

# Chapter 10

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

**Claude Belpaire<sup>1</sup>, Geert Goemans<sup>1</sup>, Caroline Geeraerts<sup>1</sup>, Paul Quataert<sup>1</sup> and Koen Parmentier<sup>2</sup>**

1 - Research Institute for Nature and Forest, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium

2 - Institute for Agricultural and Fisheries Research (ILVO Fisheries), Ankerstraat 1, B-8400 Oostende, Belgium

This chapter is in press as:

Belpaire, C., Goemans, G., Geeraerts, C., Quataert, P., Parmentier, K., 2008. Pollution fingerprints in eels as models for the chemical status of rivers. *ICES Journal of Marine Science* 65, in press.

## Summary

The 2006 EU Water Framework Directive has proposed to monitor a selection of priority substances in the aquatic phase, including lipophilic substances. However, there are strong arguments for measuring the latter 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 which 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, one set from a small catchment area to investigate site-specific profiles, and one from seven large Flemish rivers to investigate river-specific profiles. The pollution profiles of persistent organic pollutants 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. The results highlight the usefulness of eel as a bioindicator for monitoring pollution with lipophilic chemicals like polychlorinated biphenyls and organochlorine pesticides in rivers. As such, eel may be used effectively within the monitoring programme for a selection of priority substances referred to in the Water Framework Directive.

---

## **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 to measure most of these in the aquatic phase. If based on analysis of water samples only, establishing a framework for the management of lipophilic compounds to restore freshwater ecosystems is inadequate and inappropriate, because many of these chemicals are difficult to analyse in water as measurements generally remain below the detection limit (Belpaire and Goemans, 2007a). There is a growing awareness that lipophilic compounds should be measured preferably 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 like 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 bioindicator of chemical pollution (Bruslé, 1991; de Boer and Hagel, 1994; Belpaire and Goemans, 2007a).

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

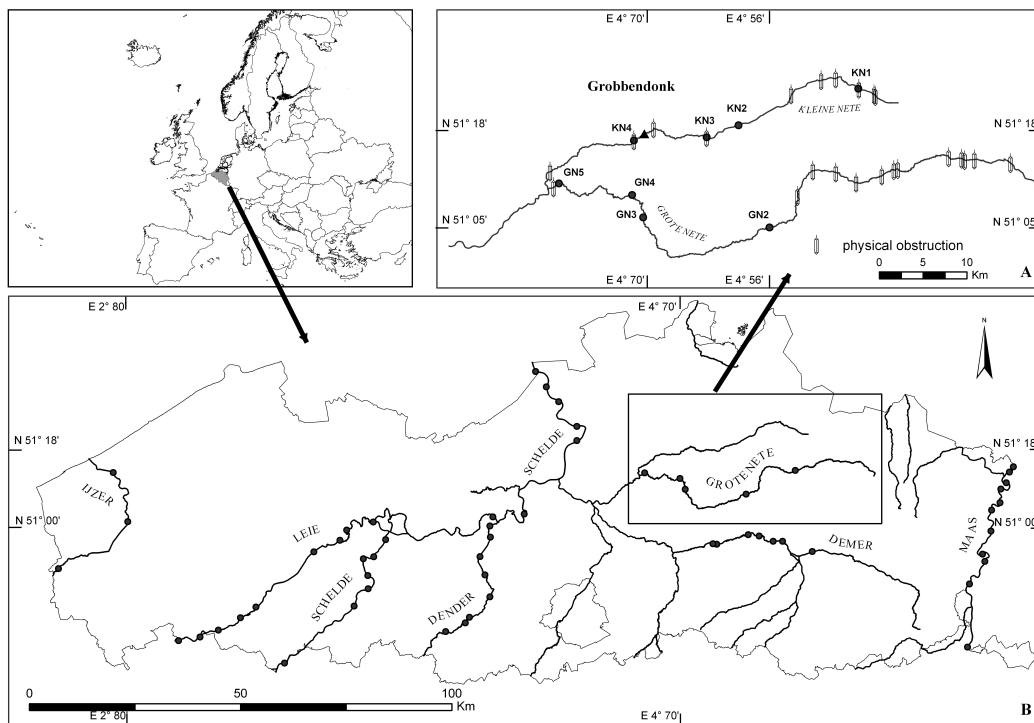
Belpaire and Goemans (2007a) indicated through various examples that eels may be used to pinpoint sources of pollution, and discussed their value as a tool for monitoring environmental contamination, both on local and international scales. Belpaire and Goemans (2007b) discussed how eels may be used to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

While many studies have reported spatial differences in contaminant loads within or among basins, few attempts have been made to investigate to which 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 is on the spatial scale at which differences may be detected: is the contaminant fingerprint of yellow eel caught at a specific site sufficiently representative to assess 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 one set 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 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<sup>2</sup> 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 8 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 and larger dataset comprising 450 eels from seven rivers (1996-2005) was selected to investigate the variation in river-specific profiles of pollution.



**Figure 10.1.** Location of sampling sites on: A. the Grote Nete and Kleine Nete in the Nete basin with indication of physical obstructions; and B. on seven rivers in Flanders (IJzer, Leie, Schelde, Dender, Grote Nete, Demer and Maas).

(1) The river Nete basin represents a small part of the Schelde basin (northern part of Belgium) and consists of two main tributaries, the Kleine Nete and Grote Nete (Figure 10.1A). Both are relatively small lowland rivers with bream-zone fish assemblages (Huet, 1959). The 50 km-long Kleine Nete has been fragmented by ten physical obstacles to ensure water control for agricultural purposes. Up to the water mill 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 84 km-long Grote Nete had originally 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 10.1, Figure 10.1A) were selected, four on the Kleine Nete (KN1 - KN4) and four on the Grote Nete (GN2 - GN5; a fifth, most upstream site GN1 was not retained as it was not possible to catch eels during the 2002-2003 campaigns). Distance between adjacent sampling sites varied between 4.2 and 20.8 km. The aim was to collect 10 yellow eels per site in the length range between 35 and 45 cm, but limited catches obliged us to broaden the length range used. Mean length per site ranged between 33.9 cm and 40.4 cm (range 28.6-49.4 cm). Tukey tests indicate that sample means from the downstream sites KN3 and KN4 in the Grote Nete were significantly larger than from the other sites (Table 10.1).

**Table 10.1.** Information on sampling sites (code, locality and distance from source - D) along the rivers Grote Nete (GN) and Kleine Nete (KN) and on the eel samples taken (date, number sampled - N, length - L, results 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	Date	N	L (cm)	T	W (g)
		km			mean±S.E. (min-max)		mean±S.E. (min-max)
GN2	Westerlo	45.0	19/03/2003	4	35.2±0.7 (34.2 - 36.2)	a	74±6 (58 - 83)
GN3	Itegem	65.8	19/03/2003	10	34.5±1.2 (30.9 - 44.3)	a	71±9 (43 - 149)
GN4	Bevel	70.2	19/03/2003	2	33.9±0.1 (33.7 - 34.0)	a	52±1 (36 - 60)
GN5	Lier	82.5	18/03/2003	6	35.7±3.1 (28.6 - 49.0)	a	84±26 (34 - 203)
KN1	Dessel	5.2	04/04/2002	9	34.7±1.9 (29.5 - 47.2)	a	64±13 (34 - 150)
KN2	Olen	21.9	19/03/2003	10	34.5±1.2 (30.9 - 44.3)	a	71±9 (43 - 149)
KN3	Herentals	26.1	18/09/2003	10	39.9±1.3 (33.2 - 43.9)	b	108±12 (58 - 173)
KN4	Bouwel	36.7	25/09/2003	10	40.4±1.5 (34.2 - 49.4)	b	110±19 (58 - 224)

(2) The second dataset comprises samples from seven rivers constituting Flanders' major river systems (Figure 10.1B): 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 10.2). Because most rivers are transboundary with the Netherlands, France or Wallonia, only part of the total river stretches could be sampled. In total, 450 eels from 58 sites have been analysed, but the number sampled per river varied considerably (Table 10.2). Again, it was not always possible to catch individuals within the target size range (35-45 cm) and in many cases smaller or larger specimens had to be included (range 25.2-76.5 cm). Mean length per river ranged between 35.7 cm and 48.4 cm, eels from the Grote Nete, IJzer and Schelde being significantly smaller than those from the other five rivers and also pairwise being significantly different according to the Tukey test (Table 10.2).

**Table 10.2.** Information on the samples taken from the seven rivers in Flanders (sampling date, number of sites per river - n, number sampled - N, mean length - L, results 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)	T	W (g)
				mean±S.E. (min-max)		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 of 100 m length, both river banks being sampled. In the other rivers, sampling sites were 250 m long. 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 7 indicator congeners in bold). 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  $\alpha$  and  $\gamma$  hexachlorocyclohexanes (HCH) were determined. Full description of the analytic methodology and quality assurance is given in Goemans and Belpaire (2004) and Maes *et al.* (2008). Concentrations are expressed in  $\mu\text{g.kg}^{-1}$  lipid weight (LW). The detection limit (DL) for both PCBs and pesticides was  $0.5 \mu\text{g.kg}^{-1}$  LW.

### Statistical analysis

Statistical analyses were performed with S-PLUS 6.2 Professional. The Tukey test was carried out to test 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 were different among sites (KN and GN) or among the seven rivers (all samples from different sites and years combined). Results are presented as means  $\pm$  standard deviation (SD) and a p-value <0.05 was considered statistically significant. Box-and-Whisker plots illustrate the concentrations of selected contaminants by site or river.

To analyse if individual eels with deviating pollution profiles were present in the dataset, a divisive hierarchical cluster analysis was performed. Hierarchical cluster analysis groups quantitative variables that are similar to one another, 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 like Sum PCBs and Sum DDT were not used in the analysis. A canonical discriminant analysis was carried out to ascertain whether pollution profiles of individual specimens could be discriminated on the basis of sampling site or river. Canonical discriminant analysis is a dimension-reduction technique related to principal component analysis 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

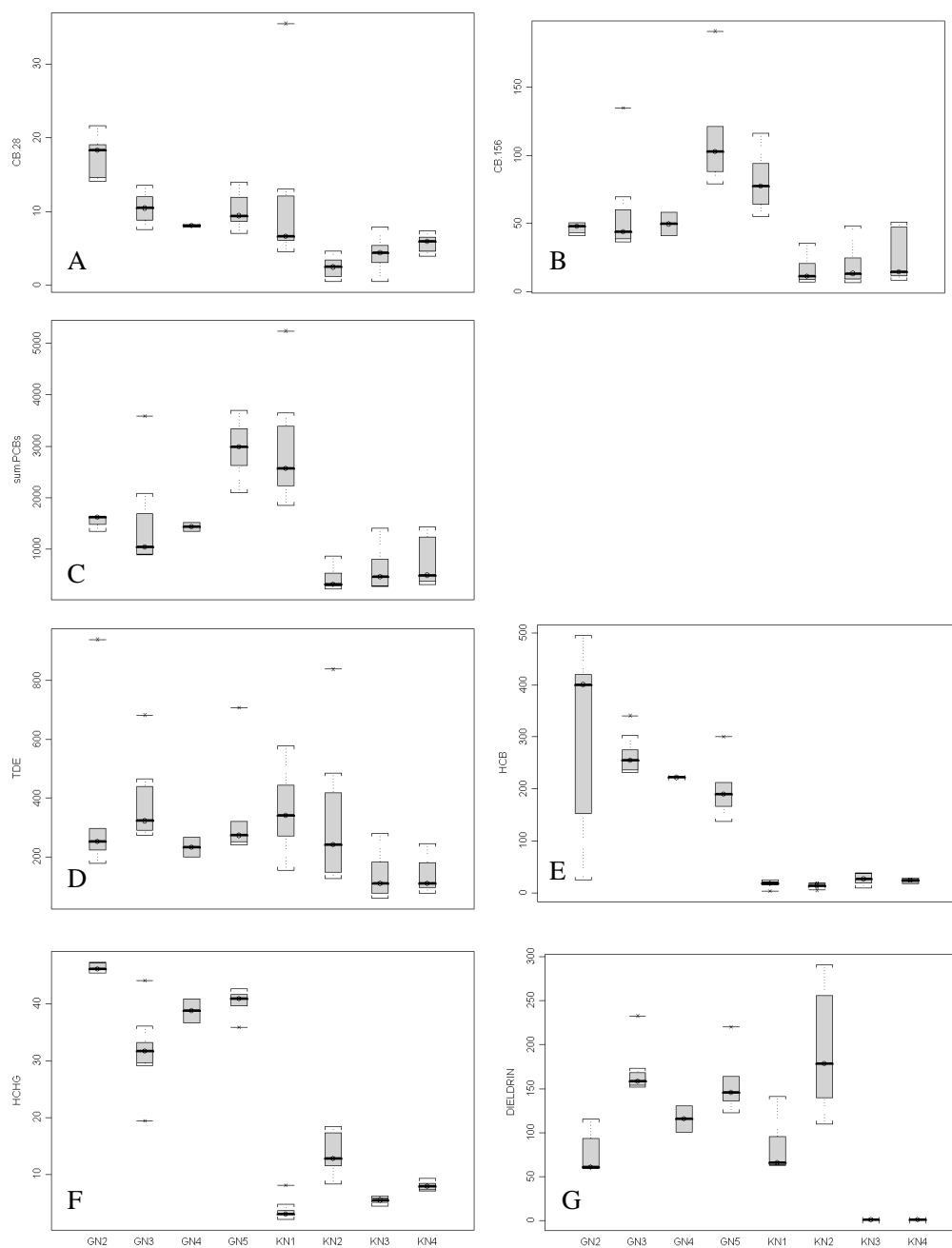
### Site-specific analysis

MANOVA showed that the contaminant loads of eels were significantly different ( $p < 0.01$ ), both between the two rivers and among all sites. Figure 10.2 shows the variations in specific contaminant loads over the eight sites. PCB concentrations were generally higher in the Grote Nete (mean Sum PCBs =  $1867 \pm 927 \mu\text{g.kg}^{-1}$  LW, range 885-3690) than in the Kleine Nete ( $1126 \pm 1155 \mu\text{g.kg}^{-1}$  LW, range 221-5238). In both rivers, the lower-chlorinated PCBs (e.g. PCB 28, Figure 10.2A) were higher at the most-upstream locations. For the higher-chlorinated PCBs (e.g. PCB 156, Figure 10.2B), the situation is similar in the Kleine Nete, with eels from KN1 being more contaminated than those from more-downstream sites. Conversely, in the Grote Nete, the most-downstream site is more contaminated. Concentrations of *p,p'*-DDD (Figure 10.2D) and *p,p'*-DDE (and also Sum DDT) show a similar trend in their distribution: decreasing in the Kleine Nete in the downstream direction, whereas concentrations in the Grote Nete tend to increase in the downstream direction. However, *p,p'*-DDT shows low concentrations in the upstream site of both rivers, increasing in the second site and tending to decrease again in the most downstream sites. HCB concentrations (Figure 10.2E) 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 most upstream site (GN2) and decreased in the downstream direction. Also for  $\gamma$ -HCH, concentrations were higher in eels from the Grote Nete, but without a consistent trend along the river (Figure 10.2F). Overall,  $\alpha$ -HCH concentrations were lower, being highest in the most-upstream site and decreasing to the DL in the three downstream sites of the Grote Nete. In the Kleine Nete,  $\alpha$ -HCH concentrations were detectable in eels from all four sites, but were highest in KN2. Dieldrin levels (Figure 10.2G) were under the DL for KN3 and KN4, and quite variable at all other sites.

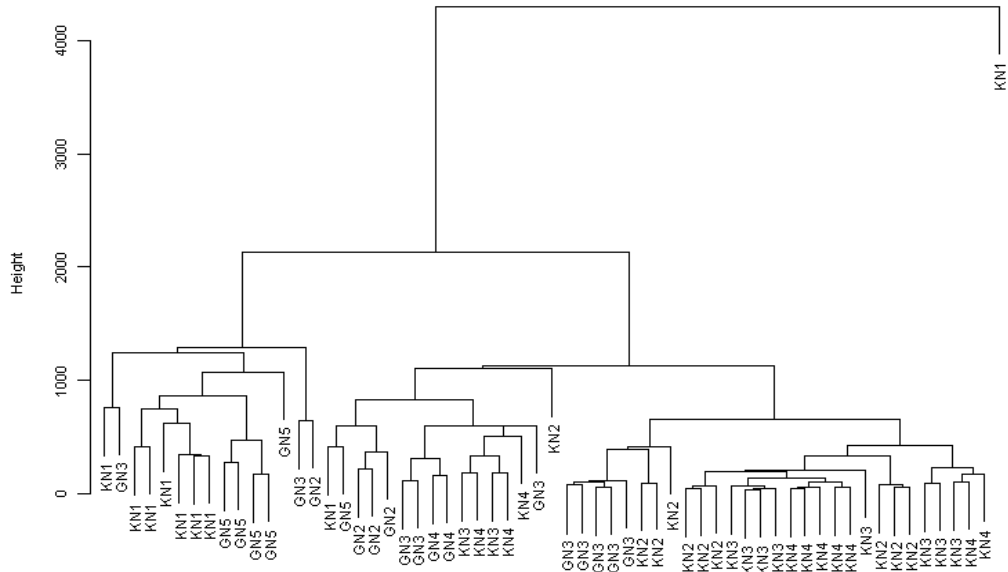
Divisive hierarchical cluster analysis on the basis of PCB and OCP concentrations in individual eels (Figure 10.3) suggests two major clusters separating eels from KN1 and GN5 from the other sites. One eel originating from KN1 (length 36.6 cm, weight 55 g) had an aberrant pollution profile compared to all other eels having extremely high and outlying concentrations ( $\mu\text{g.kg}^{-1}$  LW) of PCB 138 (1452), PCB 153 (2096), PCB 180 (913) and *p,p'*-DDE (3529).

The canonical discriminant analysis (CDA) was run twice on the contaminant data, once with the data of the outlying eel of KN1 included and once excluding this eel. Both biplots showed the same image: most individuals congregate according to

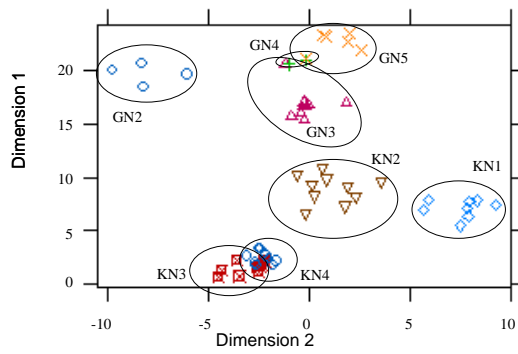
the site where they had been collected. However, in the biplot including the outlier, the KN1 cluster was more isolated from the other clusters, and therefore it was considered more appropriate to leave the outlier out. The first two dimensions of the CDA explained 74% of the total variance (Figure 10.5). Eels within each tributary are more similar in their pollution profile than eels from different tributaries, indicating a river-specific contaminant pressure.



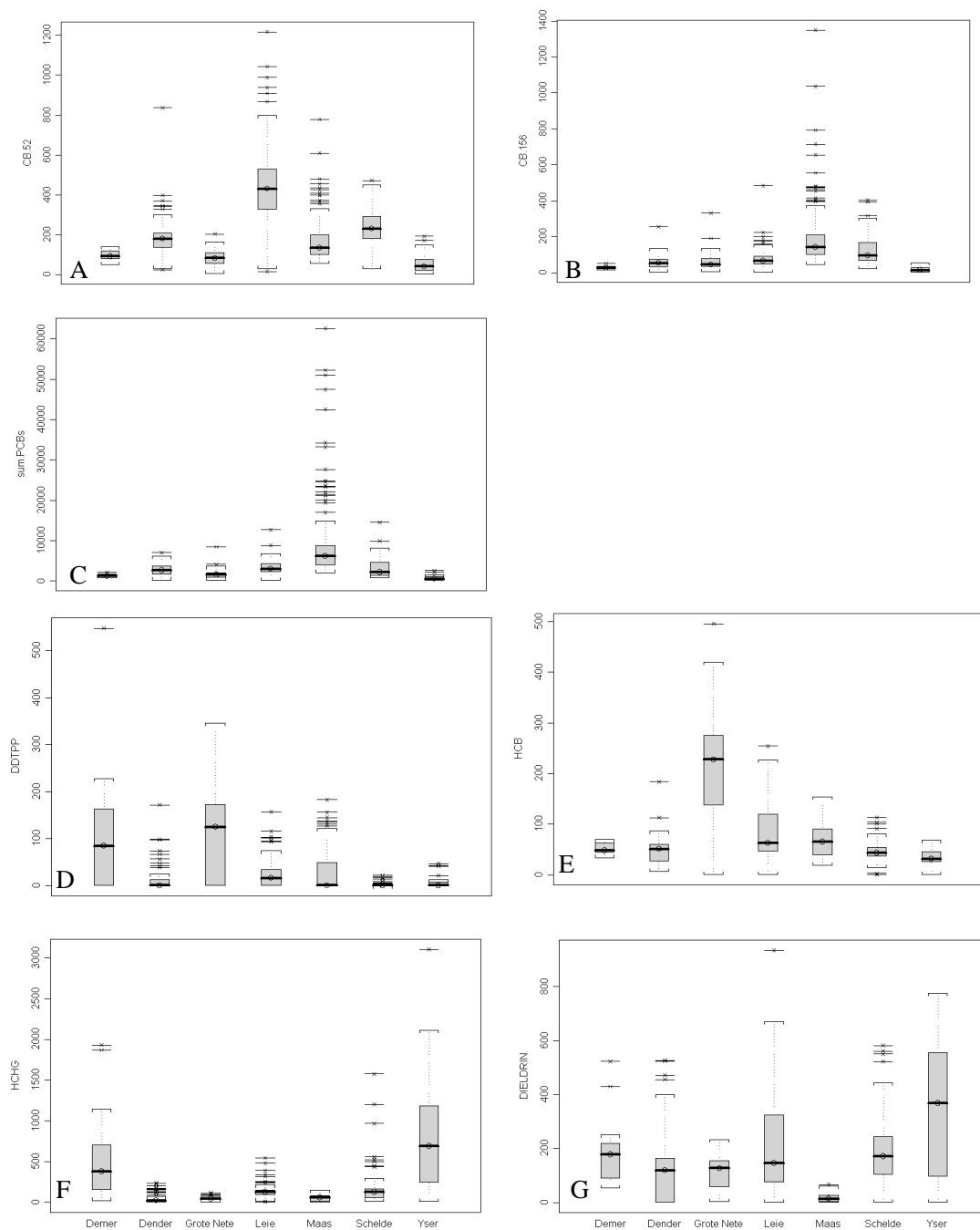
**Figure 10.2.** Box-and-Whisker plots (minimum, first quartile, median, second quartile, maximum and eventual outliers ) for (A-C) PCB and (D-G) OCP concentrations ( $\mu\text{g.kg}^{-1}$  LW ) in eels from eight sites on the Grote Nete and Kleine Nete: A. PCB 28; B. PCB 156; C. Sum PCBs; OCP concentrations - D. TDE (*p,p'*-DDD or 1,1'-(2,2 dichloroethylidene)bis [4-chlorobenzene]; E. HCB; F.  $\gamma$  – HCH; and G. dieldrin. The outlier from KN1 (see text) is included, and apparent in A and C.



**Figure 10.3.** Cluster analysis of eels collected at eight sites in the Grote Nete and Kleine Nete on the basis of their PCB and OCP concentrations ( $n = 61$ ).



**Figure 10.4.** Canonical discriminant analysis of eels collected at eight sites in the Grote Nete and Kleine Nete on the basis of their PCB and OCP concentrations ( $N = 61$ ). The outlier from KN1 is excluded from this analysis.

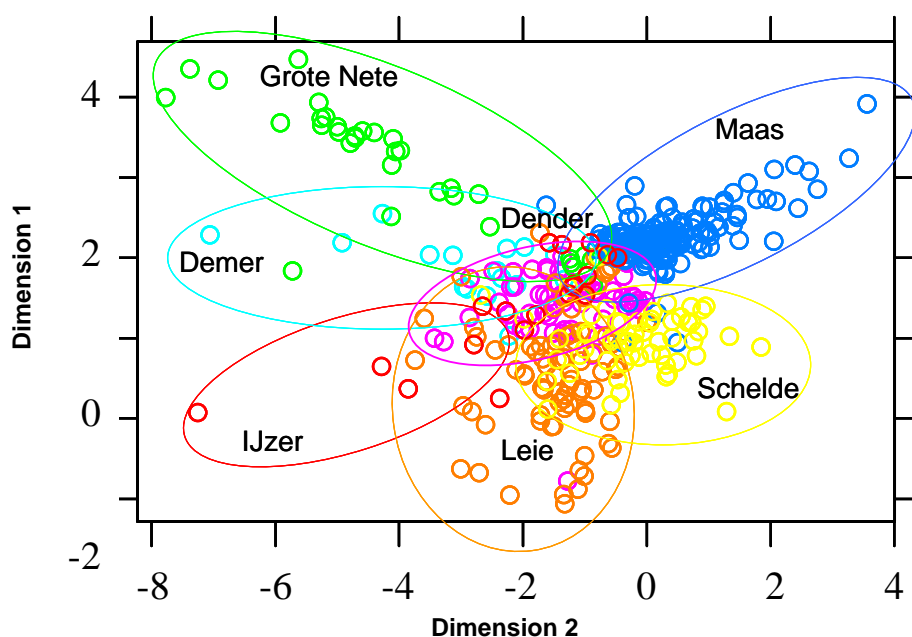


**Figure 10.5.** Box-and-Whisker plots (minimum, first quartile, median, second quartile, maximum and eventual outliers ) for (A-C) PCB and (D-G) OCP concentrations ( $\mu\text{g}\cdot\text{kg}^{-1}$  LW) in eels from seven rivers in Flanders: A. PCB 28; B. PCB 156; C. Sum PCBs; D.  $p,p'$ -DDT or dichlorodiphenyltrichloroethane ; E. HCB; F.  $\gamma$ -HCH; and G. dieldrin.

### River-specific analysis

Analysis of the variation in the contaminant load through MANOVA showed 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 10.5B) are most prominently present in the Maas, whereas the IJzer and Demer have the lowest concentrations. The lower-chlorinated PCB congeners (PCB 28, Figure 10.5A) were most prominent in the Leie, but also 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) showed 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 high concentrations in the Grote Nete and Demer compared to the other five river systems (Figure 10.5D). HCB concentrations varied considerably among rivers with highest concentrations found in the Grote Nete (Figure 10.5E). Both  $\alpha$ - and  $\gamma$ -HCH were prominently present in the IJzer and Demer, but low in the other rivers (Figure 10.5F). Dieldrin reached the highest concentration in the IJzer (Figure 10.5G).

Even though the data set 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 10.6) showed 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 center, the clusters diverge in different directions 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 where exactly or in which year the sample was taken.



**Figure 10.6.** Canonical discriminant analysis of eels from seven rivers in Flanders on the basis of PCB and OCP concentrations ( $n = 450$  eels from 56 sites).

## Discussion

The samples from the Kleine Nete and Grote Nete show that contaminant concentrations may vary considerably among individuals collected at the same location. However, specific contaminants varied systematically among sites, even over relatively short distances <5 km (Figure 10.2). For instance, considerable differences were observed for both isomers of HCH, dieldrin and some DDT metabolites between KN2 and KN3 and for PCB 31,  $\gamma$ -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. Numerous small brooks, creeks and ditches discharge in the two rivers and these may be responsible for specific pollution.

One KN1 eel showed a completely aberrant pollution profile (Figure 10.3), not only when compared to other eels from the same site, but also compared to 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, 180) and  $p,p'$ -DDE were extremely high. An explanation for this exceptional contaminant load is lacking. Home-range studies indicate that most eels are generally recaptured close to their initial capture site, but some may be caught more than several kilometers from the initial site (Lafaille *et al.*, 2005). This particular eel might represent one of these non-sedentary, erratic eels ('nomads') described by Feunteun *et al.* (2003), may have been released by a fisherman, or could have been present in a batch of restocked coarse fish. When monitoring chemicals in yellow eels, one has to 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.

**Table 10.3.** Mean muscle-tissue concentration ( $\pm$  SD and range in brackets;  $\mu\text{g.kg}^{-1}$  lipid weight) of hexachlorobenzene (HCB) and  $p,p'$ -DDT and its derivatives  $p,p'$ -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 $\pm$ 221 (25-495)	70 $\pm$ 114 (9-241)	399 $\pm$ 362 (178-940)	472 $\pm$ 320 (251-946)	0.09 $\pm$ 0.10 (0.03-0.25)
GN3	10	264 $\pm$ 34 (232-341)	213 $\pm$ 72 (140-346)	375 $\pm$ 126 (273-683)	576 $\pm$ 273 (375-1258)	0.39 $\pm$ 0.10 (0.22-0.55)
GN4	2	222 $\pm$ 1.2 (220-222)	138 $\pm$ 11.5 (129-146)	233 $\pm$ 46 (200-265)	612 $\pm$ 69.2 (562-660)	0.23 $\pm$ 0.01 (0.22-0.23)
GN5	6	199 $\pm$ 57 (137-300)	152 $\pm$ 57 (100-262)	344 $\pm$ 180 (241-707)	534 $\pm$ 82 (430-634)	0.28 $\pm$ 0.07 (0.23-0.41)
KN1	8 <sup>1</sup>	18 $\pm$ 6 (3-26)	3.0 $\pm$ 2.2 (0.5-7.6)	359 $\pm$ 144 (155-577)	904 $\pm$ 343 (350-1524)	0.0033 $\pm$ 0.0014 (0.0004-0.0050)
KN2	10	14 $\pm$ 3 (6-19)	176 $\pm$ 118 (79-468)	310 $\pm$ 220 (127-839)	389 $\pm$ 195 (179-720)	0.45 $\pm$ 0.12 (0.21-0.65)
KN3	10	26 $\pm$ 10 (9-39)	81 $\pm$ 40 (40-169)	129 $\pm$ 73 (58-280)	374 $\pm$ 237 (154-886)	0.24 $\pm$ 0.06 (0.12-0.33)
KN4	10	24 $\pm$ 7 (19-29)	101 $\pm$ 54 (55-196)	137 $\pm$ 57 (76-245)	352 $\pm$ 185 (206-739)	0.29 $\pm$ 0.04 (0.23-0.34)

<sup>1</sup> Excluding one eel from KN1 with outlying analytic results (see text).

Another factor contributing to the variability may be the size of the eel sampled. Collecting 10 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 10.1A), and poor water quality. Belpaire *et al.* (2003) reported that eel may be caught at only 18% of the sites on rivers and brooks and that abundance is usually low (1-5 individuals/100 m electrofishing). To obtain sufficient data, eels from a broader size range had to be included. This may to some extent have biased the results, because in general larger eels may be expected to have a larger pollution load than smaller specimens. However, as revealed by principal component analysis, length has 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 over Flanders (2526 eels from 365 sites) amount to a mean of  $5.89 \pm 8.91$  (range 0.002–192)  $\mu\text{g.kg}^{-1}$  on a muscle wet weight basis. In comparison, the HCB concentrations in the Grote Nete (21-53  $\mu\text{g.kg}^{-1}$  muscle wet weight) were relatively high, especially in the upstream part. This indicates a local source of pollution, even though this chemical has been banned in 1974. Another pesticide banned from agricultural application in 1974 is DDT. Nevertheless, DDT and its metabolites are still present in quite large quantities in eels from both rivers (Table 10.3). The relative proportion of the breakdown products compared to *p,p'*-DDT provides some striking results. DDT/DDE amounts to 0.003 and 0.09 at the most upstream sites of the two rivers (KN1 and GN2, respectively), peaks at the second-most upstream site (KN2 and GN3) at 0.45 and 0.39, respectively, to decrease again in the downstream sites. This would suggest that there are recent sources of pollution by DDT in the upstream parts. Goemans *et al.* (2003) reported that DDT and its metabolites are present in non-neglectable amounts in most eels over Flanders. Unexpectedly, Maes *et al.* (2008) observed in a trend analysis (1994-2005) that concentrations of *p,p'*-DDT had increased over time, while 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 eel may 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 bioindicator. The discriminating power among sites over a geographical range is a measure of the efficiency of the bioindicator. Univariate analysis of the variations in specific contaminants gives clear indications of their presence in the river systems. However, to evaluate 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 evaluate intra- and inter-site variability in pollution profiles in individual eels sampled within a small catchment area, with sites lying 20 km apart at maximum. 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 to evaluate intra-site variability.

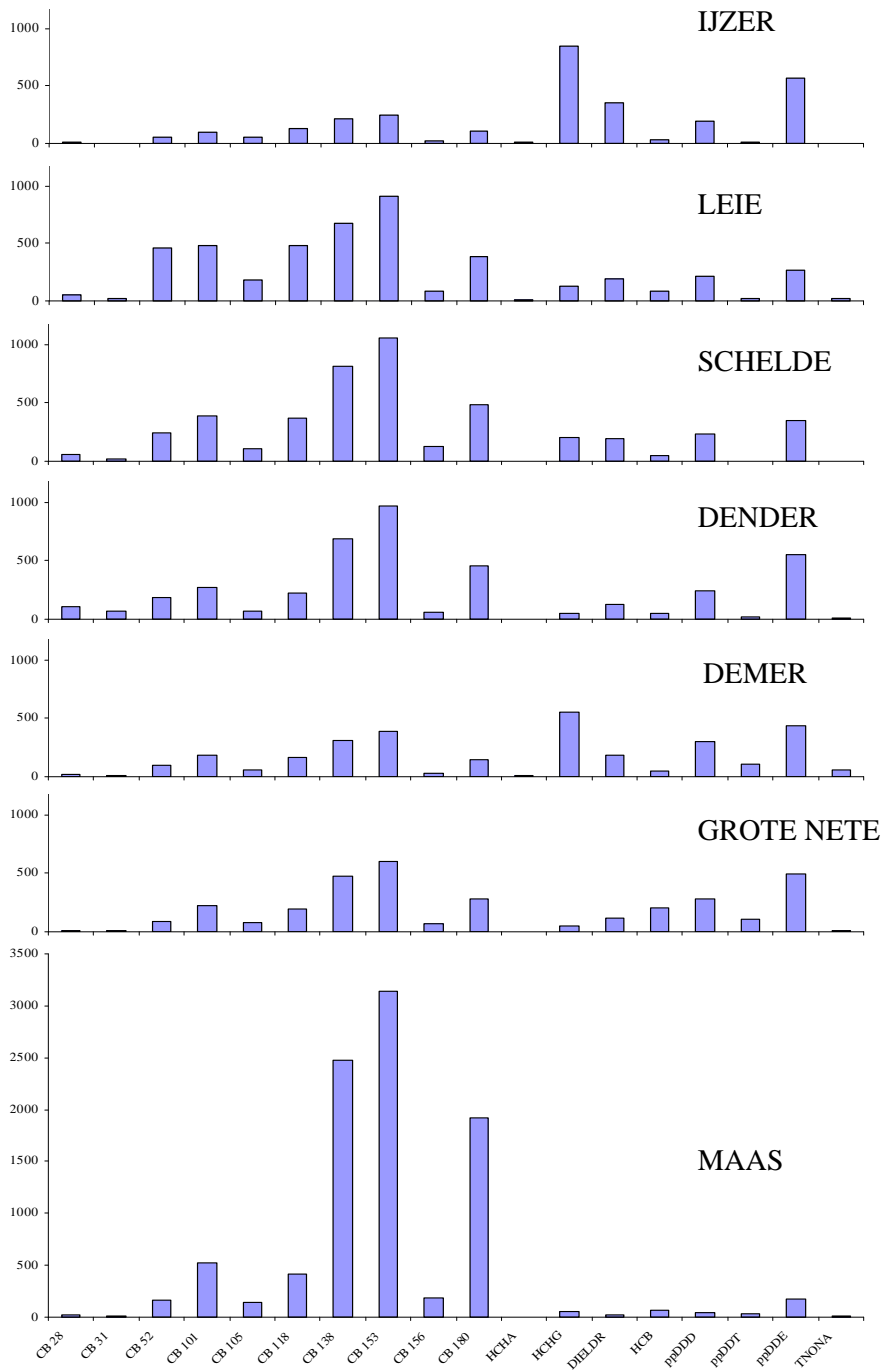
The CDA (Figure 10.4) yielded rather conclusive results: all eels from the same site clustered closely together, even when distance between sample sites was less than 5 km. Apparently, site-specific aquatic pollution by lipophilic compounds can be tracked in eels. Also, within each tributary, site-clusters congregate, indicating

river-specific contaminant pressure. From these results, we conclude that the contaminant fingerprint of yellow eels, after filtering out outliers, is representative for the environmental quality (in terms of the local load with lipophilic chemicals) of the site where it was caught. We tried to compare these bioaccumulation data in eel with measurements of the same contaminants carried out during monitoring of water and sediment quality in the two Nete basins by the Flemish Environmental Agency. However, just because these chemicals are lipophilic, they are hard to trace in the water phase or even in sediments (Table 10.4). 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 is totally dependent on biomonitoring.

Similar results to ours regarding small-scale differences were obtained studying pollution profiles in eels in a canal and under lacustrine conditions. Belpaire and Goemans (2007b) reported spatial and temporal differences in pollution load within a 14 km-long Belgian canal. Belpaire *et al.* (2001) observed variation among eels caught in four different parts of Lake Schullen (90 ha), as well as significant differences in lindane ( $\gamma$ -HCH) concentrations in their muscle tissue. All these observations are in line with the conclusion from ecological studies on home ranges that foraging movements of yellow eels are mostly restricted to a few hundred meters (Baras *et al.*, 1998; Lafaille *et al.*, 2005). Such a small home range would explain why yellow eels serve as good indicator species for monitoring site-specific pollution pressure.

**Table 10.4.** 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). Number of sites (n), period of sampling and number of measurements (N) are also indicated. The detection limits are 1 or 2 ng l<sup>-1</sup> for water (dependent of the substance), 0.05 ng g<sup>-1</sup> dry matter for sediment and 0.5 ng g<sup>-1</sup> lipid weight for eel. Water and sediment data were provided by the Flemish Environment Agency (VMM).

Substance	Water		Sediment		Eel	
	3 sites, 2000-2007		73 sites, 2000-2006		8 sites, 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
Alpha-HCH	0	118	0	130	74	88
Gamma-HCH	16	246	2	130	100	88
<i>p,p'</i> -DDT	0	115	8	130	77	88
<i>p,p'</i> -DDE	0	107	31	130	100	88
<i>p,p'</i> -DDD	0	112	22	130	100	88
dieldrin	0	110	4	130	78	88



**Figure 10.7.** Pollution fingerprints based on means of PCB and OCP concentrations ( $\mu\text{g.kg}^{-1}$  LW) in eels from seven rivers in Flanders.

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 indicates 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 with the geographical positions of the (sub-)basins (Figure 10.1B), the most-western catchment (IJzer) being most distinct from the most-eastern Maas catchment. Within the centrally-positioned Schelde, adjacent subbasins take up adjacent positions in the clustering: the adjacent basins of Demer and Grote Nete as well as those of Schelde and Leie, have more comparable profiles (despite their distinctness) than any of these with the Dender, which is located in between. While subbasins indicate, overall, distinct contaminant profiles, similarities between subbasins suggest geographical gradients in contaminant pressure that might well result from variations in land use. An increasing west-east gradient in PCB-contamination in eel in Flanders has been reported before by Maes *et al.* (2008).

Figure 10.7 summarizes the averaged river-specific pollution fingerprints observed in eels. These observations are generally in line with Maes *et al.* (2008), who reported high  $\alpha$ - and  $\gamma$ -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. The approach of using this bioindicator for lipophilic substances might be used more effectively within the monitoring programme of the Water Framework Directive than using indicators derived from concentrations in the water phase (Chapter 13).

## Acknowledgements

The authors gratefully acknowledge Tom De Boeck, Yves Maes and Gerlinde Van Thuyne from INBO for database and GIS support and Thierry Onckelinx and Pieter Verschelde for statistical support. We thank all people involved in eel sampling and analysis. We are grateful to Dirk Roos and Ward De Cooman from VMM (Vlaamse Milieumaatschappij, the Flemish Environment Agency) for providing the water and sediment quality data. We acknowledge Niels Daan, Willem Dekker and an anonymous referee for their critical comments on an earlier version of the manuscript.

## References

- Baras, E., Jeandrain, D., Serouge, B., Philippart, J.-C., 1998. Seasonal variations in time and space utilization by radiotagged yellow eels *Anguilla anguilla* (L.) in a small stream. *Hydrobiologia* 371/372, 187-198.
- Belpaire, C., Derwich, A., Goemans, G., Van Thuyne, G., Cooreman, K., Guns, M., Ollevier, F., 2001. Intra lake spatial variations in pollution patterns of eel *A. anguilla*. In: Aida, K., Tsukamoto, K., Yamauchi, K., (eds.), Proceedings of the International Symposium 'Advances in Eel Biology'. University of Tokyo, 28-30 Septembre 2001. p.170-174.
- Belpaire C., Goemans G., Van Thuyne G., Verreycken H., Maes, J., 2003. Eel Fisheries and Management in Flanders, Belgium: Status and Trends. International Eel symposium, Quebec, 10-15 august, 2003. Available at <http://www.giuliodedeo.it/AFS-Index.html>

- Belpaire, C., Goemans, G., 2007a. Eels: contaminant cocktails pinpointing environmental pollution. *ICES Journal of Marine Science* 64, 1423-1436.
- Belpaire, C., Goemans, G., 2007b. The European eel *Anguilla anguilla*, a rapporteur of the chemical status for the Water Framework Directive? 12 years of monitoring in Flanders. *Vie et Milieu - Life and Environment* 57 (4), 235-252.
- Bruslé, J., 1991. The eel (*Anguilla* sp) and organic chemical pollutants. *The Science of the Total Environment* 102, 1-19.
- CEC, 2006a. Proposal for a Directive of the European parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC (presented by the Commission) {COM(2006) 398 final}{SEC(2006) 947} Commission of the European Communities, Brussels, 17.7.2006 COM(2006) 397 final 2006/0129 (COD).
- CEC, 2006b. Opinion of the Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE) on "The Setting of Environmental Quality Standards for the Priority Substances included in Annex X of Directive 2000/60/EC in Accordance with Article 16 thereof " Adopted by the CSTEE during the 43rd plenary meeting of 28 May 2004. European Commission, Health & Consumer Protection Directorate-General, Directorate C – Public Health and Risk Assessment C7 – Risk Assessment Brussels, C7/GF/csteeop/WFD/280504 D(04).
- de Boer, J., Hagel, P., 1994. Spatial differences and temporal trends of chlorobiphenyls in yellow eel (*Anguilla anguilla*) from inland waters of the Netherlands. *The Science of the Total Environment* 141, 155-174.
- Feunteun, E., Laffaille, P., Robinet, T., Briand, C., Baisez, A., Olivier, J.M., Acou, A., 2003. A review of upstream migration and movements in inland waters by anguillid eels: towards a general theory. In: Aida, K., Tsukamoto, K., Yamauchi, K., (eds.), Eel biology. Springer Verlag, Tokyo, p.191-213.
- Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentennet, 1994-2001: gehalten aan polychloorbifenyleen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollutant monitoring network 1994-2001: polychlorine biphenyls, organochlorine pesticides and heavy metals in eel]. Report of the Institute for Forestry and Game Management, IBW.Wb.V.R.2003.99. 169p.
- Goemans, G., Belpaire, C., 2004. The eel pollutant monitoring network in Flanders, Belgium. Results of 10 years monitoring. *Organohalogen Compounds* 66, 1834-1840.
- Huet, M., 1959. Profiles and biology of western European streams as related to fish management. *Transactions of the American Fisheries Society*, 88: 155–163.
- Knights, B., 1997. Risk assessment and management of contamination of eels (*Anguilla* spp.) by persistent xenobiotic organochlorine compounds. *Chemical Ecology* 13, 171-212.
- Laffaille, P., Acou, A., Guillouët, J., 2005. The yellow European eel (*Anguilla anguilla* L.) may adopt a sedentary lifestyle in inland freshwaters. *Ecology of Freshwater Fish* 14, 191-196.
- Maes, J., Belpaire, C., Goemans, G., 2008. Spatial variations and temporal trends between 1994 and 2005 in polychlorinated biphenyls, organochlorine pesticides and heavy metals in European eel (*Anguilla anguilla* L.) in Flanders, Belgium. *Environmental Pollution* 153, 223-237.
- Robinet, T., Feunteun, E., 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265-277.
- Van Overmeire, I., Pussemier, L., Hanot, V., De Temmerman, L., Hoenig, M., Goeyens, L., 2006. Chemical contamination of free-range eggs from Belgium. *Food Additives & Contaminants* 23 (11), 1109-1122.