Pollution fingerprints in eels as models for the chemical status of rivers

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The 2006 EU Water Framework Directive (WFD) proposed the monitoring of a selection of priority substances in the aquatic phase, including lipophilic substances. However, there are strong arguments for measuring lipophilic substances in biota. Yellow eel is a good candidate because it is widespread, sedentary, and accumulates many lipophilic substances in its muscle tissue. Several authors have described the indicative value of measured concentrations, yet few studies have investigated to what extent the spectrum of contaminants present characterizes the local environmental pollution pressure. To evaluate the value of the pollution profile of an eel as a fingerprint of the chemical status of the local environment, two datasets were selected from the Flemish Eel Pollutant Network database. The pollution profiles in individual eels along a river (even at distances <5 km) proved to be significantly different. Analysis of pooled contaminant data from multiple sites and sampling years within rivers allows characterization of river-specific chemical pressures. These results highlight the usefulness of eels as bio-indicators for monitoring pollution with lipophilic chemicals, such as polychlorinated biphenyls and organochlorine pesticides, in rivers. As such, eels may be used effectively within the monitoring programme for a selection of priority substances referred to in the WFD.

Keywords: bio-indicator, European eel, Flanders, pollution fingerprints, Water Framework Directive.

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Introduction

In 2006, the Water Framework Directive (WFD) proposed the monitoring of a selection of priority substances in selected water bodies of EC Member States (CEC, 2006a). Despite the lipophilic character of many of these substances, the proposal prescribes measuring most of them in the aquatic phase. If based only on the analysis of water samples, establishing a framework for the management of lipophilic compounds to restore fresh-water ecosystems is inadequate and inappropriate, because many of these chemicals are difficult to analyse in water because measurements generally remain below the detection limit (DL; Belpaire and Goemans, 2007a). Awareness is growing that lipophilic compounds should preferably be measured in, and environmental quality standards should be set for, biota (CEC, 2006b). An increasing number of studies has focused on the use of anguillid eels to monitor harmful substances (Belpaire and Goemans, 2007b), with the emphasis on lipophilic compounds such as polychlorine biphenyls (PCBs) and organochlorine pesticides (OCPs), which accumulate in the fat of this lipid-rich species. Several reports describe specific ecological and physiological features of the eel that support its use as a bio-indicator of chemical pollution (Bruslé, 1991; de Boer and Hagel, 1994; Belpaire and Goemans, 2007a).

Since the 1990s, many countries have used eels in monitoring the contaminant load in the environment. Bruslé (1991) published a review on contamination with heavy metals, OCPs, and PCBs in different eel species. Knights (1997) and Robinet and Feunteun (2002) documented the use of eels during their non-migratory phase (yellow eel) to monitor xenobiotics. Belpaire and Goemans (2007b) provide a summary of recently published EC reports. In the Netherlands and Belgium, nationwide monitoring networks have been operational since 1977 and 1994, respectively. In other EC countries, biomonitoring studies on local scales have been undertaken or are in progress.

Using various examples, Belpaire and Goemans (2007a) indicated that eels can be used to pinpoint sources of pollution, and discussed the eels' value as a tool for monitoring environmental contamination, on both local and international scales. Belpaire and Goemans (2007b) discussed how eels can be used to evaluate the chemical status of the aquatic environment in the WFD context.

Although many studies have reported spatial differences in contaminant loads within or among basins, few attempts have been made to investigate to what extent the spectrum of contaminants identified characterizes the local pollution pressure. Our objective is to explore how these spectra vary within and among sites and river systems in Flanders, Belgium. The specific question raised refers to the spatial scale at which differences can be detected: is the contaminant fingerprint of yellow eels caught at a specific site sufficiently representative to permit assessment of the environmental quality of that site? To this end, two datasets were selected from the Flemish Eel Pollutant Monitoring Network database, one

set from a relatively small catchment area and the other from seven major Flemish river systems.

Material and methods

Study area

The data have been generated by the Flemish Eel Pollutant Monitoring Network operated by the Research Institute for Nature and Forest (INBO) since 1994. This network uses the yellow eel as a biomonitor for the presence of contaminants in public water bodies. This monitoring programme covers both running and stagnant waters over a total area of ca. 13 500 km^2, and up to and including 2005, 2946 eels have been sampled on 365 sites. We selected two sets of data on PCBs and OCPs from riverine environments only. One set included contaminant data from 61 eels collected at eight different sites within a small catchment area (Nete basin, 2002/2003) to investigate small-scale variations in individual and grouped pollution profiles by site. The other, larger dataset, comprising 450 eels from seven rivers (1996–2005), was selected to investigate the variation in river-specific pollution profiles.

(i) The River Nete basin represents a small part of the Schelde basin (northern Belgium) and consists of two main tributaries, the Kleine Nete and Grote Nete (Figure 1a). Both are relatively small, lowland rivers with bream-zone fish assemblages (Huet, 1959). The Kleine Nete, 50 km in length, has been fragmented by ten physical obstacles, to ensure water control for agricultural purposes. Up to the watermill and weir of Grobbendonk, the river is influenced by the tide; upstream of this weir, it is a slow-moving river with luxuriant vegetation. The Grote Nete, 84 km in length, originally had a strong, meandering course, but many interventions have taken place for agricultural purposes and water control. The river is fragmented by 13 physical obstacles. Eight sampling sites (Table 1; Figure 1a) were selected, four on the Kleine Nete (KN1–KN4) and four on the Grote Nete (GN2–GN5; farthest upstream, a fifth site, GN1, was eliminated because it proved impossible to catch eels during the 2002/2003 campaigns). The distance between adjacent sampling sites varied between 4.2 and 20.8 km. The aim was to collect ten yellow eels per site, ranging in length between 35 and 45 cm, but limited catches obliged us to broaden the length range used. Mean length per site ranged between 33.9 and 40.4 cm (range: 28.6–49.4 cm). Tukey tests indicated that sample means from the downstream sites KN3 and KN4 in the Grote Nete were significantly larger than from the other sites (Table 1).

(ii) The second dataset includes samples from seven rivers constituting Flanders' major river systems (Figure 1b): one river in the IJzer basin (IJzer), five rivers in the Schelde basin (Leie, Schelde, Dender, Grote Nete, and Demer), and one river in the Maas basin (Maas). The number of sites per river varied between 3 (IJzer) and 12 (Schelde; Table 2). Because most rivers are transboundary with the Netherlands, France, or Wallonia, only part of the rivers' total stretches could be sampled. In total, 450 eels from 58 sites have been analysed, but the number sampled per river varied considerably (Table 2). Again, it was not always possible to catch individuals within the target size range (35–45 cm), and often, smaller or larger specimens had to be included (range: 25.2–76.5 cm). Mean length per river ranged between 35.7 and 48.4 cm, eels from the Grote Nete and IJzer being significantly smaller than those from the other five rivers and also pairwise being significantly different according to the Tukey test (Table 2).

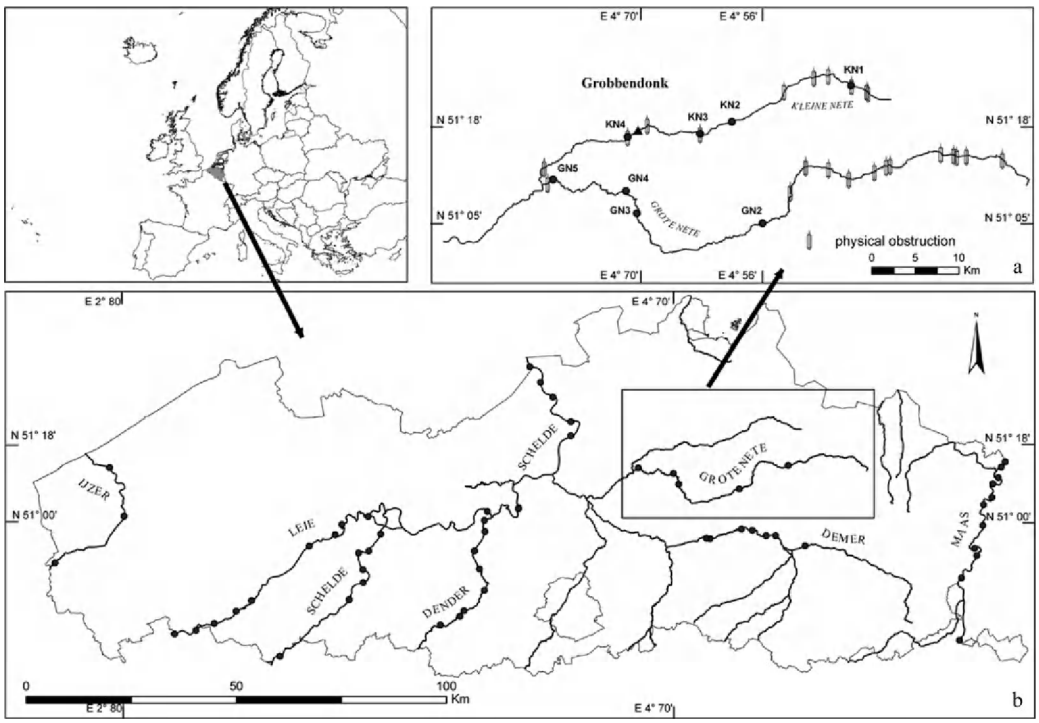

Figure 1. Location of sampling sites in Belgium on: (a) the Grote Nete and Kleine Nete in the Nete basin with an indication of physical obstructions; and (b) on seven rivers in Flanders (IJzer, Leie, Schelde, Dender, Grote Nete, Demer, and Maas).

Table 1. Information on sampling sites and eel samples taken along the rivers Grote Nete (GN) and Kleine Nete (KN): code, locality, distance from source (*D*), sampling date, number sampled (*N*), length (*L*), results of Tukey test for 95% overlap in confidence intervals (T; samples with the same letter indicate no significant difference in means), and weight (*W*).

Code	Locality	*D* (km)	Date	*N*	*L* (cm) mean ± s.e. (min–max)	T	*W* (g) mean ± s.e. (min–max)
GN2	Westerlo	45.0	19 March 2003	4	35.2 ± 0.7 (34.2–36.2)	a	74 ± 6 (58–83)
GN3	Itegem	65.8	19 March 2003	10	34.5 ± 1.2 (30.9–44.3)	a	71 ± 9 (43–149)
GN4	Bevel	70.2	19 March 2003	2	33.9 ± 0.1 (33.7–34.0)	a	52 ± 1 (36–60)
GN5	Lier	82.5	18 March 2003	6	35.7 ± 3.1 (28.6–49.0)	a	84 ± 26 (34–203)
KN1	Dessel	5.2	04 April 2002	9	34.7 ± 1.9 (29.5–47.2)	a	64 ± 13 (34–150)
KN2	Olen	21.9	19 March 2003	10	34.5 ± 1.2 (30.9–44.3)	a	71 ± 9 (43–149)
KN3	Herentals	26.1	18 September 2003	10	39.9 ± 1.3 (33.2–43.9)	b	108 ± 12 (58–173)
KN4	Bouwel	36.7	25 September 2003	10	40.4 ± 1.5 (34.2–49.4)	b	110 ± 19 (58–224)

Table 2. Information on the samples taken from the seven rivers in Flanders: sampling period, number of sites per river (*n*), number sampled (*N*), mean length (*L*), results of Tukey test for 95% overlap in confidence intervals (T; samples with the same letter indicate no significant difference in means), and weight (*W*).

River	Period	*n*	*N*	*L* (cm) mean ± s.e. (min–max)	T	*W* (g) mean ± s.e. (min–max)
IJzer	2000–2005	3	20	39.1 ± 1.9 (30.5–60.8)	c	130 ± 25 (50–511)
Leie	1996–2003	9	79	46.8 ± 1.3 (28.5–76.5)	a	230 ± 22 (32–997)
Schelde	1998–2004	14	59	43.2 ± 1.1 (29.0–73.0)	b	175 ± 19 (36–926)
Dender	2000–2005	9	61	44.7 ± 1.1 (27.3–68.0)	b	183 ± 15 (33–554)
Grote Nete	2000–2003	5	35	35.7 ± 0.8 (28.6–49.0)	d	79 ± 7 (34–203)
Demer	1999–2003	7	16	48.4 ± 2.9 (25.2–63.7)	a	274 ± 40 (35–520)
Maas	1997–2005	11	180	46.4 ± 0.6 (31.0–69.2)	a	196 ± 8 (40–601)

Sampling and analysis

Eels were collected by electrofishing or fyke-netting. In the Nete basin, sites were defined as river stretches 100 m in length, sampling both riverbanks. In the other rivers, sampling sites were 250 m in length. Length and weight of the fish were recorded. In the laboratory, fillets were wrapped in aluminium paper (cleaned with hexane 99%) and stored at −20°C. Chemical analyses for PCBs and OCPs were carried out by the Institute for Agricultural and Fisheries Research in Ostend. Ten PCB congeners were analysed (IUPAC numbers **28,** 31, **52, 101,** 105, **118, 138, 153,** 156, and **180**). Results were also expressed as Sum PCBs (representing the sum of the seven indicator congeners shown here emboldened). The OCPs measured were hexachlorobenzene (HCB), trans-Nonachlor (TNONA), DDT (*p,p′*-DDT or dichlorodiphenyltrichloroethane), and its breakdown products [*p,p′*-DDD or 1,1′-dichloro-2,2-bis(4-chlorophenyl)ethane and *p,p′*-DDE or 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene]. Sum DDT was calculated including its metabolites DDE and TDE (DDD). Cyclodienes included dieldrin, endrin, and aldrin. The α- and γ-hexachlorocyclohexanes (HCH) were determined. A full description of the analytical methodology and quality assurance is given in Goemans and Belpaire (2004) and Maes *et al.* (2008). Concentrations are expressed in $\mu g\,kg^{-1}$ lipid weight (LW). The DL for both PCBs and pesticides was 0.5 $\mu g\,kg^{-1}$ LW.

Statistical analysis

Statistical analyses were performed with S-PLUS 6.2 Professional. The Tukey test was carried out to determine if mean length differed significantly between sites or rivers. Multivariate analysis of variance (MANOVA) was used to ascertain whether there was statistical evidence that the pollution profiles of the eel samples differed among sites (KN and GN) or among the seven rivers (all samples from different sites and years combined). Results are presented as means ± s.d., and a *p*-value of <0.05 was considered statistically significant. Box-and-whisker plots illustrate the concentrations of selected contaminants by site or river.

To analyse whether individual eels with deviating pollution profiles were present in the dataset, a divisive hierarchical cluster analysis was performed. Hierarchical cluster analysis groups similar quantitative variables and represents this grouping in a dendrogram. In the divisive method, we used the Euclidean dissimilarity measure to compute the cluster-to-cluster distance. Aldrin and endrin (too many missing values or values under the DL) and derived variables such as Sum PCBs and Sum DDT were not used in the analysis. A canonical discriminant analysis (CDA) was carried out to ascertain whether pollution profiles of individual specimens could be discriminated based on sampling site or river. CDA is a dimension-reduction technique related to principal component analysis (PCA) and canonical correlation, deriving linear combinations of the quantitative variables that provide maximal separation between the groups (sites in the first dataset, rivers in the second).

Results

Site-specific analysis

MANOVA revealed that the contaminant loads of eels were significantly different ($p < 0.01$), both between the two rivers and among all sites. Figure 2 shows the variations in specific contaminant loads over the eight sites. PCB concentrations were generally higher in the Grote Nete (mean sum PCBs = 1867 ± 927 $\mu g\,kg^{-1}$ LW, range: 885–3690) than in the Kleine Nete (1126 ± 1155 $\mu g\,kg^{-1}$ LW, range: 221–5238). In both rivers, the

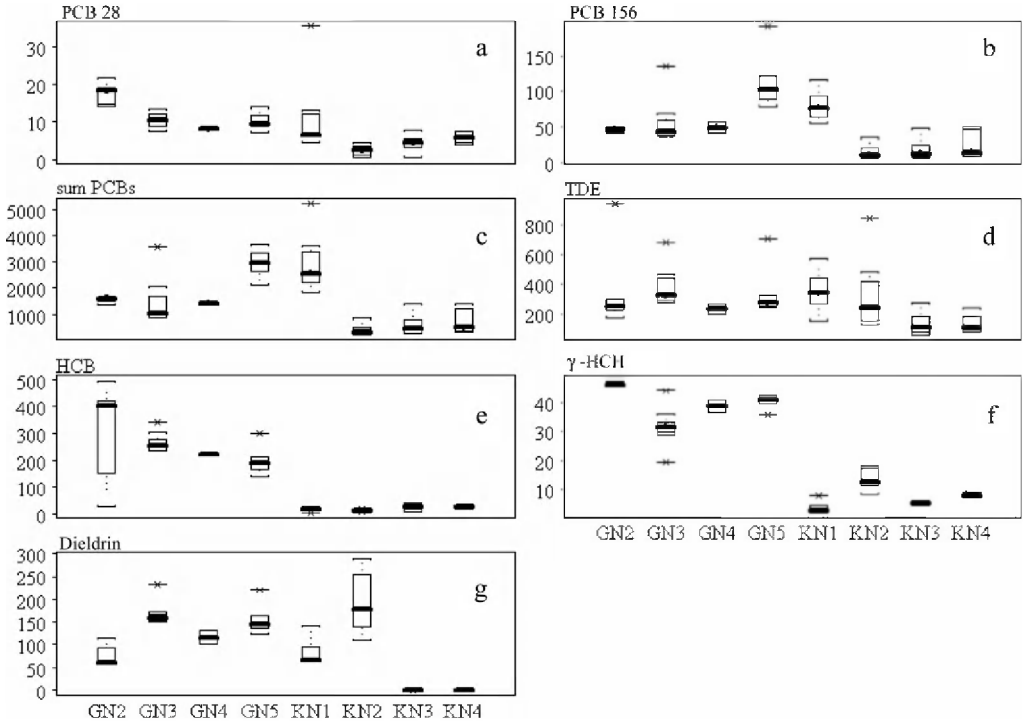

Figure 2. Box-and-whisker plots (minimum, first quartile, median, second quartile, maximum, and eventual outliers) for (a–c) PCB and (d–g) OCP concentrations (μg kg^{-1} LW) in eels from eight sites on the Grote Nete and Kleine Nete: (a) PCB 28; (b) PCB 156; (c) sum PCBs; OCP concentrations; (d) TDE (*p,p′*-DDD) or 1,1′-(2,2-dichloroethylidene)bis [4-chlorobenzene]; (e) HCB; (f) γ-HCH; and (g) dieldrin. The outlier from KN1 (see text) is included and apparent in a and c.

lower-chlorinated PCBs (e.g. PCB 28; Figure 2a) were higher at the locations farthest upstream. For the higher-chlorinated PCBs (e.g. PCB 156; Figure 2b), the situation was similar in the Kleine Nete, eels from KN1 being more contaminated than those from sites farther downstream. Conversely, in the Grote Nete, the site farthest downstream was more contaminated. Concentrations of *p,p′*-DDD (Figure 2d) and *p,p′*-DDE (and also Sum DDT) reveal a similar trend in their distribution: decreasing in the Kleine Nete in the downstream direction, whereas concentrations in the Grote Nete tended to increase in the downstream direction. However, low concentrations of *p,p′*-DDT were found in the upstream site of both rivers, increasing in the second site and tending to decrease again in the sites farthest downstream. HCB concentrations (Figure 2e) were very different between the two rivers, being low in the Kleine Nete and much higher in all sites of the Grote Nete. The mean value was very high in the site farthest upstream (GN2) and decreased in the downstream direction. Also for γ-HCH, concentrations were higher in eels from the Grote Nete, but without a consistent trend along the river (Figure 2f). Overall, α-HCH concentrations were lower, being highest in the site farthest upstream and decreasing to the DL in the three downstream sites of the Grote Nete. In the Kleine Nete, α-HCH concentrations were detectable in eels from all four sites but were highest in KN2. Dieldrin levels (Figure 2g) were under the DL for KN3 and KN4, and quite variable at all other sites.

Divisive hierarchical cluster analysis based on PCB and OCP concentrations in individual eels (Figure 3) suggests two major clusters separating eels from KN1 and GN5 from the other sites. One eel originating in KN1 (length 36.6 cm, weight 55 g) had an aberrant pollution profile compared with all other eels, having extremely high and outlying concentrations (μg kg^{-1} LW) of PCB 138 (1452), PCB 153 (2096), PCB 180 (913), and *p,p′*-DDE (3529).

The CDA was run twice on the contaminant data, once including the data on the outlying eel of KN1 and once excluding this eel. Both biplots revealed the same image: most eels congregate according to the site where they had been collected. However, in the biplot including the outlier, the KN1 cluster was more isolated from the other clusters, and therefore it was considered more appropriate to leave the outlier out. The first two dimensions of the CDA explained 74% of the total variance (Figure 4). Eels within each tributary are more similar in their pollution profile than eels from different tributaries, indicating a river-specific contaminant pressure.

River-specific analysis

MANOVA analysis of the variation in the contaminant load revealed significant differences ($p < 0.001$) among all rivers. The variation in concentrations of selected compounds shows that the higher-chlorinated PCBs (e.g. PCB 156; Figure 5b) are most prominently present in the Maas, whereas the IJzer and Demer have the lowest concentrations. The lower-chlorinated PCB congeners (PCB 28; Figure 5a) were most prominent in the Leie, but also appeared in the Schelde and Maas, with lowest values recorded from the IJzer. As was the case in the site-specific analysis, *p,p′*-DDD and *p,p′*-DDE (and also sum DDT) revealed similar distributions (not shown). The lowest values were recorded in eels from the Maas and the highest values in those from the Dender, Demer, and Grote Nete. The boxplot of *p,p′*-DDT, however, indicates higher concentrations in the Grote Nete and Demer than in the other five river systems (Figure 5d). HCB

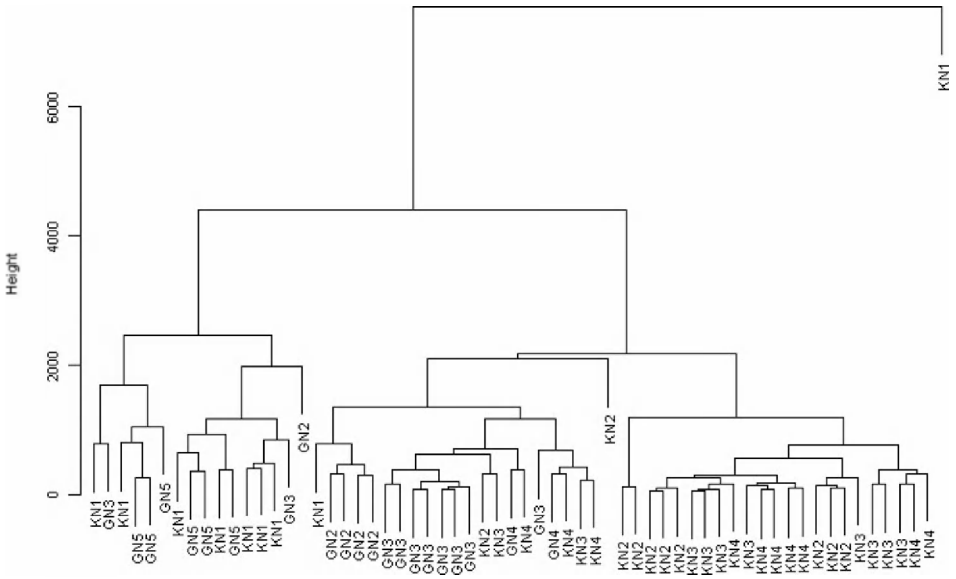

Figure 3. Cluster analysis of eels collected at eight sites in the Grote Nete and Kleine Nete based on their PCB and OCP concentrations ($N = 61$).

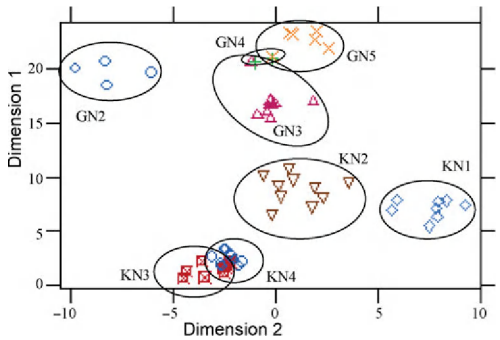

Figure 4. CDA of eels collected at eight sites in the Grote Nete and Kleine Nete based on their PCB and OCP concentrations ($N = 60$; excludes the outlier from KN1).

concentrations varied considerably among rivers, with highest concentrations in the Grote Nete (Figure 5e). Both α- and γ-HCH were prominent in the IJzer and Demer, but low in the other rivers (Figure 5f). Dieldrin reached the highest concentration in the IJzer (Figure 5g).

Although the dataset for the seven rivers contained data from 58 sites collected over long stretches of rivers (sometimes >100 km) and in different years over a decade (1996–2005), the discriminant analysis (Figure 6) revealed clear clusters for all rivers. The first two dimensions explained 57% of the variance. As a consequence of occasionally high values in all rivers, many observations appear to be scaled down towards the centre. Although they do overlap in the centre, the clusters diverge towards the periphery. This suggests that different rivers are characterized by different combinations of PCB and OCP components, although the absolute concentrations may differ according to exactly where or in which year the sample was taken.

Discussion

The samples from the Kleine Nete and Grote Nete demonstrate that contaminant concentrations can vary considerably among individuals collected at the same location. However, specific contaminants varied systematically among sites, even over relatively short distances of <5 km (Figure 2). For instance, considerable differences were observed for both isomers of HCH, dieldrin, and some DDT metabolites between KN2 and KN3, and for PCB 31, γ-HCH, *p,p′*-DDD, *p,p′*-DDT, dieldrin, and HCB between GN3 and GN4. Variations at such a small spatial scale can only be explained by the sedentary behaviour of eels and by apparent variations in pollution pressure within short river stretches. Many small brooks, creeks, and ditches discharge into the two rivers and may be responsible for specific pollution.

One KN1 eel showed a completely aberrant pollution profile (Figure 3), not only when compared with other eels from the same site but also compared with all other eels from the Nete basin. Despite its relatively small size of 36.6 cm, concentrations of the higher-chlorinated PCBs (especially PCB 138, 153, and 180) and *p,p′*-DDE were extremely high. There is no explanation for this exceptional contaminant load. Home-range studies indicate that most eels are generally recaptured close to their initial capture site, but some may be caught more than several kilometres from the initial site (Laffaille *et al.*, 2005). This particular eel might represent one of the non-sedentary, erratic eels ("nomads") described by Feunteun *et al.* (2003), may have been released by a fisher, or could have been present in a batch of restocked coarse fish. When monitoring chemicals in yellow eels, one must be aware that a small proportion may not reflect the site-specific pollution load, but statistical tools such as cluster analysis can help to identify and remove atypical eels.

Another factor contributing to the variability may be the size of the eel sampled. Collecting ten yellow eels in the range of 35–45 cm at each site is not easy in Flanders. Stock densities in these riverine systems are low because of low recruitment, the presence of multiple migration barriers (Figure 1a), and poor water quality. Belpaire *et al.* (2003) reported that eels may be caught at only 18% of the sites on rivers and brooks and that abundance is usually low (1–5 eels per 100 m electrofishing). To obtain sufficient data, eels from a broader size range had to be included.

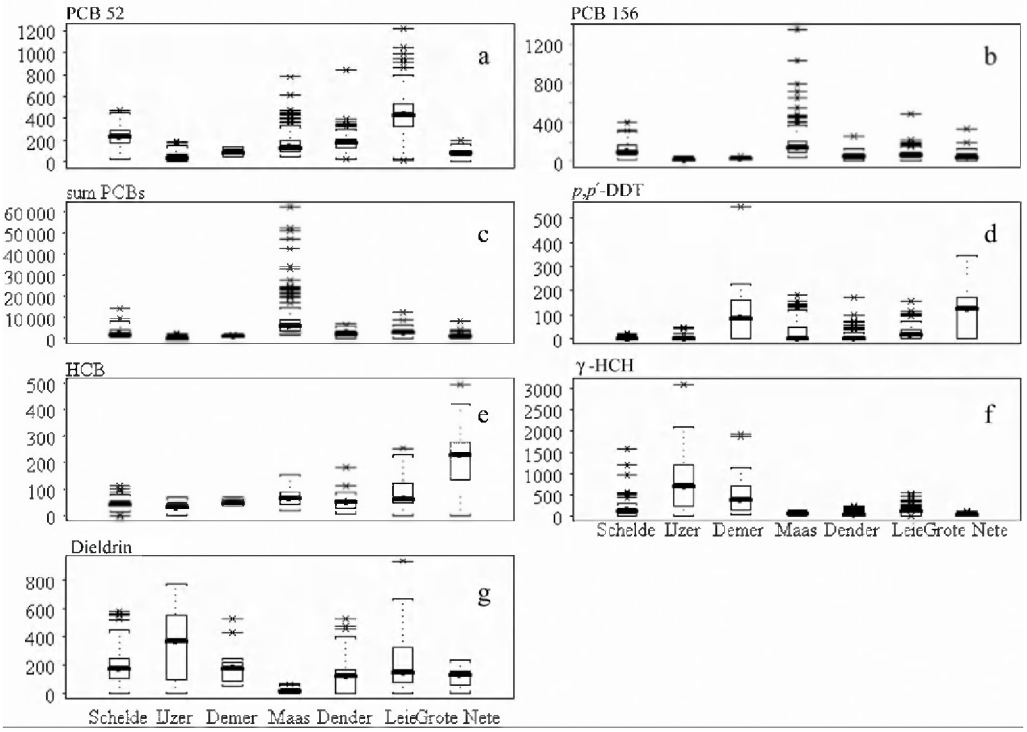

Figure 5. Box-and-Whisker plots (minimum, first quartile, median, second quartile, maximum, and eventual outliers) for (a–c) PCB and (d–g) OCP concentrations ($\mu g\,kg^{-1}$ LW) in eels from seven rivers in Flanders: (a) PCB 28; (b) PCB 156; (c) Sum PCBs; (d) *p,p′*-DDT or dichlorodiphenyltrichloroethane; (e) HCB; (F) γ-HCH; and (g) dieldrin.

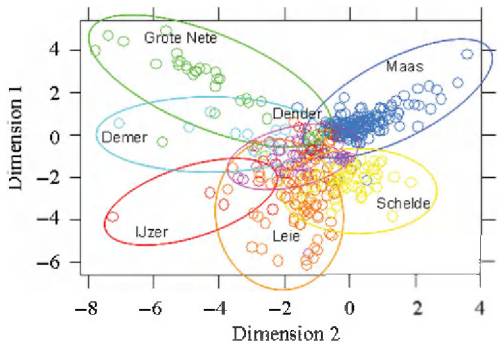

Figure 6. CDA of eels from seven rivers in Flanders based on PCB and OCP concentrations ($N = 450$ eels from 56 sites).

To some extent, this may have biased the results because, in general, larger eels can be expected to have a larger pollution load than smaller specimens. However, the PCA revealed that length makes only a minor contribution to the variance (Nete dataset: 13% for the first two principal components; seven rivers dataset: 14%).

Maes *et al.* (2008) reported that HCB concentrations in eels throughout Flanders (2526 eels from 365 sites) amount to a mean of 5.89 ± 8.91 (range 0.002–192) $\mu g\,kg^{-1}$ on a muscle–wet-weight basis. In comparison, the HCB concentrations in the Grote Nete (21–53 $\mu g\,kg^{-1}$ muscle–wet-weight) were relatively high, especially upstream. This indicates a local source of pollution, although this chemical was banned in 1974. Also banned from agricultural application in 1974 is the pesticide DDT. Nevertheless, DDT and its metabolites are still present in quite large quantities in eels from both rivers (Table 3). The relative proportion of the breakdown products compared with *p,p′*-DDT provides some striking results. DDT/DDE amounts to 0.003 and 0.09 at the sites of the two rivers farthest upstream (KN1 and GN2, respectively), peaks at the second-farthest upstream site (KN2 and GN3) at 0.45 and 0.39, respectively, to decrease again in the downstream sites. This suggests that there are recent sources of pollution by DDT upstream. Goemans *et al.* (2003) reported that DDT and its metabolites are present in considerable amounts in most eels throughout Flanders. Unexpectedly, Maes *et al.* (2008) observed in a trend analysis (1994–2005) that concentrations of *p,p′*-DDT had increased over time, whereas its metabolites had been reduced significantly, implying that not all stock has been depleted and suggesting that DDT was being applied again. This conclusion has been corroborated by Van Overmeire *et al.* (2006), who analysed DDT and derivatives in eggs obtained from free-ranging hens from private owners in Belgium. The DDT/DDE ratio observed indicated recent use of DDT as insecticides in henhouses. Our observations illustrate how chemical monitoring in eels can pinpoint local sources of specific pollution.

An efficient biomonitor should reflect the specific contaminant pressure at a certain site, and variations in this pressure among sites should be reflected in variations in the concentrations measured in the bio-indicator. The discriminating power among sites over a geographical range is a measure of the efficiency of the bio-indicator. Univariate analysis of the variations in specific contaminants gives clear indications of their presence in the river systems. However, in evaluating the usefulness of eels as a pollution indicator, our objective was to explore to what extent the total spectrum of contaminants is indicative for a specific site and to what extent individual pollution profiles vary within and between sites. To our knowledge, this study is the first to

Table 3. Mean muscle-tissue concentration (± s.d. and range in parenthesis; µg kg^{-1} lipid weight) of hexachlorobenzene (HCB) and *p,p′*-DDT and its derivates *p,p′*-DDD, and *p,p′*-DDE in eels sampled (*N*) at eight sites along the Grote Nete and Kleine Nete (2002/2003). The proportion DDT:DDE is also indicated.

Site	*N*	HCB	*p,p′*-DDT	*p,p′*-DDD	*p,p′*-DDE	DDT:DDE
GN2	4	273 ± 221 (25–495)	70 ± 114 (9–241)	399 ± 362 (178–940)	472 ± 320 (251–946)	0.09 ± 0.10 (0.03–0.25)
GN3	10	264 ± 34 (232–341)	213 ± 72 (140–346)	375 ± 126 (273–683)	576 ± 273 (375–1 258)	0.39 ± 0.10 (0.22–0.55)
GN4	2	222 ± 1.2 (220–222)	138 ± 11.5 (129–146)	233 ± 46 (200–265)	612 ± 69.2 (562–660)	023 ± 0.01 (0.22–0.23)
GN5	6	199 ± 57 (137–300)	152 ± 57 (100–262)	344 ± 180 (241–707)	534 ± 82 (430–634)	0.28 ± 0.07 (0.23–0.41)
KN1	8*	18 ± 6 (3–26)	3.0 ± 2.2 (0.5–7.6)	359 ± 144 (155–577)	904 ± 343 (350–1 524)	0.0033 ± 0.0014 (0.0004–0.0050)
KN2	10	14 ± 3 (6–19)	176 ± 118 (79–468)	310 ± 220 (127–839)	389 ± 195 (179–720)	0.45 ± 0.12 (0.21–0.65)
KN3	10	26 ± 10 (9–39)	81 ± 40 (40–169)	129 ± 73 (58–280)	374 ± 237 (154–886)	0.24 ± 0.06 (0.12–0.33)
KN4	10	24 ± 7 (19–29)	101 ± 54 (55–196)	137 ± 57 (76–245)	352 ± 185 (206–739)	0.29 ± 0.04 (0.23–0.34)

*Excluding one eel from KN1 with outlying analytical results (see text).

Table 4. Percentage of measured concentrations of lipophilic substances in river water, sediment, and eels from the Grote Nete and Kleine Nete basins above the detection limit (%>DL).

Substance	Water *n* = 3, 2000–2007		Sediment *n* = 73, 2000–2006		Eel *n* = 8, 2002/2003	
	%>DL	*N*	%>DL	*N*	%>DL	*N*
PCB 28	0	95	8	130	85	88
PCB 31	0	100	6	118	85	88
PCB 52	0	116	16	130	99	88
PCB 101	0	113	38	130	100	88
PCB 118	0	109	37	130	100	88
PCB 138	0	114	47	130	100	88
PCB 153	0	109	47	130	100	88
PCB 180	0	115	48	130	100	88
HCB	0	106	5	112	100	88
α-HCH	0	118	0	130	74	88
γ-HCH	16	246	2	130	100	88
p,p′-DDT	0	115	8	130	77	88
p,p′-DDE	0	107	31	130	100	88
p,p′-DDD	0	112	22	130	100	88
dieldrin	0	110	4	130	78	88

Number of sites (*n*), period of sampling, and number of measurements (*N*) are also indicated. The detection limits are 1 or 2 ng l^{-1} for water (dependent on the substance), 0.05 ng g^{-1} dry matter for sediment, and 0.5 ng g^{-1} lipid weight for eels. Water and sediment data were provided by the Flemish Environment Agency (VMM).

evaluate intra- and intersite variability in pollution profiles in individual eels sampled within a small catchment area, with sites lying a maximum of 20 km apart. Most work describing such variations has been done on larger geographical scales. Furthermore, many studies present results obtained from the analysis of pooled samples from each site (Belpaire and Goemans, 2007b), and thus are of no use in evaluating intrasite variability.

The CDA (Figure 4) yielded rather conclusive results: all eels from the same site clustered closely together, even when the distance between sample sites was <5 km. Apparently, site-specific aquatic pollution by lipophilic compounds can be tracked in eels. Also, within each tributary, site clusters congregate, indicating river-specific contaminant pressure. From these results, we conclude that the contaminant fingerprint of yellow eels, after filtering out outliers, is representative of the environmental quality (for the local load with lipophilic chemicals) of the site where it was caught. We tried to compare these bioaccumulation data in eels by measuring the same contaminants during monitoring of water and sediment quality in the two Nete basins by the Flemish Environmental Agency. However, because these chemicals are lipophilic, they are hard to trace in the water phase or even in sediments (Table 4). Only lindane is to some extent detectable in water, whereas in sediment, mainly the higher-chlorinated PCBs are sometimes detectable, but only in a minority of the cases. These observations clearly illustrate that the pollution pressure cannot be measured independently and that an effective strategy to measure the input of these lipophilic contaminants depends completely on biomonitoring.

Results similar to ours of small-scale differences were obtained studying pollution profiles in eels in a canal and under lacustrine conditions. Belpaire and Goemans (2007b) reported spatial and

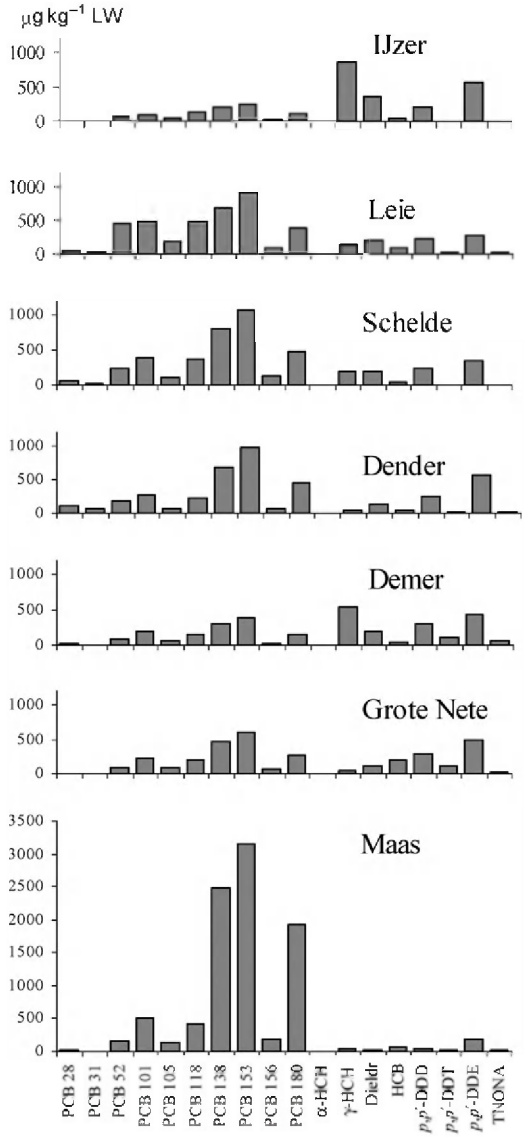

Figure 7. Pollution fingerprints based on means of PCB and OCP concentrations (µg kg^{-1} LW) in eels from seven rivers in Flanders.

temporal differences in pollution load in a Belgian canal 14 km long. Belpaire *et al.* (2001) observed variation among eels caught in four different parts of Lake Schulen (90 ha), as well as significant differences in lindane (γ-HCH) concentrations in their muscle tissue. All of these observations are in line with the conclusion of ecological studies on home ranges that foraging movements of yellow eels are mostly restricted to a few hundred metres (Baras *et al.*, 1998; Laffaille *et al.*, 2005). Such a small home range would explain why yellow eels serve as good indicator species for monitoring site-specific pollution pressure.

Although site-specific pollution profiles may be quite different among years, as shown for eels sampled in a canal in 1991 and 1995 (Belpaire and Goemans, 2007b), the results of the CDA of samples collected over several years clearly indicate that the profiles in the different rivers vary consistently. The position of the clusters for the three major catchment areas (IJzer, Schelde, and Maas basins) match the geographical positions of the (sub-)basins (Figure 1b), the westernmost catchment (IJzer) being most distinct from the easternmost Maas catchment. Within the centrally positioned Schelde, adjacent sub-basins take up adjacent positions in the clustering: the adjacent basins of the Demer and Grote Nete, as well as those of the Schelde and Leie, have more comparable profiles (despite their distinctness) than any of these with the Dender, which is located in between. Although overall, sub-basins reveal distinct contaminant profiles, similarities between sub-basins suggest geographical gradients in contaminant pressure that might well result from variations in land use. An increasing west–east gradient in PCB contamination in Flanders eels was reported by Maes *et al.* (2008).

Figure 7 summarizes the averaged river-specific pollution fingerprints observed in eels. These observations are generally in line with Maes *et al.* (2008), who reported high α- and γ-HCH and dieldrin concentrations in the IJzer basin, and the highest PCB concentrations in the Maas basin. We conclude that the yellow-eel stage can serve as an excellent environmental indicator of both small-scale (km) and large-scale (catchment area) pollution loads of rivers with lipophilic chemical substances. An approach using this bio-indicator for lipophilic substances might prove more effective in the monitoring programme of the WFD than using indicators derived from concentrations in the water phase.

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