 5-α-HCH), diluted 1:1 with Milli Q water, mixed with formic acid, sonicated for 20 min and extracted using solid-phase extraction cartridges (3 mL/60 mg Oasis HLB, Waters). After elution with 5 mL dichloromethane, the extracts were cleaned on 500 mg acidified silica. Analytes were eluted with 8 mL hexane:dichloromethane (1:1, v:v), evaporated to near dryness, and reconstituted in 100 μL iso-octane.

PBDEs, MeO-PBDEs, HCHs and chlordanes were analysed using a gas chromatography-mass spectrometer (GC-MS) operated in electron capture negative ionisation mode, equipped with a 30 m × 0.25 mm × 0.25 μm DB-5 capillary column (J&W Scientific). The ion-source temperature was 170 °C. The MS was used in the SIM mode with two ions monitored for each compound in specific windows, while ions m/z = 79 and 81 were monitored for MeO-PBDEs and PBDEs during the entire run. Two microlitres of the extract were injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 mL/min).

PCBs, HCB and DDTs (p,p′-DDT, p,p′-DDE and p,p′-DDD) were analysed by GC–MS in electron impact ionisation mode, equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE). The ion-source temperature was 230 °C. The MS was used in SIM mode with two ions monitored for each PCB homologue group or compound in specific windows. Two microlitres of the extract were injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 mL/min).

Multi-level calibration curves (r² > 0.9999) in the linear response interval of the detector were created for quantification.

Quality control was performed by regular analyses of procedural blanks and random injection of standards, spiked samples and solvent blanks. The quality control scheme was also assessed by regular participation in inter-laboratory comparison exercises organised by the Arctic Monitoring and Assessment Programme (persistent organic pollutants in serum), with obtained values deviating by no more than 20% from the consensus values. The mean recoveries ± standard deviation (SD) of the internal standards CB 143, BDE 77 and 5-α-HCH were 87 ± 8%, 92 ± 5% and 84 ± 8%, respectively. For analytes detected in the procedural blanks, the mean procedural blank value was subtracted from the result. After blank subtraction, the limit of quantification (LOQ) was set at three times the SD of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for S/N = 10. LOQs for the analysed contaminants are given in Table 1.

2.4. Analyses of stable isotopes

Freeze-dried blood cells, serum and feathers (tips of feathers were used to avoid variability; see Bontempo et al., 2014) were ground to a powder using a mortar and pestle and loaded into tin boats. All feathers analysed were black. The high melanin content of black feathers leads to a depletion on 13C and 15N (Michalk et al., 2010). However, as all feathers showed similar pigment levels the depletion was consistent over all samples. Stable isotope measurements were performed using an isotope ratio mass spectrometer (V.G. Optima, Micromass) coupled to an N-C-S elemental analyser (Carlo Erba) for automated analyses. Stable isotope abundances are expressed in delta (δ) notation as the deviation from standards in parts per thousand (‰), according to the following equation:

\[
δX = \left[ \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right] \times 1000
\]

where X is 13C or 15N and R is the corresponding ratio 13C/12C or 15N/14N.

Standard values were based on the Vienna Pee Dee Belemnite (v-PDB) for δ13C measurements and atmospheric nitrogen for δ15N measurements. Reference materials were IAEA-N1 (δ15N = 0.4 ± 0.2‰) and IAEA CH-6 (sucrose) (δ13C = −10.4 ± 0.2‰). Internal standards (glycine) were inserted into all runs at regular intervals to calibrate the system and to assess drift over time. SDs of internal standard replicates were 0.1‰ and 0.3‰ for carbon and nitrogen, respectively.

2.5. Statistical analyses

All statistical analyses were performed using the open source software package R version 3.0.2 (RDevelopment Core Team, 2013). Samples with levels below LOQ were assigned a value of f × LOQ, with f as the proportion of measurements with levels above the LOQ or the detection frequency (Voorspoels et al., 2002). The same compounds were tested in feathers and serum, except for PCBs 28 and 52, which were only tested in feathers, and PCB 156 and p,p′-DDD, which were only tested in serum. Compounds for which > 50% of the measurements were < LOQ for a given location or tissue were excluded from statistical analyses. The following substances were therefore excluded from analysis in feathers: PCBs 28, 52, 99, 101, 105 (Oland only), 128 (Oland only), 146, 149, 183 (Oland only); OxC; TN; CN; γ-HCH; and all PBDE congeners. The following substances were excluded from analysis in serum: PCB 101 (Oland only); OxC (Oland only); TN; p,p′-DDT; p,p′-DDD (Oland only); α-HCH; γ-HCH; PBDEs 28, 47 (Oland only), 49, 99 (Oland only), 153, 154, 183; 2-MeO-BDE68; and 6-MeO-BDE47.
When comparing ΣPCBs, ΣHCHs, HCB, ΣPBDEs and ΣDDTs between tissues or locations, only compounds that were found in >50% of individuals in terms of either tissue or location, respectively, were included; i.e., if a compound was present in <50% of tissue samples or <50% of birds from one location, that compound was excluded from the analysis for the other tissue or location. Thus, only compounds for which tissue or location measurements were possible or where it was possible to insert a value using the formula given above were analysed.

Profiles of PCB congeners were analysed by computing the relative contribution of each congener to the sum of all PCBs. ΣPCBs, ΣHCHs, HCB and ΣDDTs were compared between the two sampling locations using Student’s t-tests, after checking the homogeneity of variances (Sokal and Rohlf, 1981). Differences in the composition of different compounds and congeners in each tissue were detected by performing detrended correspondence analyses, including a permutation test with 1,000 permutations (DECORANA; Hill and Gauch, 1980; Oksanen and Minchin, 1997).

Relationships between contaminant levels in feathers and serum were examined. Because we detected significant differences in both levels and composition of contaminants between sites, we used linear mixed effect models with site as a random factor (Venables and Ripley, 2002; Faraway, 2006). Tests were conducted using library lme4 (Bates and Maechler, 2009). We determined if the test compound accumulated more in feathers (slope > 1) or in serum (slope < 1) by computing confidence intervals. Confidence intervals including the slope 1 indicated that differences in accumulation were non-significant. To test for relationships between ΣPCBs, ΣHCHs, HCB, ΣPBDEs, ΣDDTs and δ13C and δ15N, we adopted the same approach used to compare levels of contaminants between tissues, except that no confidence intervals were computed because we were only interested in regressions between contaminants and isotope profiles.

δ13C and δ15N values in different tissues were analysed by computing mean values and 25% and 95% percentiles for each tissue and site separately. Isotopic enrichment between diet and consumer tissues is a consequence of isotopic fractionation during the assimilation process (Ponsard and Averbuch, 1999). No fractionation factors for δ13C and for δ15N are currently available for oystercatchers and we therefore used the values given for dunlin (Calidris alpina) whole blood, i.e., 1.3 for δ13C and 2.9 for δ15N (Evans Ogden et al., 2004). To the best of our knowledge, these are the only available values for shorebirds.

All values were log-transformed (log 10 (x + 1)) to match a normal distribution and to allow for parametric tests.

### 3. Results

#### 3.1. Differences in contaminant levels and composition between locations and tissues

Oystercatchers in both locations showed comparably high levels of PCBs in feathers and blood, respectively, whereas contamination with PBDEs was very low in both areas and tissues (Table 1; Fig. 2). Among all the tested substances, PCB 153 showed by far the highest values, both in blood (4.02 ± 1.85 ng/ml (Oland); 12.29 ± 4.37 ng/ml (Elbe)) and feathers (2.3 ± 1.38 ng/g (Oland); 9.99 ± 5.95 ng/g (Elbe) (Table 1). However, as indicated by the large SDs, PCB contamination varied considerably between individuals, particularly at the Elbe site (Table 1). Most PBDE congeners in serum were only slightly above or < LOQ. PBDEs in feathers from the riverine site were < LOQ in most cases, but slightly higher in feathers from the non-riverine location. Only BDE 100 was detectable in levels significantly above LOQ in serum in both locations (Fig. 2e). PBDE values were 100–1000 times

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>River Elbe</th>
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<th>Oland</th>
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<td>Serum</td>
<td>Feathers</td>
<td>Serum</td>
<td>Feathers</td>
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<td>PCB 99</td>
<td>1.46 ± 0.39 (0.1)</td>
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<td>0.38 ± 0.13 (0.1)</td>
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<td>PCB 105</td>
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<td>0.21 ± 0.30 (0.4)</td>
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<td>PCB 118</td>
<td>1.57 ± 0.1</td>
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<td>0.87 ± 0.05</td>
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<td>PCB 138</td>
<td>5.92 ± 2 (0.05)</td>
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<td>PCB 146</td>
<td>2.90 ± 0.96 (0.3)</td>
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<td>0.94 ± 0.39 (0.1)</td>
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<td>PCB 149</td>
<td>0.95 ± 0.28 (0.1)</td>
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<td>0.18 ± 0.05 (0.1)</td>
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<td>PCB 153</td>
<td>12.29 ± 4.37 (0.05)</td>
<td>9.99 ± 9.55 (0.4)</td>
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<td>PCB 170</td>
<td>1.70 ± 0.74 (0.05)</td>
<td>1.71 ± 1.26 (0.4)</td>
<td>0.60 ± 0.30 (0.05)</td>
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<td>PCB 180</td>
<td>3.92 ± 1.63 (0.05)</td>
<td>3.71 ± 3.28 (0.4)</td>
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<td>PCB 183</td>
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<td>PCB 187</td>
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<td>HCB</td>
<td>0.45 ± 0.11 (0.05)</td>
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<td>0.50 ± 0.36 (0.3)</td>
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<td>nd (0.2)</td>
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<td>pp-DDE</td>
<td>4.12 ± 1.15 (0.1)</td>
<td>4.85 ± 2.63 (0.4)</td>
<td>0.47 ± 0.19 (0.1)</td>
<td>0.99 ± 0.48 (0.4)</td>
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<td>pp-DDT</td>
<td>nd (0.1)</td>
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<td>0.05 ± 0.0 (0.1)</td>
<td>not tested</td>
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<td>β-HCH</td>
<td>nd (0.02)</td>
<td>0.29 ± 0.05 (0.2)</td>
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<td>γ-HCH</td>
<td>nd (0.02)</td>
<td>0.58 ± 0.85 (0.2)</td>
<td>0.11 ± 0.04 (0.02)</td>
<td>0.47 ± 0.19 (0.2)</td>
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<td>BDE 47</td>
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<td>nd (0.1)</td>
<td>0.01 ± 0.01 (0.02)</td>
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<tr>
<td>BDE 99</td>
<td>0.01 ± 0.01 (0.01)</td>
<td>nd (0.1)</td>
<td>0.01 ± 0.01 (0.01)</td>
<td>0.05 ± 0.19 (0.1)</td>
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<td>BDE 100</td>
<td>0.03 ± 0.01 (0.01)</td>
<td>0.10 ± 0.10 (0.1)</td>
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<td>BDE 153</td>
<td>nd (0.01)</td>
<td>nd (0.2)</td>
<td>0.02 ± 0.01 (0.01)</td>
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<td>BDE 154</td>
<td>nd (0.01)</td>
<td>nd (0.2)</td>
<td>0.01 ± 0.01 (0.01)</td>
<td>0.06 ± 0.07 (0.2)</td>
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<tr>
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<td></td>
<td></td>
<td>0.01 ± 0.00 (0.01)</td>
<td>nd (0.2)</td>
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nd – not detectable.
Fig. 2. Comparisons of (a) $\Sigma$PCBs, (b) HCB, (c) $\Sigma$DDTs, (d) $\Sigma$HCHs and (e) BDE100 between locations. Bold line: median; box: 25% confidence interval; dashed line: 95% confidence interval; open circles: outlier. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Please note different scales of y-axes.
Both locations also differed significantly with respect to the compositions of contaminants both in feathers (DECORANA: DCA1 = 0.7, DCA2 = 0.72, R² = 0.75, p < 0.001; Fig. 3a) and in serum (DECORANA: DCA1 = −0.98, DCA2 = −0.22, R² = 0.89, p < 0.001; Fig. 3b), indicating major differences in contaminant loads between the two sites. The difference between the sites was more pronounced in serum than in feathers.

Most tested compounds showed significant regressions between feathers and blood, except for CB170, CB183 and β-HCH (Table 2). There was no indication that any compound tended to accumulate more in either tissue (confidence intervals included 1 in all cases), suggesting that the accumulation of contaminants was similar in feathers and serum.

### 3.2. Profiles

It was only possible to establish a profile of different congeners for PCBs, because most PBDE congeners were < LOQ (see above). The PCB congeners 153, 138, 180 and 187 comprised by far the highest proportion of total PCBs in both locations and tissues (Table 1; Fig. 4). However, there were clear differences between the tissues in terms of most of the PCB congeners that accounted for relatively low proportions of the total PCBs. Less-chlorinated PCBs accounted for relatively higher proportions in serum from both locations, but were mostly < LOQ in feathers. PCB 146 was lower than PCB values. Furthermore, the levels of HCB, ∑DDTs and ∑HCHs were 2–10 times lower than those for ∑PCBs (Table 1; Fig. 2). Birds from the river location had significantly higher levels of all analysed contaminants compared with birds from the Oland location, both in feathers (t-tests: ∑PCBs: t = 2.97, df = 10.3, p < 0.05; HCB: t = 2.5, df = 14.5, p < 0.05; ∑DDTs: t = 4.67, df = 10.6, p < 0.001; ∑HCH: t = 4.07, df = 11.4, p < 0.01; PBDEs: not detectable; Fig. 2) and serum (t-tests: ∑PCBs: t = 5.86, df = 14.1, p < 0.001; HCB: t = 10.84, df = 13.8, p < 0.001; ∑DDTs: t = 10.92, df = 10.7, p < 0.001; ∑HCHs: t = 7.49, df = 10.6, p < 0.001; BDE 100: t = 2.88, df = 15.9, p < 0.05; Fig. 2). PCB contamination varied strongly among individual oystercatchers.
present at comparably high proportions in serum in both locations, but was undetectable in feathers (Table 1). In general, it was possible to detect most of the tested compounds in serum, but these were often < LOQ in feathers.

3.3. Differences in stable isotopes between tissues and locations

There were clear differences in δ13C and δ15N values between the two locations (Fig. 5). Individual values of levels in red blood cells and feathers scattered much stronger for the river site compared with the Oland site. Birds from Oland displayed lower δ15N values in all tested tissues compared with birds from the Elbe estuary. Furthermore, there were clear differences between the three tissue types in birds from Oland, with δ15N values in blood cells and serum being much lower than in feathers. δ13C values were higher in feathers than blood cells, and the lowest δ13C values were found in serum. In contrast, there were only slight differences among tissue types for birds from the river location, and values of stable isotopes in feathers were very similar to those in serum (Fig. 5). Blood cells displayed lower δ15N values than the other two tissue types. There was a positive correlation between δ13C and δ15N in blood cells for the river site (linear model: t=8.54, df=9, p < 0.001) and serum (linear model: t=5.6, df=9, p < 0.001).

3.4. Relationships between stable isotopes and contaminants

There was no significant relationship between levels of any class of contaminants in feathers or serum and δ13C or δ15N, respectively, except for BDE 100, which was significantly related to δ15N (Table 3).

4. Discussion

4.1. Contamination of the eastern Wadden Sea reflected by oystercatchers

Oystercatchers feed on a variety of benthic organisms (Hulscher, 1996; Schwemmer et al., 2012) and thus belong among the higher trophic levels of the food web in the Wadden Sea. Piscivorous seabirds such as terns are the top-predators in this ecosystem and have been shown to exhibit higher levels of contaminants than benthivorous birds such as oystercatchers (Becker and Muñoz Cifuentes, 2004; Becker and Dittmann, 2009). Nevertheless, the filter-feeding prey organisms that make up a high proportion of the diet of oystercatchers provide a good reflection of the contaminant load of the ecosystem.

The results of the present study demonstrated significant differences in both the levels and composition of contaminants in oystercatchers between a location influenced by one of Europe’s largest river systems and a non-riverine site. Currents within the North Sea transport riverine water from the Elbe in a north-westerly direction (BSH, 2014), and thus away from the sampled non-riverine site, supporting the idea that river discharge may be responsible for the significant differences between the two locations. Although PCB pollution has fallen recently (Bakker et al., 2009), these contaminants persist in organisms at higher trophic levels. Similar differences in contaminant levels between sites close to the River Elbe and non-riverine sites were found in egg shells of oystercatchers and piscivorous common terns (Sterna hirundo) (Beyerbach et al., 1993; Becker and Muñoz Cifuentes, 2004; Becker and Dittmann, 2009). Our data show that blood and feathers can also be used to demonstrate regional differences in organic contaminant loads in top predators on a small spatial scale, as previously shown for heavy metal contamination (Thompson and Dowling, 1999).

In general, levels of all PCB congeners were higher in serum than in feathers; indeed several compounds were undetectable in feathers but present at least at low levels in serum. However, the overall levels of all compounds, both in serum and feathers, were significantly lower compared with the levels in egg shells of oystercatchers and common terns from the Wadden Sea (Becker and Muñoz Cifuentes, 2004; Becker and Dittmann, 2009) and riverine

| Table 3 |

Linear regressions between contaminant levels (sum of all substance classes) in feathers/serum and δ13C/δ15N, respectively.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Slope</th>
<th>Intercept</th>
<th>t</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB (F)</td>
<td>2946</td>
<td>28,188</td>
<td>0.73</td>
<td>0.302</td>
<td>0.02</td>
</tr>
<tr>
<td>PCB (S)</td>
<td>921.3</td>
<td>39,942.5</td>
<td>0.38</td>
<td>0.634</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HCH (F)</td>
<td>74.8</td>
<td>172.9</td>
<td>0.45</td>
<td>0.505</td>
<td>0.01</td>
</tr>
<tr>
<td>HCH (S)</td>
<td>71.3</td>
<td>162.3</td>
<td>0.61</td>
<td>0.095</td>
<td>0.04</td>
</tr>
<tr>
<td>HCB (F)</td>
<td>36.1</td>
<td>1314.3</td>
<td>0.27</td>
<td>0.871</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HCB (S)</td>
<td>9.3</td>
<td>95.2</td>
<td>0.45</td>
<td>0.673</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DDT (F)</td>
<td>134.8</td>
<td>1269.2</td>
<td>0.27</td>
<td>0.656</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DDT (S)</td>
<td>502.7</td>
<td>416.2</td>
<td>0.49</td>
<td>0.586</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>BDE 100 (F)</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>BDE 100 (S)</td>
<td>2.72</td>
<td>68.2</td>
<td>1.16</td>
<td>0.182</td>
<td>0.06</td>
</tr>
</tbody>
</table>

F – feathers; S – serum; nt – not tested.

Fig. 5. δ13C and δ15N values for feathers (diamonds), serum (triangle) and scotched cells (circles) from birds from the River Elbe and Oland sites.
sites on the German mainland (Exo et al., 1998). Levels of all contaminants in feathers (except PBDEs, which were not significantly lower) were within the range of values reported for a set of terrestrial and aquatic bird species from Belgium (Jaspers et al., 2007). Previous studies have demonstrated that contaminant levels may differ strongly between different bird species and tissue types, with feathers having relatively low levels of contamination (Jaspers et al., 2006, 2007; Voorrips et al., 2006).

One aim of this study was to elucidate the level of pollution by PBDEs, which are not currently included in the monitoring schemes in the Wadden Sea and which have not yet been adopted as an Ecological Quality Objective (Dittmann et al., 2011). Our results indicated that PBDEs did not represent a problem in the food chain of the German North Sea, given that neither birds from the non-riverine nor the riverine site showed significant amounts. The only congener found at > LOQ was BDE 100 in serum, though levels of this congener were low compared with levels in aquatic and terrestrial bird species from elsewhere (Voorrips et al., 2006; Jaspers et al., 2006, 2007; Crosse et al., 2012).

4.2. PCB profiles and differences in contaminant loads between tissues

There were clear differences in PCB profiles between the tissues, with less-chlorinated PCBs accounting for relatively higher proportions in serum from birds in both locations. This is in contrast with earlier studies that showed that feathers had higher proportions of less-persistent compounds than internal organs (Dauwe et al., 2004; Jaspers et al., 2007). There might be higher levels in serum in contrast to feathers or internal organs because the liver had no opportunity to metabolise the compounds in serum. Levels of PCB congeners may also differ between feathers and serum because of external contamination of feathers, or contamination from the uropygial gland (e.g., Dauwe et al., 2002, 2004). However, these issues are beyond the scope of the current study.

Contaminant levels in feathers reflect the levels and composition of congeners in blood during the period of feather formation, which might differ from the composition in the blood during the period of blood sampling. Oystercatchers moult most of their feathers after the breeding period (Bauer et al., 2005). Although oystercatchers are comparatively resident birds (Hulscher et al., 1996), some individuals might have left the breeding areas to moult and overwinter at different sites, which could contribute to the differences in contaminant levels between feathers and serum. However, this is only likely to be relevant in some individuals, given that our data showed significant relationships between the levels of most PCB congeners in feathers and serum. Furthermore, no compound accumulated significantly more in one tissue than another, suggesting that feathers were moulted in the breeding area, and that only the less-persistent compounds were not transferred into feather tissues.

The dietary spectrum of oystercatchers from the Wadden Sea is relatively small (Schwemmer et al., 2012) compared with other marine bird species such as gulls (Kubetzki and Garthe, 2003), and levels of contaminants are thus less likely to differ between different time periods. This would further explain the high correlation between serum and feather levels.

4.3. Differences in stable isotopes between tissues and sites

Persistent contaminants accumulate in the higher trophic levels of the food chain. We therefore hypothesised that contaminant levels would be higher with increasing values of δ15N; furthermore, we hypothesised higher contaminant levels with increasing values of δ13C as those would indicate a higher degree of foraging in the potentially more contaminated marine environment (Fry, 2006; Inger and Bearhop, 2008; Cea et al., 2014). However, we found no indication of such a relationship in either feathers or blood. Jaspers et al. (2007) described the same situation in feather and muscle tissues in a set of terrestrial and aquatic bird species from elsewhere. The most likely explanation for the lack of a relationship between contaminant levels and δ15N is that the diets of individual oystercatchers were too similar to promote strong differences in nitrogen values. In fact, oystercatchers may specialise on a particular diet in a given region (Bunskoike et al., 1996). Sample sizes were also relatively small and the apparent absence of a link between stable isotope values and contaminant levels should thus be regarded with care.

High δ15N values in coastal birds such as oystercatchers indicate that they feed on prey originating from higher trophic levels, while low δ13C values indicate prey of terrestrial origin (Inger and Bearhop, 2008; Cea et al., 2014). We found higher δ15N values and lower δ13C in feathers, compared with serum and blood cells, at the Oland site. We also found lower δ13C values in blood at the Elbe site compared with Oland. Isotope ratios in feathers reflect the diet during the period of feather growth, whereas isotope ratios in blood reflect the diet several days before blood sampling (e.g., Evans Ogden et al., 2004). We therefore conclude that oystercatchers from Oland fed more on terrestrial prey with a lower trophic origin during the breeding period (i.e., period of blood sampling) compared with the period of feather growth and compared with birds from the Elbe location. Differences in δ13C between the two sites may be associated with differences in primary producers (such as phytoplankton). However, there are some indications that the diets of the oystercatchers differed between the two sites; Kolze (2014) found that oystercatchers from Oland consumed a relatively high proportion of terrestrial prey items (i.e., mostly insects) during the breeding period, which they collected on pastures within the breeding sites. In contrast, oystercatchers from other sites on the German Wadden Sea coast are known to feed on an almost exclusively marine diet (Schwemmer et al., 2012; Schwemmer et al., 2014). This difference is clearly reflected in the terrestrial signatures of serum from birds from the Oland site, compared with individuals from the river mouth.

The high δ15N and δ13C values in feathers from the Oland site indicate that the individuals have left the pastures during feather formation and are consuming a more marine diet. The reasons for the stronger terrestrial component in the diet of birds breeding on a Hallig, an island in the centre of the Wadden Sea, are currently unknown. However, there are some indications that the marine prey base around the colony on Oland is currently insufficient to sustain a high number of chicks, and the adults therefore supplement their diet with terrestrial prey (Kolze, 2014; own unpublished data).

The greater use of terrestrial prey in individuals from Oland may explain the lower levels of contamination at this site, because the birds make less use of the potentially more-contaminated marine diet. However, we found no relationship between contaminant levels and δ13C, suggesting that contaminant loads are independent of the degree of terrestrial feeding. It is also possible that the overall levels of contamination were too low to show proper relationships between stable isotope values and contaminants.

The stable isotope signatures of birds breeding at the riverine site indicate an intermediate nutrition. However, the relatively high variance may reflect strong individual diet specialisation.

5. Conclusions

In conclusion, our results suggest that the outflow of one of Europe’s largest river systems still shows significant levels of...
contamination that accumulate in the tissues of a predator at the top end of the marine food chain. Although levels of PCBs and DDT are generally much lower than in the past (Becker and Muñoz Cifuentes, 2004; Becker and Dittmann, 2009), values increased again in the short-term from 2008 to 2011, indicating that it might be difficult to meet Ecological Quality Objectives (Dittmann et al., 2011). Trends in contaminant levels in benthivores such as the oystercatcher should thus continue to be monitored within the established schemes. Although PBDEs were not detected in significant amounts in the current study, it might be advisable to include these compounds in the monitoring process.

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References


