Article

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Factors influencing the bioaccumulation of persistent organic pollutants in food webs of the Scheldt estuary

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Abstract

Concentrations of several persistent organic pollutants (POPs: PCBs, PBDEs, OCPs) in aquatic species from the Scheldt estuary were related with factors (body size, lipids, trophic position) possibly influencing their bioaccumulation. Stable nitrogen isotope ratios ($\delta^{15}N$) were used as a measure for trophic position. A decreasing trend in POP levels towards the sea was observed. For POP concentrations in sediments, this trend could be attributed to a dilution effect from mixing with seawater. However, concentrations in biota more downstream were higher than expected after taking into account the dilution effect, possibly due to differences in bioavailability. Tissue concentrations were correlated with the lipid content in biota, but not with body size. Biomagnification was only significant for some PCB congeners and *p,p'*-DDE at the most marine sampling location (Terneuzen, L1) and for *p,p'*-DDD and BDE 100 at the second sampling location (Bath, L2). A significant decreasing relationship was found for ɣ-HCH concentrations with increasing $\delta^{15}N$ at Terneuzen. For Antwerpen (L3), no significant relationships were detected. TMFs ranged from 0.64 for ɣ-HCH up to 1.60 for PCB 194. These results suggest that biomagnification was more important in the marine part of the estuary, although the presence of multiple carbon sources at the freshwater side might have led to an underestimation of the influence of trophic position.

Keywords

Persistent organic pollutants; Polychlorinated biphenyls (PCBs); Polybrominated diphenyl ethers (PBDEs); Stable carbon and nitrogen isotopes; Biomagnification; Scheldt estuary

Introduction

Intensive industrial and agricultural activities have caused the worldwide introduction of organic chemicals in the aquatic environment. Man-made chemicals, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs), can persist for many years in these environments, hence forming a possible health threat for wildlife and humans through bioaccumulation[1-3]. The bioaccumulation of persistent organic pollutants (POPs), which mostly have a lipophilic character, into aquatic biota is believed to be mainly driven by two processes. The first process is the direct partitioning of chemicals between the organism's body and the abiotic environment, also called bioconcentration. The second process is dietary uptake[4]. However, for lipophilic POPs (log K_{ow} > 5) bioconcentration is considered to be of less importance for most fish when compared to dietary uptake[5]. If the chemical concentration in a consumer exceeds the concentration in his diet, and if the absorption rate exceeds the elimination from the body through biotransformation, growth and reproductive loss, biomagnification occurs. In this case, the POP level in an aquatic species will be influenced by its trophic position in the local food web. Consequently, top predators tend to contain the highest body burdens of pollutants and may also suffer the highest risk for adverse health effects[6].

To understand the importance of trophic transfer in relation to the fate of pollutants in the food web and quantify the extent of biomagnification, the first step is to determine the trophic positions of the species in the food web. A frequently used method for this is the analysis of stable isotope ratios, as the isotopic signature of an animal reflects its assimilated diet[7, 8]. By measuring stable isotope ratios as well as the pollution levels in several species, it is possible to identify and quantify biomagnification within a food web[9].

The present study was conducted in the Scheldt estuary (the Netherlands – Belgium). The river Scheldt is a lowland-river which has its source in St. Quentin (France), flows

63 through Belgium and flows into the North Sea in Vlissingen (the Netherlands). The river has a
64 total length of 355 km and the tidal effects reach 160 km upstream, until Ghent[10]. With a total
65 catchment area of 22000 km², the river receives water from dense populated and
66 industrialized areas, enriching the estuary with nutrients and pollutants, including trace
67 metals[11] and POPs[3, 12], making the Scheldt one of the most polluted estuaries in Europe.
68 Nonetheless, the estuary is of great ecological value, for example because of its function as
69 nursery room for demersal fish species, as breeding area of the harbor seal (*Phoca vitulina*)[13],
70 and because of the international importance for seabird conservation[14]. For this reason it is
71 essential to establish the fate of man-made chemicals in the estuary and their possible effects
72 in the food webs.

73 In this paper, concentrations of POPs in different aquatic species from the Scheldt
74 estuary were measured and related with their stable isotope ratios, as a measure for trophic
75 position, to see whether POPs are biomagnified through the food web in this estuary. The
76 study was conducted at three different locations along the salinity gradient to compare the fate
77 of POPs in freshwater versus saltwater conditions. Tissue POP concentrations were also
78 linked with other factors (body size, lipid content), possibly influencing the bioaccumulation
79 of POPs.

80

Material and Methods

2.1 Sample collection

In June 2011, samples were collected at three locations along the Scheldt Estuary (Fig. 1): Terneuzen (51°35'N 3°88'E), Bath (the Netherlands, 51°40'N 4°21'E) and Antwerpen (Belgium, 51°23'N 4°39'E). Fish, crab and shrimp species were collected by means of fyke fishing (INBO, Research Institute for Nature and Forest) and trawl fishing with the vessel *Zeeleeuw* (VLIZ, Flanders Marine Institute). Other invertebrates were sampled on the shore by hand at low tide. Filamentous algae were collected from rocks. An overview of the collected species is given in Table 1. More detailed data on the lipid content, length and weight of the collected samples is provided in Table SI-1 of the Supporting Information (SI). Suspended particulate matter (SPM) was collected by filtration of surface water with a vacuum pump over glass fiber filters (VWR International, pore size 0.7 μm). Because of limited sample size, no POP analyses could be performed on SPM samples. The top layer (10 cm) of the surface sediment was sampled manually from the shores at low tide. At each location, three replicates were taken. TOC (total organic carbon) was determined through Loss on Ignition (LOI). To this, the sediment subsamples were incinerated at 550 °C for 4 h and weight loss was determined[15].

Before freezing and dissection, the organisms were kept for depuration in filtered locally collected river water (0.2 μm) for 24h. A part of the caudal musculature of the fish was sampled to perform stable isotopes and POP analyses. For smaller fishes, crabs and shrimps, the whole musculature was homogenized for analysis. Soft tissues of other invertebrates were analyzed as a whole. For POP analysis, tissues from shrimps, mollusks and bristle worms were pooled to get an adequate sample size. Stable isotopes in shrimps, mollusks and bristle worms were determined in individual samples from the same area, which

were not analyzed for POPs. Filamentous algae were rinsed to remove sand and organisms. All samples were frozen (-20°C) until analysis.

2.2 POP analysis

The following POPs were targeted for analysis in all samples: 33 PCB congeners (IUPAC numbers: CB 18, 28, 44, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 151, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, 206, 209), 7 PBDEs (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, 183), DDXs (*o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT), chlordanes (TC, CC, TN, OxC), HCHs (α-, β-, γ-hexachlorocyclohexane) and HCB (hexachlorobenzene). PBDE 209 was measured only in the sediment samples due to low expected concentrations in biota relative to the rather high method LOQ[3, 16]. A detailed description of the methods used for POP analysis and quality control is described in Van Ael et al.[3] and is provided as SI.

2.3 Stable isotope analysis

To indicate the trophic position of the collected species, carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were measured. For this, muscle tissues (fish, crabs, shrimps) or whole soft body (mollusks, bristle worms, *Chaetogammarus marinus*) tissues were freeze dried and ground to a powder with mortar and pestle. From fish tissues with a high lipid content, lipids were extracted by rinsing the samples with chloroform:methanol (2:1, v/v). SPM samples, that potentially contained carbonates, were acidified by placing them during 24h under a glass jar with fuming HCl (37%), to remove calcareous material[8]. Samples were measured before and after acidification or removal of lipids. Stable isotope ratios were determined using a mass spectrometer (VG Optima, Isoprime, UK) equipped with an elemental analyzer (Carlo Erba, Italy) for combustion and automated analysis. Carbon and

nitrogen isotope ratios were expressed as δ values (‰) relative to the Vienna PeeDee Belemnite (vPDB) standard and to atmospheric N_2 respectively. IAEA-N1 ($\delta^{15}N$ = 0.4 ± 0.2‰) and IAEA C-6 ($\delta^{13}C$ = -10.8 ± 0.2‰) were used as reference materials. Standard deviations for multi-batch replicated measurements (N = 22) of one fish muscle sample were ±0.19 and ±0.25 ‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

2.4 Statistical analysis

POP concentrations below the LOQ were substituted by a value of LOQ*f (detection frequency). After testing the normality and homogeneity of variances, concentrations were log-transformed where necessary. Differences between locations were detected by using One-way ANOVA with Tukey test or Student's t-test. The level of statistical significance was set at $p < 0.05$. Pearson correlation was applied to determine the influence of lipid content and body size (length and weight) on the bioaccumulation of POPs.

The dilution effect of mixing with seawater, which may cause lower sediment and biota POP concentrations more downstream in the estuary, was tested by normalizing the POP concentrations for salinity. Therefore, the POP concentrations were multiplied by the ratio of salinity at (Site X/Anwerpen). Salinity values used: Terneuzen, 27.3; Bath, 15.6; Antwerpen, 3.4. Ratios used: (Terneuzen/Antwerpen) = 8.1; (Bath/Antwerpen) = 4.7; (Antwerpen/Antwerpen) = 1. Salinity values are year averages from a monitoring database from Rijkswaterstaat, a part of the Dutch Ministry of Infrastructure and the Environment, and from the Flemish Environment Agency (VMM).

Differences between locations in $\delta^{13}C$ values in the same species were detected by using Student's t-test.

Regression analyses was used to study the relationship between POP concentrations and stable isotope values (individual data), as a tracer for the trophic position. The calculation

155 of trophic levels (TLs) has been used in several studies to indicate trophic magnification. To
156 derive the TL from $\delta^{15}N$ values the following equation is often used[17]:

157 $$TL_{(consumer)} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/ \Delta\delta^{15}N$$

158 where $\Delta\delta^{15}N$ is the trophic enrichment factor or the shift in $\delta^{15}N$ between consecutive TLs[17].
159 Trophic magnification factors (TMFs) are than calculated from the slope of the regression
160 between log-transformed concentrations of pollutants and the trophic levels[18]. However, the
161 trophic enrichment factor ranges between 3‰ and 5‰ and can be variable, depending on
162 species, diet, tissue and physiology[19, 20], making it questionable to apply a fixed value for
163 $\Delta\delta^{15}N$. For this reason TLs were not calculated in this study[21]. TMFs are calculated as the
164 antilog with base 10 of the slope from the regression between log-transformed concentrations
165 of pollutants and $\delta^{15}N$ values[22].

166 Multiple regressions models were constructed to analyse the combined effect of lipid
167 content and $\delta^{15}N$.

168 Statistical analyses were performed using GraphPad Prism 6.00 (GraphPad Software,
169 Inc).

170

Results and discussion

3.1 POP concentrations

Median (+ range) POP concentrations and lipid content in biota and sediment samples are reported in Table 1. Average TOC content (± SD) in the sediment measured 2.7 ± 1.5%, 2.5 ± 1.1% and 4.7 ± 2.5% for Terneuzen, Bath and Antwerpen respectively. Sediment PCB concentrations were low, ranging from 1.8 up to 58.7 ng/g dw. PCB levels in biota samples were highest in European eel (*Anguilla anguilla*), ranging from 846 up to 2190 ng/g ww. Lowest PCB concentrations were found in common periwinkle (*Littorina littorea*) (from 17.6 up to 28.0 ng/g ww). Median PCB concentrations in Baltic tellin (*Macoma balthica*), European sprat (*Sprattus sprattus*) and European eel exceeded the maximum limit for the sum of the 6 indicator PCBs (75 ng/g ww; indicated in bold), as set by European legislation[23]. Concentrations in European eel also exceeded the consumption limit of 300 ng/g for muscle meat of wild caught eel[24]. PCB 153 was the most dominant congener in all species (12 – 28% of ∑PCBs), followed by PCB 138 and 149. Only in filamentous algae, PCB 149 was most abundant (11%). PCB 18, 205, 206 and 209 were not frequently detected in all samples.

From the PBDE congeners, PBDE 209 was dominant in the sediment (99.7%), while in the tissues, BDE 47 was most abundant (32-69%, with PBDE 209 not measured in tissues). Total PBDE concentrations in the sediment ranged from 1.70 up to 575 ng/g dw. Median tissue PBDE concentrations were lowest in brown shrimp (*Crangon crangon*) and common periwinkle (0.01 ng/g ww) and highest in European eel (8.76 ng/g ww).

Chlordanes were not detected in the sediment and only in low concentrations in biota samples: medians from below detection limit up to 5.67 ng/g ww in European eel (Table 1). Trans-chlordane was the most detected chlordane, although in European eel, oxychlordane reached the highest concentrations. *p,p'*-DDE was the most detectable DDT congener. ∑DDT ranged from 0.56 ng/g ww in brown shrimp up to 49.3 ng/g ww in European eel.

196 The contaminant levels measured in the present study were relatively high compared
197 to studies from other regions. PCB concentrations in European eel were higher than
198 concentrations detected in eels from the Garigliano river in Italy (119.7-2156 ng 7 ICES
199 PCB/g lw; present study: 3851-5670 ng 7 ICES PCB/g lw))[25] and from five Irish rivers (13.7-
200 197 ng 7 ICES PCB/g lw)[26]. Detected PCB levels in European eel were within the ranges
201 previously measured in Flanders (Belgium) by Belpaire et al.[27] (11.4-7753 ng 7 ICES PCB/ng
202 ww). In the present study however, higher average concentrations were measured in eels from
203 Antwerpen (Belpaire et al., 2011: 513.4 ng 7 ICES PCB/g ww; present study: average 814.5
204 ng 7 ICES PCB/g ww). A French study from Bragigand et al.[28] showed lower PBDE levels in
205 European eels from the Loire estuary (0.13-0.57 ng BDE 47/g ww; present study: 4.40-11.0
206 ng BDE 47/g ww) and comparable levels in eels from the Seine estuary (2.67-7.84 ng BDE
207 47/g ww).
208 PCB concentrations in European sprat from the Polish Baltic Sea were five times
209 lower (average 20.8 ng 6 ICES PCBs/g ww; present study: average 102 ng 6 ICES PCBs/g
210 ww)[29]. PBDE concentrations in pike-perch (*Sander lucioperca*) from the present study were
211 higher than in pike-perch from the Baltic Sea (average 0.57 ng/g ww for 15 PBDE congeners;
212 present study: average 1.88 ng/g ww for 7 PBDE congeners)[30]. Ragworm (*Nereis*
213 *diversicolor*) contained higher PBDE concentrations than ragworms from the Loire and the
214 Seine estuary (0.03-0.12 ng BDE47/g ww; present study 0.15-0.62 ng BDE 47/g ww)[28]; and
215 *Nereis virens* from the St. Lawrence estuary, Canada (average 0.18 ng BDE 47/g ww)[31].
216 PBDE levels detected in brown shrimp were lower than previously reported for the North Sea
217 by Boon et al.[32] (average of 37 ng BDE 47/g lw) and for the Scheldt by Voorspoels et al.[16]
218 (0.2-8.3 ng ∑6 PBDE/g ww). Van Leeuwen and de Boer measured comparable levels of
219 PBDEs in sole, brown shrimp, blue mussels and pike-perch in Dutch rivers and lakes.
220 However, concentrations in European eel (0.4-81 ng BDE47/g ww; present study: 4.4-11 ng

221 BDE47/g ww) and European flounder (4.4-11 ng BDE47/g ww; present study: 0.09-2.17 ng
222 BDE47/g ww) were higher[33].

223 Similar concentrations were measured in a previous study in organisms from the same
224 system (European flounder: median 46.1 ng 6 ICES PCBs/g ww; present study: 42.5 ng 6
225 ICES PCBs/g ww; Common sole: median 17.2 ng 6 ICES PCBs/g ww; present study: 22.2 ng
226 6 ICES PCBs/g ww).[3]

227 The concentration of each individual contaminant was highest in European eel. Eel
228 species are known for their ability to accumulate lipophilic substances[2]. They are carnivorous
229 predators and compared to other fish species, they show very high lipid values (average of
230 18.6 % in the present study).

231 The tissue concentrations (ww) of several POPs in the aquatic biota were significantly
232 correlated with their lipid content. Correlations were very strong for biota collected in
233 Antwerpen ($p<0.0008$). From the 55 analyzed POPs, 50 compounds showed a correlation
234 with the lipid content (with $r^2>0.2$). This data set included the European eel, which is known
235 for its high lipid content. When eels were excluded from the data set, POP concentrations and
236 lipid content were less correlated ($p<0.0391$, 14 significant correlations). In samples from
237 Bath, tissue concentrations of 22 POPs were significantly correlated with the lipid content
238 ($p<0.0006$). In Terneuzen, 29 POP congeners showed significant correlations between tissue
239 concentrations and lipid content ($p<0.0045$). Table SI-2 lists all significant correlations (p and
240 r^2).

241 The individual length and weight of fish and crabs have been plotted against tissue
242 concentrations of POPs. Positive significant relationships were found for most compounds
243 (ww) in European flounder near Antwerpen ($0.002<p<0.033$) and for some PCB congeners
244 (ww) in smelt (*Osmerus eperlanus*) from Bath ($0.014<p<0.033$). For the other species no
245 significant correlations were detected. Increasing POP concentrations with increasing body

size were found for PCBs and PBDEs in salmon (*Oncorhynchus sp.*)[34] and for PBDEs in striped bass (*Morone saxstilis*) and catfish (*Pilodictus olivaris* and *Ictalurus punctatus*)[35]. As body size increases, the elimination rates for lipophilic compounds via direct partitioning through the water decreases because of a reduced exchange surface[5, 36]. However, in the present study, few correlations were significant. The body size has influence on the bioaccumulation process, but the effect appears to be overwhelmed by other factors, like trophic position and lipid content.

Higher POP concentrations were generally found more upstream from the estuary. Although this trend was not statistically significant in sediment samples, a significant difference was found in the tissues of European flounder (*Platichthys flesus*) ($0.007 \leq p \leq 0.353$), shore crab (*Carcinus maenas*) (for PCBs, $0.002 \leq p \leq 0.017$) and bristle worm (Polychaeta; Terneuzen: *Arenicola marina*; Bath and Antwerpen: *Nereis diversicolor*) ($0.035 \leq p \leq 0.047$) (Fig. 2). This indicates that the POP levels are higher more upstream of the estuary, probably caused by the vicinity of the city of Antwerpen, which is highly industrialized and urbanized. Furthermore, the Scheldt receives waste waters from other large cities like Brussels. This observation has been described before in other studies[3, 16, 37]. More downstream in the estuary, lower environmental pollution levels could be attributed to a dilution effect, because of a wider riverbed and the increasing mixing with seawater. Since the salinity can be used as a measure for the dilution with seawater, it can be tested if the dilution effect is responsible for the decreasing trend in POP levels. If POP concentrations in sediments are normalized for salinity, the decreasing trend towards the North Sea gets minimalized and the normalized concentrations get more or less constant (Fig. 2). This means that the dilution of the river water explains lower POP concentrations in the sediments towards the sea. However, normalizing the POP concentrations in bristle worm and European flounder for salinity (Fig. 2) does not have the same effect. Biota from Terneuzen (L1) and

Bath (L2) contain higher POP levels than expected from the dilution gradient, in contrast to concentrations in the sediment. When performing the same normalization on lipid weight POP concentrations, the same results were obtained. Possible POP sources more downstream in the estuary such as industrial factories in Terneuzen or the Ghent-Terneuzen canal, could cause local higher POP concentrations. However, these are not reflected in the sediment POP concentrations. For European flounder, possible migration from one location in the estuary to another must be taken into account. Nevertheless, a significant difference in tissue concentrations of POPs from the different locations was observed. The differences in bioaccumulation along the salinity gradient may be caused by variation in the bioavailability. Moreover, this also implies that sediment concentrations are poor predictors of bioaccumulation, because pollutant levels were much higher in the downstream parts of the estuary than expected on the basis of the sediment concentrations.

3.2 Isotopic compositions

The isotopic compositions of the samples collected at the three sampling locations are reported in Figure 3. With an average value of -26.4‰, $\delta^{13}C$ values for SPM samples were comparable with previously reported values for the Scheldt estuary[38, 39]. SPM from the riverine part of the Scheldt is more ^{13}C depleted (-27.7‰) than SPM from the marine side (-25.3‰), indicating the input of riverine and terrestrial organic matter in the upper estuary[39]. Consumers $\delta^{13}C$ values ranged from -27.6‰ for pike-perch in Antwerpen up to -17.3‰ for common periwinkle in Terneuzen. Freshwater species, such as pike-perch, had slightly more ^{13}C-depleted values when compared to marine species. Species at the bottom of the food web, such as Oligochaeta and Baltic tellin ($p = 0.0002$) had less depleted $\delta^{13}C$ values when sampled in Terneuzen (L1) compared with two other locations (Bath, L2 and Antwerpen, L3). The available carbon sources in Terneuzen were probably mainly marine sources, which are

296 typically less $\delta^{13}C$ depleted than those of freshwater[8, 40], while the two other locations receive
297 more terrestrial and riverine input.

298 The large variation in $\delta^{13}C$ values in the present study indicates consumers are feeding
299 on different carbon sources and may also be part of different food webs. For this reason, $\delta^{15}N$
300 values are site-specific and were used as an overall indication of site-specific trophic position.

301 Mean consumer $\delta^{15}N$ values ranged from 13.7‰ for mud shrimp (*Corophium*
302 *volutator*) in Terneuzen (L1) to 21.6‰ for ragworm in Antwerpen (L3), although these values
303 for ragworm were exceptionally high. In general, invertebrates such as the bivalves blue
304 mussel (*Mytilus edulis*) and Baltic tellin showed the lowest $\delta^{15}N$ values. Highest $\delta^{15}N$ values
305 were detected in carnivorous fish. Filamentous algae showed relatively high $\delta^{15}N$ values,
306 especially the samples collected at Terneuzen (L1).

307

308 *3.3 Influence of trophic position on bioaccumulation*

309 Several PCB congeners and *p,p'*-DDE showed a significant increase in log-
310 transformed lipid weight concentrations with increasing $\delta^{15}N$ values (Table 2, Figure 4) for
311 the samples collected at Terneuzen (L1), indicating a positive relationship between pollution
312 level and trophic level. A significant decreasing trend was found for ɣ-HCH concentrations
313 with increasing $\delta^{15}N$. In Bath (L2), significant relationships were only found for *p,p'*-DDD
314 and BDE 100. For Antwerpen (L3), no significant relationships were detected. TMFs were
315 higher than 1, except for the TMF for ɣ-HCH, indicating biomagnification in the Scheldt
316 estuary. TMFs ranged from 0.64 for ɣ-HCH up to 1.60 for PCB 194 (Table 2).

317 As mentioned above, the large variation in $\delta^{13}C$ values in the present study indicates
318 consumers are feeding on different carbon sources and may be part of different food webs.
319 However, the significant relationship observed still suggest biomagnification of the selected

compounds. This could mean that the $\delta^{15}N$ values of the baseline are similar between sources at one site, making the delta 15N values an overall indication of site-specific trophic position.

The biomagnification of PCBs has been described before in food webs at various locations, such as a marine food web in Norway[41], in fish of the sub-alpine Como Lake in Italy[42], in arctic food webs[41, 43] and in a freshwater food web from China[44]. The TMFs found in the present study for PCBs are lower than TMFs found in the Iroise Sea (Western Brittany) and the Seine Bay (1.9-17.3)[45], Congo River Basin (1.72-2.93)[9], the Northwater Polynya marine food web (1.7-10.7)[46], lakes in Canada and the north eastern US (1.3-8.0)[47] and from a freshwater food web from South China (0.75-5.10)[44].

In the present study, biomagnification of PCBs was linked with the degree of chlorination of the PCB congeners. Regressions were only significant for hexa- to octa-PCBs, which also possess higher log K_{ow} than lower chlorinated congeners. However, this statement probably holds only for non-metabolizable PCB congeners. The same trend was also reported by Skarphedinsdottir et al.[48] in a food web near the coast of Iceland. Yu et al.[49] described a parabolic relationship between the TMFs of PCBs for freshwater fish and the log K_{ow}, with largest TMFs at log K_{ow} of 6.89. In the present study however, the greatest TMF was found for PCB 194, which has a log K_{ow} greater than 6.89 (log K_{ow} = 7.8). PCBs were clearly more biomagnified than PBDEs, which only showed a significant relationship with $\delta^{15}N$ in case of PBDE 100, with a TMF of 1.17. The lower biomagnification potential of PBDEs was previously reported[50, 51]. Although Kelly et al.[51] demonstrated the biomagnification of BDE 47 in a marine food web, they found that the TMF for BDE 47 was much lower than the TMFs of comparable PCBs.

No biomagnification was found for HCHs. For ɣ-HCH, a significant decreasing relationship of concentration with increasing $\delta^{15}N$ values was observed (Table 2). When compared to PCBs or DDTs, HCHs have a lower log K_{ow}, indicating that they may have a

345 lower bioaccumulation potential. This limited bioaccumulation of HCHs has been
346 documented in several studies[21, 41]. Other authors have stated before that species ecology has a
347 minor influence on the bioaccumulation of substances with log K_{ow}< 5[4, 52]. Moreover, some
348 fish species, such as shorthorn sculpin (*Myoxocephalus scorpius*), have the ability to rapidly
349 eliminate ɣ-HCH after oral exposure[53], which can result in lower concentrations in their
350 predators.

351 Significant multiple regressions models were constructed for the combined effect of
352 lipid content and $\delta^{15}N$. For PCB 153 and *p,p'*-DDE at Terneuzen, the regression resulted in
353 the following equations: [PCB153]=2.91 x $\delta^{15}N$ + 11.5 x Lipid with p<0.0001 and R^2=0.55;
354 [*p,p'*-DDE]=0.55 x $\delta^{15}N$ + 1.99 x Lipid with p<0.0001 and R^2=0.58. For ɣ-HCH at
355 Terneuzen, no significant model was found. For *p,p'*-DDD and BDE100 at Bath, the
356 following equations were obtained: [*p,p'*-DDD]=0.063 x $\delta^{15}N$ + 1.16 x Lipid with p<0.0001
357 and R^2=0.75; [BDE100]=0.013 x $\delta^{15}N$ + 0.11 x Lipid with p<0.0001 and R^2=0.42.

358 The results of this study indicate that biomagnification is more pronounced in the
359 marine part of the estuary, as stronger relationships between POP level and $\delta^{15}N$ values were
360 found closest to the sea. At the most downstream sampling location (L1, Terneuzen), the
361 available food sources will be mainly from marine origin, in contrast with the other locations,
362 where the input from riverine and terrestrial carbon sources is larger. The input from riverine
363 and terrestrial sources is indicated by more depleted $\delta^{13}C$ values for SPM. Therefore, the
364 carbon sources available for the food web of Terneuzen will be more restricted. At the two
365 upstream locations, consumers might be feeding on more different carbon and nitrogen
366 sources. In this case, the stable isotope values may not be perfectly comparable with each
367 other among species, because of a difference in carbon and nitrogen sources and so,
368 biomagnification might also be more difficult to detect. For this reason, the influence of
369 trophic position on the bioaccumulation might be underestimated. This may explain why more

370 significant relationships are found at the most downstream sampling location, Terneuzen,
371 where there are fewer carbon and nitrogen sources.
372
373 In conclusion, the contamination levels of POPs detected in the tissues of aquatic
374 species from the Scheldt estuary were relatively high compared to concentrations found in
375 other studies, making the Scheldt one of the most polluted estuaries in Europe. A decreasing
376 trend in POP levels towards the sea was observed. For POP concentrations in sediments, this
377 trend could be attributed to a dilution effect from mixing with seawater. However,
378 concentrations in biota more downstream were higher than expected after taking into account
379 the dilution effect, possibly due to differences in bioavailability. Regression of $\delta^{15}N$ results
380 with logged, lipid normalized concentration data showed more pronounced biomagnification
381 at the marine site, although the presence of multiple carbon sources at the freshwater side may
382 have led to an underestimation of the influence of the trophic level.
383

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390

391 ## Supporting Information Available

392 The first paragraph in the Supporting Information gives a detailed description of the methods
393 and quality control used for POP analysis. Table SI-1 presents mean lipid content, weight and
394 total length of the collected species, together with median concentrations, separated per
395 location. Table SI-2 lists all significant correlations between tissue concentrations (ww) of
396 several POPs in the aquatic biota and their lipid content. Table SI-3 presents the mean $\delta^{13}C$
397 and $\delta^{15}N$ values (‰) of all samples per location. This information is available free of charge
398 via the Internet at http://pubs.acs.org/.

399

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570

571 Table 1. Median and range of POP concentrations in sediment (ng/g dw) and biota (ng/g ww; filamentous green algae expressed in
572 ng/g dw) from the Scheldt estuary, together with lipid content (%), number of measurements (N: individual measurements or pools for
573 POP analysis per sampling site, Terneuzen/Bath/Antwerpen) and the tissue analyzed. ICES PCBs are the 6 indicator PCBs: 28, 52,
574 101, 138, 153, 180. n.d. = not detected.

Sample		N (T/B/A)	Tissue	Lipid (%)	∑PCB	∑ICES PCB	PCB 153	∑PBDE	BDE47	∑DDT	*p,p'*-DDE	HCB	∑HCH	∑chlordanes
Sediment		2/2/2		-	4,28	1,58	0,49	56,8	0,02	0,21	0,08	0,02	0,35	n.d.
					(1,75 - 58,7)	(0,43 - 21,5)	(0,08 - 4,62)	(1,70 - 575)	(0,02 - 0,28)	(0,15 - 4,45)	(0,05 - 0,96)	(0,02 - 0,19)	(0,10 - 0,74)	
Filamentous algae		1/1/1		-	32,7	9,60	2,30	0,96	0,47	2,51	1,22	0,13	1,05	0,13
					(2,52 - 32,7)	(0,47 - 10,8)	(0,07 - 2,75)	(0,41 - 1,52)	(0,19 - 0,59)	(0,30 - 2,83)	(0,17 - 1,53)	(0,07 - 0,22)	(0,83 - 1,64)	(0,07 - 0,15)
Mollusks														
Mytilus edulis	Blue mussel	4/0/0	whole	2,51	94,9	41,1	18,1	0,59	0,21	3,75	2,40	0,03	0,22	0,23
				(2,20 - 2,97)	(79,5 - 111)	(34,5 - 48,2)	(15,2 - 21,3)	(0,49 - 1,06)	(0,17 - 0,29)	(3,02 - 4,81)	(1,90 - 3,04)	(0,03 - 0,13)	(0,17 - 0,24)	(0,19 - 0,27)
Macoma balthica	Baltic tellin	0/2/0	whole	2,80	249	**103**	37,2	1,71	0,60	11,2	7,03	0,24	0,38	0,87
				(2,73 - 2,86)	(245 - 253)	**(101 - 105)**	(36,5 - 37,8)	(1,62 - 1,80)	(0,55 - 0,65)	(10,8 - 11,7)	(6,72 - 7,35)	(0,24 - 0,24)	(0,33 - 0,44)	(0,84 - 0,90)
Littorina littorea	Common periwinkle	4/0/0	whole	0,82	22,8	10,4	3,97	0,01	n.d.	0,70	0,59	0,03	0,16	n.d.
Polychaeta				(0,69 - 1,06)	(17,6 - 28,0)	(7,84 - 12,5)	(3,19 - 4,87)	(0,01 - 0,05)		(0,60 - 0,82)	(0,49 - 0,67)	(0,03 - 0,10)	(0,10 - 0,19)	
Nereis diversicolor	Ragworm	0/2/3	whole	1,34	93,8	42,7	18,2	0,86	0,31	4,74	2,78	0,17	0,40	0,54
				(1,05 - 1,65)	(50,6 - 142)	(22,9 - 65,7)	(9,34 - 30,4)	(0,46 - 1,71)	(0,15 - 0,62)	(2,59 - 9,37)	(1,50 - 5,13)	(0,10 - 0,34)	(0,10 - 0,90)	(0,21 - 1,14)
Arenicola marina	Lugworm	3/0/0	whole	1,00	22,4	9,60	4,02	0,24	0,04	0,85	0,58	n.d.	0,10	n.d.
				(0,99 - 1,02)	(18,1 - 30,3)	(7,99 - 13,3)	(3,17 - 5,38)	(0,20 - 0,35)	(0,04 - 0,07)	(0,80 - 0,90)	(0,55 - 0,62)		(0,10 - 0,36)	
Crustacea														
Chaetogammarus marinus		1/1/0	whole	1,29	33,6	15,2	5,20	0,26	0,15	0,69	0,40	n.d.	2,02	n.d.
				(1,10 - 1,47)	(33,5 - 33,7)	(14,4 - 16,0)	(5,17 - 5,24)	(0,13 - 0,39)	(0,10 - 0,20)	(0,39 - 0,98)	(0,29 - 0,50)		(0,75 - 3,28)	
Crangon crangon	Brown shrimp	2/4/0	muscle	0,84	25,3	10,7	3,95	0,01	0,01	0,56	0,56	n.d.	0,03	n.d.

Sample		N (T/B/A)	Tissue	Lipid (%)	∑PCB	∑ICES PCB	PCB 153	∑PBDE	BDE47	∑DDT	*p,p'*-DDE	HCB	∑HCH	∑chlordanes
				(0,77 - 1,08)	(17,8 - 61,9)	(7,68 - 28,4)	(2,78 - 11,3)	(0,01 - 0,06)	(0,01 - 0,06)	(0,40 - 0,86)	(0,40 - 0,86)		(0,03 - 0,25)	
Carcinus maenas	Shore crab	2/11/0	muscle	0,63	84,1	41,5	20,3	0,22	0,15	1,50	1,46	0,06	0,06	n.d.
				(0,51 - 0,88)	(30,4 - 125)	(16,8 - 63,4)	(8,43 - 28,9)	(0,11 - 0,51)	(0,05 - 0,35)	(0,51 - 2,29)	(0,47 - 2,01)	(0,06 - 0,26)	(0,06 - 0,45)	
Eriocheir sinensis	Chinese mitten crab	0/3/4	muscle	0,61	80,5	43,2	18,6	1,22	0,84	2,39	2,35	0,19	0,06	1,23
				(0,39 - 0,97)	(40,5 - 336)	(22,0 - **187)**	(8,18 - 69,6)	(0,82 - 4,55)	(0,51 - 3,64)	(1,52 - 7,07)	(1,48 - 7,03)	(0,06 - 0,73)	(0,06 - 0,34)	(0,27 - 2,47)
Fish														
Platichthys flesus	European flounder	5/5/6	muscle	0,72	90,4	42,5	16,2	0,60	0,40	2,23	1,72	0,10	0,05	0,08
				(0,44 - 1,89)	(34,0 - 344)	(15,7 - **155)**	(6,29 - 65,8)	(0,19 - 3,32)	(0,09 - 2,17)	(1,14 - 11,9)	(0,81 - 8,43)	(0,06 - 0,49)	(0,05 - 0,39)	(0,08 - 0,60)
Solea solea	Common sole	1/4/6	muscle	0,60	47,7	22,2	8,07	0,18	0,06	1,26	0,90	n.d.	0,05	n.d.
				(0,45 - 1,02)	(29,3 - 107)	(13,7 - 51,8)	(4,79 - 20,2)	(0,13 - 0,51)	(0,04 - 0,18)	(0,58 - 3,66)	(0,38 - 2,65)		(0,05 - 0,13)	
Osmerus eperlanus	Smelt	2/7/0	muscle	1,19	76,1	37,1	14,1	0,67	0,45	3,05	2,16	0,21	0,05	0,08
				(0,92 - 1,74)	(41,9 - 235)	(18,6 - **105)**	(7,44 - 44,3)	(0,31 - 1,64)	(0,19 - 1,05)	(1,84 - 11,4)	(1,27 - 7,77)	(0,13 - 0,44)	(0,05 - 0,12)	(0,08 - 0,41)
Sprattus sprattus	European sprat	0/1/0	muscle	0,96	210	**102**	48,0	1,00	0,55	8,47	7,34	0,12	0,17	n.d.
Sander lucioperca	Pike-perch	0/2/3		0,53	140	65,4	25,8	1,32	0,76	5,02	3,86	0,15	0,15	0,08
				(0,49 - 1,00)	(96,2 - 290)	(45,6 - **137)**	(18,2 - 55,4)	(0,64 - 4,69)	(0,35 - 1,96)	(3,19 - 8,62)	(2,51 - 7,06)	(0,06 - 0,21)	(0,05 - 0,74)	(0,08 - 0,16)
Trisopterus luscus	Pouting	3/4/0	muscle	0,66	24,5	11,3	4,46	0,14	0,05	0,84	0,64	0,06	0,05	0,08
				(0,28 - 0,76)	(9,5 - 48,9)	(3,88 - 22,2)	(1,71 - 9,03)	(0,13 - 0,17)	(0,04 - 0,08)	(0,55 - 1,15)	(0,38 - 0,88)	(0,06 - 0,18)	(0,05 - 0,13)	(0,08 - 0,17)
Myoxocephalus scorpius	Shorthorn sculpin	2/6/0	muscle	0,83	30,1	18,8	8,68	0,22	0,13	0,95	0,79	0,06	0,05	n.d.
				(0,63 - 1,14)	(12,1 - 43,4)	(6,59 - 25,9)	(3,02 - 12,3)	(0,13 - 0,31)	(0,04 - 0,22)	(0,57 - 1,58)	(0,41 - 1,42)	(0,06 - 0,15)	(0,05 - 0,14)	
Anguilla anguilla	European eel	0/0/6	muscle	18,6	1290	**645**	285	8,76	5,85	49,3	32,3	3,92	2,24	5,67
				(9,16 - 23,2)	(846 - 2193)	**(433 - 1102)**	(191 - 512)	(7,52 - 18,1)	(4,40 - 11,0)	(36,8 - 89,3)	(28,2 - 60,4)	(2,10 - 5,67)	(1,06 - 3,74)	(3,29 - 7,14)

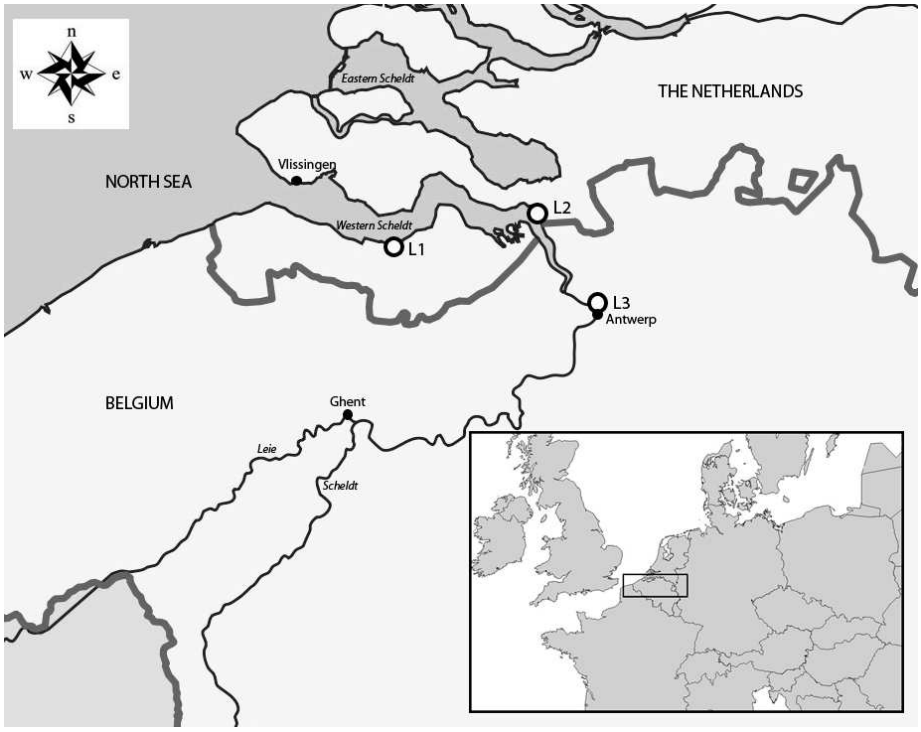
575

576 Table 2. Statistics for the significant linear regression between log-transformed POP
577 concentrations and $\delta^{15}N$ values, together with the corresponding TMFs.

Location	Compound	p	r^2	Slope	TMF
Terneuzen, L1	PCB 118	0.016	0.190	0.061	1.15
N=30	PCB 153	0.018	0.184	0.065	1.16
	PCB 138	0.042	0.139	0.057	1.14
	PCB 128	0.049	0.131	0.056	1.14
	PCB 156	0.004	0.259	0.081	1.21
	PCB 183	0.043	0.138	0.058	1.14
	PCB 180	< 0.001	0.423	0.110	1.29
	PCB 170	< 0.001	0.450	0.126	1.34
	PCB 199	0.034	0.205	0.120	1.32
	PCB 194	0.001	0.416	0.203	1.60
	p,p'-DDE	0.023	0.171	0.068	1.17
	□-HCH	0.001	0.371	-0.191	0.64
Bath, L2	*p,p'*-DDD	0.016	0.110	0.105	1.27
N=52	PBDE 100	0.013	0.129	0.068	1.17

578 Only results with $r^2 > 0.1$ are shown.

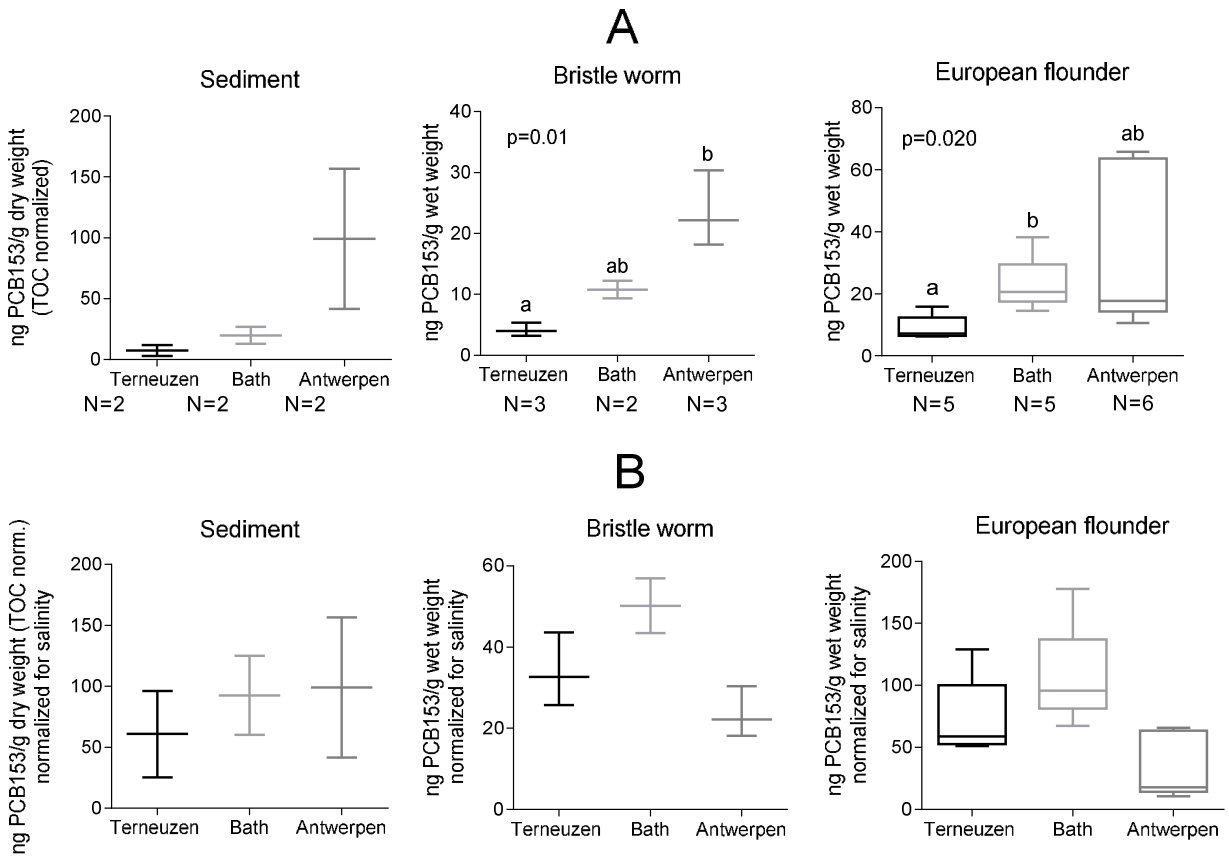
579



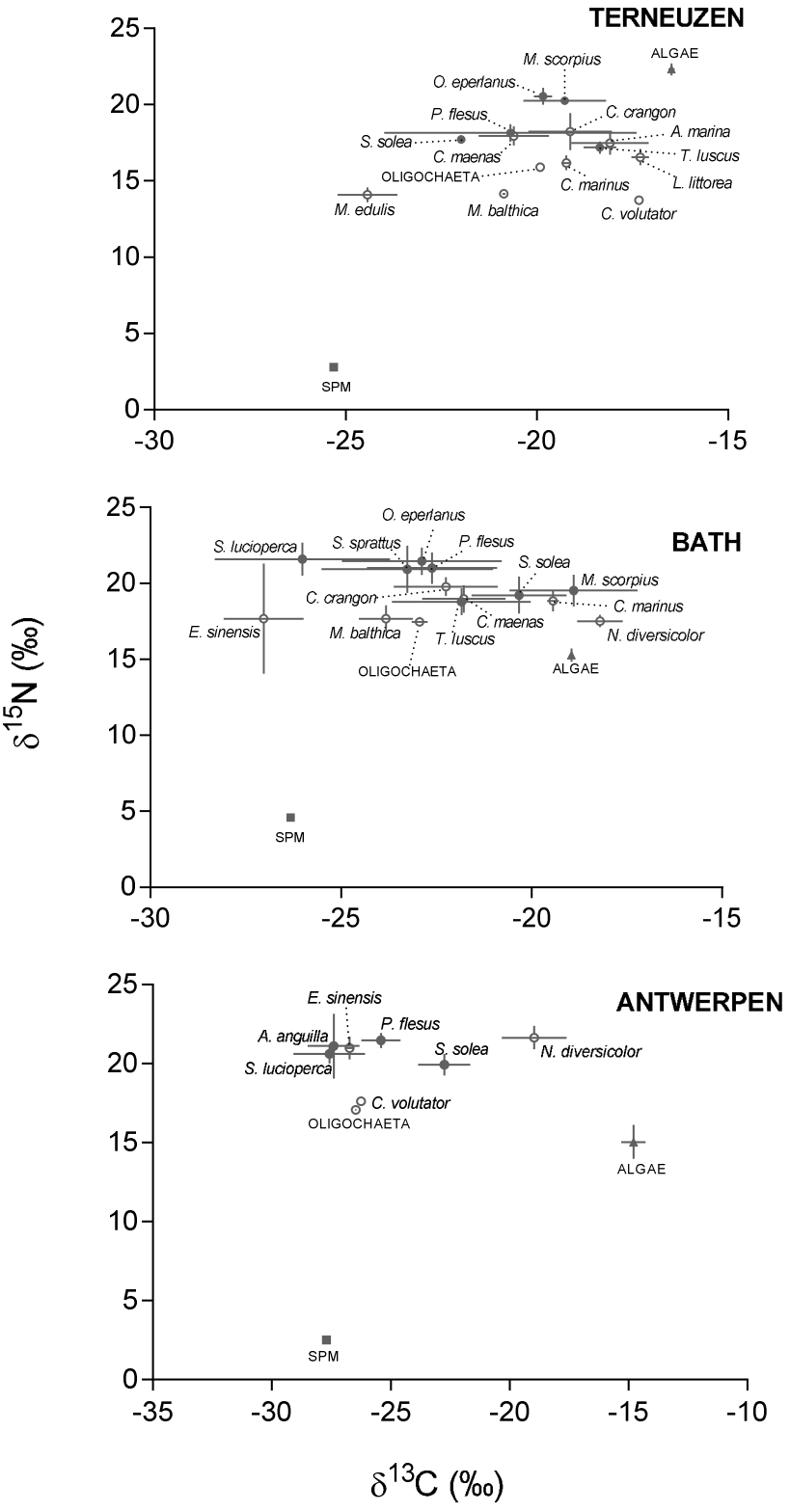
580

581 Fig. 1. Sampling locations along the Scheldt estuary: 1-Terneuzen; 2-Bath (the

582 Netherlands); 3-Antwerpen (Belgium)

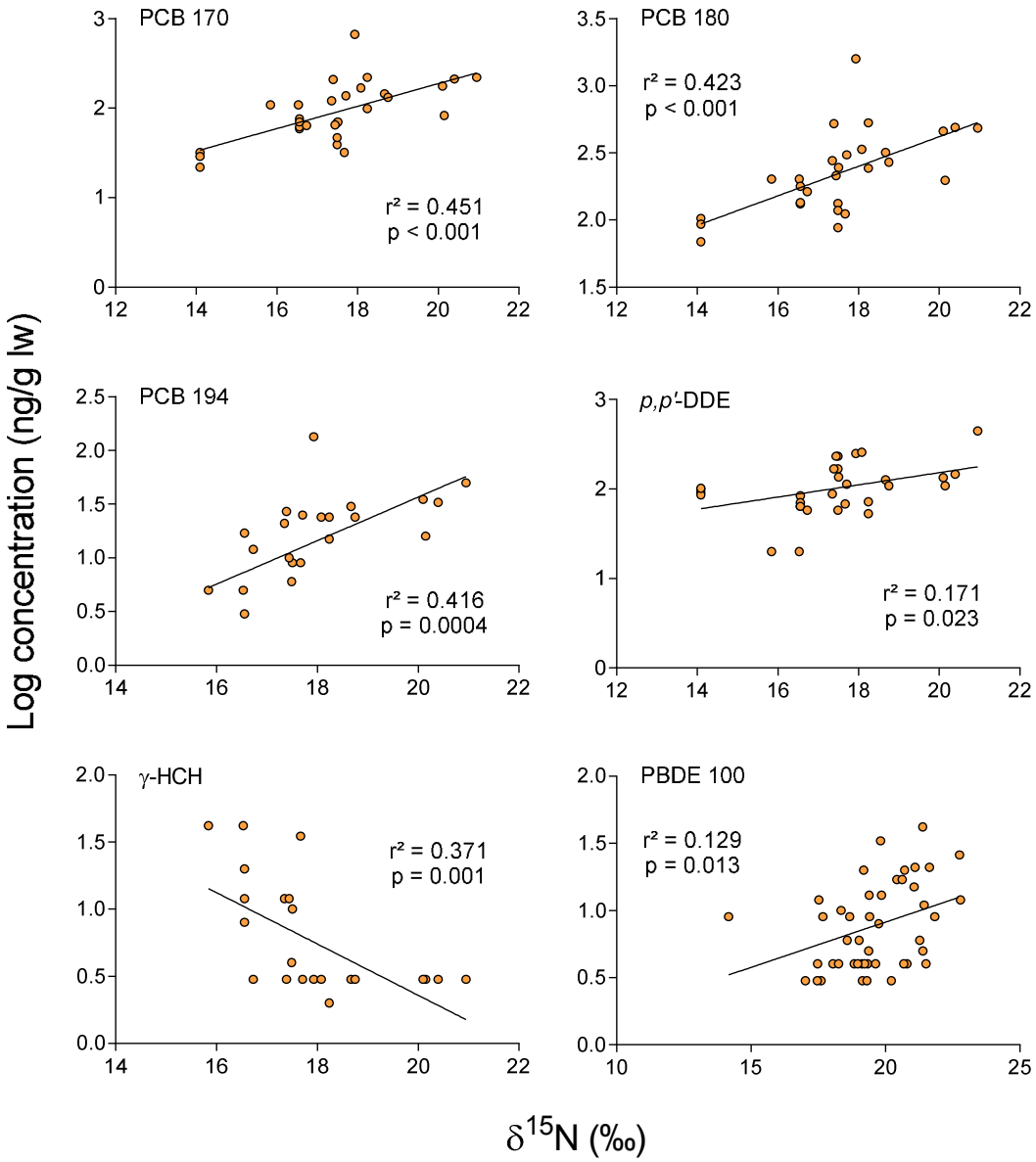


583
584 Fig. 2. Boxplots of CB153 concentrations in sediment (TOC normalized), bristle worm
585 and European flounder, A) before and B) after normalization for the ratio of salinity at
586 (Site X/Antwerpen). Salinity values used: Terneuzen, 27.3; Bath, 15.6; Antwerpen, 3.4.
587 Ratios used: (Terneuzen/Antwerpen) = 8.1; (Bath/Antwerpen) = 4.7;
588 (Antwerpen/Antwerpen) = 1. Salinity values are year averages from a monitoring
589 database from Rijkswaterstaat, a part of the Dutch Ministry of Infrastructure and the
590 Environment, and from the Flemish Environment Agency (VMM). The relations between
591 POP concentrations in sediment and biota from the same locations, with taking into
592 account sediment characterstics (TOC and grain size) were studied in Van Ael *et al.*,
593 2012[3].
594



595
596 Fig. 3. Stable isotope signature (mean ± SD) for all samples at the three sampling
597 locations, with $\delta^{15}N$ indicating the trophic level of the organisms. Symbols: ● fish, ○
598 invertebrates, ▲ filamentous algae, ■ SPM.

599



600

601 Fig. 4. Linear regression between log-transformed POP concentrations (ng/g lw) and $\delta^{15}N$

602 values from biota samples from Terneuzen (N = 30) and Bath (PBDE 100; N = 52).

603

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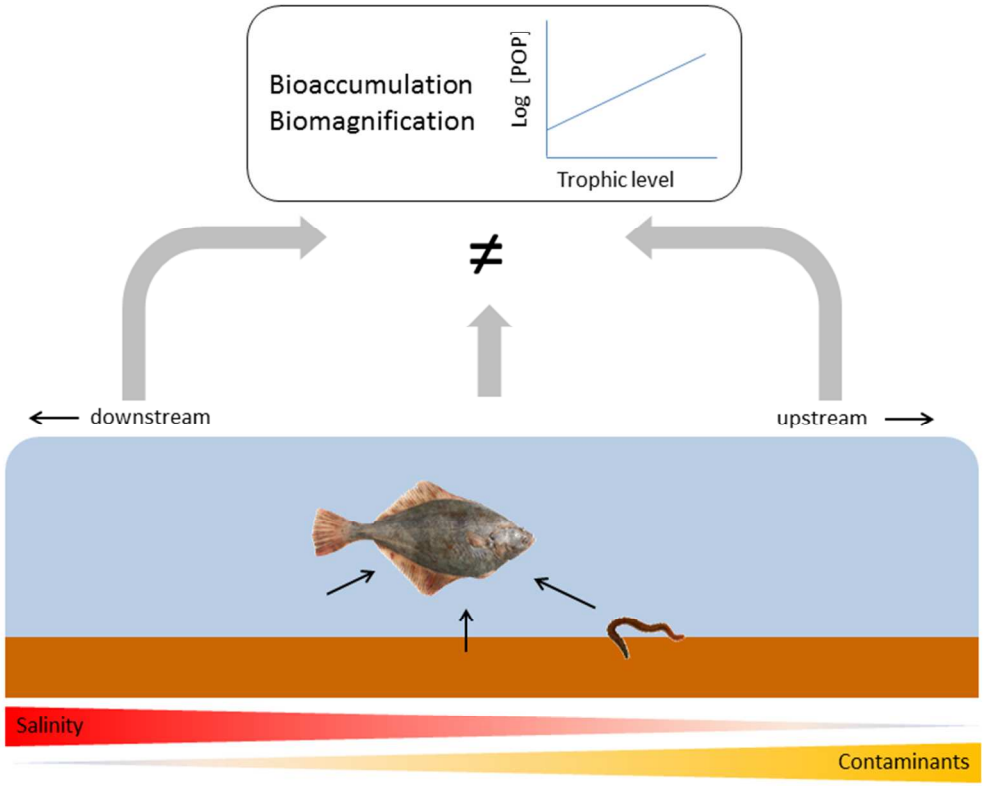
Bioaccumulation
Biomagnification
Log [POP]
Trophic level
≠
downstream
upstream
Salinity
Contaminants

605