Laboratorium voor Aquatische Ecologie
en Evolutiebiologie
Laboratorium voor Diversiteit en
Systematiek van Dieren
Charles Deberiotstraat 32
B-3000 Leuven, België
Aquabio@bio.kuleuven.be

2008

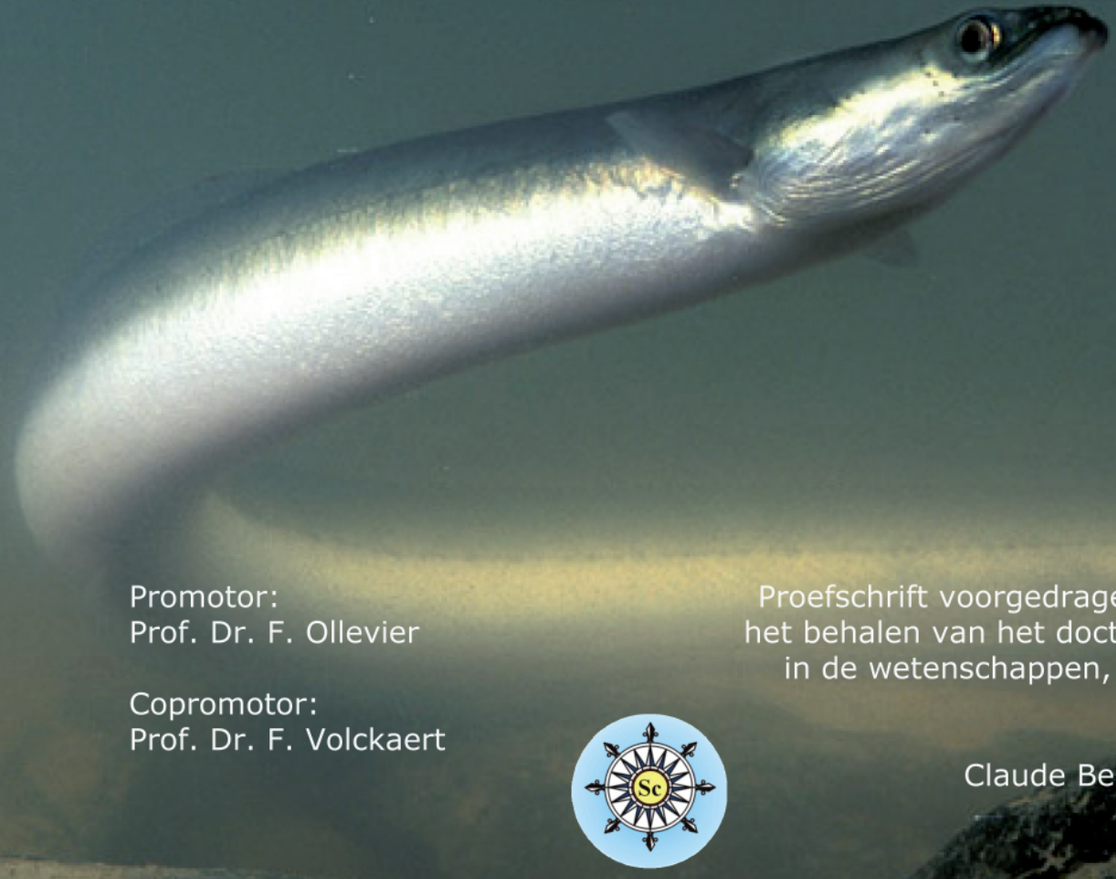
Claude Belpaire **Pollution in eel. A cause of their decline?**

KATHOLIEKE UNIVERSITEIT
LEUVEN

Faculteit Wetenschappen

Departement Biologie
Laboratorium voor Aquatische Ecologie
en Evolutiebiologie
Laboratorium voor Diversiteit en
Systematiek van Dieren

Pollution in eel.
A cause of their decline?



Promotor:
Prof. Dr. F. Ollevier

Copromotor:
Prof. Dr. F. Volckaert

Proefschrift voorgedragen tot
het behalen van het doctoraat
in de wetenschappen, 2008

Claude Belpaire

Author/Editor: Claude Belpaire

Published by:
Instituut voor Natuur- en Bosonderzoek - INBO
Duboislaan 14, 1560 Groenendaal
www.inbo.be

E-mail: Claude.Belpaire@inbo.be

Cite as:
Belpaire, C., 2008. Pollution in eel. A reason for their decline? Ph.D. thesis Catholic University of Leuven, INBO.M.2008.2. Instituut voor Natuur- en Bosonderzoek, Brussels, 459 pages, III annexes.

D/2008/10.705/30
INBO.M.2008.2
ISBN 978-90-8649-184-1

Layout: Claude Belpaire
Printed by: Management ondersteunende diensten van de Vlaamse overheid
Photos front and back cover: © INBO (Vilda – Rollin Verlinde)

English title: Pollution in eel. A cause of their decline?
Nederlandse titel: Verontreiniging in paling. Een oorzaak van zijn achteruitgang?

Faculteit Wetenschappen
Afdeling Ecologie en Systematiek der Dieren
Laboratorium voor Aquatische Ecologie en Evolutiebiologie
Laboratorium voor Diversiteit en Systematiek van Dieren
Charles Deberiotstraat 32
B-3000 Leuven, België

Katholieke
Universiteit
Leuven

Promotor : Prof. Dr. Frans Ollevier
Copromotor : Prof. Dr. Filip A.M. Volckaert
Katholieke Universiteit Leuven
Laboratorium voor Aquatische Ecologie en Evolutiebiologie
Laboratorium voor Diversiteit en Systematiek van Dieren

Overige leden van de examencommissie :

Dr. Willem Dekker
Dr. Gregory Maes
Dr. Joachim Maes
Prof. Dr. Jos Snoeks
Dr. Jurgen Tack
Prof. Dr. Guido van den Thillart

Het onderzoek voorgesteld in dit proefschrift werd uitgevoerd aan het Instituut voor Natuur- en Bosonderzoek en werd gesteund door de Vlaamse Milieumaatschappij, het Agentschap voor Natuur en Bos en de Vlaamse Overheid .

Dit proefschrift bevat niet gepubliceerde wetenschappelijke gegevens. Bijgevolg mag niets van dit proefschrift op welke wijze ook, gebruikt of publiek gemaakt worden zonder voorafgaande schriftelijke toestemming van de auteur.

The research described in this thesis was carried out at the Institute for Nature and Forest Research - INBO, Groenendaal, Belgium.

Katholieke Universiteit Leuven
Faculteit Wetenschappen
Departement Biologie
Laboratorium voor Aquatische Ecologie en Evolutiebiologie
Laboratorium voor Diversiteit en Systematiek van dieren

Pollution in eel.
A cause of their decline?

Verontreiniging in paling.
Een oorzaak van zijn achteruitgang?

Promotor :
Prof. Dr. Frans Ollevier
Copromotor :
Prof. Dr. Filip A.M. Volckaert

Proefschrift voorgedragen tot het behalen
van de graad van Doctor in de Wetenschappen
door:
Claude Belpaire

"Peaking in POPs"

Eels, weird and inscrutable
Scaping to deep Sargasso.
For years, unfathomed, ungrasped, un-understood
Now counted with anxiety, elvers, returning in ones,
Scientists agonizing. Why now so few?
Some cut flesh in a machine.
Biologist and chemist
Stared at the POP speaking
And understood silver eels' sorrow .

Claude Belpaire & Robert Rosell

For Ranec, Tom & Lut

Table of Contents

		page
	Table of Contents	i
	Acknowledgements	iii
	List of Figures	vi
	List of Tables	ix
	List of Abbreviations and Acronyms	xi
	Abstract	xv
	Samenvatting	xvii
Part I	**Introduction**	**1**
Chapter 1	Introduction	3
Part II	**Status and trends of contaminants in eels**	**13**
Chapter 2	Spatial variation and temporal trends of polychlorinated biphenyls, organochlorine pesticides and heavy metals	15
Chapter 3	Brominated flame retardants in eels from River Scheldt	45
Chapter 4	Volatile organic compounds in yellow eel	65
Part III	**Effects of contaminants on the eel**	**83**
Chapter 5	Effects of contaminants on the eel: a review	85
Chapter 6	Decreasing eel stocks: survival of the fattest	115
Chapter 7	Pollution, condition and genetic variability	145
Chapter 8	Vitellogenin in eel	173
Part IV	**The use of the European eel as an indicator of pollution**	**189**
Chapter 9	Intra lake spatial variations in pollution patterns of eel	191
Chapter 10	Pollution fingerprints in eels as models for the chemical status of rivers	201
Chapter 11	Congener profiles in eel as a method of tracing the origin of PCB contamination.	221
Chapter 12	Eels: contaminant cocktails pinpointing environmental pollution	229
Chapter 13	Eels as chemical bioindicators for the Water Framework Directive?	255
Chapter 14	Towards a European Eel Quality Database	289
Part V	**Contaminants in eel and human health**	**299**
Chapter 15	Polychlorinated biphenyl exposure through eel consumption in recreational fishermen	301

Part VI **Conclusions and perspectives** **315**

Chapter 16 Summary, conclusions and recommendations 316

Annex I **Maps** **353**
Annex II **Data tables** **389**
Annex III **List of publications** **453**

Acknowledgements

This work would not have been possible without the help of a large group of people, as diverse as society can be: researchers, professors, field workers, laboratory assistants, technicians, analysts, designers, fisheries managers, directors, administrators, librarians, secretaries, students, maintenance personnel accountants, HRM and IT supporters and even politicians. I have appreciated their help during the many years of the genesis of this book. Naming all of them would lead me much too far and would make me very uncomfortable as I risk to forget many of them. Several of them became good friends.

I remember the first discussion with my promotor Prof. Dr. Frans Ollevier who invited Willy Verdonck and myself – twenty-five years ago now – to work on a study on growth and culture of fish at his fish laboratory, which at that time consisted of us three and some fishtanks. This invitation was for me the introduction to the eel, this fascinating fish, which has passionated me during all this years. Frans and I went a long way together, joined on our way, by a bunch of motivated young researchers studying diverse aspects of the eel, and it has always been a pleasure for me to work with them. Thank you for all this, Frans.
My gratitude goes also to my co-promotor Prof. Dr. Filip Volckaert, for the cooperation during recent years and for his scientific advice.
Sincere thanks also to all the other members of my jury, Dr. Willem Dekker, Dr. Gregory Maes, Dr. Joachim Maes, Prof. Dr. Jos Snoeks, Dr. Jurgen Tack and Prof. Dr. Guido van den Thillart, for their willingness to read this work and for their constructive comments.

The very first eel samples for measuring contamination were taken during my years at university, but unfortunately, they never have been analysed, due to a lack of funding. Later on, I took a new start, and this work was carried out at INBO, the Research Institute for Nature and Forest. I thank our institute and its managers and ex-managers for giving me the opportunity to carry out this work, especially Ir. Jos Van Slycken, who willingly helped me during many years in my search for funding. Additional funding was provided through various research projects with ANB (Flemish Forest and Nature Agency), VMM (Flemish Environment Agency) and OVAM (Public Waste Agency of Flanders).

The analyses of the contaminants were performed by external laboratories, ILVO (Sea Fisheries Department, Institute for Agricultural and Fisheries Research) at Ostend and CODA (Veterinary and Agrochemical Research Centre) at Tervuren, but also laboratories at the universities of Antwerpen (Laboratory for Ecophysiology, Biochemistry and Toxicology), Gent (Laboratory of Environmental Toxicology and Aquatic Ecology), Leuven (Laboratory for Animal Diversity and Systematics) and Liège (Physical Chemistry, Mass Spectrometry Laboratory) and at IMARES (Netherlands Institute for Fisheries Research, IJmuiden). I would like to thank all of them for their excellent contribution.

During my work I came in contact with many scientists in Belgium and far abroad. Many Belgian universities were interested to join the monitoring network with their specific expertise, and it was a pleasure for me to cooperate with all of them. I have very good memories of lengthy e-mail discussions I had with many of the (co-)authors of the papers presented here. A lot of other people helped during the redaction and are acknowledged in the papers. The annual meetings of the ICES/EIFAC Working Group on Eel assembling world leading scientists on eel were very stimulating, and from the early beginning,

This is part of the current team of technicians, administrators and biologists from the Groenendaal site at the Research Institute for Nature and Forest. Many of them were personally involved in my research by catching eels, preparing samples, technical and administrative support, scientific advice, etc. Without their support this work would never have been possible.
From left to right, below: Tom De Boeck, Johan Moysons, Danny Bombaerts, Caroline Geeraerts, Jan Breine, Gerlinde Van Thuyne, Ilse Simoens, Ann Verheyden
Upper row: Hugo Verreycken, Jikke Janssens, Yves Maes, Franky Dens, Yves Verhaegen, Isabel Lambeens, Jean-Pierre Croonen, Kathleen Peirsman, Adinda De Bruyn, Claude Belpaire, Alain Vanderkelen.

these meetings sharpened my view and made me conscious of the fact that science and management are two very different issues, notwithstanding their final goal ('*saving the eel from decline*') may be common.

To allow me to use some of their photographs and drawings to illustrate this thesis, I thank especially Gerard Alsteens (GAL), Ward De Cooman, David Carss, Rollin Verlinde, Cathy Wilcox, Deutsche Presse-Agentur, Peter Van Hoof, Gerlinde Van Thuyne and Geert Goemans.

Writing this book implied considerable field work, which required technical assistance by experienced and trained field collaborators. I was very lucky to be able to count on the every-day commitment of a team of motivated field workers making an all-out effort to have good and reliable samples; the current team consists of Daniel Bombaerts, Jean-Pierre Croonen, Adinda De Bruyn, Marc De Wit, Franky Dens, Jikke Janssens, Christian Langsberg, Johan Moysons, Alain Vanderkelen and Yves Verhaegen. A special word for Nico Van Pee who unfortunately has left us much too early.

Coordination of the field work, processing of the samples, tissue storage, and data and database management constitutes the foundation of this work. For this I could benefit from the indispensable support of Tom De Boeck, Isabel Lambeens, Yves Maes and Kathleen Peirsman. It was really a pleasure to know I could rely on them for this essential work. Also Filip Coopman and Ann Verheyden helped me a lot with many administrative jobs, whereas Lymke Janssens did vital work for the final layout.

And - of course - warm feelings of gratitude to my direct team colleagues, Jan Breine, Gerlinde Van Thuyne, Ilse Simoens, Hilde Verbiest and Hugo Verreycken. Not only for providing me with eel samples and for scientific discussions or any other professional activities, but - especially - for their confidence and pleasant cooperation. Thank you Hugo, for last-minute editing work. Finally, I would like to thank my two team-brothers in crime, Geert Goemans and Caroline Geeraerts, who also have been bitten by the *eel contaminant virus*. Geert did tremendous work for the Flemish Eel Pollution Monitoring Network. It was a tough day hearing he had chosen for a new challenge, a transatlantic job dealing with his other passion, insect taxonomy. Caroline took over his job and I wish her much success and perseverance on the eel contamination footpath. Both of them helped me a lot during last year's rush to finalize this thesis.

Thank you all, and it is my strong conviction that - finally - we will succeed to elucidate the enigma of the decline of the Atlantic eel species, and it is my deepest wish that our grandchildren will be able to see many fat eels with low contaminant body burdens swimming their way from our Flemish rivers to the Sargasso Sea.
I hope that, to some extent, this work will have contributed to this.

List of Figures

		page
1.1.	The life cycle of the European eel with indication of major life stages (after Dekker, 2000).	5
1.2.	Time-series of monitoring glass eel recruitment in European rivers. Each series has been scaled to its 1979–1994 average.	6
2.1.	Geographic position of Flanders in Europe and location of the sampling points of the eel pollutant monitoring network.	19
2.2.	Spatial distribution of the average eel muscle tissue concentration of ∑PCB, lindane, α-HCH and dieldrin over the different river basins in Flanders (Belgium).	24
2.3.	Spatial distribution of the average eel muscle tissue concentration of endrin, ∑DDT, HCB and transnonachlor (T-nona) over the different river basins in Flanders (Belgium).	25
2.4.	Spatial distribution of the average eel muscle tissue concentration of four heavy metals over the different river basins in Flanders (Belgium).	26
2.5.	Temporal trends in average eel muscle tissue concentration of ∑DDT, ∑PCB, HCB and PCB 153 at sampling stations that were sampled more than once between 1994 and 2005.	29
2.6.	Temporal trends in average eel muscle tissue concentration of lindane, dieldrin, endrin and α--HCH at sampling stations that were sampled more than once between 1994 and 2005.	30
2.7.	Temporal trends in average eel muscle tissue concentration of three heavy metals at sampling stations that were sampled more than once between 1994 and 2005.	31
3.1.	Basin of the River Scheldt and situation of the sampling area nearby Oudenaarde.	48
3.2.	Geographical variation of sum PBDEs and total HBCD levels (ng/g lw) of all analyzed fish samples (A) and eel samples (B).	53
3.3.	Geographical variation of sum PCBs (ng/g lw) of all analyzed fish samples.	54
3.4.	Average PBDE congener profiles in eel, carp and perch samples.	55
3.5.	Typical LC–MS chromatogram for an eel sample from location L5.	57
4.1.	Sampling locations in the region of Flanders (Belgium).	68
4.2.	Total ion count GC-MS chromatogram for eel sample No. 5 and extracted ion chromatogram (*m/z* 164+165) for tetrachloroethene.	70
4.3.	Percentage of positive samples for the detected VOCs in order of abundance.	73
4.4.	Box and whisker plot of the detected VOCs in eel samples.	74
4.5a.	Comparison of the concentrations of selected CHCs in tissues of marine species and freshwater eel.	76
4.5b.	Comparison of the concentrations of selected BTEX in tissues of marine species and freshwater eel.	77
4.6.	Comparison of the concentrations of tetrachloroethene, chloroform and ΣBTEX for the various sampling stations.	78
5.1.	A simplified conceptual model of the effects of pollution exposure on the population structure of the European eel, *A. anguilla*.	90
6.1.	Sampling locations for measuring the fat contents in yellow eel.	118
6.2.	Morphological parameters of the yellow eels of the three year groups in Belgium. Mean lengths and weights of the yellow eels from Belgium, over the three year groups between 1994 and 2006, analysed for muscle fat content.	120
6.3.	Temporal trend in fat contents in yellow eels in Flanders. a, Decreasing fat contents and condition factor in yellow eels in Flanders (Belgium) between 1994 and 2006. **b,** Frequency distribution of lipid content in Flemish yellow eel from the three periods.	122
6.4.	Temporal trend in fat contents in yellow eels in The Netherlands. a, Decreasing fat contents in yellow eels in The Netherlands between 1977 and 2004. **b,** Frequency distribution of lipid content in yellow eel from the Netherlands during the six periods.	123
6.5.	Temporal trends in fat contents in yellow eels from four water bodies of different typology.	124
6.6.	Time-series of glass eel recruitment in Europe (WG Eel, 2007) and of muscle lipid	134

contents in yellow eels from The Netherlands.
7.1. *Anguilla anguilla*: Flanders with sampling sites along three Belgian river basins; Yser: Y1, Y2, Y3, Y4, Y5; Scheldt: S1, S2, S3, S4, S5, S6, S7; Meuse: M1, M2, M3, M4. 149
7.2. Level of heavy metal pollution (IMBI) in *Anguilla anguilla*. a) Per sampling site within river basin; b) Histogram: the "HIGH" and "LOW" group are defined from the bimodal distribution of IMBI values. 154
7.3. Boxplots representing relative condition index (CI ± S.D.), hepatosomatic index (HSI ± SD) and level of heavy metal pollution (IMBI ± SD) of *Anguilla anguilla* for each river basin. ** = $p < 0.01$ 155
7.4. Correlation between heavy metal bioaccumulation (IMBI) of *A. anguilla* and a) condition index (CI) with $R = - 0.24$; $p = 0.039$; b) hepatosomatic index (HSI) with $R = - 0.20$; $p = 0.09$ for all individuals ($N = 73$). 156
7.5. Allozymatic genetic variability of *A. anguilla*. Boxplots representing mean (± SE) a) allelic richness (AR, t-test; $p = 0.01$), b) observed heterozygosity ($H_{O;}$ t-test; $p = 0.03$) locus-by- locus and c) Multi-locus Heterozygosity (MLH, ANOVA, $p = 0.05$) comparisons between "HIGH" and "LOW" heavy metal pollution groups. 160
7.6. Correlation between relative heavy metal bioaccumulation (IMBI) and multi-locus heterozygosity (MLH) in *A. anguilla* for allozymes and microsatellites. 162
8.1. Location of the different sampling sites (the inset top left is Europe). 177
8.2. (A) A typical gel after protein electrophoresis of plasma of ethinylestradiol-exposed eel (EE2) and solvent control eel (S.C.) and (B) a typical Western blot gel. 181
8.3. Relative plasma vitellogenin (VTG) content of the eels sampled at different locations in Flanders, represented as mean ± standard deviation. The number of fish is given for each sampling site. 183
9.1. Position of Lake Schulen in Flanders with the indication of the different zones. 194
9.2. Concentrations of Sum PCBs, mercury, dieldrin, lindane, cadmium and Sum DDT in individual eels from the different zones. The concentrations are expressed in ng/g fat weight except for the heavy metals (in ng/g body weight). Dotted lines correspond to Belgian consumption standards. 196
9.3. Factor analysis of contaminant concentrations in the eels of the different zones of Lake Schulen. 197
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). 204
10.2. Box-and-Whisker plots for (A-C) PCB and (D-G) OCP concentrations 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; E. HCB; F. γ – HCH; and G. dieldrin. 209
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. 210
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. 210
10.5. Box-and-Whisker plots for (A-C) PCB and (D-G) OCP concentrations 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. 211
10.6. Canonical discriminant analysis of eels from seven rivers in Flanders on the basis of PCB and OCP concentrations. 212
10.7. Pollution fingerprints based on means of PCB and OCP concentrations ($\mu g.kg^{-1}$ LW) in eels from seven rivers in Flanders. 216
11.1. Scatter plots of different indicator PCB-congeners to the sum of the 6 remaining indicator PCBs. 224
11.2. Variation in location specific ratio of CB 118 to the sum of the indicator PCBs. Selection of 337 eels from 7 water bodies in Flanders, Belgium. 225
11.3. Temporal shift in relative fraction of PCB 118 compared to the sum of the indicator PCBs. 226
12.1. The Flemish Eel Pollutant Monitoring Network. Geographical distribution of sampling stations (*n* = 357). 232
12.2. Concentrations of 1,2-dichlorobenzene in eels collected at 20 sites in Flanders (1996–1998). 234
12.3. Concentrations of 1,2-dichlorobenzene in water collected monthly at 40 sites in Flanders (2005). 235

12.4. Concentrations of 1,2-dibromo-3-chloropropane in eels collected at 20 sites in Flanders (1996–1998). 236
12.5. Concentrations of BTEX compounds in eels collected at 20 sites in Flanders (1996–1998). 238
12.6. Concentrations of (a) PBDEs and (b) HBCD in eels collected at 18 sites in Flanders. 240
12.7. Concentrations of lindane in eels collected at 357 sites in Flanders (1994–2005). 241
12.8. Concentrations of cadmium in eels collected at 333 sites in Flanders (1994–2005). 242
13.1. Distribution map of the European eel. 258
13.2. Percentage of individual yellow eel measurements above the detection limit for 10 PCB congeners and 10 organochlorine pesticides and 9 heavy metals in eels from Flanders collected in the period 1994-2005. 260
13.3. Concentration of Sum PCBs over various trophic levels in Lake Weerde (Flanders) in 2001 (spring). 261
13.4. Cluster analysis based on the PCB and OCP (lipid weight basis) profiles of 129 yellow eels from 30 sites in Flanders sampled between 1994 and 1998. 264
13.5. Discriminant analysis on the concentrations of 16 PCBs and OCPs (on lipid weight basis) in 31 eels from three zones in the Boudewijnkanaal from 1991 (11 eels) and 1995 (20 eels). 265
13.6. Factor analysis of contaminant concentrations (PCBs and OCPs) in 17 yellow eels from the zones A, B, C and D in Lake Schulen. 266
13.7. Correlation between the concentration of Sum PCBs measured simultaneously in predator fish species, roach, invertebrates and macrophytes, compared to the concentration of Sum PCBs in eel. 267
13.8. Sampling sites of the Eel Pollutants Monitoring Network in Flanders and geographical distribution of quality classes in Flemish eels for Sum PCBs (N = 351 sites, 1994-2005). 274
13.9. Distribution of Sum PCB quality classes in Flemish eels (N = 351 sites, 1994-2005). 275
13.10. Status of heavy metals in eel in Flanders (after Peeters *et al*., 2006 in Flanders environmental report 2006). 275
13.11. Mean Sum PCB values in eel from 351 sites in Flanders (1994-2005): distribution between quality classes and comparison with threshold values for action or target values as proposed by Belpaire and Goemans (2004). 276
13.12. Possibilities of combined use of monitoring contaminants in the eel. 279
15.1. Box and Whisker plots for concentrations of the Σ_7 iPCBs (ng/g wet weight), analysed in commercially available eel and in eel in Flemish waterbodies. 306
15.2. Fitted cumulative distribution functions for the concentrations of the Σ_7 iPCBs (ng/g wet weight) for eel in Flemish water bodies and commercially available eel. 307
15.3. Cumulative distribution functions of the estimated intake of the Σ_7 iPCBs (ng/kg bw/d) for the background population and the recreational fishermen for the different consumption scenarios (A1, A2, B1, B2). 308
16.1. Position of sampling sites of the Eel Pollution Monitoring Network (n=376). 325
16.2. Sum PCBs (means) in predatory fish in 62 locations over Flanders (2001-2005). 326
16.3. Sampling sites of the Eel Pollution Monitoring Network and the river Sediment Monitoring Network. 328
16.4. Example of a query for contamination data in eel from a site on River Maas (VIS, 2008). 331
16.5. Localisation of stretches of rivers or canals where a total catch and release obligation for all fish species was established in 2002. 335
16.6. Schematic representation of the proposed integration of the bioaccumulation data in biota with sediment quality evaluation for prioritizing water bodies for clearing out sediment for ecological or ecotoxicological reasons. 336

List of Tables

		page
2.1.	Mean eel life history statistics and mean muscle tissue concentration and range (ng g^{-1} wet weight) of different pollutants in muscle tissue sampled in surface waters of Flanders (Belgium).	23
2.2.	Linear mixed models results and diagnostics of the regressions of the concentration of contaminants and pesticides based in wet weight against time.	28
2.3.	Reported concentrations of PCBs in *Anguilla anguilla* in Europe.	34
2.4.	Reported concentrations of organochlorine pesticides in *Anguilla anguilla* in Europe.	35
2.5.	Reported concentrations of heavy metals in *Anguilla anguilla* in Europe.	36
2.6.	Maximum residue and contaminant levels (MRL) in eel as adopted by different European regulations and proportion of eel (%) captured in this study with tissue concentrations higher than this maximum.	38
3.1.	Overview of the investigated fish samples. Values in brackets represent the number of individual fish samples used to compose a pool.	49
3.2.	Concentrations of total HBCDs (ng/g wet weight) in 6 eel samples from 2000 and 2006. Each sample has been analysed using 3 GC-MS and 2 LC-MS different procedures.	56
3.3.	Mean (or median) concentrations of BDE 47 and PCB 153 in eel samples from various studies. When available, standard deviation or ranges are also given.	58
3.4.	Estimated dietary intake from different food groups (ng/day) in Belgium.	60
4.1.	Overview of sampling stations and sampled eel.	69
4.2.	Set of 52 VOCs studied and relevant analytical information	71
4.3.	Concentrations (ng/g ww) of VOCs detected in freshwater eel.	72
4.4.	Correlation matrix for BTEX compounds.	73
4.5.	Comparison between observed VOC concentrations (ng/g) and HC5 values (ng/g) calculated according to van Leeuwen *et al*. (1992)	80
6.1.	Different scenarios of hypothetical calculations of the energy remaining for reproduction (ER) and reproductive potential (RP) in female eels by arrival at their spawning ground.	133
7.1.	Average heavy metal concentration per river basin of *Anguilla anguilla* L.	153
7.2.	Allozymatic and microsatellite genetic variability of *Anguilla anguilla* L. in the three river basins and in the LOW and HIGH pollution group.	158
7.3.	Multiple regression analysis relating individual heavy metal bioaccumulation to condition and multi-locus heterozygosity.	161
8.1.	Summary of sampling sites and date of collection, mean length (± standard deviation) of the eels and organochlorine and PCB load.	178
8.2.	Weight, length, condition factor, plasma protein content and plasma vitellogenin content of eel exposed to 10 µg ethinylestradiol / L compared with controls.	180
9.1.	List of pollutants measured	195
10.1.	Information on sampling sites (code, locality and distance from source) along the rivers Grote Nete and Kleine Nete and on the eel samples taken (date, number sampled, length, and weight).	205
10.2.	Information on the samples taken from the seven rivers in Flanders (sampling date, number of sites per river, number sampled, mean length, and weight).	206
10.3.	Mean muscle tissue concentration of hexachlorobenzene 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).	213
10.4.	Percentage of detectable measurements in water, river sediment and eels from the Grote Nete and Kleine Nete basins. Period, number of sites and number of measurements is indicated.	215
12.1.	Concentrations of 5 VOCs in water and eel from Flanders (Belgium). Data from the Flemish Environment Agency and Roose *et al*. (2003) respectively.	235
12.2.	Potential characteristics of a biomonitor appropriate for the monitoring of hazardous substances in the aquatic environment.	245

12.3. WFD substances mentioned under CEC (2006), and available data from measurements of Flemish eels. 248
13.1. Concentrations of PCB 153, lindane and cadmium measured simultaneously in water, sediment, suspended solids and eel at 5 stations in Flanders (2001). 259
13.2. Overview of recent reports describing bioaccumulation data of various chemicals in *Anguilla anguilla* within EC countries. 269
13.3. Reference values and boundary values of the quality classes for a series of heavy metals, PCB congeners and organochlorine pesticides as defined in the EPMN. 273
14.1. Quality Database. Overview of quality elements included in the European Eel. For condition and contaminants data are presented as minimum, maximum and mean values. 293
14.2. Number of records of eel quality data reported by European countries and compiled by WG Eel (2007) in the European Eel Quality Database. 293
15.1. Estimated intake of the Σ_7 iPCBs (ng/kg bw/d) for the background population and the recreational fishermen. The estimates for the fishermen are presented for the different consumption scenarios (A1, A2, B1, B2). 308
16.1. Overview of EPMN related research, cooperating laboratories or institutes and results valorising maximally eel samples. 323
16.2. Description of data from the EPMN available in VIS (February 2008). 325
16.3. SWOT analysis of the Eel Pollution Monitoring Network. 330

List of Abbreviations

AAS	atomic absorption spectrometry
AchE	acetyl cholinesterase
ALP	alkali labile phosphate
ALT	alanine aminotransferase activity
A/M	axial modulation
AMAP	arctic monitoring assessment programme
amu	atomic mass unit
ANOVA	analysis of variance
AR	allelic richness
As	arsenic
ATP	adenosine triphosphate
BaP	benzo[a]pyrene
BB	body burden
BCF	bioconcentration factor
BCIP/NBT	5-bromo-4-chloro-3-indolyl phosphate / nitro blue tetrazolium
BDE	brominated diphenylether
BFR	brominated flame retardant
BKPME	bleached kraft pulp mill effluent
BNF	beta-naphtoflavone
BTEX	benzene, toluene, ethylbenzene and xylene
BW	body weight
CA	carbonic anhydrase
Ca	calcium
$CaCO_3$	calcium carbonate
CAGE	cellulose acetate gel electrophoresis
cAMP	adenosine 3',5'-cyclic monophosphate
CAT	catalase
CB	chlorinated biphenyls
Cd	cadmium
$CdCl_2$	cadmium chloride
CDA	canonical discriminant analysis
CDNB	1-chloro-2,4-dinitrobenzene
CEC	commission of the European Communities
Cf	condition factor
CHCs	chlorinated hydrocarbons
CI	condition index
CITES	Convention on International Trade in Endangered Species of Wild Flora and Fauna
CODA	Veterinary and Agrochemical Research Centre, Tervuren (Centrum voor Onderzoek in Diergeneeskunde en Agrochemie)
COT	cost of transport
Cr	chromium
Cu	copper
CYP1A	cytochrome P450 1A
DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane)
DDE	1,1-dichloro-2,2- bis(4-chlorophenyl)ethene).
DDT	dichlorodiphenyltrichloroethane

DF	degrees of freedom
DHAA	dihydroabietic acid
DL	detection limit
DNA	deoxyribose nucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
DVZ	Sea Fisheries Department, Ostend (Departement voor Zeevisserij)
E2	17β-estradiol
EC	European Commission
ECD	electron capture detector
ECNI	electron capture negative ionization
EEQD	European Eel Quality Database
EE2	ethinylestradiol
EI	electron ionisation
EIFAC/ICES	European Inland Fisheries Advisory Commission / International Council for the Exploration of the Sea
ENA	erythrocytic nuclear abnormalities
EPA	environmental Protection Agency (U.S.A.)
EPMN	Eel Pollutant Monitoring Network
EQSs	environmental quality standards
ER	energy remaining for reproduction
ETHA	ethacrynic acid
EROD	ethoxyresorufine-O-deethylase
EtOH	ethyl alcohol
EVEX	Eel Virus European X
EQSs	Environmental Quality Standards
FFQ	Food Frequency Questionnaire
GC	gas chromatography
GC-ECNI-MS	gas chromatograph- electron capture negative ionisation- mass spectrometer
GC-MS	gas chromatograph-mass spectrometer
gDNA	genomic deoxyribose nucleic acid
GDR	German Democratic Republic (former East Germany)
GF-AAS	graphite furnace atomic absorption spectrometry
GPX	glutathione peroxidase
GSH	reduced glutathione
G_{ST}	Coefficient of genetic differentiation
GST	Glutathione S-transferase
HBCD	hexabromocyclododecane
HC5	the hazardous concentration which is unlikely to cause harm to more than 5% of the aquatic community
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
HCHG	gamma-hexachlorocyclohexane (lindane)
H_E	expected heterozygosity
HFC	Heterozygosity Fitness Correlation
Hg	mercury
$HgCl_2$	mercury chloride
HMW	high molecular weight
HNO_3	nitric acid
HPI	hypothalamic pituitary interrenal axis
H_2O_2	hydrogen peroxide
H_O	observed heterozygosity
HSI	hepato-somatic index

H&W	Hardy-Weinberg
IAEA	International Atomic Energy Agency
IBI	Index of Biotic Integrity, fish index
IBW	Institute for Forestry and Game Management (Instituut voor Bosbouw en Wildbeheer)
ICP-OES	inductive coupled plasma-optical emission spectrometry
IMBI	individual (multi) metal bioaccumulation index
INBO	Research Institute for Nature and Forest (Instituut voor Natuur- en Bosonderzoek)
iPCB	indicator PCB
ITCs	internal toxic concentrations
IUPAC	International Union of Pure and Applied Chemistry
IWT	Institute for the Promotion of Innovation by Science and Technology in Flanders (Instituut voor de aanmoediging van innovatie door Wetenschap & Technologie in Vlaanderen)
K	potassium
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography / tandem massaspectrometry
LC50	median lethal concentration
LOD	limit of detection
LOQ	limit of quantification
LPO	lipid peroxidation
LW	lipid weight
MANOVA	multivariate analysis of variance
MAHs	monoaromatic hydrocarbons
MFO	mixed function oxygenase
Mg	magnesium
$MgCl_2$	magnesium chloride
MLH	multi-locus heterozygosity
MLH-A	multi-locus heterozygosity allozymes
MLH-M	multi-locus heterozygosity microsatellites
MNA	mean number of alleles per locus
MoBB	margin of body burden
MRL	maximum residue limit
MT	methallothionein
Na	sodium
NaAc	sodium acetate
Ni	nickel
NIST	US National Institute for Standards and Technology
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentrations
OC	organochlorine
OCPs	organochlorine pesticides
OP	organophosphate
OSPAR	The Convention for the Protection of the Marine Environment of the North-East Atlantic (the “OSPAR Convention”) was opened for signature at the Ministerial Meeting of the Oslo and Paris Commissions in Paris on 22 September 1992.
P	P-value (Statistics) or Level of polymorphism (genetics)
PAC	polycyclic aromatic compound
PAHs	polyaromatic hydrocarbons
Pb	lead
PBDEs	polybrominated diphenylethers

PBS	phosphate buffered saline
PBST	PBS + 0.1% tween
PCBs	polychlorinated biphenyls
PCDD/Fs	polychlorinated dibenzodioxins/furans
PCR	polymerase chain reaction
PFOS	perfluorooctane sulphonic acid
PCP	pentachlorophenol
POPs	persistent organic pollutants
QSARs	quantitative structure–activity relationships
QUASIMEME	Quality Assurance of information for marine environmental monitoring in Europe
REACH	registration, evaluation and authorization of chemicals
RP	reproductive potential
SAS	Statistical Analysis Software
SD	standard deviation
SE	standard error
Se	selenium
SIM	selected ion-monitoring
S/N	signal/noise
SPSD	Scientific support Plan for a Sustainable Development
STECF	Scientific, technical and economic committee for fisheries of the EC
SWOT	a SWOT analysis includes analysis of strengths, weaknesses, opportunities and threats
TDE or DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane)
TDI	Tolerated Daily Intake
TEF	toxic equivalence factor
TEQ	toxic equivalent
TG	Tris-Glycine
TM	Tris-Maleate
T-nona	trans-nonachlor
V	Volt
VITO	Flemish Institute for Technological Research (Vlaamse Instelling voor Technologisch Onderzoek)
VMM	Flemish Environmental Agency (Vlaamse Milieu Maatschappij)
VOC	Volatile organic compound
VTG	vitellogenin
WFD	Water Framework Directive
WHO	World health organisation
WW	wet weight
Zn	zinc
∑PCB, Σ_7 iPCBs	sum of seven indicator PCBs (28, 52, 101, 118, 138, 153, 180)

Pollution in eel.
A cause of their decline?

ABSTRACT. The European eel *Anguilla anguilla* (L.) is a widespread, panmictic and catadromous fish, widely distributed over Europe, with an important economic value for fisheries. The population is waning, as shown through recruitment monitoring in European rivers. The state of the stock is now considered below safe biological limits and a recent European regulation urges for stock protection measures. Although many potential causes have been suggested, the reasons for this dramatic decline remain unknown.

As the eel is a long-lived, carnivorous, benthic and lipid-rich species, it is particularly prone to the accumulation of noxious chemical compounds, especially lipophilic contaminants like polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and brominated flame retardants (BFRs). At the Research Institute for Nature and Forest, we set up a monitoring network (Eel Pollution Monitoring Network, EPMN) and measured contaminants in the eel over Flanders during a 14-year research programme. Between 1994 and 2007 more than 3000 eels from 376 locations were analysed for PCBs, OCPs, heavy metals and some other compounds.

We demonstrated that eels in their yellow stage are very suitable chemical bioindicators; contaminant profiles in those eels are fingerprints of the contamination pressure on the site where they grow up. Monitoring of contaminants in Flanders is based on measuring chemicals in water and sediments, but many analytical results of lipophilic compounds like PCBs and OCPs such as DDT, drins or hexachlorobenzene, fall under the detection limit, whereas in eel, those compounds are detectable in nearly all cases. We therefore strongly recommend a critical assessment of the monitoring strategy of chemical substances in our aquatic environment, both at a Flemish and an international scale, within the European Water Framework Directive.

Our results generated a status report and distribution maps of eel pollution for some 30 substances. Most substances are present all over Flanders, but there is considerable variation between river basins, dependent on land use. Contaminant analysis in eel is able to pinpoint specific pollution sources, like some volatile organic compounds in very specific locations, very high BFR levels in eels from areas with intensive textile industry, or high lindane levels in some rivers under agricultural pressure. We could demonstrate that banned chemicals like DDT are still in use in some places. Within the study period, trend analysis indicated significant reductions in PCBs and many OCPs. Also for some heavy metals (lead, arsenic, nickel and chromium), concentrations decreased in the eel, but this was not the case for cadmium and mercury.

Self-caught eels are much esteemed by fishermen, but considering the eel's high contaminant body burden, consumption constitutes a potential risk for human health. After reporting our results, several measures were taken, such as a temporary catch and release obligation for eels caught between 2002 and 2006, and the legal enforcement of a maximum concentration for PCBs in fish and fisheries products. On ca 75 % of the sites, PCB levels in eel exceed however this legal upper limit. The intake of PCBs through consumption of eel by recreational fishermen was compared

with the intake of a background population through a probabilistic approach. PCB intake seems to be at a level of high concern, and body burden in fishermen in Flanders might reach levels of toxicological relevance. Currently, human health protection is not assured, and we recommended more stringent measures from policy makers.

We assessed potential impacts of contaminants on the eel population. Despite a very high internal load of endocrine disrupters, we did not find any effects on vitellogenin levels in immature yellow eel. However, a significant negative correlation between heavy metal pollution load and condition was observed, suggesting an impact of pollution on the health of sub-adult eels. In strongly polluted eels a reduced genetic variability was observed. We further demonstrated that fat stores and condition decreased significantly during the last 15 years in eels in Flanders and The Netherlands, jeopardizing a normal migration and successful reproduction of this long-distance migrator. We hypothesize that pollution is a major driver for this decrease in fat reserves. These findings are of utmost importance for eel management, and may represent a key element in the search for understanding the causes of the decline of the eel. It may well be that the Darwinian evolutionary theory on the survival of the fittest in eel-terms has to be interpreted as *the survival of the fattest*.

Verontreiniging in paling.
Een oorzaak van zijn achteruitgang?

SAMENVATTING. De Europese paling *Anguilla anguilla* (L.) is een ruim verspreide, panmictische en katadrome vissoort. Deze soort vertegenwoordigt een belangrijke economische waarde voor de visserij en komt voor over een groot deel van het Europese continent. Uit monitoring van de hoeveelheid glasaal die de Europese rivieren optrekt, wordt afgeleid dat de palingpopulatie in zeer ernstige mate afgenomen is. De toestand van de stock wordt momenteel beschouwd als beneden de biologische veiligheidsgrenzen en een recente Europese regelgeving vraagt dringend om beschermingsmaatregelen. Alhoewel verschillende mogelijke oorzaken voor de achteruitgang gesuggereerd werden, kon een wetenschappelijk gefundeerde verklaring nog niet gegeven worden.

Paling is een langlevende, carnivore, bentische en vetrijke soort, die bijzonder gevoelig is voor opstapeling van lipofiele contaminanten zoals polychloorbifenylen (PCB's), organochloorpesticiden (OCP's) en gebromeerde vlamvertragers (brominated flame retardants, BFR's). Aan het Vlaamse Instituut voor Natuur- en Bosonderzoek (INBO) werd een meetnet over Vlaanderen opgezet, waarbij over een onderzoeksperiode van 14 jaar contaminanten in paling gemeten werden. Zo werden tussen 1994 en 2007 meer dan 3000 palingen afkomstig van 376 meetstations, geanalyseerd op PCB's, OCP's, zware metalen en een selectie van andere stoffen.

Wij toonden aan dat de paling in het gele-aalstadium een zeer geschikte chemische bio-indicator is; het contaminantprofiel in paling is een vingerafdruk van de pollutiedruk op de meetplaats waar de paling opgroeit. Monitoring van contaminanten in het aquatisch milieu is in Vlaanderen gebaseerd op metingen in water en sediment, maar veel metingen van lipofiele stoffen zoals PCB's en OCP's zoals DDT, de drins en hexachloorbenzeen vallen onder de detectielimiet. In paling echter zijn deze stoffen in haast alle gevallen meetbaar. Het is daarom wenselijk om de meetstrategie voor deze verontreinigende stoffen in ons aquatisch milieu kritisch door te lichten, zowel op Vlaams als op internationaal niveau, in de context van de verplichtingen van de Europese Kaderrichtlijn Water.

Met de resultaten van onze analyses werd de toestand van een 30-tal verontreinigende stoffen beschreven en werden kaarten gemaakt van hun verspreiding in Vlaanderen. De meeste stoffen zijn algemeen verspreid en alom aanwezig in Vlaamse paling. De mate waarin deze stoffen aangetroffen worden is afhankelijk van het rivierbekken. De analyse van contaminanten in paling laat ons toe om specifieke verontreinigingshaarden aan te duiden. In gebieden met intensieve textielindustrie werden zeer hoge BFR-gehaltes gemeten en in gebieden met hoge landbouwdruk werden hoge lindaanconcentraties aangetroffen. Er kon worden aangetoond dat sinds lang verboden stoffen zoals DDT, op sommige plaatsen nog steeds gebruikt worden. Een trendanalyse binnen de studieperiode toont aan dat de

gehaltes aan PCB's en sommige OCP's significant dalen. Ook de gehaltes van sommige metalen (lood, arseen, nikkel en chroom) blijken in paling af te nemen. Dit is evenwel niet het geval voor cadmium en kwik.

Zelfgevangen paling is sterk gegeerd door sportvissers, omwille van de hoge concentraties aan allerlei vervuilende stoffen vormt de consumptie van deze paling echter een mogelijk gevaar voor de volksgezondheid. Verschillende maatregelen werden dan ook reeds genomen: een tijdelijk meeneemverbod voor alle gevangen paling tussen 2002 en 2006, en de uitvaardiging van een consumptienorm voor PCB's in vis en afgeleide producten. Op ca. 75% van de meetplaatsen overschrijden de PCB-gehaltes in paling deze wettelijke consumptienorm. Via een inschatting werd de PCB-belasting bij sportvissers die zelfgevangen paling consumeren, vergeleken met deze bij een populatie niet-vissers. PCB-inname bij vissers is een reden tot grote bezorgdheid en er wordt verwacht dat PCB-opstapeling er dermate hoog kan zijn dat toxicologische effecten niet uitgesloten mogen worden. Momenteel wordt de bescherming van de volksgezondheid onvoldoende gewaarborgd, het is daarom wenselijk om meer doortastende beleidsmaatregelen te nemen.

Mogelijke effecten op de paling van de contaminantbelasting werden eveneens onderzocht. Ondanks de zeer hoge opstapeling van endocrien verstorende stoffen, werden geen afwijkende vitellogenineconcentraties in het immature gele-aalstadium gemeten. Wel werd er een significant negatieve correlatie vastgesteld tussen zware-metaalbelasting en conditie, hetgeen wijst op een impact op de gezondheid van de paling. In de groep van sterk verontreinigde palingen werd een verminderde genetische variabiliteit waargenomen. We konden bovendien aantonen dat het vetgehalte en de conditie van de palingpopulatie significant afnemen tijdens de laatste 15 jaar, zowel in Vlaanderen als in Nederland. Dit brengt de migratie en de voortplanting van deze trekvis in het gedrang. Deze afname in energiereserve lijkt ons te wijten aan de invloed van verontreinigende stoffen. Dergelijke resultaten zijn belangrijk voor het internationaal palingbeheer, en spelen mogelijk een sleutelrol in het onderzoek naar de oorzaken van de achteruitgang van de soort. Wellicht moet de Darwiniaanse evolutietheorie in palingtermen geïnterpreteerd worden als '*the survival of the fattest*'.

Part I

Introduction

A glasseel (total length ca. 7 cm) from the Nieuwpoort monitoring station at the river IJzer mouth (Belgium). Annual levels of glasseel recruitment have dropped from ca. 500 kg in the 1970s to 1-5 kg after 2000.

Photo: Claude Belpaire

Chapter 1

Introduction

Summary

This work is the result of a 14-year research programme (1994-2007) carried out at the Research Institute for Nature and Forest (formerly the Institute for Forestry and Game Management).

In this introduction we present our rationale: the study of an endangered fish species particularly prone to bioaccumulation of contaminants and possibly also very sensitive to the effects of pollution. To this end, a monitoring network for contamination in the eel was set up and developed.

The objectives of this study are: (1) to give a comprehensive overview of status and trends of these chemicals in the eel in Flanders, (2) to study the effects of pollution in eels, (3) to assess the potential of the eel as a chemical bioindicator in regional and international context, and (4) to estimate health risks for eel consumers.

The European eel: a waning population

Life cycle

The European eel *Anguilla anguilla* is a marine fish with a complex life cycle (Figure 1.1). Reproduction takes place in the Sargasso Sea (central North Atlantic Ocean) at some 5000 to 8000 km from European continental waters where the eel grows up. After hatching, the larvae (leptocephali) grow and drift with oceanic currents towards the European continent. At the continental shelf edge leptocephali metamorphose into glass eel. Depending on the latitude these glass eel enter European estuaries during winter or early spring. They pigment and either swim up the rivers in search for a suitable habitat to grow up or grow up in the coastal zone. This growing phase as yellow eels takes 3 to 20 years, dependent on gender (on average 5.9 years for males and 8.7 years for females) and local environmental variables (Vøllestad, 1992). There is a marked sexual dimorphism with respect to size. Males have a mean length of 32-46 cm, whereas female mean size at maturity varies between 45 and 86 cm (Vøllestad, 1992). Among fishes eels present extremely high lipid contents (Tesch, 2003). These energy stores are essential for fulfilling their journey back to the spawning area. At the end of their growing phase, eels go through some morphological and physiological changes and become silver eels. In fall or early winter these silver eels leave continental waters to start their journey to the Sargasso Sea. While swimming, gonadal development is continued and further maturation takes place. Spawning takes place from March to June along frontal zones approximately between 48° to 74° W longitude and between 23° and 30° N latitude (McCleave *et al*. 1987).

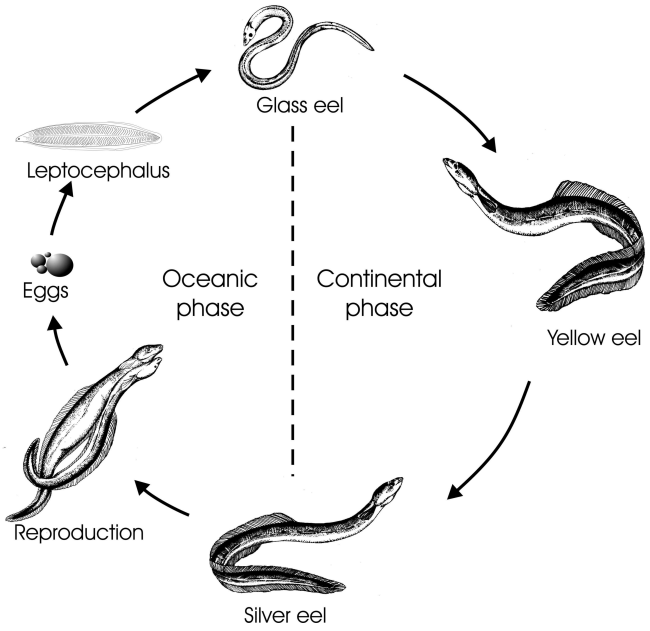


Figure 1.1. The life cycle of the European eel with indication of major life stages (after Dekker, 2000).

Decreasing trend and international action

Since the beginning of the 1980s the stocks of the European eel are in steep decline. Fisheries yields of both yellow and silver eels have declined in most European countries. Monitoring series of glass eel quantities ascending European rivers and estuaries have dropped to *ca.* 1 % of the quantities observed during the 1970s and before. The EIFAC/ICES Working Group on Eel (2007) compiled recruitment data from 21 river catchments in 12 countries. These data, shown in Figure 1.2, incorporate catch statistics, and fishery-independent surveys.

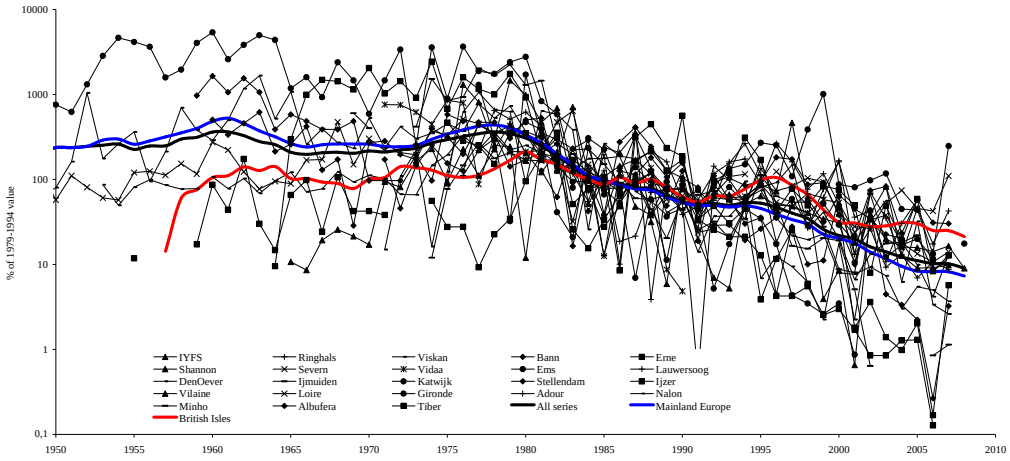


Figure 1.2. Time-series of glass eel recruitment in European rivers. Each series has been scaled to its 1979–1994 average. Source ICES-EIFAC Working Group on Eel (2008 unpublished).

Reasons for this abrupt decline have not been firmly established. Many potential causes have been suggested: climate change and oceanic variability (current changes, decrease in productivity) influencing (the migration of) the leptocephali; destruction of habitats in freshwater; physical obstruction of the migration by dams, sluices, pumps and hydropower; overfishing of glass eel, yellow eel and silver eel; infestations by introduced pathogens (parasites like *Anguillicola crassus*, and viruses like EVEX), predation and pollution (see also Chapter 16).

Finally, 25 years after the first warnings following the recruitment decrease in the early 1980s, issued by the Working Group on Eel, the international community took action. ICES (2001) considered the status of the stock as outside safe biological limits and concluded that current fisheries is not sustainable. In 2003, scientists in eel biology from 18 countries assembled at the International Eel Symposium 2003 organized in conjunction with the 2003 American Fisheries Society Annual Meeting in Québec unanimously raised an urgent alarm and asked for immediate action to save the declining eel resources (Québec Declaration of Concern). In 2007, the European eel was added to the UN CITES Appendix II list, putting it under tight trading restrictions, and rated "critically endangered" on a Red List of species compiled by experts of the World Conservation Union. The Council of the European Union established in September 2007 a framework and measures for the recovery and sustainable use of the stock of European eel (EC 2007). The Council also requires the preparation of national eel management plans (EMPs). If EC member countries fail to prepare and implement these plans, fisheries will be obliged to close.

Simultaneously to the development of the EMPs by national fisheries managers, the international scientific community searches frenetically for evidence explaining the decrease of the stocks. Recently, benefitting from new scientific evidence, pollution received increasing attention as possible cause for eel decline.

Pollution: old and new contaminants

Old and new contaminants

Over 30 000 chemicals are in use in Europe, and may pose a threat to aquatic life. Only for a small number of them the potential impacts have been assessed through thorough toxicological and environmental testing (de Boer and Leonards, 2006). Especially on substances such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and heavy metals, impact studies have been carried out demonstrating their toxicity. PCBs and OCPs are persistent organic pollutants that are regulated under international agreements in order to reduce or eliminate their use and release into the environment. Heavy metals of concern include, amongst others, cadmium, lead and mercury because of their toxicity and their potential to bioaccumulate. Many old substances, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs), which have been demonstrated as toxic, have been banned during the 1970s. However due to their persistence, they still can be traced in the various compartments of our environment. Also in our aquatic environment their presence is still a reason for concern (MIRA, 2007b,c).

A large number of new, emerging contaminants came in use during the last 30 years (e.g. brominated flame retardants, perfluorinated compounds, dioxins, polyaromatic hydrocarbons) (de Boer and Leonards, 2006). For many of them potential adverse effects have not been extensively studied in the environment. Newly manufactured compounds are just being introduced into the environment without any knowledge of possible side effects. Analytical methods for measuring relevant concentrations in the environment have only just been developed (de Boer and Leonards, 2006).

Flanders, a pollution black spot?

Pollution levels in Flanders are relatively high in comparison with other areas in Europe. In 2006, only 26% of the river water quality (Prati oxygen index) was categorized as '*non-polluted*' (7%) or '*acceptable quality'* (19%). River sediments are heavily polluted: 76% of the sites classified through the Triad methodology were qualified as '*contaminated'* (33%) or '*heavily contaminated'* (43%) (MIRA, 2007a). Monitoring ecological river quality shows similar results; a fish based index qualified 794 Flemish river sites: 65% were '*critical'* (45%) or '*bad'* (20%) and 31% were defined as '*reasonable'.* Only 4% of the river sites had '*good'* quality whereas not one of them could be classified as '*excellent'* (MIRA, 2007a). Persistent organic pollutants (POPs) are markedly present in the Flemish environment. Many pesticides are being measured in water, but the percentage detected varied considerably by substance (from 0 to 92%) (MIRA, 2007b). In river sediments organochlorine pesticides (OCPs) and PCBs were '*deviating'* or '*strongly deviating*' from the reference value in respectively 11% and 28% of the cases (MIRA, 2007b,c). In terrestrial organisms like songbirds, hedgehogs and foxes, OCPs are omnipresent (MIRA, 2007b).

An EU report about the burden of dioxin in human milk shows a medium concentration of 34.7 pg TEQ/g fat for Flanders in 1994, placing this region at the top

of the world population (Dujardin *et al*., 2001). PCBs and polychlorinated dibenzo-p-dioxin and dibenzofuran (PCDD/F) concentrations in human serum from Flanders are higher than in other countries (Koppen *et al*., 2002). There also seems to be considerable regional variation in contamination level. Schroijen *et al*. (2008, in press) measured contaminants in 1679 adolescents from Flanders, residing in nine areas with different patterns of pollution. Significant regional differences in internal lead, cadmium, PCBs, DDE (*p,p'*-DDE or 1,1-dichloro-2,2- bis(4-chlorophenyl)ethene) and hexachlorobenzene (HCB) exposure were observed in function of area of residence.

Effects in fish and eel

Many studies deal with specific toxicological and physiological effects of contamination in fish. Pollution may impact fish through many possible mechanisms (see e.g. Chapter 5, Figure 5.1). Specific chemicals are known to induce endocrine disruption, as some of these compounds have biological effects similar to those of the steroid hormone estrogen (Turner and Sharpe, 1997). Sexual disruption and development of ovotestes have been reported in freshwater and marine fish in Europe (Jobling *et al*., 1998). Berckmans *et al*. (2007) have demonstrated that also in Flanders endocrine disruption is widely spread. In ca. 50% of the cases, testes of male roach showed female characteristics and fish had increased vitellogenin levels. The development of healthy populations seems to be hampered by contaminant pressure, presumably not only in roach, but in all fish species. Probably POPs constitute a major threat to aquatic life, but also heavy metals seem to have detrimental impacts on fish as documented by Bervoets *et al*. (2005). They demonstrated a clear relation between metal load in Flemish rivers and a low fish community quality (IBI).

Impacts of contaminants have been reported also in European eel. As a marine fish, spending a considerable part of its life cycle in continental waters, especially the fresh water phase of the eel, the yellow eel, has been object of studies of contaminants. Numerous reports show that the yellow eel is prone to bioaccumulation of hazardous substances. An increasing number of studies recently focus on effects of pollution at the individual level (detoxification, tissue damages, endocrine response, immune response, genotype). In yellow eel, gonads are not yet fully developed, while silver eels mature during their oceanic migration to the spawning grounds. Consequently, potential impacts of contaminants on reproduction physiology are difficult to study as maturing silver eels are not being caught. New experimental opportunities will only appear when artificial reproduction of the eel has been optimized. However, contaminants have also been suggested as a crucial factor in the decline of the species (Robinet and Feunteun, 2002). Palstra *et al.* (2006) reported disturbed reproduction caused by a high bioaccumulation of PCBs in artificially reproduced eel. Differences in development and survival of larvae showed a significant negative correlation with the TEQ levels in the gonads, even at levels far below the maximal allowable level for fish consumption. It was suggested that current gonadal levels of dioxin-like contaminants, including PCBs, in eels from most European locations impair normal embryonic development. Palstra *et al.* (2006) consider it likely that dioxin-like PCBs contributed to the collapse of the European eel populations.

Flanders' Eel Pollutant Monitoring Network

A major outcome of our work has been the development of the Flemish Eel Pollutant Monitoring Network (EPMN), and most of the results presented here are generated through this network. The monitoring network was initiated in 1994 and is operated and managed by the Research Institute for Nature and Forest (INBO). It uses the eel in its yellow phase to monitor the environmental chemical status in Flemish water bodies. At the moment the network includes ca. 350 locations covering the region of Flanders (see Chapter 16 Figure 16.1), including rivers, canals, polder waters and closed water bodies. Eel muscle tissues are analysed for a series of hazardous substances like PCBs, OCPs and heavy metals. At a number of sites additional substances are being analysed (e.g. brominated flame retardants, volatile organic pollutants (VOCs), endocrine disruptors, dioxins, perfluorooctane sulfonic acids (PFOSs), and polycyclic aromatic compounds. More details and output of the EPMN are described in several chapters.

Objectives and general outline

Our research focuses on the study of pollution in the European eel in its yellow phase, living in the inland waters of Flanders. The research programme has joined her primary scientific goals to a strong applied aspect of monitoring contamination and advising policy makers. The research is positioned within the international frameworks of the management of contamination (the Water Framework Directive and REACH) and the international eel restoration plans. On the national level our research serves the environmental management of pollution and the protection of human health.

The foundation of our research is constituted by the Flemish Eel Pollution Monitoring Network. The development of this network has been an major objective of our work and the data and results generated through this network form the basis of our analyses. Our study is thus mainly conceived following an empirical approach, benefitting from a large quantity and unique series of data, which definitely constitutes its strength. But this approach, being dependent on the EPMN, also entails some restrictions. It hampers planning and design of experiments to study process-thriven interactions and causalities between contamination pressure in the environment and the accumulation in and effects on the eel.

The collection, description and analyses of data of contamination in the eel and the use of the eel as an indicator of pollution constitute the main focus of our work. Nevertheless we choose not to restrict our objectives to report on the monitoring of status and trends of contamination in eel, but included results of studies oriented toward the potential effects on eel as well as the risks for humans consuming (polluted) eels. We believe this combined approach certainly will support the international community in their efforts to seek the most optimal management measures for restoration of the eel stock.

In the following chapters we will focus on several specific objectives:

1. - We want to characterise the chemical status of our water bodies by measuring the body burden of selected contaminants in eel. Local pollution sources may be targeted and it might be possible to select some chemicals of special concern when considering their bioaccumulation capacity. Many management measures have been initiated to diminish the levels of contaminants in our environment e.g. phasing

out PCBs from our industrial applications, banning specific pesticides, or using lead free fuel. By monitoring these compounds over a longer period it might be possible to detect some trends over time, and to study the effects of those management measures. The concentration of some contaminants in eel might decrease while for other substances body burden might increase. It may be possible to target new emerging threats. An important aspect is to include recommendations for contaminant management and monitoring in Flanders or world wide. In Chapter 2 we analyse the spatial and temporal variation in polychlorinated biphenyls, organochlorine pesticides and heavy metals measured in eel, while in Chapters 3 and 4 the presence of respectively brominated flame retardants and volatile organic compounds is described.

2. - Chemicals may affect the viability of fish communities in various ways. In high concentrations they may be directly toxic for organisms, but at lower concentrations they can negatively influence diverse physiological functions, resulting in endocrine disruption and disturbed reproduction. We studied the presence of several contaminants within the immature yellow eel stage. Even in this immature stage adverse effects on the eels' health may be apparent. If eels are to some extent affected by pollution, is it then conceivable that there could be a causal relation with stock decline? The effects of contaminants in the eel have been studied in Chapters 5 to 8.

3. - Eel is proposed as a sensitive chemical bioindicator. A bioindicator is a species used to monitor the health of an ecosystem; a chemical bioindicator indicates the status or effects of (specific) chemicals in an ecosystem. Our objective was to ascertain if eel adequately reflects the chemical load in our aquatic ecosystems and to analyse the advantages and constraints of using this species as a chemical bioindicator. This is discussed within Chapters 9 to 12. The Water Framework Directive (WFD) urges to monitor the chemical status of our water bodies. We wanted to know if eel is appropriate to use in this framework, and if we can recommend the use of the eel for fingerprinting pollution in a regional and an international context (Chapter 13 and 14). In general terms, monitoring contamination in aquatic biota may represent an added value compared to classical analysis of water and river sediments.

4. - The initial impetus of this work was inspired by the concern for human health. Consuming eels might give reason for concern, as eels turned out to have heavy pollution loads. We quantify the risk of eating feral eels from Flanders and issue recommendations to policy makers. The results warrant special communication actions. This issue is discussed in parts of Chapters 2 and 3 and in Chapter 15.

Following these objectives, the content of this book has been organised in four parts:

In a first part (Part II - Status and trends of contaminants in eels), a comprehensive overview of the status and trends of a variety of chemicals in eels in Flanders is provided. Emphasis is put on polychlorinated biphenyls, organochlorine pesticides and heavy metals, but also on a series of new and less known chemicals like brominated flame retardants and volatile organic substances. In the annex a cartographic representation of the major contaminants is provided, updating the report of Goemans *et al.* (2003) for the 2002-2005 period.

A second part (Part III - Effects of contaminants on the eel) discusses the effects of pollution in eels, including a literature review and the results of a study on

the potential effects of xenoestrogens by analysis of the plasma vitellogenin content. Furthermore, the effects of pollution and relationships with energy reserves, condition and genetic variability are dealt with in two papers.

The third part (Part IV - The use of the European eel as an indicator of pollution) focuses on the sensitivity of the eel as a chemical bioindicator. The use of the eel for fingerprinting pollution in regional and international context is discussed. Special emphasis is given to monitoring the chemical quality for the Water Framework Directive. As eel quality benefits from increasing attention from national and international eel managers a proposal has been initiated to develop a European Eel Quality Database.

In the fourth part (Part V - Contaminants in eel and human heath) health risks for eel consumers were estimated, especially with regard to PCB exposure in recreational anglers.

Finally, a last chapter summarizes results and conclusions. This chapter also includes an overview of the achieved results of the Eel Pollution Monitoring Network and related studies. It also comprises management and communication issues, and concludes with final recommendations for future work.

References

Berckmans, P., Witters, H., Goemans, G., Maes, J., Belpaire, C., 2007. Ondersteunend studiewerk en verdere karakterisatie van de Vlaamse toestand inzake hormoonverstoring: vraagstelling inzake ecologische relevantie. Study for the Flemish Environmental Agency (VMM). Vlaamse Instelling voor Technologisch Onderzoek, Mol, VITO Report 2007/TOX/R071 and Instituut voor Natuur- en Bosonderzoek, Brussel, INBO Report R.2007.37.

Bervoets, L., Knaepkens, G., Eens, M., Blust, R., 2005. Fish community responses to metal pollution. *Environmental Pollution* 138, 338-349.

de Boer J, Leonards P.E.G., 2006. Contaminants in Marine Food Chains: Old Patents and New Pollutants. ICES Annual Science Conference, Maastricht, CM 2006/G:04.

Dekker, W. 2000. The fractal geometry of the European eel stock. *ICES Journal of Marine Science* 57, 109-121.

Dujardin, M., Narbonne, J.F., Alexander, S., 2001. The Belgian “Dioxin” crisis. In : Robertson, L.H. & Hansen, L.W., eds. PCBs: recent advances in environmental toxicology and health effects. University Press of Kentucky, Kentucky. pp. 155-159.

EC 2007. Council Regulation (EC) No 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. *Official Journal of the European Union* 22.9.2007 L 248/17-23.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001 : gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollution 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.2003.99.169 p.

ICES, 2001. Report of the EIFAC/ICES Working Group on Eels. ICES CM 2001/ACFM:03. 87 pp.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. *Environmental Science & Technology* 32(17): 2498–2506.

Koppen, G., Covaci, A., Van Cleuvenbergen, R., Schepens, P., Winneke, G., Nelen, V., Van Larebeke, N., Vlietinck, R., Schoeters, G., 2002. Persistent organochlorine pollutants in human serum of 50–65 years old women in the Flanders Environmental and Health Study (FLEHS). Part 1: Concentrations and regional differences. *Chemosphere* 48, 811–825.

McCleave, J.D., Kleckner, R.C., Castonguay, M., 1987. Reproductive sympatry of American and European eels and implications for migration and taxonomy. *American Fisheries Society Symposium* 1, 286–297

MIRA, 2007a. Milieurapport Vlaanderen, MIRA Achtergronddocument 2007, Kwaliteit oppervlaktewater, De Cooman W., Peeters B., Theuns I., Vos G., Lammens S., Debbaudt, W., Timmermans G., Meers, B., Van Erdeghem M., Van Wauwe P., Callebaut R., Barrez I., Van den Broeck S., Emery J., Van Volsem S., Bursens K., Van Hoof K., D'Heygere T., Soetaert H., Martens K., Baten I., Goris M., Haustraete K., Breine J., Van Thuyne G., Belpaire C., Smis A., Vlaamse Milieumaatschappij, http://www. milieurapport.be.

MIRA, 2007b. Milieurapport Vlaanderen, MIRA Achtergronddocument 2007, Verspreiding van bestrijdingsmiddelen, Claeys, S., Steurbaut, W., Theuns, I., De Cooman, W., De Wulf, E., Eppinger, R., D'hont, D., Dierckxens, C., Goemans, G., Belpaire, C., Wustenberghs, H., den Hond, E., Peeters, B., Overloop, S., Vlaamse Milieumaatschappij, http://www. milieurapport.be.

MIRA, 2007c. Milieurapport Vlaanderen, Achtergronddocument 2007, Verspreiding van PCB's, M. Wevers, M., Voorspoels, S., Covaci, A., Schepens, P., D'Havé, H., De Coen, W., Van Gerven, T., Vandecasteele, C., Goemans, G., Belpaire, C., Van Hooste, H., MIRA H. Van Hooste, Vlaamse Milieumaatschappij, http://www. milieurapport.be.

Palstra, A.P., Ginneken, V.J.T. van, Murk, A.J., Thillart, G.E.E.J.M. van den, 2006. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93, 145-148.

Robinet, T., Feunteun, E., 2002. Sublethal effects of exposure to chemical compounds : a cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265-277.

Schroijen, C., Baeyens, W., Schoeters, G., Den Hond, E., Koppen, G. Bruckers, L., Nelen, V., Van De Mieroop, E., Bilau, M., Covaci, A., Keune, H., Loots, I. , Kleinjans, J., Dhooge, W., Van Larebeke, N., 2008 in press. Internal exposure to pollutants measured in blood and urine of Flemish adolescents in function of area of residence. *Chemosphere*, doi:10.1016/j.chemosphere.2007.11.053.

Tesch, F.-W., 2003. The eel. Third edition. Blackwell Science Ltd, Oxford, U.K., 408p.

Turner, K.J., Sharpe, R.M., 1997. Environmental oestrogens – present understanding. *Reviews of Reproduction* 2, 69–73.

Vøllestad, L. A., 1992. Geographic variation in age and length at metamorphosis of maturing European eel: environmental effects and phenotypic plasticity. *Journal of Animal Ecology* 61, 41–48.

WG Eel, 2007. Report of the 2007 Session of the Joint EIFAC/ICES, Working Group on Eels, FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Bordeaux, 3–7 September 2007, EIFAC Occasional Paper No. 38, ICES CM 2007/ACFM:23, Draft, 524 p. Available from www.ices.dk/reports/ACFM/2007/WGEEL/WGEEL_07_draft.pdf

Part II

Status and trends of contaminants in eel

Electrofishing for eels …
Photo: Peter Van Hoof

Chapter 2

Spatial variations and temporal trends in polychlorinated biphenyls, organochlorine pesticides and heavy metals

Joachim Maes[1,2], Claude Belpaire[1] and Geert Goemans[1]

1 - Research Institute for Nature and Forest (INBO), Duboislaan 14, B-1560 Groenendaal-Hoeilaart.

2 - Present address: Flemish Institute for Technological Research (VITO), Integrated Environmental Studies, Boeretang 200, B-2400 Mol, Belgium.

This chapter is published as:

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

Summary

In Flanders, the northern region of Belgium, European yellow eel muscle tissue was used as an indicator of environmental and potential human dietary exposure by hazardous chemicals of surface waters and sediments. Between 1994 and 2005, over 2800 eel captured at 365 stations were analysed for PCBs, pesticides and heavy metals. Contamination of eel in Flanders fell within the range of reported concentrations in other watersheds of Western Europe. A spatial analysis of the data demonstrated that the variation in pollutant concentration tended towards higher values. This was especially evident for PCBs, lindane, endrin, dieldrin and DDE. The concentration of almost all banned substances decreased significantly during the study period.

Introduction

The widespread occurrence of hazardous chemicals in the environment remains of major concern for wildlife and human health. Many chemicals, even banned ones, persist in the environment and continue to accumulate in ecosystems. In Europe, concern about the release of chemicals into the environment was shared by the European Parliament and the Council which adopted a far reaching Commission proposal aimed at ensuring greater safety in the manufacture and use of chemical substances (European Commission, 2006a). The new system REACH, which stands for registration, evaluation and authorization of chemicals, will ensure that gaps in existing information on hazardous properties of chemicals are filled. In this renewed political context, the monitoring of chemicals in the environment and its ecosystems remains of crucial importance in order to produce data that serve as a baseline against which future policy results may be evaluated.

This paper presents a synthesis of a routine monitoring programme that started in 1994 aimed at following the tissue concentration of hazardous chemicals in eel in Flanders, Belgium. Data were available for contamination of eel by polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and heavy metals. PCBs and OCPs are persistent organic pollutants that are regulated under international agreements in order to reduce or eliminate their use and release into the environment. Heavy metals of concern include, amongst others, cadmium, lead and mercury because of their toxicity and their potential to bioaccumulate.

The yellow eel was selected as a bio-monitor for the aquatic environment for a number of reasons. Eels in the yellow stage are premature individuals. Eels do not reproduce in freshwater. Therefore, body burdens are not affected by a reproduction cycle and associated changes in lipid metabolism. Further, yellow eel have a high lipid content, increasing with age and reaching a maximum prior to silvering and emigration. They generally show life-long accumulation and low depuration rates (Larsson *et al*., 1991; Tulonen and Vuorinen, 1996; Knights, 1997; Daverat *et al*., 2006). Yellow eel are carnivores, widespread in all aquatic habitats, benthic, often burrowed in the bottom, tolerant to pollution and sedentary (Mason and Barak, 1990; Van der Oost *et al*., 1996; Ashley *et al*., 2003; Linde *et al*., 2004). Home range may be larger in tidal estuaries than in freshwater habitats (Parker, 1995). Seasonal movements possibly occur while also the occurrence of erratic eels (‘nomads’) has been reported (Feunteun *et al*., 2003). These typical life history characteristics warrant the use of eel as an indicator for the presence of hazardous chemicals in the environment and, in particular, of those substances with a low solubility in water. The hypothesis is that the eel tissue concentration and body burden reflect well environmental exposure and that tissue concentrations are related to pollution levels of prey species, surface waters and sediments.

A second important advantage of the use of eel as bio-indicator of chemicals in the environment relates to the consequences of eel consumption for human health. Eel consumption is a definite pathway of human exposure to persistent organic chemicals and heavy metals (Harrad and Smith, 1999). Also in Flanders, explicit concern was raised in order to warn of the health hazard associated with the consumption of eel and other predatory fish species by recreational fishermen (Hoge Gezondheidsraad, 2005). Many of these chemicals are considered potential carcinogens and some are believed to disturb metabolic and endocrine functions of the human body (European Environment Agency, 2005).

In addition, the European Commission (2006b) recently proposed a system to monitor a selection of priority substances and to report the chemical status of water bodies in order to protect aquatic life and human health. An important task was

therefore to demonstrate the suitability of bio-indicators, such as the European eel (*Anguilla anguilla* L.), as models for evaluating the chemical status of surface waters which was required by the water framework directive. Eel contaminant profiles, especially for lipophylic substances, appeared to be a fingerprint of the contamination pressure of a specific site (Knights 1997, Belpaire and Goemans, 2007)

So far, studies on the pollution of eels in Flanders focused on targeted research actions (Roose *et al*., 2003; Versonnen *et al*., 2004; Goemans and Belpaire, 2004, 2005; Hoff *et al*., 2005; Maes *et al*., 2005; Belpaire and Goemans, 2007). A general synthesis reporting on all the data that were collected since 1994 has been provided as a report in Dutch (Goemans *et al*., 2003). This paper presents the first general description of the concentration of hazardous chemicals in the European eel in Flanders, the northern part of Belgium. Spatial information was provided at the river basin level while a selection of temporal data was retained in order to investigate temporal trends in the tissue concentration.

Materials and methods

Field sampling and analysis of the lipid content

Between 1994 and 2005, 2 839 eel were captured. Eel were always caught between March and October. Total annual catch varied between 25 eel in 1996 and 732 in 2000. On average, 237 eel were captured each year. Yellow eel were sampled at 365 different stations using fyke nets or an electrofisher. Stations were characterised as rivers or brooks, canals, polder water courses or closed water bodies such as old meanders, ponds or lakes. Stations were situated in all 11 river basins (Figure 2.1). Between 1994 and 2005, 91 stations were visited twice for sampling; 16 stations were sampled 3 times, 6 stations were sampled 4 times and 2 stations were sampled 5 times. One station (Lake Weerde, a man-made water body) was sampled 8 times between 1997 and 2005.

After capture, eel were sorted according to life history stage and only yellow eel were placed in cooling units for live transport to the laboratory. At the lab, eel were measured , weighed and stored at –20°C for sub sequent tissue analysis. From each individual eel six samples of muscle tissue (10 g wet weight each) were removed, labelled and frozen again at –20°C. Two sa mples, originating from the mid part of the body, were analysed for heavy metals, OCPs and PCBs. Lipid was extracted and quantified using the Bligh & Dyer method (1959)**.** The other samples were stored as back up.

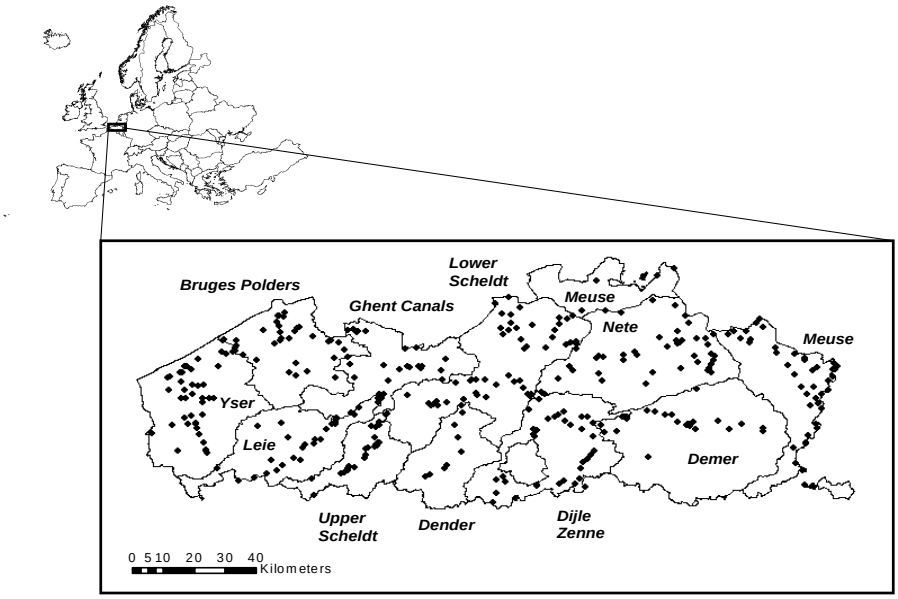


Figure 2.1. Geographic position of Flanders in Europe and location of the sampling points of the eel pollutant monitoring network. The different sub basins are indicated using their English names (if available)**.**

Chemical analysis

Tissue samples were analysed for PCBs, several pesticides and nine heavy metals. Pesticides included two hexachlorocyclohexanes, three cyclodienes, hexachlorbenzene and three chloroethanes.

PCBs were used as insulating fluids in transformers, occur as plasticizers, oil and paint additives or as by-products of combustion. Tissue samples were analysed for 10 different congeners identified according to IUPAC numbers 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180. Seven congeners are considered as indicator PCBs (28, 52, 101, 118, 138, 153, 180). In Belgium, the sum of these seven indicator PCBs (further abbreviated as ∑PCB) was used in national legislation to ensure food safety. The concentration of ∑PCB in food products may not exceed 75 ng g^{-1} wet weight. Hexachlorocyclohexanes were used as insecticides. Here, we report on α-HCH and γ-HCH (lindane), which were banned in Belgium in 2002. Cyclodienes in this study include dieldrin, endrin and chlordane. The use of dieldrin has been prohibited since 1974 while the use of endrin has never been authorized at all. Chlordane is a mixture of different components of which only trans-nonachlor was assessed. The use of the latter substance by agriculture has been prohibited since 1981 but non agricultural use was allowed until 1998. Hexachlorobenzene (HCB) was formerly used as an insecticide and was banned in 1974. Concentrations of three chloroethans (*p,p'*-DDD, *p,p'*-DDT, *p,p'*-DDE) were measured and their sum used as a proxy of total DDT (∑DDT). DDTs have been banned since 1974 in case of agricultural application and since 1976 for all other uses. Finally, the levels of nine heavy metals (cadmium, lead, mercury, chromium, nickel, copper, zinc, arsenic, selenium) were determined.

Determination of PCBs and pesticides

Fish tissue was extracted using the Bligh & Dyer method (1959). The extract was evaporated (Rotavapor) and a minimum of 100 mg lipid was dissolved in hexane and applied on an aluminum oxide chromatography column. After elution with hexane, the lipid free eluate was evaporated and applied on a silica gel chromatography column. PCB congeners, *p,p'*-DDE and HCB were isolated after elution with hexane. After elution with diethylether/hexane (10/90) the remaining organochlorine pesticides were isolated. Both fractions were evaporated to 1 ml, after addition of an internal standard (tetrachloronaphtalene) and separated by GC using a Rtx-5ms capillary column (60 m x 0.25 mm x 0.25 µm), with helium as a carrier gas and an electron capture detector (ECD). The detection limit for both PCBs and pesticides was 0.5 ng g^{-1} lipid weight.

Determination of heavy metals

Fish muscle tissue (between 3 and 5 g) was placed in an oven for 12 h at 450°C. Once cooled, 100 µl HNO_3 was added and the analyte was dried again at 450°C for one hour. Subsequently, 1 ml HNO_3 was added to the ash and diluted using distilled water.

Trace elements of Cr, Ni, Cu, Zn, Cd and Pb in solution were analysed using ICP-OES (Spectra AA-400 with Zeeman correction, Varian). The detection limits for each of these metals varied: 2 ng g^{-1} wet weight for Cd and Pb, 10 ng g^{-1} wet weight for Ni, 35 ng g^{-1} wet weight for Cr and 100 ng g^{-1} wet weight for Cu and Zn.

As and Se were determined using GF-AAS. Prior to analysis, fish tissue was heated in a medium of 5 ml HNO_3 and 3 ml H_2O_2 and afterwards diluted in distilled water. The detection limits for As and Se were 10 and 35 ng g^{-1} wet weight, respectively.

Hg was quantified using AAS (AMA 254 mercury analyser, Altec). Hg was detected if the concentration was higher than 10 ng g^{-1} wet weight.

Quality assurance

Analysis were carried out at two different Belgian research institutes (DVZ, the Sea Fisheries Department, Ostend and CODA, the Veterinary and Agrochemical Research Centre, Tervuren). Quality assurance consisted of the analysis of procedural blanks, reproducibility and repeatability tests, injection of standard solutions as unknowns, and analysis of certified reference material. Both institutes routinely analyse samples in the framework of the international proficiency testing scheme QUASIMEME (Quality Assurance of information for marine environmental monitoring in Europe) for organochlorines in biological samples and participate in intercalibration studies organised by the IAEA (International Atomic Energy Agency). Internal quality assurance at DVZ was realised by monthly analyses using certified reference materials. GC equipment was calibrated every 60 samples and every 20 samples, two standards were analysed. Quality of heavy metal analyses performed at CODA were assured using reference materials and blanks every 12 samples (daily for Hg). ICP-OES and GF-AAS equipment was calibrated every 15 samples.

Data storage and statistical analysis

Eel contaminant data are stored in a database as a concentration in ng g^{-1} wet body weight. A unique identification number was assigned to each individual eel followed by a location code (linked to geographical information of the sampling station), sampling data (e.g. fishing date and procedure), length (cm), weight (g) and lipid content (as a percentage of wet weight). Concentrations of organic pollutants were imported on a lipid weight basis and converted into wet weight concentrations using lipid content as a conversion factor. Heavy metals were always expressed on a wet weight basis. Calculations used half the detection limit when a below detection limit reading was observed.

A general eel pollution profile was assessed using the average, the range and standard deviation based on all samples. Spatial analysis of sampling sites and the correlation structure between chemicals was investigated by arranging the data according to river basin. A linear mixed model was used to infer general trends in eel muscle tissue concentration over time and to test the null hypothesis that the slope of the trend line was not significantly different from zero. For this analysis, individual eel data based on wet weight were station-averaged and normalized using a $\log_{10}$ transformation. Only those stations that were sampled more than once were retained in the analysis. A paired t-test would be an appropriate statistic test if each station was sampled twice on two fixed dates. In this study, the data were, however, largely unbalanced with sets of two, three, four, five or eight repeated measurements, taken at different times and over different time intervals, hence the choice for a mixed model. The mixed model can be considered as a distinct linear regression for each set of clustered data with fixed and random, hence mixed, regression coefficients. In the model, time was considered as a fixed factor while the different stations constitute the random factor. The mixed model that was fitted through the data was a random slopes and random intercepts model and has the following form:

$$(\text{eq.1}) \quad \text{Log}_{10}[C+1] = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i}) \times (\text{Year} - 1994) + \varepsilon_i$$

Where β_0 and β_1 are regression parameters which were the same for all stations and b_0 and b_1 were station-specific regression coefficients; C was the concentration of a chemical substance on a wet weight basis. Essentially, we are only interested in the value for β_1 which describes the average trend over time. It was assumed that all random effects b_i are normally distributed with mean zero and variance $\sigma^2_{station}$. The error term with residuals ε_i is normally distributed with mean zero and variance σ^2. $\sigma^2_{station}$ is a 2×2 covariance matrix containing d_{11} the variance of the random intercepts, d_{22} the variance of the random slopes and $d_{12} = d_{21}$ which stands for the covariance between the random intercepts and the random slopes. Note that the covariance is related to the correlation between the random intercepts and the random slopes.

The MIXED procedure in SAS (SAS Institute Inc, 1999) was used to find parameter values for equation 1. The best solution was found using the restricted maximum likelihood estimator. It follows that mixed models are not interpreted in terms of explained variance (R^2). Inference for the parameters β_0 and β_1 was based on the Wald statistic. In the model, it was assumed that the covariance matrix was unstructured by entering the SAS statement “type = un” in the code. Verbeke and Molenberghs (2000) give statistical details and examples.

Results

Length, weight and bioaccumulation

A total of 2 839 eels was analysed for at least one of the substances listed in Table 2.1. Mean length and weight of eel included in this analysis were 41.8 ± 9.3 cm and 153.5 ± 152.7 g, respectively. Lipid content of the total body weight averaged 14.9 ± 10.2%. Eel tissue concentrations (mean, range, standard deviation) of PCBs, OCPs and heavy metals are presented in Table 2.1.

PCBs were ubiquitous in eel. The average concentration of the sum of the seven indicator PCBs was 605 ng g^{-1} wet weight, exceeding the Belgian limit for human consumption (75 ng g^{-1} wet weight) almost by one order of magnitude (Table 2.1). Note the high standard deviation and the maximum concentration of almost 12 500 ng g^{-1} wet weight. The distribution of ∑PCB in eel was positively skewed, so the probability of capturing highly contaminated eel was higher than could be expected in a normal distribution. 17% of all individuals displayed a total PCB concentration of > 1 000 ng g^{-1} wet weight and 1.7% with concentration higher than 5 000 ng g^{-1} wet weight. Note that the highest levels were found for PCBs 138, 153 and 180 which are particularly recalcitrant compounds (Knights, 1997).

Concentrations of the biocide lindane ranged between 0.01 and 2 225 ng g^{-1} wet weight with an average concentration of 27.9 ng g^{-1} wet weight. Concentrations of α-HCH averaged 0.64 ng g^{-1} wet weight. Dieldrin and endrin, once used as insectides but banned since 1974, were detected in >90% of all eel analysed. Values of dieldrin averaged 15.6 ng g^{-1} wet weight with a maximum of 389 ng g^{-1} wet weight; mean endrin concentration was 1.4 ng g^{-1} wet weight. The fungicide hexachlorobenzene was detected in all eel at an average concentration of 5.9 ng g^{-1} wet weight (max 192 ng g^{-1} wet weight). DDTs were present in all fish with ∑DDT varying between 1.5 and almost 4 000 ng g^{-1} wet weight. The distributions of pesticide concentrations were also positively skewed.

Eel carried significant concentrations of heavy metals in their muscle tissue. Concentrations of mercury, cadmium and lead, substances for which maximum limits apply on a European level, averaged 116, 15.8 and 81 ng g^{-1} wet weight, respectively. Maximum observed concentrations for each of these metals exceeded or were well over the European maximum residue limit (MRL). Eel also exhibited extreme values for other heavy metals (Table 2.1), exceeding the average concentration by an order of magnitude, demonstrating the high potential of eel for severe contamination.

Spatial variability in the tissue concentration

Figures 2.2-2.4 summarize the spatially resolved pollutant averages on a river basin level (Figure 2.1). Two major findings emerged: some pollutants were evenly spread while others clearly peaked in selected river basins and the variance around the mean was high due to the presence of high concentrations in some specific sites. The contamination of eel by different pesticides was most notable in the basin of river Yser. This was particularly evident for lindane with a basin average of 262.9 ± 552.7 ng g^{-1} wet weight. Also α-HCH and dieldrin peaked in this river basin with averages that were clearly higher than the overall average tissue concentration (Table 2.1). PCB contamination more or less increased along a west-east gradient and reached a maximum in the basin of River Meuse where an average ∑PCB of nearly 800 ng g^{-1} wet weight was detected. DDT in eel muscle tissue peaked in the Upper Scheldt and Demer river basins, while Cd and Pb pollution was typical for the

River Nete. Pollution levels of As and Hg, as well as other pesticides such as HCB and chlordane, were more evenly spread throughout the entire region.

Table 2.1. Mean eel life history statistics and mean muscle tissue concentration and range (ng g^{-1} wet weight) of different pollutants in muscle tissue sampled in surface waters of Flanders (Belgium). Number of eel (n), mean, range (minimum – maximum) and standard deviation (SD) were calculated for the period 1994-2005.

	n	Mean	Minimum	Maximum	SD
Eel life history data					
Length	2 839	41.79	19.2	102.30	9.28
Weight (g)	2 838	153.46	11.7	2 284.00	152.69
Lipid content (%)	2 528	14.92	0.52	57.59	10.18
Substance					
PCBs					
PCB 28	2 525	6.44	0.0035	292.65	15.11
PCB 31	2 525	3.04	0.0037	211.84	7.97
PCB 52	2 526	30.61	0.0087	624.36	53.82
PCB 101	2 526	55.55	0.0272	1 505.79	104.77
PCB 105	2 526	18.12	0.0104	478.12	34.81
PCB 118	2 526	57.13	0.2904	2 076.45	112.23
PCB 138	2 526	149.69	0.5805	2 924.25	295.65
PCB 153	2 526	211.89	1.0423	5 098.68	430.33
PCB 156	2 525	13.98	0.0263	352.71	25.17
PCB 180	2 525	93.48	0.1250	2 131.50	180.93
∑PCB	2 524	604.99	3.5213	12 455.38	1 118.56
Pesticides					
α-HCH	2 528	0.64	0.1	16.94	1.32
Lindane	2 527	27.89	0.0109	2 225.46	131.68
Dieldrin	2 528	15.63	0.0046	388.78	30.21
Endrin	2 446	1.39	0.0026	495.83	11.59
HCB	2 526	5.89	0.0026	191.95	8.91
TDE	2 528	26.26	0.0108	568.46	41.36
p,p'-DDT	2 528	3.19	0.0037	187.81	9.58
p,p'-DDE	2 526	61.77	0.1007	3 422.63	112.73
∑DDT	2 528	90.77	1.5149	3 995.42	148.27
Trans-nonachlor	2 528	1.43	0.0026	52.03	2.73
Aldrin	548	1.11	0.0056	14.11	2.21
Heavy metals					
Mercury	2 769	116.62	5.0	1 185	98.89
Cadmium	2 809	15.75	1.0	2 474	62.21
Lead	2 802	81.17	1.0	3 453	172.17
Copper	2 117	909.73	50.0	436 000	10 006.90
Zinc	2 117	25 864.79	1 200.0	243 100	15 919.30
Nickel	2 117	207.83	5.0	16 300	692.00
Chromium	2 117	254.51	17.5	13 690	455.97
Arsenic	1 410	168.13	14.0	1 805	176.72
Selenium	1 410	753.93	25.0	5 098	499.75

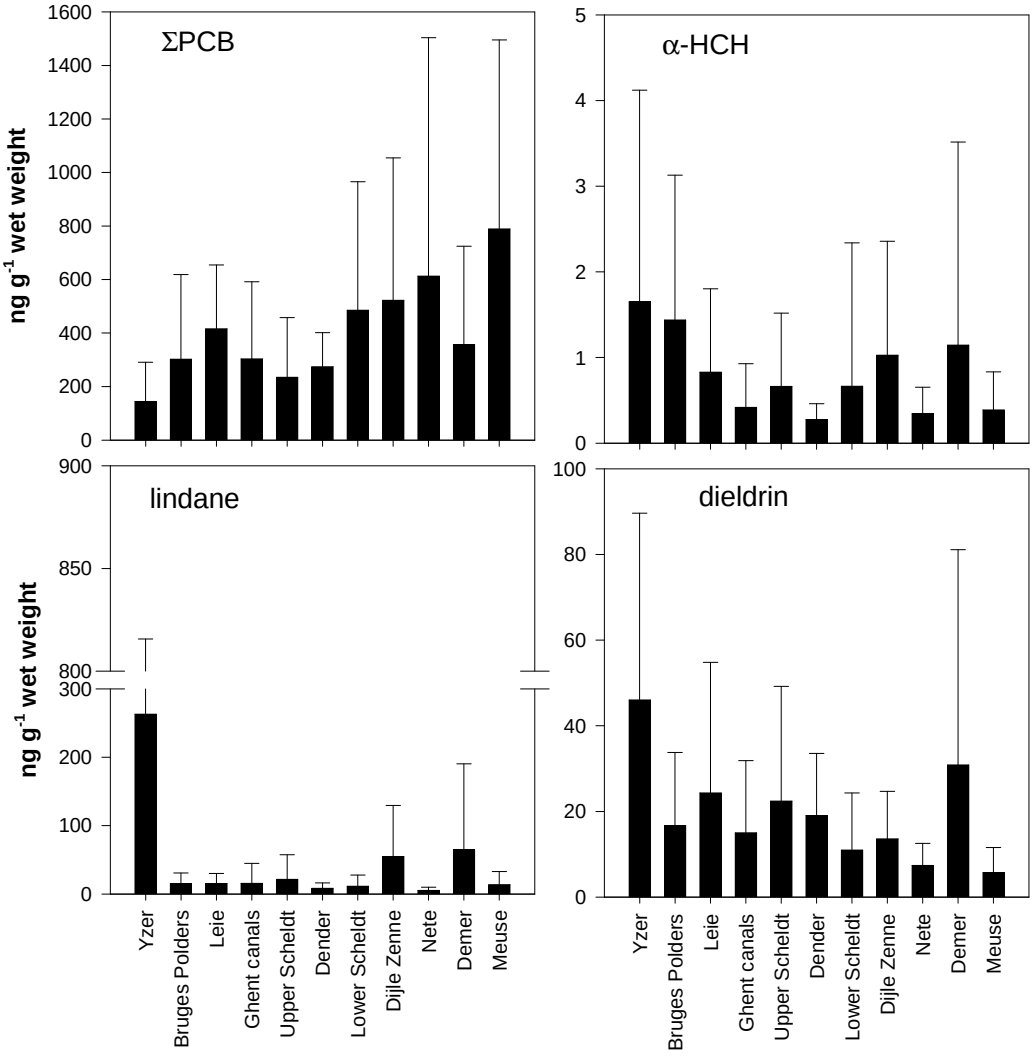


Figure 2.2. Spatial distribution of the average eel muscle tissue concentration of ∑PCB, lindane, α-HCH and dieldrin over the different river basins in Flanders (Belgium).

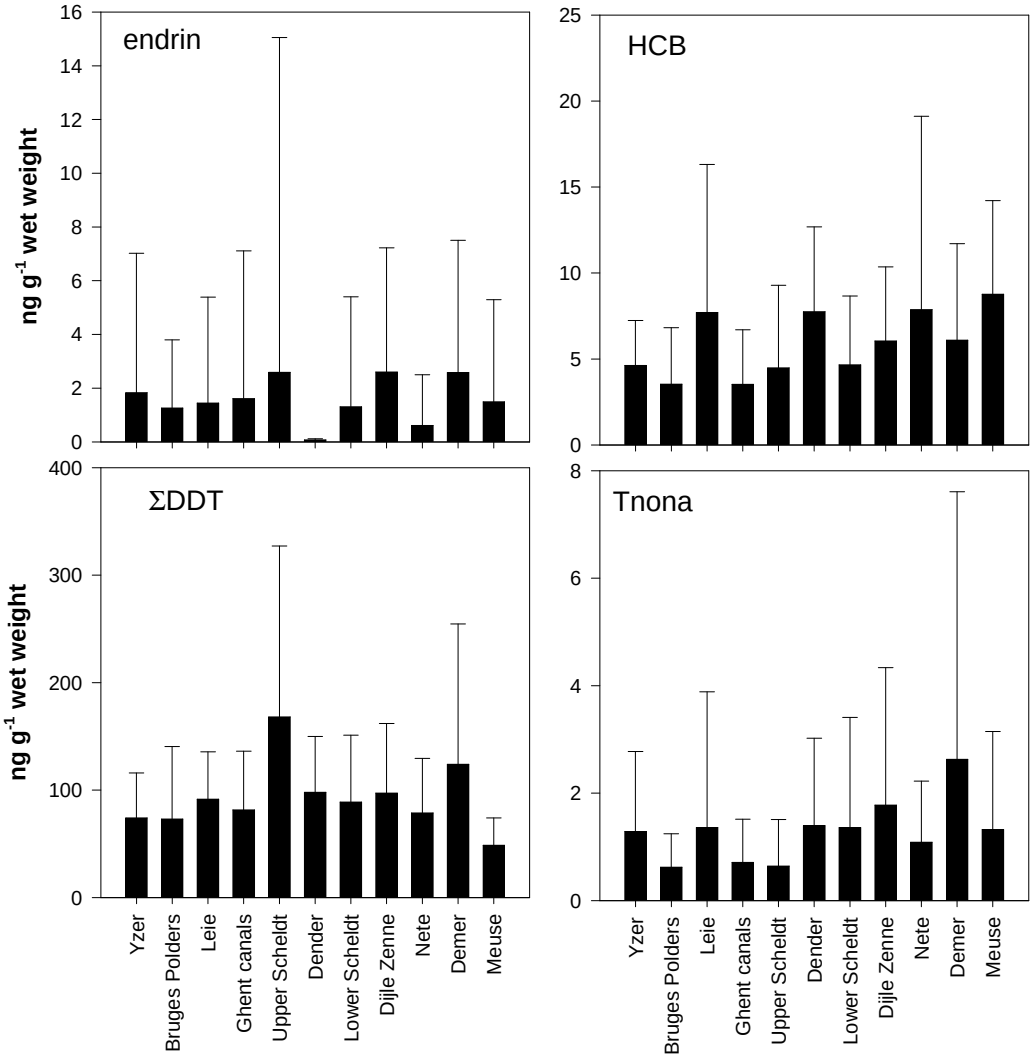


Figure 2.3. Spatial distribution of the average eel muscle tissue concentration of endrin, ∑DDT, HCB and trans-nonachlor (Tnona) over the different river basins in Flanders (Belgium).

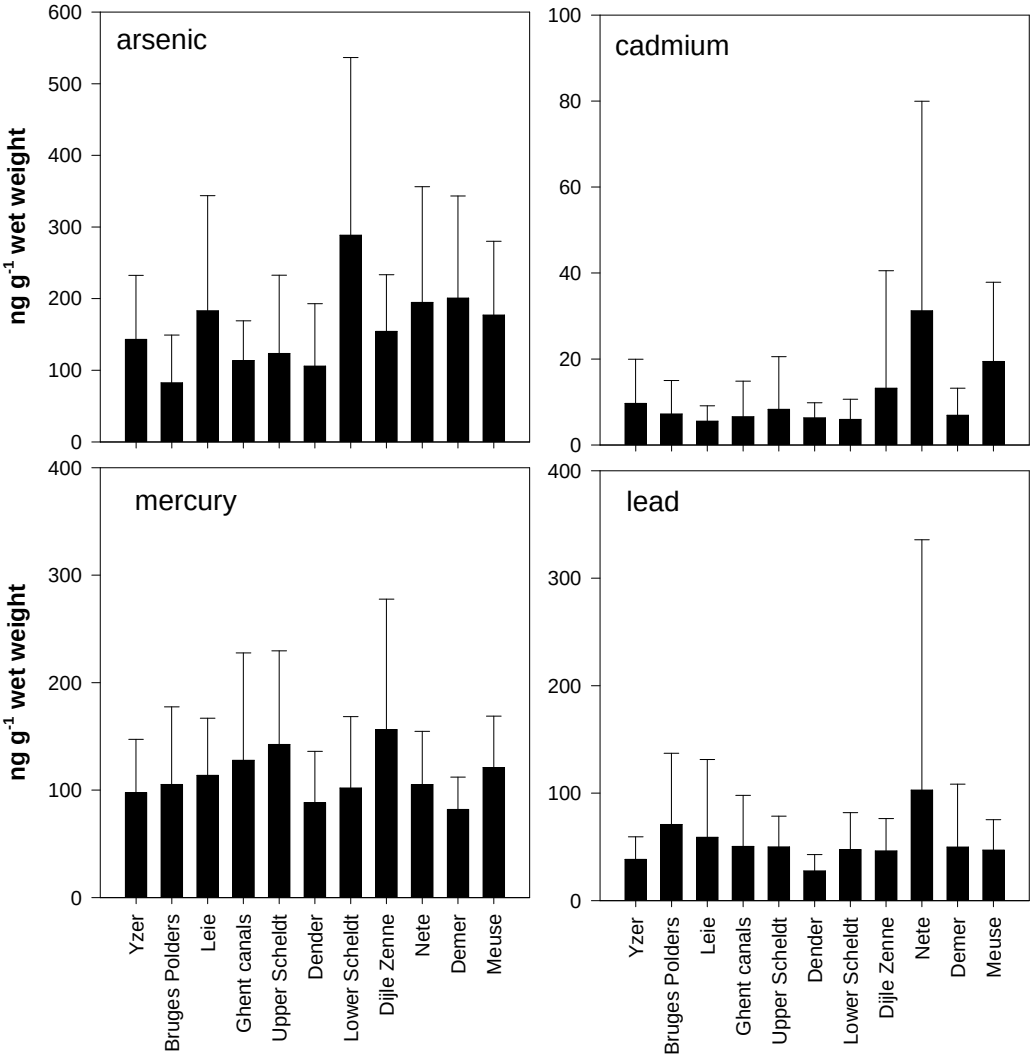


Figure 2.4. Spatial distribution of the average eel muscle tissue concentration of four heavy metals over the different river basins in Flanders (Belgium).

Temporal trends in the tissue concentration

Sampling took place more than once at 116 stations, and these data were useful to investigate time trends in eel pollutant concentration. Sampling station-averaged tissue concentration data in a general linear mixed model was used to infer an average time trend of pollutant contamination. Station-averaged time profiles and the fitted regression line for several organochlorine pollutants and four metals between 1994 and 2005 are presented in Figures 2.5-2.7. Model parameters (intercept and slope) and diagnostics are given in Table 2.2. There were significant reductions in the average wet weight concentration of all PCB congeners, nearly all pesticides and four metals. In Table 2.2, the variance present in the data set was partitioned into the variance due to the random intercepts, variance due to the random slopes, covariance between these parameters and, finally, the variance of the residuals. Generally, most of the variance was due to the random intercepts, which corresponds to variability in tissue concentration amongst the different sampling stations. Further, the covariance between intercepts and slopes was invariantly negative. This means that in stations where pollutant concentrations were initially above average, the rate of reduction was more pronounced than in stations with initially below-average concentrations.

All PCB congeners had significantly negative slopes indicating their gradual reduction in the freshwater environment. Figures 2.5 presents the station-averaged time profiles as well as the modelled trend line for PCB 153 and ∑PCB. Based on the regression model, the back calculated average ∑PCB concentration of eel was 770.6 ng g^{-1} wet weight in 1994 while for 2005, the regression model predicted an average concentration of 125.3 ng g^{-1} wet weight. It follows that the PCB concentration of eel decreased with a modelled rate of 15% per year.

Also concentrations of most pesticides decreased significantly over time. This was especially evident for α-HCH and lindane (Figure 2.6). Similar reductions were modelled for HCB, dieldrin and endrin. Unexpectedly, concentrations of *p,p'*-DDT increased over time but this effect was countered by significant reductions of the metabolites *p,p'*-DDE and *p,p'*-DDD (=TDE). As a result also ∑DDT decreased significantly.

The most notable reductions in the tissue concentration of heavy metals were observed for lead (Figure 2.7), arsenic, nickel and chromium. However, for the latter three metals, the trend may be biased as data were available only since 2000. The selenium concentration increased significantly but, similarly, data were only available since 2000. No trend was observed in the concentrations of mercury and cadmium (Figure 2.7).

Table 2.2. Linear mixed models results and diagnostics of the regressions of the concentration of contaminants and pesticides based in wet weight against time. This shows for each pollutant the total number of samples, the model intercept β_0 and slope β_1, the t-value and significance level *P* corresponding to the slope and the partitioning of the variance over d_{11} (variance of the random intercepts), d_{22} (variance of the random slopes), d_{12} (covariance between random intercepts and random slopes) and σ. (residual variance). Scatterplots are presented in Figures 2.5-2.7. No model was constructed for aldrin due to limited data.

	n	β_0	β_1	t	*P*	d_{11}	d_{12}	d_{22}	σ
PCBs									
PCB 28	256	0.8376	-0.04317	-5.21	<0.0001	0.3195	-0.02793	0.003641	0.03427
PCB 31	256	0.6425	-0.03547	-5.18	<0.0001	0.1921	-0.01783	0.002404	0.02465
PCB 52	256	1.6996	-0.08075	-11.29	<0.0001	0.2484	-0.00309	0.001051	0.04559
PCB 101	256	1.9204	-0.07721	-10.61	<0.0001	0.2519	-0.00428	0.000721	0.05274
PCB 105	256	1.4554	-0.06607	-11.84	<0.0001	0.1775	-0.00559	0.000584	0.02855
PCB 118	256	1.9132	-0.0696	-11.22	<0.0001	0.2062	-0.00521	0.000744	0.03487
PCB 138	256	2.2845	-0.07387	-10.11	<0.0001	0.3354	-0.00876	0.000652	0.05414
PCB 153	256	2.3323	-0.05965	-8.03	<0.0001	0.3275	-0.0077	0.000726	0.05494
PCB 156	256	1.1426	-0.04289	-7.51	<0.0001	0.1887	-0.00342	0.000319	0.03424
PCB 180	256	1.9934	-0.06046	-8.18	<0.0001	0.316	-0.0056	0.000505	0.05767
∑PCB	256	2.8874	-0.07143	-10.13	<0.0001	0.2781	-0.00333	0.000549	0.05103
Pesticides									
α-HCH	256	0.4736	-0.04087	-11.06	<0.0001	0.05387	-0.00471	0.000318	0.01793
Lindane	256	2.118	-0.1653	-18.18	<0.0001	0.4585	-0.02463	0.000964	0.09491
Dieldrin	256	1.7184	-0.1215	-10.4	<0.0001	0.2883	-0.03071	0.004721	0.1112
Endrin	249	0.6146	-0.06479	-7.04	<0.0001	0.4175	-0.04744	0.005242	0.05075
HCB	256	1.0777	-0.04906	-8.67	<0.0001	0.1294	-0.0041	0.000435	0.03263
TDE	256	1.4564	-0.03919	-3.14	0.0022	0.4285	-0.05093	0.007281	0.1009
p,p'-DDT	256	-0.4265	0.1082	9.74	<0.0001	0.2697	-0.04518	0.007798	0.0659
p,p'-DDE	256	2.0327	-0.05871	-9.85	<0.0001	0.1329	-0.00755	0.000672	0.03453
∑DDT	256	2.0939	-0.04403	-7.19	<.0001	0.1041	-0.00442	0.000384	0.04192
Trans-nonachlor	256	-0.2373	0.07239	8.64	<.0001	0.1892	-0.02658	0.004179	0.0356
Heavy Metals									
Mercury	266	2.0107	-0.0008	-0.16	0.8708	0.08724	-0.00648	0.000637	0.02176
Cadmium	268	0.9845	-0.00143	-0.17	0.8645	0.1502	-0.00323	<0.0001	0.09567
Lead	268	1.8097	-0.02813	-3.16	0.0021	0.09827	-0.00915	0.001597	0.09254
Copper	191	2.6312	0.009044	1.2	0.2351	0.07991	-0.00456	<0.0001	0.03505
Zinc	191	4.4179	-0.00777	-1.29	0.2033	<0.0001	-0.00289	0.000823	0.01489
Nickel	191	2.4514	-0.08422	-4.55	<.0001	0.6791	-0.0376	<0.0001	0.21715
Chromium	191	2.5072	-0.02356	-2.32	0.0237	0.02888	-0.00125	<0.0001	0.05499
Arsenic	160	2.5802	-0.05309	-3.95	0.0003	0.03658	-0.00548	<0.0001	0.02548
Selenium	160	2.3095	0.05859	6.21	<.0001	0.1111	-0.00418	<0.0001	0.02592

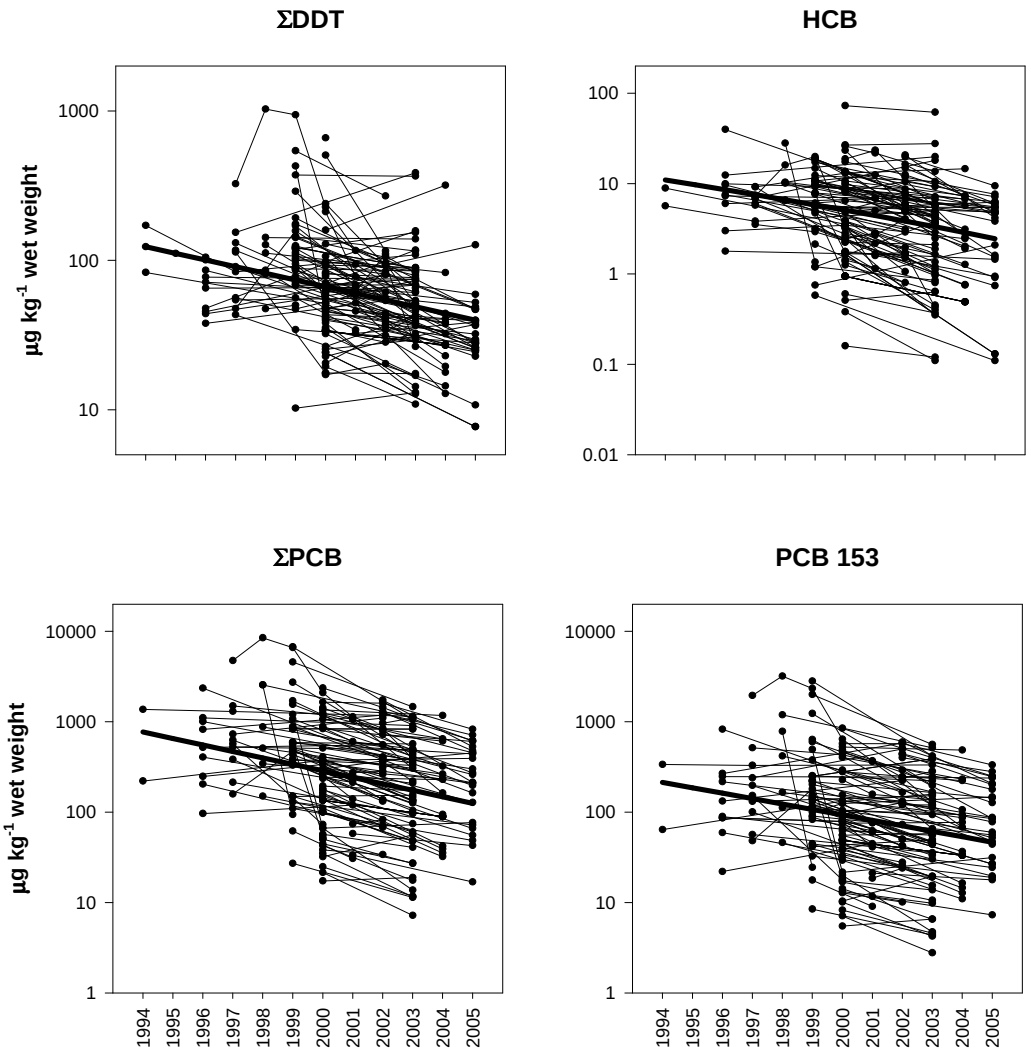


Figure 2.5. Temporal trends in average eel muscle tissue concentration of ∑DDT, ∑PCB, HCB and PCB 153 at sampling stations that were sampled more than once between 1994 and 2005. The bold line represents the average time trend which was modelled using a linear mixed model. See Table 2.2 for the intercept and slope.

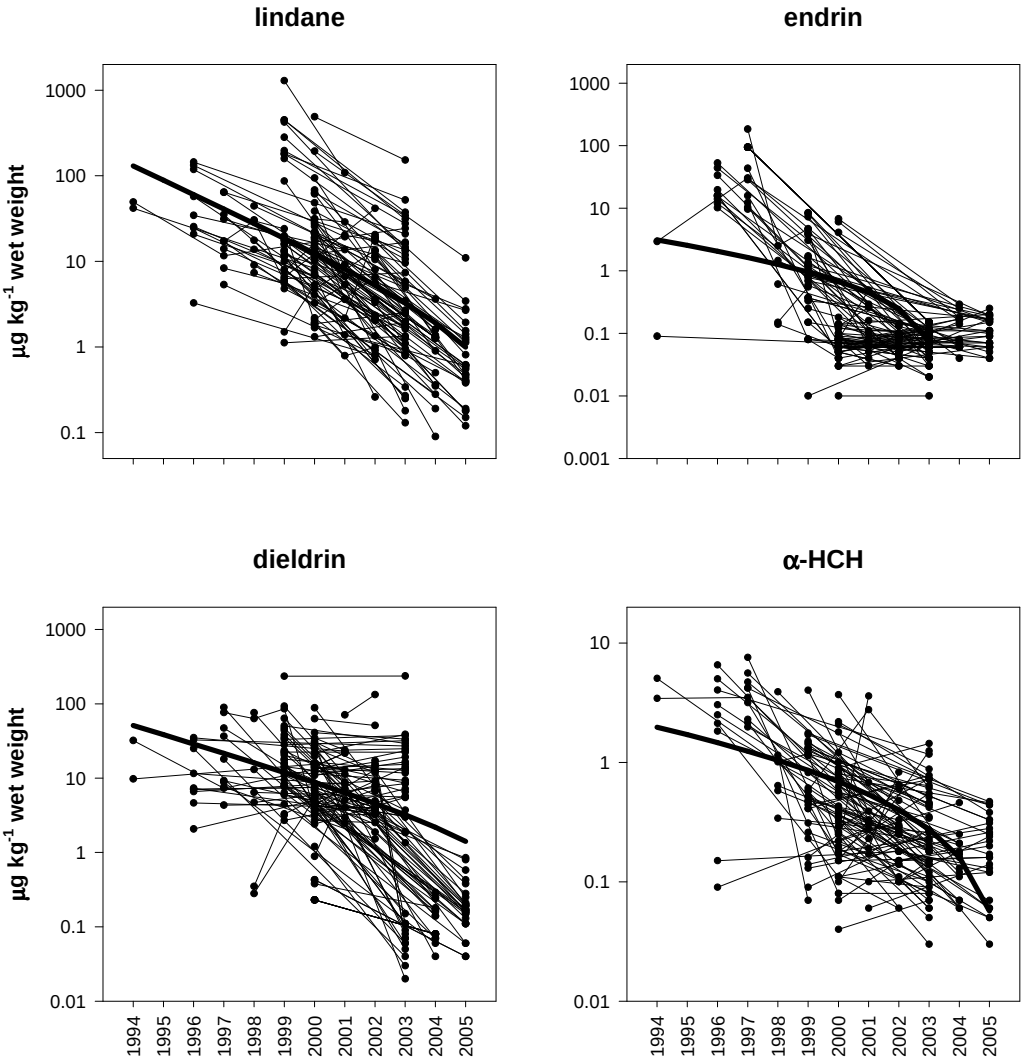


Figure 2.6. Temporal trends in average eel muscle tissue concentration of lindane, dieldrin, endrin and α-HCH at sampling stations that were sampled more than once between 1994 and 2005. The bold line represents the average time trend which was modelled using a linear mixed model. See Table 2.2 for the intercept and slope.

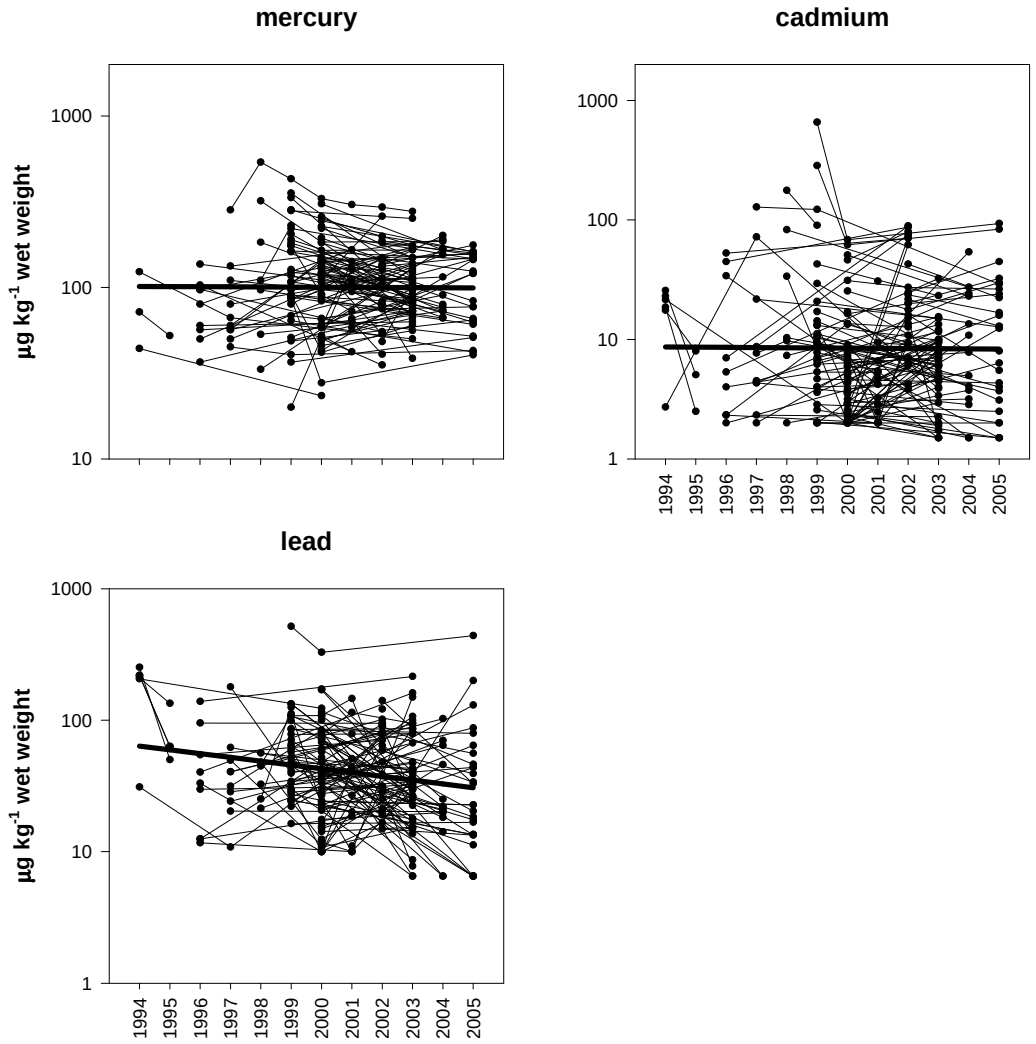


Figure 2.7. Temporal trends in average eel muscle tissue concentration of three heavy metals at sampling stations that were sampled more than once between 1994 and 2005. The bold line represents the average time trend which was modelled using a linear mixed model. See Table 2.2 for the intercept and slope.

Discussion

Belgium, and in particular its northern region Flanders, suffers substantial environmental problems. As a result, the country performs poorly in international studies. Based on a recently established environmental performance index (Esty *et al*., 2006), Belgium ranks 39th globally, last within the EU-25 and 26th out of 29 OECD member countries. Essentially, environmental problems in Flanders relate to the high human population density coupled with a historical lack of proper land use planning. The landscape is characterized by a patchy distribution of urbanization, industry, agriculture and nature. The high population density, as well as intensive livestock production, and the economical development of chemical industries result in a high pressure on the environment while at the same time, the fragmentation of the landscape greatly reduces the possibilities of targeted actions and effective environmental management. In particular, the management of water resources is an issue of concern. Belgium lags behind in the EU and faces serious water challenges. An additional problem is the trans-boundary aquatic pollution from neighbouring countries via the main rivers (Scheldt and Meuse).

This paper compares the pollution profile of European eel caught in Flemish inland waters with profiles reported elsewhere in Europe, using peer-reviewed papers that presented quantitative contaminant data in eel muscle tissue. Where appropriate, results were averaged while concentrations below detection limits were not considered. The results of this literature survey, as well as references, were listed in Tables 2.3-2.5. This comparison is not complete so we refer to Bruslé (1989) for additional concentration ranges of heavy metals and to Robinet and Feunteun (2002) for mean concentrations and ranges of different synthetic chemicals in yellow European eel muscle tissue. Further, the comparison between our results and published data was likely biased. Almost all the references that are included in Tables 2.3-2.5 focus on particular sites, where contamination was suspected, or are limited to single rivers. In addition, reported concentrations are based on sample sizes that were considerably smaller than in this study. Arguably, it was appropriate to also include our basin-specific and individual maximum values in this comparison.

Average PCB contamination levels of Flemish eel fall within concentrations reported for Western Europe. Data obtained from literature refer to inland waters of Spain, Luxembourg, UK and The Netherlands. Average PCB concentrations in this study were higher than those reported for River Turia (Spain), the Severn (UK) and the Sur (Luxembourg) but comparable to those of eel caught in the waters nearby Amsterdam (The Netherlands). Eel caught in River Moselle (Luxembourg) indicated heavy PCB contamination. Individual maximum levels of the different PCB congeners that were found in this study were, however, at least one order of magnitude higher than the average concentrations given in Table 2.3. The proportion of samples analysed in this study that exceeded the maximum reported average concentration based on the literature reports varied between 1.1% for PCB 52 and 10.4% for PCB 156 (Table 2.3). This demonstrates that eel experienced substantial exposure during the study period. In particular, eel captured in the basin of River Meuse were highly loaded with PCBs. River Meuse runs through an important industrial area including energy production and power transformation industries, which are possible historical sources of PCB contamination. The PCB data reported for the Netherlands by de Boer and Hagel (1994) proved to be particularly relevant to this analysis given the similarities in sampling design. Our study differs with De Boer and Hagel (1994) in that the Dutch monitoring programme mainly focused on contaminated sites in the rivers Rhine and Meuse, sampled between 1977 – 1990. Our data were collected 15 years later and also include smaller sized rivers and brooks, where exposure was assumed to be lower. Yet, we argue that the data presented in De Boer and Hagel

(1994) may be used as a baseline against which the present PCB pollution in Flemish eels can be evaluated. Average values reported in this study for individual PCB congeners were four times lower than the average results presented in De Boer and Hagel (1994). Then again, it appeared that 7.7% of our tissue samples tested for PCB 153 exceeded the baseline value based on De Boer and Hagel (1994) (Table 2.3). For PCB 156 and PCB 180, the proportion of eel with total concentrations above the average concentrations reported by De Boer and Hagel were 10.4% and 13%, respectively. So, in spite of decreasing concentrations, still a significant proportion of eel that was present in Flemish inland waters during the study period had a relatively high PCB concentration.

Reported contaminant concentrations in European eel by different pesticides were variable (Robinet and Feunteun, 2002). Apart from endrin, average concentrations in the present study did not exceed any of the means reported for other surface waters in Europe (Table 2.4). However, pesticide contamination in eel muscle tissue was not evenly distributed in Flanders with high levels of lindane and dieldrin in the basin of River Yser and above average DDT concentrations in the basin of the Upper Scheldt. Using these basin-averaged data (Figures 2.2-2.3), it appeared that pesticide tissue concentrations reported in this study are at the higher end relative to values reported for the rest of Europe. Most data in Table 2.4 refer to results for DDT derivatives and lindane. The incidence of the latter pesticide was highest in the Severn and in the delta of River Rhone. In Flanders, lindane and dieldrin peaked in the basin of River Yser, where land use is predominantly agriculture. Ten sampling sites showed tissue concentrations >100 ng g^{-1} wet weight, evidencing high local body burden in fish. *p,p'*-DDE varied in Europe between 3.9 and 187.9 ng g^{-1} wet weight and averaged 56 ng g^{-1} wet weight which was considerably lower than the average that was observed in the basin of the Upper Scheldt.

Only limited information of contamination by heavy metals in eel tissue was available in the literature. Bruslé (1990) reviewed metal contamination ranges in eel. Again, average concentrations do not differ much from reported ones as the pollution was very much focused in particular river basins. This was especially evident for Cd and Pb, which peaked in the basin of River Nete. The pollution in this river basin can be related to the presence of different non-ferrous industries producing zinc, cadmium and copper. As a consequence, heavy metals have been widespread in the local environment.

In summary, average contamination of eel in Flanders falls within the range of reported concentrations in other watersheds of Western Europe. However, spatial partitioning of the data demonstrated that the variation in pollutant concentration was positively skewed. This was especially evident for PCBs, lindane, endrin, dieldrin and DDE. A similar conclusion was made for heavy metals.

Table 2.3. Reported concentrations of PCBs (ng g^{-1} wet weight) in *Anguilla anguilla* in Europe. Numbers refer to different congeners. The bottom line presents the proportion of eel (%) captured in this study of which tissue concentrations exceeded the maximum value reported in the cited studies (Max)

Sampling site	Year	28	31	52	101	105	118	138	153	156	180	Reference
Amsterdam area (The Netherlands)	1991	17.63		28.53	38.49		74.93	115.37	112.90		38.65	Van der oost (1996)
Inland waters (The Netherlands)	1977-1990	28.71	12.33	261.44	354.90	49.08	226.17		570.93	29.56	181.98	de Boer and Hagel (1994) [1]
River Moselle (Luxemburg)	1996-1997						179.40	694.60	783.90		335.70	Dauberschmidt and Hoffmann (2001)
River Severn (UK)	1996			8.65	6.30	3.75	12.65	26.60	28.15	1.95	12.05	Harrad and Smith (1999)
River Sur (Luxemburg)	1996-1997						5.70	29.00	39.40		16.80	Dauberschmidt and Hoffmann (2001)
River Turia (Spain)	2000	0.75		2.16	2.99		2.74	12.30		0.35	2.43	Bordajandi *et al*. (2003)
Proportion of eel in Flanders > Max	1994-2005	6.2%	6.1%	1.1%	1.9%	6.9%	3.8%	3.6%	4.9%	10.4%	5.5%	

[1] Value represents average of the data

Table 2.4. Reported concentrations of organochlorine pesticides (ng g^{-1} wet weight, unless indicated) in *Anguilla anguilla* in Europe. T-nona refers to transnonachlor.

Sampling site	Year	α-HCH	Lindane	Dieldrin	Endrin	HCB	TDE	*pp'*-DDT	*pp'*-DDE	*t*-DDT	T-nona	Reference
Amsterdam area (The Netherlands)	1991	39.1	1.3	0.6	0.4	59.1	0.6	47.3	97.7			Van der oost (1996) [1]
Ebro delta (Spain)	1985						48.8	15.8	35.9			Ruiz and Llorente (1991)
River Moselle (Luxemburg)	1996-1997		37.4						187.9			Dauberschmidt and Hoffmann (2001)
Orbetello lagoon (Italy)	2002		10.0			0.1			3.9	4.4		Corsi *et al*. (2005) [1]
Po delta (Italy)	1994					0.2	19.9	4.2	27.4			Bressa (1997) [1]
River Severn (UK)	1996		2210.1									Harrad and Smith (1999) [1]
River Sur (Luxemburg)	1996-1997		53.1						42.8			Dauberschmidt and Hoffmann (2001)
River Turia (Spain)	2000						8.5	7.0	29.9	45.3		Bordajandi *et al*. (2003) [1]
Vaccares lagoon (France)	1996-1997		120.0	0.6		5.6			6.1	107.6		Roche *et al*. (2000) [1 2]
Vaccares lagoon (France)	1998		266.7	54.7								Roche *et al*. (2002) [1 2]
River Vanajavesi (Finland)	1990-1993	2.5	4.8			3.8				93.8	3.8	Tulonen and Vuorinen (1996)
Welsh rivers (UK)	1993		10.0				6.0	6.0	22.8			Weatherley *et al*. (1997)

[1] value represents average of the data
[2] dry weight basis

Table 2.5. Reported concentrations of heavy metals in *Anguilla anguilla* (ng g^{-1} wet weight) in Europe.

Sampling site	Year	Mercury	Cadmium	Lead	Copper	Zinc	Arsenic	Reference
River Turia (Spain)	2000		4.9	101.8	977	16950	227.9	Bordajandi *et al.* (2003)
River Gironde (France)	2001	170			150	10200		Durrieu *et al.* (2005)
Mersey estuary (UK)	1991-1993	962			884	24000	1100	Collings *et al.* (1996) [1]
River Ferrerias and River Raices (Spain)	No date given	278	24.5	33.25	218.75			Linde *et al.* (2004) [1]

[1] Value represents average of the data

Trends in eel contamination

In this paper, evidence was presented that the tissue concentration of some persistent chemicals in European eel has declined. Time series of the tissue concentration of PCBs and several pesticides showed a negative time trend. This conclusion was based on the application of linear mixed models for longitudinal data. This method was preferable to regressing annual averaged concentrations over time since the data were clustered according to sampling stations and hence, not independent from each other. The analysis demonstrated that river basins and sampling sites had clearly different pollution profiles.

The observed decline of PCBs in eel tissue was in agreement with other studies reporting on time series of contaminants in fish. PCBs were banned from the EU in 1985 and since then, several time series have indicated decreasing levels of contamination. A well known example was the decreasing trend of PCBs in human breast milk in Sweden (Noren and Meironyte, 2000). In teleosts, significant declines are reported for Spanish commercial fishes between 1995 and 2003 (Gomara *et al*., 2005), in salmonids of Lake Michigan between 1972 and 1994 (Lamon *et al*., 1998) and in Arctic char for the period 1960-1996 in Lake Vattern, Sweden (Lindell *et al*., 2001). In Flanders, concentrations of ∑PCB in eel tissue were shown to have decreased by 15% per year. This rate was in agreement with other studies in fish (Lindell *et al*., 2001). Also the time series of lindane, α-HCH, dieldrin, endrin, and HCB showed that bans and environmental policies lead to decreased concentrations.

A notable exception to this general decrease in persistent organic pollutants was *p,p'*-DDT. The linear model indicated an increase while at the same time, *p,p'*-DDD and *p,p'*-DDE showed significant decreases. However, it appeared that *p,p'*-DDT decreased between 1994 and 2001 while concentrations increased again after 2002. At first sight, the ratio between DDE and DDT was in all eel analysed >1, suggesting that remaining DDT had not been recently reapplied. However, at some locations in Flanders (Kanaal Dessel Schoten, Handzamevaart and Ieperkanaal) the ratio between DDE and DDT rapidly decreased over a few years by an order of magnitude of three. Such a steep decrease, even if the ratio was higher than one, probably indicates recent application of DDT and shows that not all stock was depleted. These results, as well as the recent observation of the human blood samples, particularly of the juvenile population living outside urban areas, still contain DDT (Steunpunt Milieu en Gezondheid, 2006) should urge regional policy makers to make a serious attempt in order to collect remaining stock of banned pesticides.

Mercury, cadmium and lead are heavy metals of special concern as they tend to bioaccumulate in the body. This study showed that only the concentration of lead in eel muscle tissue was consistently decreasing between 1994 and 2005, which possibly related to the gradual changeover from leaded to unleaded fuels and a reduction of industrial emissions. Cadmium and mercury, however, remain common environmental pollutants in the industrialized region of Belgium as there was no evidence that exposure of eel to these metals was decreasing. For other metals, data were available only since 2000 so a continuation of the sampling programme is necessary to confirm the observed trends.

Eel as pathway of human exposure to pollutants

Both European and national legislative initiatives have established a framework on maximum residue and contaminant levels in or on food and feed of plant and animal origin including the Regulation (EC) No 396/2005 of the European Parliament and of the Council (European Commission, 2005). The maximum pesticide residue level (MRL) in foodstuffs is 0.01 mg kg^{-1}. This general limit is applicable by default, i.e. in all cases where an MRL has not been specifically set for

a product or product type. Definitive tolerances will be listed in Annex II of the regulation which is yet to be published. Until then, MRLs for pesticides in products of animal origin established by Council Directive 86/363/EEC, as amended, are in force (European Commission, 1986). For cadmium, lead and mercury, levels have been established by Commission regulation (EC) No 466/2001 (European Commission, 2001). In February 2006, the European Commission (2006c) revised the maximum levels for dioxins and dioxin-like PCBs in foodstuffs (Commission regulation (EC) No 199/2006). These limits evidently apply in Belgium, a member state of the EU. However, the maximum limit for PCBs differs in Belgium in that the sum of the seven indicator PCBs was used to adopt a maximum level. The European legislative framework was used in this paper in order to assess the consumption quality of eel (Table 2.6). In particular, the proportion of eel analysed in this study that exceeded maximum residue or contaminant limits was calculated.

Table 2.6. Maximum residue and contaminant levels (MRL) in eel as adopted by different European regulations and proportion of eel (%) captured in this study with tissue concentrations higher than this maximum.

Substance	MRL in eel or fish	Proportion of non compliant eel (%)
Pesticides (EEC/86/1986)		
Endrin	0.01 mg kg^{-1} wet weight	3.8
Dieldrin	0.2 mg kg^{-1} wet weight	0.4
α-HCH	0.02 mg kg^{-1} wet weight	0
Lindane	0.2 mg kg^{-1} wet weight	2.3
Sum of DDTs	1 mg kg^{-1} wet weight	0.5
HCB	0.1 mg kg^{-1} wet weight	0.1
Trans-nonachlor	0.05 mg kg^{-1} wet weight	0
Heavy metals (EC/466/2001)		
Mercury	1 mg kg^{-1} wet weight	0
Lead	0.4 mg kg^{-1} wet weight	2.4
Cadmium	0.1 mg kg^{-1} wet weight	1.0
Dioxines and PCBs (EC/199/2006)		
Sum of dioxins	4 pg WHO-TEQ g^{-1} wet weight	-
Sum of dioxines and dioxinlike PCBs	12 pg WHO-TEQ g^{-1} wet weight	32.0 [2]
Sum of 7 indicator PCBs	0.075 mg kg^{-1} wet weight [1]	75.4

[1] Belgian MRL
[2] based on a regression equation between PCB153 and PCB TEQ (see text)

Relative to maximum quantities as adopted by legislation, it appears that eel tissue was, in general terms, compliant with European regulations for pesticides and heavy metals. The incidence of different pesticides in fish tissue was related to land use, so concentrations of lindane and dieldrin peaked in the western part of the country (the basin of River Yser) which has intensive horticulture. In that basin 14% of eels are non-compliant for lindane.

PCB concentrations in eel muscle tissue remain problematic. About 76% of the analysed individuals and 78% of the sampling stations exceeded the maximum level for human consumption. This maximum was based on the sum of seven indicator PCBs which, in Belgium, was fixed at 75 ng g^{-1} wet weight basis for fish. This limit was established after the Belgian dioxin crisis in 1999. In the spring of 1999, dioxin was introduced into the Belgian food chain via contaminated animal fat that was used in animal feeds. Due to the subsequent awareness of the public to food safety issues, the consumption of eel caught in public waters by anglers was prohibited. However, this ban was lifted again in December 2005. In February 2006, the European Commission established a maximum level for the sum of dioxins and furans in muscle meat of eel (4.0 pg WHO-PCDD/F-TEQ g^{-1} wet weight) as well as a maximum for the sum of dioxins, furans and dioxin-like PCBs (12.0 pg WHO-PCDD/F-PCB-TEQ g^{-1} wet weight) (European Commission, 2006c). Only two PCB congeners that were included in this study have toxic equivalent factors for human risk assessment. Neither the most toxic dioxin-like PCBs (congeners 126 and 169) nor dioxins and furans were monitored in this study. Therefore, it was not possible to directly assess the potential risks of consuming eel. As an alternative, De Boer *et al*. (1993) demonstrated a highly significant, empirical relationship between the concentration of PCB congener 153 in ng g^{-1} wet weight and pg PCB TEQ g^{-1} fresh weight. Here, we used their equation to assess the risk of eel consumption. In this study, eel showed an average PCB 153 concentration of 223 ng g^{-1} wet weight, which corresponds to 17.1 pg PCB TEQ g^{-1} wet weight. So even without accounting for the presence of dioxins and furans, an average sample of muscle meat of eel captured in surface waters exceeds the maximum by a factor of two.

A meal consisting of 100 gram would result in a dietary uptake of 24 pg TEQ g^{-1} body weight for an adult person weighing 70 kg. It follows that dietary exposure to PCBs by eating wild eel caught by angling exceeds the tolerable weekly intake that was advanced by the Scientific Committee on Food of the EU, which is 14 pg TEQ kg^{-1} body weight (Communication/593/2001). These results do not take into account the average dietary intake of dioxins and dioxin-like PCBs in the EU which was in the range of 1.2-3 pg kg^{-1} body weight per day. From that perspective, the consumption of eel caught in the wild should continue to be discouraged.

Acknowledgements

We thank all scientific and technical personnel at INBO for field sampling and sample preparation. A special thanks goes out to Kris Cooreman, Mark Raemaekers and Koen Parmentier from DVZ in Ostend and to Mark Guns, Hervé Baeten and Jos Liboton from CODA in Tervuren for the analyses of our samples. The preparation of the samples for analyses would not have been possible without the enthousiastic and constructive organization and comments from Omer Decock (DVZ). We are sure he is fully enjoying his retirement. A final version of the manuscript was edited by Dr. Russell Poole (Marine Institute, Newport, Ireland).

References

Ashley, J.T.F., Horwitz, R., Steinbacher, J.C., Ruppel, B., 2003. A comparison of congeneric PCB patterns in American eels and striped bass from the Hudson and Delaware River estuaries. *Marine Pollution Bulletin* 46, 1294-1308.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37, 911-917.

Belpaire, C., Goemans, G., 2007. Eels: contaminant cocktails pinpointing environmental pollution. *ICES Journal of Marine Science* 64, 1423-1436.

Bordajandi, L.R., Gomez, G., Fernandez, M.A., Abad, E., Rivera, J., Gonzalez, M.J. 2003. Study on PCBs, PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the River Turia (Spain). *Chemosphere* 53, 163-171.

Bressa, G., Sisti, E., Cima, F., 1997. PCBs and organochlorinated pesticides in eel (*Anguilla anguilla* L) from the Po Delta. *Marine Chemistry* 58, 261-266.

Bruslé, J., 1990. Effects of heavy metals on eels, *Anguilla* sp. *Aquatic Living Resources* 3, 131-141.

Collings, S.E., Johnson, M.S., Leah, R.T., 1996. Metal contamination of angler-caught fish from the Mersey Estuary. *Marine Environmental Research* 41, 281-297.

Corsi, I., Mariottini, M., Badesso, A., Caruso, T., Borghesi, N., Bonacci, S., Iacocca, A., Focardi, S., 2005. Contamination and sub-lethal toxicological effects of persistent organic pollutants in the European eel (*Anguilla anguilla*) in the Orbetello lagoon (Tuscany, Italy). *Hydrobiologia* 550, 237-249.

Dauberschmidt, C., Hoffmann, L., 2001. Distribution of persistent lipophilic contaminants in fish from the Grand Duchy of Luxembourg. *Bulletin of Environmental Contamination and Toxicology* 66, 222-230.

Daverat, F., Limburg, K.E., Thibault, I., Shiao, J.C., Dodson, J.J., Caron, F., Tzeng, W.N., Iizuka, Y., Wickstrom, H., 2006. Phenotypic plasticity of habitat use by three temperate eel species *Anguilla anguilla, A. japonica* and *A. rostrata*. *Marine Ecology Progress Series* 308, 255-269.

de Boer, J., Hagel, P., 1994. Spatial differences and temporal trends of chlorobifenyls in yellow eel (*Anguilla anguilla*) from inland waters of The Netherlands. *Science of the Total Environment* 141, 155-174.

de Boer, J., Stronck, C.J.N., Traag, W.A., Van der Meer, J., 1993. Non-ortho and mono-ortho substituted chlorobiphenyls and chlorinated dibenzo-p-dioxins and dibenzofurans in marine and fresh-water fish and shellfish from The Netherlands. *Chemosphere* 26, 1823-1842.

Durrieu, G., Maury-Brachet, R., Girardin, M., Rochard, E., Boudou, A., 2005. Contamination by heavy metals (Cd, Zn, Cu, and Hg) of eight fish species in the Gironde estuary (France). *Estuaries* 28, 581-591.

Esty, D.C., Levy, M.A., Srebotnjak, T., de Sherbinin, A., Kim, C.H., Anderson, B. 2006. Pilot 2006 Environmental Performance Index. New Haven, Yale Center for Environmental Law & Policy.

European Commission, 1986. Council Directive 86/363/EEC of 24 July 1986 on the fixing of maximum levels for pesticide residues in and on foodstuffs of animal origin. *Official Journal of the European Union* L221, 43-47.

European Commission, 2001. Commission regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union* L70, 1-13.

European Commission, 2005. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and

amending Council Directive 91/414/EEC. *Official Journal of the European Union* L70, 1-16.

European Commission, 2006a. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Official Journal of the European Union* L396, 1-849.

European Commission, 2006b. 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).

European Commission, 2006c. Commission Regulation (EC) No 199/2006 of 3 February 2006 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. *Official Journal of the European Union* L32, 34-46.

European Environmental Agency, 2005. Environment and health. EEA report 2005/10. Luxembourg, Office for Official Publications of the European Communities.

Feunteun, E., Lafaille, 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, Tokyo, p.191-213.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. Groenendaal, Belgium, Institute for Forestry and Game Management, 59p.

Goemans, G., Belpaire, C., 2004. The eel pollutant monitoring network in Flanders, Belgium. Results of 10 years monitoring. *Organohalogen Compounds* 66, 1834-1840.

Goemans, G., Belpaire, C., 2005. Congener profiles in European eel (*Anguilla anguilla* L.) as a method of tracing the origin of PCB contamination. *Organohalogen Compounds* 67, 1304-1307.

Gomara, B., Bordajandi, L.R., Fernandez, M.A., Herrero, L., Abad, E., Abalos, M., Rivera, J., Gonzalez, M.J. 2005. Levels and trends of polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) in Spanish commercial fish and shellfish products, 1995-2003. *Journal of Agricultural and Food Chemistry* 53, 8406-8413.

Harrad, S., Smith, D. 1999. Eel consumption as a pathway of human exposure to PCBs. *International Journal of Environmental Health Research* 9, 31-37.

Hoge Gezondheidsraad, 2005. Advies van de Hoge Gezondheidsraad – Schatting van de inname van PCB's door sportvissers en het hieraan verbonden gezondheidsrisico. (Advice No HGR 7747 – brought out on February 23, 2005 and validated on March 9, 2005).

Hoff, P.T., Van Campenhout, K., de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., Goemans, G., Belpaire, C., Blust, R., De Coen, W., 2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Environmental Pollution* 137, 324-333.

Knights, B., 1997. Risk assessment and management of contamination of eels (*Anguilla* spp.) by persistent xenobiotic organochlorine compounds. *Chemistry and Ecology* 13, 171-212.

Lamon, E.C., Carpenter, S.R., Stow, C.A., 1998. Forecasting PCB concentrations in Lake Michigan salmonids: A dynamic linear model approach. *Ecological Applications* 8, 659-668.

Larsson, P., Hamrin, S. and Okla, L., 1991. Factors determining the uptake of persistent pollutants in an eel population (*Anguilla anguilla* L.). *Environmental Pollution* 69, 39-50.

Linde, A.R., Sanchez-Galan, S., Garcia-Vazquez, E., 2004. Heavy metal contamination of European eel (*Anguilla anguilla*) and brown trout (*Salmo trutta*) caught in wild ecosystems in Spain. *Journal of Food Protection* 67, 2332-2336.

Lindell, M.J., Bremle, G., Broberg, O., Larsson, P., 2001. Monitoring of persistent organic pollutants (POPs): Examples from Lake Vattern, Sweden. *Ambio* 30, 545-551.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: Relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology* 73, 99-114.

Mason, C.F., Barak, N.A.E., 1990. A catchment survey for heavy metals using the eel (*Anguilla anguilla*). *Chemosphere* 21, 695–699.

Noren, K., Meironyte, D., 2000. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere* 40, 1111-1123.

Parker, S.J., 1995. Homing ability and home-range of yellow-phase American eels in a tidally dominated estuary. *Journal of the Marine Biological Association of the United Kingdom* 75, 127-140.

Robinet, T.T., Feunteun, E.E., 2002. Sublethal effects of exposure to chemical compounds: A cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265-277.

Roche, H., Buet, A., Jonot, O., Ramade, F., 2000. Organochlorine residues in european eel (*Anguilla anguilla*), crucian carp (*Carassius carassius*) and catfish (*Ictalurus nebulosus*) from Vaccares lagoon (French National Nature Reserve of Camargue) - effects on some physiological parameters. *Aquatic Toxicology* 48, 443-459.

Roche, H., Buet, A., Ramade, F., 2002. Accumulation of lipophilic microcontaminants and biochemical responses in eels from the Camargue Biosphere Reserve. *Ecotoxicology* 11, 155-164.

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U.A.T., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *Journal of Environmental Monitoring* 5, 876-884.

Ruiz, X., Llorente, G.A., 1991. Seasonal variation of DDT and PCB accumulation in muscle of carp (*Cyprinus carpio*) and eels (*Anguilla anguilla*) from the Ebro delta, Spain. *Vie et Milieu* 41, 133-140.

SAS Institute Inc, 1999. SAS/STAT Software User's guide, Version 7. Cary, NC, SAS Institute Inc.

Steunpunt Milieu en Gezondheid, 2006. Vlaams humaan biomonitoringsprogramma milieu en gezondheid (2002-2006) jongeren campagne. Resultaten rapport bestemd voor intermediairen. Unpublished report.

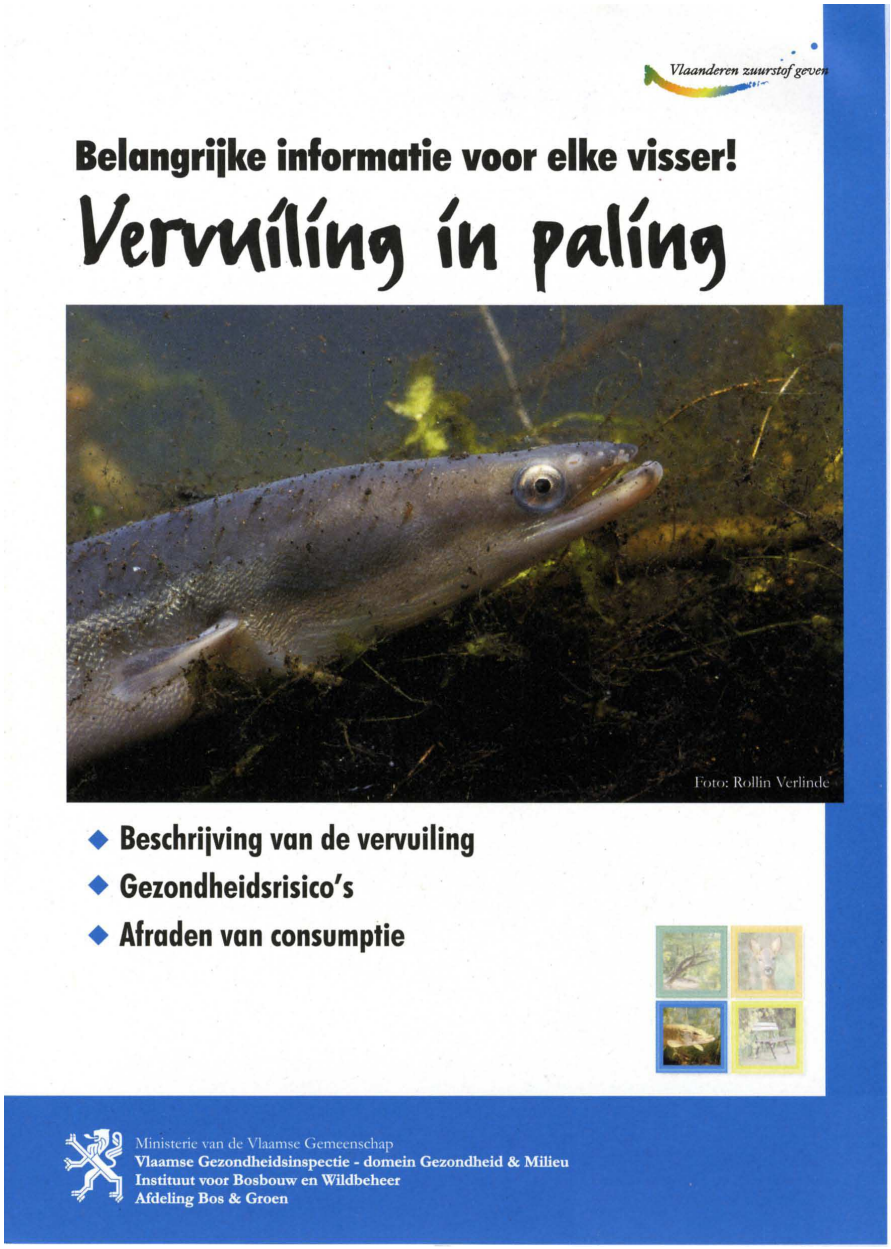
Tulonen, J., Vuorinen, P.J., 1996. Concentrations of PCBs and other organochlorine compounds in eels (*Anguilla anguilla*, L) of the Vanajavesi watercourse in southern Finland, 1990-1993. *Science of the Total Environment* 187, 11-18.

Van Der Oost, R., Opperhuizen, A., Satumalay, K., Heida, H., Vermeulen, N.P.E., 1996. Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*) .1. Bioaccumulation: Biota-sediment ratios of PCBs, OCPs, PCDDs and PCDFs. *Aquatic Toxicology* 35, 21-46.

Verbeke, G., Molenberghs, G., 2000. Linear mixed models for longitudinal data. New York, Springer Verlag.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363-371.

Weatherley, N.S., Davies, G.L., Ellery, S., 1997. Polychlorinated biphenyls and organochlorine pesticides in eels (*Anguilla anguilla* L.) from Welsh rivers. *Environmental Pollution* 95, 127-134.



A leaflet issued in Flanders (Belgium) to discourage anglers to consume self caught eels.

Chapter 3

Brominated flame retardants in eels from River Scheldt

Laurence Roosens[1], Alin Dirtu[1,2], Geert Goemans[3], Claude Belpaire[3], Adriana Gheorghe[1,4], Hugo Neels[1], Ronny Blust[5] and Adrian Covaci[1,5]

1 - Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium
2 - Department of Inorganic and Analytical Chemistry, "Al.I.Cuza" University of Iassy, Carol I Bvd. No 11, 700506 Iassy, Romania
3 - Research Institute for Nature and Forest, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium
4 - Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, Soseaua Panduri 90-92, 050663 Bucharest, Romania
5 - Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium.

This chapter is in press as:

Roosens, L., Dirtu, A., Goemans, G., Belpaire, C., Gheorghe, A., Neels, H., Blust, R., Covaci, A., 2008.
Brominated flame retardants and polychlorinated biphenyls in fish from the River Scheldt, Belgium.
Environment International, in press.

Summary

Levels of polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), and polychlorinated biphenyls (PCBs) were measured in several fish species originating from the river Scheldt (Belgium). Five sampling locations were chosen in a highly industrialized area along the river, while two ponds in the vicinity of the river served as reference sites. The present study is a follow-up of a survey performed in 2000 which reported extremely high levels of PBDEs and HBCDs in eel (*Anguilla anguilla*) collected from the same location (Oudenaarde, Flanders). The sum of tri- to hepta-BDE congeners (2 270 ± 2 260 ng/g lipid weight (LW), range 660 - 11 500 ng/g LW) and total HBCDs (4 500 ± 3 000 ng/g LW, range 390 - 12 100 ng/g LW) were one order of magnitude higher than levels usually reported from freshwater systems, indicating the presence of point sources. In most samples, levels of total HBCDs were higher than those of PBDEs, probably due to the high density of factories using HBCD as an additive flame retardant on the river Scheldt. The high values of HBCDs were confirmed by both gas- and liquid chromatography-mass spectrometry. Although BFR levels were between the highest ever reported in freshwater ecosystems, PCBs could be detected at even higher concentrations (16 000 ± 14 300 ng/g LW, range 3 900 - 66 600 ng/g LW), being among the highest levels recorded in Belgium. The inter-sampling site variation of PBDEs, HBCDs and PCBs was comparable. All locations presented similar PBDE congener profiles, with BDE 47 being the dominant congener, followed by BDE 100, BDE 99 and BDE 49, probably originating from the former use of the penta-BDE technical mixture. In order to estimate the impact of these point sources on human exposure, we further focused on eels which showed a considerable decrease in the PBDE and HBCD levels between 2000 and 2006. Due to the wide span in concentrations between the different sampling locations, a variable contribution to the total human exposure through local eel consumption was estimated. The calculated daily intake ranged from 3 ng to 330 ng PBDEs/day for normal eel consumers, but was as high as 9 800 ng PBDEs/day for anglers, which may be considered at risk.

Introduction

Due to their widespread presence in the environment and their reported possible adverse health effects, brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), have become the subject of intensive research (Birnbaum and Staskal, 2004; Covaci *et al*., 2006). Elevated PBDE levels measured in various environmental and biological samples have led to restricted use of Penta- and Octa-BDE technical mixtures in Europe (Directive 2003/11/EC). However, the Deca-BDE technical product is still used in large amounts (56 500 tons worldwide, 7 600 tons in Europe), mainly in plastic housing for electric and electronic equipment, but also in upholstery textiles (BSEF, 2007). Hexabromocyclododecanes (HBCDs) are widely used in a variety of industrial and household appliances, such as polystyrene foams and upholstery textiles, making this compound the second most used BFR in Europe (BSEF, 2007). Despite restrictions/bans on their manufacture and use in most industrialized countries since the 1970s, polychlorinated biphenyls (PCBs) can still be measured in environmental samples due to their highly lipophilic properties which make them persist in the environment, bioaccumulate through the food chain and cause potential toxic risks to humans (Domingo *et al*., 2007).

BFRs can reach the environment through leaching during production and application processes, through volatilization and leaching during use and through particulate losses during use and disposal (Darnerud *et al*., 2001). In this way, point sources often lead to contamination of adjacent aquatic systems and to increased levels in aquatic organisms, such as fish. Since fish is an important part of the human diet (Domingo 2004), the impact of point sources on human exposure have to be closely monitored. This has been recently shown by Thomsen *et al*. (2008), where contaminated fish from Lake Mjøsa, Norway contributed significantly to the human dietary exposure to PBDEs. Indeed, high concentrations of BFRs and PCBs have been previously measured in fish samples originating from the Scheldt basin and the North Sea (de Boer *et al*., 2002, Belpaire *et al*., 2003, Voorspoels *et al*., 2003, 2004; Baeyens *et al*., 2007), but human exposure profiles have yet to be calculated.

The present study aims firstly to give an overview of BFRs and PCBs concentrations in various fish species along the river Scheldt in an area of intense industrial activity (Oudenaarde, Belgium). This study is a follow-up of a survey performed in 2000 (de Boer *et al*., 2002, Belpaire *et al*., 2003) which found extremely high levels of PBDEs and HBCDs in European eel (*Anguilla anguilla*) from the river Scheldt. The second part of this article mainly focuses on eel samples, firstly to assess the impact of point sources on human exposure and secondly to exclude confounding factors, such as lipid content and trophic level, which varies between different species and therefore contribute differently to the overall BFR/PCB levels. Due to its high lipid content and predatory feeding behaviour (Dörner and Benndorf, 2003), eel is highly prone to bioaccumulate lipophilic contaminants (Ashley *et al*., 2007; Storelli *et al*., 2007). Moreover, its sedentary way of life during the yellow eel phase (Baras *et al*., 1998; Lafaille *et al*., 2005) reflects local pollution (Belpaire and Goemans, 2007a,b). Eel and other fish species were collected in 2006 from the same area and some adjacent locations. This enabled us to follow the temporal evolution of BFR levels and the spatial characterization of the BFR contamination. Additionally, the human dietary exposure through the ingestion of contaminated eel was calculated for various scenarios, which include normal fish consumers, as well as risk groups, such as local anglers.

Materials and methods

Samples

Fish samples were collected in 2006 by electrofishing and fyke fishing from 7 different locations of the Scheldt basin around the city of Oudenaarde (west of Brussels, Belgium). Two closed water bodies in the vicinity (locations L1 and L2) were included as reference areas, while other sampling locations are numbered from upstream (L3) to downstream (L7) of Oudenaarde (Figure 3.1). The distance between L3 and L7 was approximately 15 km. A pooled eel sample (3 individual fishes) from location L5 collected in 2000 was also made available for analysis.

A number of 35 (28 pooled and 7 individual) fish samples representing various trophic levels were prepared from: eel (*Anguilla anguilla*), pike (*Esox lucius*), pike-perch (*Sander lucioperca*), perch (*Perca fluviatilis*), bream (*Abramis brama*), roach (*Rutilus rutilus*), topmouth gudgeon (*Pseudorasbora parva*), carp (*Cyprinus carpio*), gibel carp (*Carassius auratus gibelio*), rudd (*Scardinius erythrophthamus*), and tench (*Tinca tinca*) (Table 3.1). Equal amounts of fish were taken to compose pooled samples, which were afterwards homogenized (using a robot mixer). Total sample weight ranged between 3.3 and 20.6 g from which approximately 2 g was taken for analysis. Fish were of variable length and weight, ranging between 9.0 - 58.6 cm and 6.7 - 1783 g, respectively. All samples were stored at –20 ℃ in tightly sealed plastic bags until analysis.

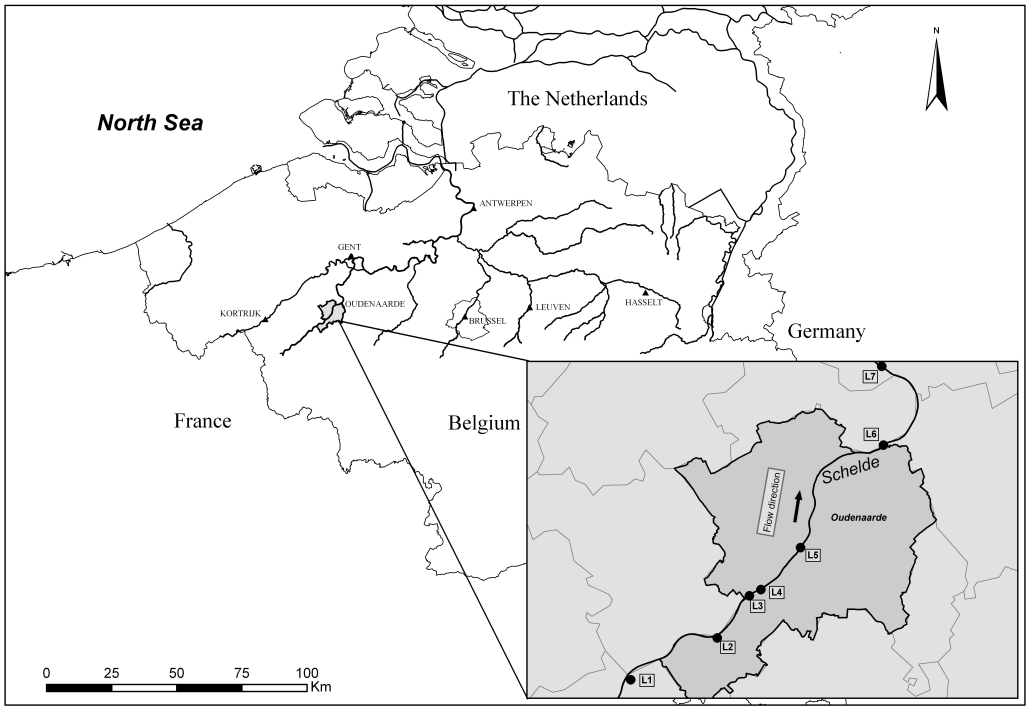


Figure 3.1. Basin of the River Scheldt and situation of the sampling area, Oudenaarde (west of Brussels). The different sampling locations are L1 (Scheyteput - Kluisbergen), L2 (Oude Schelde 'Het Veer': Oudenaarde - Melden), L3 (Schelde: Wortegem - Petegem - Molenbeek), L4 (Schelde: Oudenaarde - Scheldemeersen), L5 (Schelde: Oudenaarde), L6 (Schelde: Zingem - Zwalmbeek), L7 (Schelde: Gavere - Asper).

Table 3.1. Overview of the investigated fish samples. Values in brackets represent the number of individual fish samples used to compose a pool.

Species	Name	Total samples	Locations	N	Type (I or P)*	Lipid content (%)
Eel	*Anguilla anguilla*	10	L1	2	P(5), P(5)	0.9, 1.1
			L2	1	P(10)	6.49
			L4	1	I	13.9
			L5	3	P(5), P(5), I	18.9, 8.6, 19.0
			L6	2	P(4), P(5)	15.4, 20.1
			L7	1	P(5)	14.3
Perch	*Perca fluviatilis*	5	L1	1	I	0.97
			L3	1	I	0.63
			L4	2	P(7), I	0.80, 0.76
			L5	1	P(8)	0.54
Pike-perch	*Sander lucioperca*	4	L4	1	P(2)	0.50
			L5	1	P(6)	0.51
			L6	1	P(2)	0.31
			L7	1	P(2)	0.55
Roach	*Rutilus rutilus*	3	L3	1	P(2)	2.03
			L4	1	P(19)	0.75
			L5	1	P(13)	1.40
Carp	*Cyprinus carpio carpio*	3	L4	2	P(2), P(3)	0.59, 1.09
			L5	1	P(3)	1.30
Bream	*Abramis brama*	2	L4	1	P(15)	0.61
			L5	1	P(15)	0.77
Gibel carp	*Carassius auratus gibelio*	2	L4	1	P(10)	0.69
			L5	1	P(10)	0.79
Topmouth gudgeon	*Pseudorasbora parva*	2	L3	1	P(4)	1.40
			L4	1	P(8)	1.09
Tench	*Tinca tinca*	2	L4	1	P(2)	1.53
			L5	1	P(3)	0.94
Pike	*Esox lucius*	1	L5	1	I	0.35
Rudd	*Scardinius erythrophthamalus*	1	L3	1	I	1.07

I – individual; P - pool

Materials

PBDEs reference standards (BDE 28, 47, 49, 66, 99, 100, 153, 154, and 183) were purchased from Wellington Laboratories (Guelph, ON, Canada) and Accustandard (New Haven, CT, USA), while BDE 77 and 128, used as internal standards, were from Accustandard. Standards of individual ^{12}C-HBCD and ^{13}C-HBCD isomers were purchased from Wellington Laboratories. The following PCB congeners (IUPAC numbering) were targeted for analysis: 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 163, 170, 180, 183, 187, 196 and 199. CB 46 and 143 were used as internal standards for the quantification of PCBs. All individual PCB standards were obtained from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). All solvents used for the analysis (acetone, dichloromethane, *iso*-octane, *n*-hexane, methanol) were of SupraSolv® grade (Merck, Darmstadt, Germany). Sodium sulphate (Merck) and silica gel (0.063-0.200 mm, Merck) were pre-washed with *n*-hexane and heated overnight at 150 ℃ before use. Extraction thimbles (25 x 100 mm, Whatman®, England) were pre-extracted for 1 h with hexane/acetone (3/1; *v/v*) and dried at 100 ℃ for 1 h. Empty polypropylene columns for clean-up (25 ml) were purchased from Alltech (Lokeren, Belgium).

Sample preparation

The method used for sample extraction and clean-up has been previously described and validated (Voorspoels *et al*., 2003, 2004), and minor modifications were applied for the analysis of HBCDs. Briefly, a homogenised sample of approximately 2 g fish tissue was weighed, homogenised with anhydrous Na_2SO_4 and spiked with internal standards (PCB 46, PCB 143, BDE 77 and BDE 128). Further, the samples were extracted for 2 h by hot Soxhlet (Büchi, Flawil, Switzerland) with 100 ml hexane/acetone (3:1, *v/v*). The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h) while the rest of the extract was cleaned-up on ~8 g acidified silica and successively eluted with 20 ml hexane and 15 ml dichloromethane. The eluate was concentrated to approximately 2 ml using a rotary-evaporator and further to near dryness under a gentle nitrogen stream. The dried extract was reconstituted in 100 µl *iso*-octane and analysed for PCBs using gas chromatography-mass spectrometry (GC–MS) with electron impact ionization (EI) (method 1) and for PBDEs and HBCDs using GC–MS with electron-capture negative ionization (ECNI) (method 2).

For confirmation of HBCD levels in eel samples (containing the highest loads of pollutants), the same treatment was applied with minor modifications (e.g. the addition of internal standard ^{13}C-α-HBCD). After extraction and clean-up, the extract was analysed by GC–MS with EI (method 3) and with ECNI (method 4). The remaining extract was evaporated to dryness and reconstituted in methanol for analysis by liquid chromatography-mass spectrometry (LC–MS) (methods 5 and 6).

Analysis of PCBs (method 1)

An Agilent 6890 GC – 5973 MS system operated in EI mode was equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. One µl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) rising to 300 °C with 700 °C/min), pressure pulse 25 psi and pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as carrier gas at constant flow (1.0 ml/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (kept for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, kept for 12 min. The MS was used in the selected ion-monitoring (SIM) mode with 2 ions monitored for each PCB homologue group.

Analysis of PBDEs and HBCDs (method 2)

An Agilent 6890 GC – 5973 MS system operated in ECNI mode was equipped with a 15 m x 0.25 mm x 0.10 µm DB-5 (J&W Scientific) capillary column. The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. Helium was used as carrier gas at constant flow (1.0 ml/min) and with methane as moderating gas. The MS was operated in SIM mode and the electron multiplier voltage was set at 2100 V. One µl of the extract was injected in solvent vent mode (injector temperature at 90 °C, kept for 0.06 min, then increased with 700 °C/min to 305 °C, vent time 0.04 min, vent flow 75 ml/min). The splitless time was 1.50 min. The temperature of the DB-5 column was programmed from 90 °C, kept for 1.5 min, then increased with 15 °C/min to 295 °C, kept for 15 min. Ions *m/z* 79 and 81

were monitored for the entire run and dwell times were set to 40 ms. BDE 77 and BDE 128 were used as internal standards.

Confirmation of total HBCD levels by GC–MS

In this case, the ions $[M-Br]^-$ were monitored and this allowed the use of ^{13}C-α-HBCD as internal standard. However, the intensity of the more specific ions $[M-Br]^-$ was much lower than that of ions *m/z* = 79 and 81, which lead to a serious decrease in sensitivity. Consequently, only samples with high loads of HBCDs (eels from locations L4 through L7) could be measured using these methods.

Method 3 used the same parameters as presented for method 1 (GC–EI-MS), with the exception that only ions *m/z* = 561/563 and 573/575 were used for monitoring ^{12}C-HBCDs and ^{13}C-α-HBCD, respectively.

Method 4 used the same parameters as presented for method 2 (GC–ECNI-MS), with the exception that only ions *m/z* = 561/563 and 573/575 were used for monitoring ^{12}C-HBCDs and ^{13}C-α-HBCD, respectively.

Confirmation of individual HBCD isomers levels by LC–MS

Similar to methods 3 and 4, ^{13}C-α-HBCD has been used as internal standard for LC–MS analysis.

Method 5. Separation of α-, β-, and γ- HBCD was achieved using an Agilent 1100 LC system equipped with a Zorbax C_{18} reversed phase analytical column (50 mm x 2.1 mm i.d., 3 µm particle size). A mobile phase of (a) 10 mM ammonium acetate and (b) methanol at a flow rate of 200 µl/min was used: starting at 85% (b) hold for 6 min, then linearly increased to 100% (b) over 2 min, hold for 4 min. α-, β- and γ-HBCD were baseline separated with retention times of 3.0, 3.9 and 4.4 min, respectively. The MS system was an Agilent XL ion trap operated in the ES negative ion mode. Quantitative determination of the HBCD isomers was based on *m/z* = 640.6 and 652.4 for the native and ^{13}C-labelled HBCD isomers, respectively.

Method 6. Individual HBCD isomers were analyzed by LC-MS/MS using a method described by Abdallah *et al.* (2008). Separation of α-, β-, and γ- HBCD was achieved using a dual pump Shimadzu LC-20AB equipped with a Varian Pursuit XRS3 C_{18} reversed phase analytical column (150 mm x 2 mm i.d., 3 µm particle size). A mobile phase of (a) 1:1 water/methanol with 2 mM ammonium acetate and (b) methanol at a flow rate of 150 µl/min was used: starting at 50% (b) then increased linearly to 100% (b) over 3 min; this was held for 5 min followed by a linear decrease to 65% (b) over 2.5 min and held for 3.5 min. α-, β- and γ-HBCD were baseline separated with retention times of 9.4, 9.9 and 10.3 min, respectively. The MS system was a Sciex API 2000 triple quadrupole mass spectrometer operated in the ES negative ion mode. MS/MS detection operated in the multiple reaction monitoring mode was used for quantitative determination of the HBCD isomers based on *m/z* 640.6→ *m/z* 79 and *m/z* 652.4→ *m/z* 79 for the native and ^{13}C-labelled HBCD isomers, respectively.

Quality assurance

Multi-level calibration curves were created for the quantification and good linearity (r^2>0.999) was achieved for tested intervals that included the whole concentration range found in samples. The area ratio between the analyte and internal standard was plotted against the corresponding absolute amount ratio. The

analyte identification was based on their relative retention times to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions for GC–MS or LC–MS. A deviation of the ion intensity ratios within 20% of the mean values of the calibration standards was considered acceptable.

The extraction, clean-up and analysis procedures were validated through the regular analysis of procedural blanks, duplicate samples, recovery monitoring of spiked samples and analysis of certified material SRM 1945 (PCBs and PBDEs in whale blubber). Obtained values were deviating with less than 15 % from the certified values. The quality control scheme is also assessed through regular participation to interlaboratory comparison exercises organized by Arctic Monitoring Assessment Programme (AMAP) and the US National Institute for Standards and Technology (NIST), for which the obtained values did not vary with more than 15% from the target values. Similarly, the quality of HBCD measurements (by GC-ECNI-MS, method 2) was ensured through successful participation to the interlaboratory exercise organised by the Norwegian Institute for Public Health (Haug *et al*., 2008).

For each analyte, the mean procedural blank value was used for subtraction. The instrumental LODs and LOQs were calculated for a signal/noise (S/N) ratio equal to 3 and 10, respectively, at the chosen quantification ion(s). The method LOQs were calculated as 3 x SD of the procedural blanks, taking into account the amount of sample taken into analysis (approximately 2 g). Limit of quantification (LOQ) for individual PBDE congeners and total HBCDs (by method 2) ranged between 2 - 5 ng/g lipid weight (LW), while LOQs for PCBs ranged between 4 - 10 ng/g LW. Samples with concentrations below LOQ (which were few in number) were calculated as f*LOQ with “f” being the fraction of samples above LOQ. Recoveries for individual PBDEs and PCBs were assessed through spiking experiments and ranged between 72 and 104 %, while recoveries for α-HBCD were between 65 and 90 %.

Results and Discussion

Geographical variation

Results from the measurements of PBDEs and HBCDs (ng/g LW) are presented in Figure 3.2A (all species together) and Figure 3.2B (only eel), while PCB results are presented in Figure 3.3 (all species together). Due to the diversity in the collected species and their physiological differences, such as lipid content, feeding behaviour and degree of biomagnification for various contaminants, every species contributes differently to the overall contamination pattern at each location. Therefore, both a general overview including all species and a separate discussion of eel samples, are given. This enables us to compare an average contamination profile in a wide variety of fishes with one defined species with high lipid content and a sedentary lifestyle (eel), which would most probably be the most suitable indicator for the local pollution. L1 and L2 are not directly situated on the stream and were included in this study to test for possible atmospheric contribution to the contamination of the aquatic environment. Our results show high contamination levels along the river Scheldt (L3 through L7), but not in the ponds (L1 and L2) vicinal to the river. Atmospheric contribution to BFR contamination in water seems to be less important than the direct contamination through the water. However, it should be mentioned that other factors besides atmospheric deposition can possibly influence the BFR or PCB levels at locations L1 and L2, but this seems to be minor in importance compared to contamination at locations L3-L7. Hence, these locations (L1 and L2) can be seen as reference locations for this study. L3 and L4 are located upstream of Oudenaarde. L5 has been chosen due to the high contamination levels measured at this location in

2002 (de Boer *et al*., 2002; Belpaire *et al*., 2003). Possible point sources of contamination include local textile industry located in Oudenaarde and surroundings. L6 and L7 are both situated further downstream from the industrialized areas and serve to estimate the (more) remote influence of the textile industry.

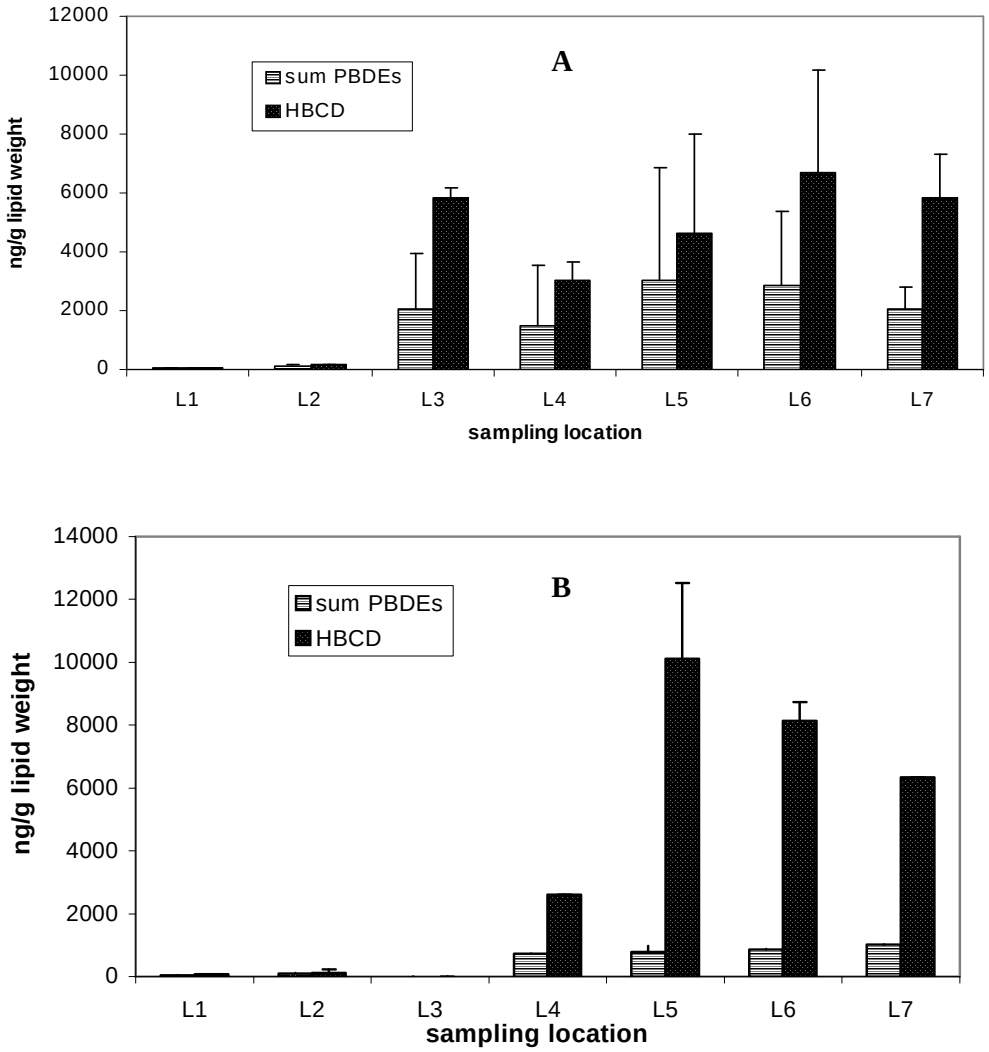


Figure 3.2. Geographical variation of sum PBDEs and total HBCD levels (ng/g LW) of all analyzed fish samples (A) and eel samples (B). Mean values are calculated for each locations, while standard deviations are indicated as error bars.

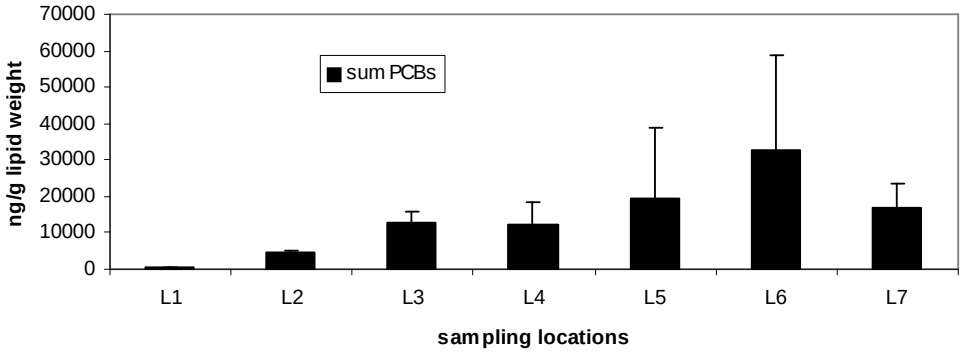


Figure 3.3. Geographical variation of sum PCBs (ng/g LW) of all analyzed fish samples. Mean values are calculated for each locations, while standard deviations are indicated as error bars.

a) All species

PBDEs and HBCDs were detected in all analyzed fish samples. The sum of PBDEs (BDE 28, 47, 49, 66, 99, 100, 153, 154, and 183) for locations L3 to L7 ranged between 660 and 11 500 ng/g LW, with mean ± SD being 2 270 ± 2 260 ng/g LW. Values of total HBCDs ranged between 390 and 12 100 ng/g LW, with mean ± SD being 4 500 ± 3 000 ng/g LW. Median concentrations for both BFRs (1 550 ng/g LW and 3 440 ng/g LW for PBDEs and HBCDs, respectively) differed only slightly from average values. Concentrations of BFRs at L1 and L2 were negligible in comparison to L3-L7, both for sum PBDEs, as for total HBCDs (L1: 40 and 70 ng/g LW, L2: 100 and 150 ng/g LW, respectively). In most samples, HBCDs were found at higher levels than PBDEs (Figure 3.2A), suggesting a high density of industrial activities which use HBCDs as FR. It should be emphasised that also distant locations L6 and L7 show high levels of HBCDs and this underlines the impact of industrialised areas on the aquatic system, both at local and regional scale.

Although PBDE and HBCD levels are on the higher end of the scale considering values reported in previous studies (see further), reported PCB levels (16 000 ± 14 300 ng/g LW, range 3 900 - 66 600 ng/g LW) were also very high. This might imply higher persistency of PCBs combined with higher pollution degree from past activities in the area.

b) Interspecies variation in PBDE and PCB profiles

In carp, eel and perch, the following PBDE congeners contributed most to the sum of PBDEs in descending order: BDE 47 > BDE 100 > BDE 99 ~ BDE 49 (Figure 3.4). The observed profile is similar to the composition observed in fish samples collected from around the world and points to the former use of the Penta-BDE formulation as a contamination source to these food webs (Luross *et al*., 2002). Eel samples, together with perch, contained high percentages of BDE 47 (~ 60%) and BDE 100 (~ 15%), together with lower percentages of BDE 99 (~ 6%) than would be expected (Ashley *et al*., 2007). Lepom *et al*. (2003) also reported 5–10 times higher BDE 100 than BDE 99 in pike-perch, bream, and eel from the Elbe river, Germany. Carp had even a lower contribution of BDE 99 to the sum PBDEs and higher

percentages of BDE 47 (Figure 3.4). PBDE patterns seem to be strongly influenced by species dependent metabolism (Ashley *et al*., 2007) and seem to be less related with sampling location. Carp is known for its capacity to metabolise BDE 99 to lower brominated BDE congeners, such as BDE 47 (Stapleton *et al*., 2004a,b; Hakk *et al*., 2003). The same can be seen for eel though to a lesser extent (Ashley *et al*., 2007).

PCB 153 was the most dominant congener, accounting for 12 % of the sum PCBs, closely followed by PCB 138 (11 %) and PCB 149 (7 %). The spatial contamination pattern was comparable with PBDEs. L1 and L2 were the least contaminated sampling sites, whereas L6 contained the highest PCB level (Figure 3.3). PCB levels accounted for the majority of the contamination.

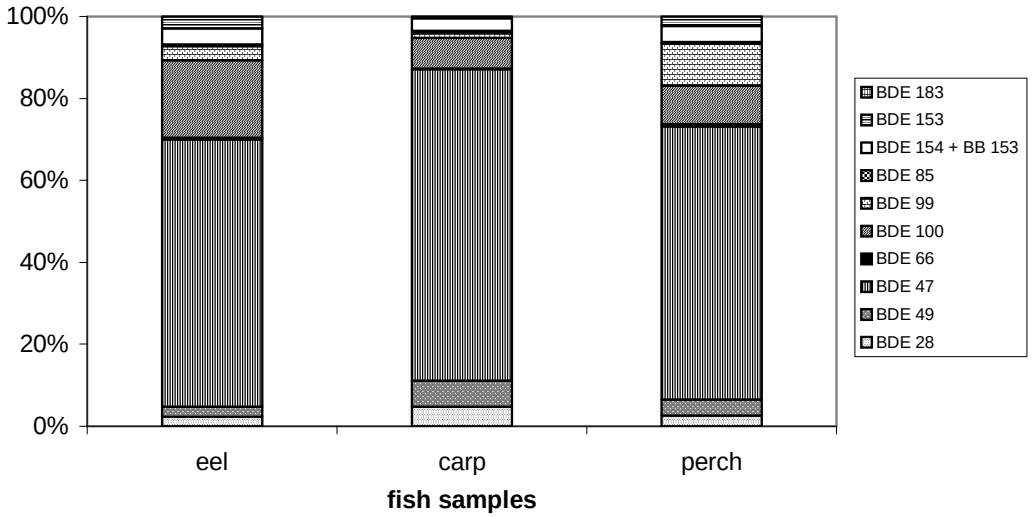


Figure 3.4. Average PBDE congener profiles in eel, carp and perch samples.

c) Eel samples

The sum PBDEs in the analysed eel samples from L4-L7 ranged between 660 and 1 010 ng/g LW (mean ± SD = 830 ± 150 ng/g LW), while total HBCD values were higher and ranged between 2 600 and 10 100 ng/ LW (mean ± SD = 7 900 ± 3 100 ng/g LW). PBDEs could be measured in every sample (Figure 3.2B). Concentrations of BFRs measured at L1 and L2 (reference area) were negligible in comparison to the other locations. When concentrating exclusively on eel data, differences in the contamination pattern can be seen. The HBCD levels in eels are higher than for the combined species, while PBDE levels seem to be lower. This probably suggests that the chosen locations are indeed more contaminated with HBCDs and that eel, as a sedentary species, is a good indicator of local pollution in comparison to other species, which have a more migratory lifestyle. Moreover, congener-specific differences in the uptake and biotransformation of PBDEs, together with a higher lipid content of eels may be responsible for the observed dissimilarities.

The sum PCBs in eel samples from locations L4-L7 ranged between 4 600 - 12 000 ng/g LW, with mean ± SD being 8 000 ± 2 700 ng/g LW. The spatial contamination pattern was comparable with PBDEs.

Temporal variation

The present study was initiated by the results of a previous survey by de Boer *et al*. (2002) reporting by GC-MS very high HBCD and PBDE concentrations (33 000 and 30 000 ng/g LW, respectively) in eels collected in 2000 from the river Scheldt at location L5. Strangely, the reanalysis of these samples by LC-MS indicate lower total HBCD levels of 266 ng/g LW (Morris *et al*., 2004). No obvious reasons regarding the analytical methods could explain this large difference in concentrations.

For the present study, a pooled sample from 3 individual eel samples originating from the same location as in 2000 was prepared once more and analysed. The HBCD and PBDE concentrations by GC-ECNI/MS were 35 000 and 26 500 ng/g LW, confirming thus the findings of de Boer *et al*. (2002). The concentrations obtained in pooled eel from 2000 are higher than the levels in the pooled eel samples from location L5 samples in 2006 (mean PBDEs: 780 ng/g LW, mean HBCDs: 10 000 ng/g LW).

An overall descending trend in the contamination with BFRs was observed from 2000 to 2006. For PBDEs, levels have decreased by a factor 35 (26 500 to 780 ng/g LW), whereas for HBCDs, the decrease was less evident, (35 000 to 10 000 ng/g LW). Note that also muscle fat content decreased considerably (See also Chapter 6). Based on these results we can conclude that fish living in this area seem to be less exposed to PBDEs than 6 years ago. This is probably due to the restriction regarding the use of the Penta-BDE technical mixture (since 2004), a better environmental management and a raising awareness concerning PBDEs. However, since there are no restrictions regarding its usage, HBCD can still be detected in large quantities, especially in aquatic environmental samples taken next to industrialized areas, where it is used in specific applications. The slight decrease in the concentrations of HBCDs in eels observed between 2000 and 2006 might indicate that HBCD is slowly being replaced by other (brominated) flame retardants for which no risk assessment is available.

Table 3.2. Concentrations of total HBCDs (ng/g wet weight) in 6 eel samples from 2000 and 2006. Each sample has been analysed using 3 GC-MS and 2 LC-MS different procedures.

Year	Location	Lipids (%)	Total HBCDs (ng/g WW)				
			Method 2 GC-MS	Method 3 GC-MS	Method 4 GC-MS	Method 5 LC-MS	Method 6 LC-MS-MS
			ECNI (79)	EI (M-Br)	ECNI (M-Br)	ion trap	triple quad.
2006	L4	13.9	360	470	420	500	610
2006	L5-pool 1	8.6	1050	640	570	710	510
2006	L5-pool 2	19.0	1420	1190	1130	1180	1090
2006	L6	15.4	1320	1160	1060	1150	890
2006	L7	14.3	900	670	620	940	440
2000	L5	24.0	8400	6900	7220	8140	7770

GC–MS vs. LC–MS

To underline the quality of the presented data, reported HBCD concentrations obtained by GC-MS (methods 2 - 4) were confirmed by LC-MS (methods 5 and 6). An overview of the obtained concentrations is given in Table 3.2. A high degree of comparability can be seen between the results issued with various methods, thus increasing the confidence in the results present in this study. The GC methods do not allow individual isomer data, but they give a very good estimation of the total HBCD concentrations. Unfortunately, for GC–MS measurements, there was tremendous loss in sensitivity (~50 times less sensitive) when specific ions *m/z* = 561 and 573, for native and ^{13}C-labelled HBCD, respectively, were used instead of *m/z* = 79 and 81. The use of ion *m/z* 561 corresponding to the ion [M-Br]$^-$ enhances the method selectivity and results in a better structural confirmation of HBCD. However, this enhancement in method selectivity is accompanied by a decrease in sensitivity as the peak at *m/z* 561 is much less intense than the "traditionally" monitored peak at *m/z* 79. Therefore, GC–MS measurements with ^{13}C-α-HBCD as internal standard could only be performed in a limited set of eel samples containing high concentrations of HBCDs. However, since most fish samples had low HBCD concentrations (due to low lipid contents), previous discussion of levels and profiles was based on results issued with method 2.

In contrast to GC, no degradation was observed for the native or ^{13}C-labelled HBCD standards when LC was used, because the analytes are not subjected to high temperatures throughout the analysis. Moreover, the LC–MS methods allow the separation of individual HBCD isomers (Figure 3.5), while the use of ^{13}C-α-HBCD improved greatly the measurements. The two LC-MS methods were similar, yet the ion-trap method was less sensitive and therefore could be applied only in samples with high concentrations (such as the analysed eel samples).

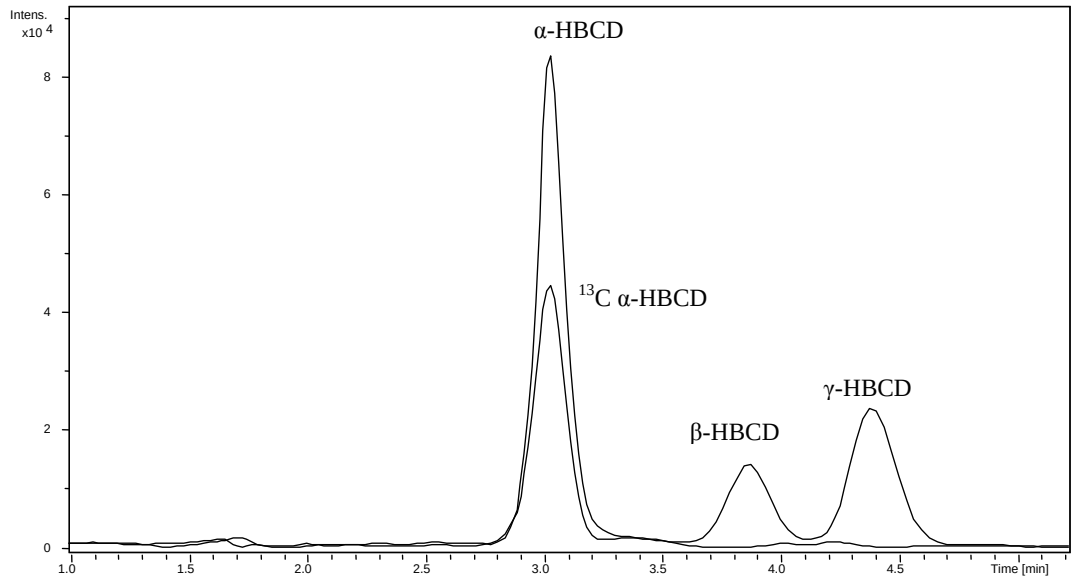


Figure 3.5. Typical LC–MS chromatogram for an eel sample from location L5.

Comparison with other studies

To compare our eel data with levels reported in other studies, only the predominant congeners, BDE 47 and PCB 153, were further discussed (Table 3.3).

Table 3.3. Mean (or median) concentrations of BDE 47 and PCB 153 in eel samples from various studies. When available, standard deviation or ranges are also given.

Country	Location	BDE 47 (ng/g WW)	Reference
Belgium	Reference location (L1)	1.56 (0)	Present study
	Oudenaarde (L5)	76.4 (15)	
Belgium	Kanaal Ieper - Yzer	2.59 (0.007)	Covaci *et al*. 2005
	Oude Maas	1.58 (0.9)	
	Zuun	1.26 (0.07)	
	Watersportbaan	10.08 (10.08)	
Japan	Inland sea of Seto	0.067 - 0.12	Akutsu *et al*., 2001
France	Loire	0.13 - 0.57	Bragigand *et al*., 2006
	Seine	2.67 - 7.84	
Country	**Location**	**PCB 153 (ng/g WW)**	**Reference**
Belgium	Reference location (L1)	6.9 (0)	Present study
	Oudenaarde (L5)	191.8 (46.9)	
Belgium	Flanders (1994-2005, n=2526) [1]	211.9 (430.3)	Maes *et al*. 2008
Belgium	Flanders (1994-2001, n=261) [2]	166.3 (1.8 - 2818)	Goemans *et al*. 2003
Italy	Adriatic sea	18.6 (2.9)	Storelli *et al*. 2007
Spain	River Turia	1.23 - 16.1	Borajandi *et al*. 2003
Germany	Berlin	202.9 (147.1)	Fromme *et al*. 1999

[1]: means of individual eels; [2]: means of mean concentration of all eels per location

Covaci *et al*. (2005) reported levels and distribution of PBDEs in eel samples originating from Flanders, Belgium. Eel liver samples were collected from 4 different locations (1 canal and 3 ponds) of which 3 locations seemed to be less contaminated with BDE 47 (levels between 1.3 – 2.6 ng/g wet weight (WW)), whereas one location had an average concentration of 10 ng/g WW. The levels reported by Covaci *et al*. (2005) are in the same range as the reference areas (L1 and L2) in the present study. Concentrations of BDE 47 in samples taken in the vicinity of Oudenaarde (mean 76 ng/g WW at L5) are one order of magnitude higher than reported elsewhere in Flanders. Akutsu *et al*. (2001) analysed eel samples collected from the inland sea of Seto, Japan. BDE 47 was the most abundant congener with levels between 0.07 – 0.12 ng/g WW. This is 10 times lower than levels reported for our reference areas L1 and L2 and several orders of magnitude lower than L3 to L7. Bragigand *et al*. (2006) monitored PBDE levels in aquatic food webs from French estuaries. Eel samples were collected in the Loire and the Seine and BDE 47 ranged between 0.13 – 0.57 and 2.7 – 7.8 ng/g WW, respectively. The levels are also much lower than the levels reported in the present study.

A recent study was performed by Ashley *et al*. (2007) on American eel species (*Anguilla rostrata*) originating from the river Delaware (USA). In total 17 eel homogenates were analysed for 27 PBDE congeners. Total PBDE concentration ranged between 1.2 and 157 ng/g WW, with two outliers of 373 and 408 ng/g WW. Concentrations of PBDEs in the river Delaware exceeded the values found in the river Scheldt, but, similar to the present study, the PBDE values were consistently an order of magnitude lower than the PCB levels.

Results from the Flemish Eel Pollutant Monitoring Network focusing mainly on PCBs and organochlorine pesticides in eels were reported by Goemans *et al*. (2003) and Maes *et al*. (2008). Goemans *et al*. (2003) reported a mean PCB 153

concentration for Flanders (1994-2001) of 166 ng/g WW. Maes *et al*. (2008) considered all eels caught and analysed in Flanders over the period 1994 to 2005. The mean PCB 153 concentration for these eels was 212 ng/g WW. Both papers show mean concentrations of PCB 153 which are much higher than our reference area (6.9 ng/g WW), but similar to the industrialized sampling locations around Oudenaarde (192 ng/g WW).

PCBs were measured in eels from the Adriatic Sea by Storelli *et al*. (2007). PCB 153 was present at 18.6 ng/g WW, higher than our reference area, but much lower than industrialized sampling locations around Oudenaarde. Bordajandi *et al*. (2003) analysed European eel from the river Turia in Spain. The PCB 153 level reported (5.9 ng/g WW) was on the low end of the results in the present study. Eel samples collected in Berlin showed average PCB 153 levels of 202 ng/g WW (Fromme *et al*. 1999). High standard deviations were due to discrimination in eel samples from Western and Eastern Berlin, resulting from the historic division of Berlin. In West Berlin, PCBs were extensively used in the past, but in the Eastern areas of the city (the former GDR) their use was limited. As a consequence, eel samples from West Berlin had higher PCB loads than those from East Berlin.

Influence of the consumption of contaminated fish on human exposure

As seen in the previous section, the concentrations of BFRs and PCBs in eel samples vary considerably according to the waters where they were collected. Since fish is an important part of human diet, the consumption of contaminated fish can lead to an unwanted increase in the body burden for the contaminants in cause.

To investigate which food items, including fish, influence human dietary intake significantly, intake of various food products in g/day were extracted from the literature (Voorspoels *et al*., 2007; Voorspoels *et al*., 2008). The mean dietary intake of PBDEs and PCBs from these different food groups were calculated by multiplying the average theoretical daily consumption of each category with the corresponding concentrations. Results are presented in Table 3.4.

The present study revealed a wide concentration range of both BFRs and PCBs in eel samples collected from the river Scheldt and with this, it has raised the question if the consumption of contaminated eel has an important impact on human exposure. Therefore, exposure profiles to PBDEs, PCBs and HBCDs through eel consumption originating from L1 (less contaminated) and L5 (most contaminated location) were calculated (Table 3.4).

Assuming that an adult consumes a daily average of 2.9 g eel (Bilau *et al*., 2007), he would be exposed to 2.5 ng PBDE/day if this fish originates from L1, whereas he would be exposed to 330 ng PBDE/day if the fish originates from L5 (130-fold difference). The same calculation can be made for HBCDs (L1: 3.2 vs. L5: 4 350 ng/day) and PCBs (L1: 119 vs. L5: 3 600 ng/day). Note that when eating the same amount of eel from the reference location L1, an average adult would be substantially more contaminated with PCBs, while eating eel from L5 would lead to a higher contamination with HBCDs. Acceptable daily intakes (ADI) have been set only for PCBs at 20 ng/kg body weight/day (WHO 2003). For an adult of 60 kg, the ADI is thus 1200 ng/day. In our case, only eel from L1 is approved for consumption, whereas eel from L5 exceeds this recommendation by a factor of three.

Keeping in mind that only average intakes were taken here into account, one could imagine extremely high levels, exceeding the reference value for risk groups, such as local anglers. Bilau *et al*. (2007) reported also consumption information for these risk groups. Two different scenarios were assumed: group A consists of fishermen who always take their catch home and eat all of it (86 g/day) and group B includes anglers who sometimes take their catch home and eat half of it (12 g/day).

Both groups eat considerably more eel than the average population (2.9 g eel/day). Fishermen A are therefore exposed to 72 or 9 800 ng PBDE/day if fish originates from L1 or L5, respectively and fishermen B to 10 or 1 360 ng PBDE/day, respectively. The same calculation can be made for HBCDs where fishermen A are exposed to 94 (L1) vs. 127 500 ng/day (L5) and fishermen B to 13 (L1) versus 18 000 ng/day. For PCBs, values exceeded the ADI for fishermen A at both locations (3 500 versus 107 000 ng/day) and for fishermen B only at L5 (14 900 ng/day). Results are summarized in Table 3.4.

Average daily consumption of freshwater fish, such as trout, pike and perch, from the lake Mjøsa (Norway), highly contaminated with PBDEs by the local industry, was around 25 g for local anglers. The mean sum PBDEs consumption was calculated as 47 ng/kg body weight/day (for an adult of 60 kg) (Thomsen *et al*., 2008). Assuming that our fishermen eat identical amounts of eel, PBDE intake resulting from fish consumption are very comparable (2 840 ng/day for eel from Oudenaarde and 2 820 ng/day for freshwater fish from lake Mjøsa).

Table 3.4. Estimated dietary intake from different food groups (ng/day) in Belgium. PBDE and PCB daily dietary intake were taken from Voorspoels *et al.* (2007) and Voorspoels *et al.* (2008), respectively.

	Estimated daily consumption[1] (g)	**PBDE intake (ng/day)[2]**	**HBCD intake (ng/day)**	**PCB intake (ng/day)[2]**
Fish and seafood	30	14	n.a.	220
Meat products	150	15	n.a.	130
Cheese	30	6.5	n.a.	83
Eggs	30	5.1	n.a.	51
Butter	5	4.1	n.a.	22
Fast food	20	2.4	n.a.	37
Total		**48**	**n.a.**	**540**
Normal consumers				
Eel location 1	2.9	2.5	3.2	119
Eel location 5	2.9	**330**	**4 350**	**3 600**
Fishermen A				
Eel location 1	86	72	94	3 500
Eel location 5	86	**9 800**	**127 500**	**107 000**
Fishermen B				
Eel location 1	12	10	13	493
Eel location 5	12	**1 360**	**18 000**	**14 900**

[1] - as taken from reference Voorspoels *et al*., 2007; [2] - upper bound intake (not detected substituted with LOQ); n.a. - not available

Conclusions

The textile industry is likely the cause of elevated BFR levels in fish from Oudenaarde on the river Scheldt. However, other sources, such as improper wastewater treatment, cannot be excluded. At all locations, HBCD had a higher contribution than PBDEs to the BFR contamination levels. Comparing these data with the same region 6 years ago, levels have decreased, but still remained higher than other locations in Flanders. Several European studies reported PBDE levels which were at least one order of magnitude lower. This is reflected in a high contribution of contaminated fish to the total dietary intake of PBDEs of the local anglers.

Contributions to the dietary intake were in the same order of magnitude as for the highly contaminated lake Mjøsa in Norway. For obvious reasons, stakeholders (fish stock managers and human health protectors) should avoid fish consumption of this part of the Scheldt with all legal and practical means. Further studies should be set up to determine how far this contaminated area extends over the whole river.

Acknowledgements

Dr. Adrian Covaci acknowledges the financial support granted by a postdoctoral fellowship by Flanders Scientific Funds for Research (FWO). We would also like to thank all the workers in the field for collecting the fish and co-workers at INBO for the preparation of the samples. Mohamed Abdallah and Dr. Stuart Harrad (University of Birmingham, UK) are acknowledged for the confirmatory analyses of HBCDs by LC-triple quadrupole MS/MS.

References

Abdallah, M., Harrad, S., Ibarra, C., Diamond, M., Melymuk, L., Robson, M., Covaci A., 2008. Hexabromocyclododecanes in indoor dust from Canada, United Kingdom and the United States**.** *Environmental Science & Technology* 42, 459-464.

Akutsu, K., Obana, H., Okihashi, M., Kitaqawa, M., Nakazawa, H., Matsuki, Y., Makino, T., Oda, H., Hori, S., 2001. GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan. *Chemosphere* 44, 1325-1331.

Ashley, J.T.F., Libero, D., Halscheid, E., Zaoudeh, L., Stapleton, H.M., 2007 Polybrominated diphenyl ethers in American Eels (*Anguilla rostrata*) from the Delaware River, USA. *Bulletin of Environmental Contamination and Toxicology* 79, 99-103.

Baeyens, W., Leermakers, M., Elskens, M., Van Larebeke, N., De Bont, R., Vanderperren, H., Fontaine, A., Degroodt, J.M., Goeyens, L., Hanot, V., Windal, I., 2007. PCBs and PCDD/Fs in fish and fish Products and their impact on the human body burden in Belgium. *Archives of Environmental Contamination and Toxicology* 52, 563-571.

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., Goemans, G., de Boer, J., Van Hooste, H., 2003. Distribution of brominated flame retardants. MIRA-T 2003, 387-394.

Belpaire, C., Goemans, G., 2007a. Eels: contaminant cocktails pinpointing environmental pollution. *ICES Journal of Marine Science* 64 (7), 1423-1436.

Belpaire, C., Goemans, G., 2007b. The European eel (*Anguilla anguilla*) a rapporteur of the chemical status for the Water Framework Directive? *Vie et Milieu- Life and Environment* 57 (4), 235-252.

Bilau, M., Sioen, I., Matthys, C., De Vocht, A., Goemans, G., Belpaire, C., Willems, J.L., De Henauw, S., 2007. Probabilistic approach to polychlorinated biphenyl (PCB) exposure through eel consumption in recreational fishermen vs. the general population. *Food Additives and Contaminants* 24, 1386-1393.

Birnbaum, L.S., Staskal, D.F., 2004. Brominated flame retardants: Cause of concern? *Environmental Health Perspectives* 112, 9-17.

Bordajandi, L.R., Gomez, G., Fernandez, M.A., Abad, E., Rivera, J., Gonzalez, M.J., 2003. Study on PCBs, PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the River Turia (Spain). *Chemosphere* 53,163-171.

Bragigand, V., Amiard-Triquet, C., Parlier, E., Boury, P., Marchand, P., El Hourch, M., 2006. Influence of biological and ecological factors on the bioaccumulation of polybrominated diphenyl ethers in aquatic food webs from French estuaries. *Science of the Total Environment* 368, 615-626.

Bromine Science and Environmental Forum (BSEF), 2007. http://www.bsef.com (accessed December 2007)

Covaci, A., Bervoets, L., Hoff, B., Voorspoels, S., Voets, J., Van Campenhout, K., Blust, R., Schepens, P., 2005. Polybrominated diphenyl ethers (PBDEs) in freshwater mussels and fish from Flanders, Belgium. *Journal of Environmental Monitoring* 7, 132-136.

Covaci, A., Gerecke, A., Law, R.J., Voorspoels, S., Kohler, M., Leslie, L., Allchin, C.R., de Boer, J., 2006. HBCD in the environment and humans: a review. *Environmental Science & Technology* 40, 3679-3688.

Darnerud, P.O., Eriksen, G.S., Johannesson, T., Larsen, P.B., Viluksela, M., 2001. Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. *Environmental Health Perspectives* 109, 49-68.

Darnerud, P.O., Atuma, S., Aune, M., Bjerselius, R., Glynn, A., Petersson, K.G., Becker, W., 2006. Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. *Food and Chemical Toxicology* 44, 1597-1606.

Directive 2003/11/EC of the European Parliament and of the Council of 6 February 2003 amending for the 24th time Council directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether and octabromodiphenyl ether). Official Journal L 042, 15/02/2003.

de Boer, J., Allchin, C., Zegers, B., Boon, J.P., Brandsma, S.H., Morris, S., Kruijt, A.W., van der Veen, I., van Hesselingen, J.M., Haftka, J.J.H., 2002. HBCD and TBBP-A in sewage sludge, sediments and biota, including interlaboratory study. Report RIVO, C033/02.

Domingo, J.L., 2004. Human exposure to polybrominated diphenyl ethers through the diet. *Journal of Chromatography A* 1054, 321-326

Domingo, J.L., Bocio, A., 2007. Levels of PCDD/PCDFs and PCBs in edible marine species and human intake: A literature review. *Environment International* 33, 397-405.

Dörner, H., Benndorf, J., 2003. Piscivory by large eels on young-of-the-year fishes: its potential as a biomanipulation tool. *Journal of Fish Biology* 62, 491-494.

Fromme, H., Otto, T., Pilz, K., Neugebauer, F., 1999. Levels of synthetic musks; bromocyclene and PCBs in eel (*Anguilla anguilla*) and PCBs in sediment samples from some waters of Berlin/Germany. *Chemosphere* 39, 1723-1735.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollution monitoring network 1994-2001: polychlorinated biphenyls, organochlorine pesticides and heavy metals in eel]. Report of the Institute for Forestry and Game Management, IBW. Wb.V.R.2003.99., 169p.

Hakk, H., Letcher, R.J., 2003. Metabolism in the toxicokinetics and fate of brominated flame retardants-a review. *Environment International* 29, 801-828.

Haug, L.S., Thomsen, C., Liane, V.H., Becher, G., 2008. Comparison of GC and LC determinations of hexabromocyclododecane in biological samples – Results from two interlaboratory comparison studies. *Chemosphere*, in press.

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.

Lepom, P., Karasyova, T., Sawal, G., 2003. Polybrominated diphenyl ethers in liver and muscle tissue of freshwater fish from Germany. *Organohalogen Compounds* 61, 135-138.

Luross, J.M., Alaee, M., Sergeant, D.B., Cannon, C.M., Whittle, D.M., Solomon, K.R., Muir, D.C.G., 2002. Spatial distribution of polybrominated diphenyl ethers and polybrominated biphenyls in lake trout from the Laurentian Great Lakes. *Chemosphere* 46, 665-672.

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.

Morris, S., Allchin, C., Zegers, B., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P.G., van Leeuwen, S.P.J., de Boer, J., 2004. Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environmental Science & Technology* 38, 5497-5504.

Stapleton, H.M., Letcher, R.J., Li, J., Baker, J.E., 2004a. Dietary accumulation and metabolism of polybrominated diphenyl ethers by juvenile carp (Cyprinus carpio). *Environmental Toxicology and Chemistry* 23, 1939-1946.

Stapleton, H.M., Letcher, R.J., Baker, J.E., 2004b. Debromination of polybrominated diphenyl ether congeners BDE 99 and BDE 183 in the intestinal tract of the common carp (*Cyprinus carpio*). *Environmental Science & Technology* 38, 1054-1061.

Storelli, M.M., Barone, G., Garofalo, R., Marcotrigiano, G.O., 2007. Metals and organochlorine compounds in eel (*Anguilla anguilla*) from the Lesina lagoon, Adriatic Sea (Italy). *Food Chemistry* 100, 1337-1341.

Thomsen, C., Knutsen, H.K., Liane, V.H., Froshaug, M., Kvalem, H.E., Haugen, M., Meltzer, H.M., Alexander, J., Becher, G., 2008. Consumption of fish from a contaminated lake strongly affects the concentrations of polybrominated diphenyl ethers and hexabromocyclododecane in serum. *Molecular Nutrition and Food Research*, in press.

Voorspoels, S., Covaci, A., Schepens, P., 2003. Polybrominated diphenyl ethers in marine species from the Belgian North Sea and the Western Scheldt estuary: Levels, profiles, and distribution. *Environmental Science & Technology* 37, 4348-4357.

Voorspoels, S., Covaci, A., Maervoet, J., De Meester, I., Schepens, P., 2004. Levels and profiles of PCBs and OCPs in marine benthic species from the Belgian North Sea and the Western Scheldt estuary. *Marine Pollution Bulletin* 49, 393-404.

Voorspoels, S., Covaci, A., Neels, H., Schepens, P., 2007. Dietary PBDE intake: A market basket study in Belgium. *Environment International* 33, 93-97.

Voorspoels S, Covaci, A, Neels, H., 2008. PCB intake through the diet: Mass production vs. organically grown. *Environmental Toxicology and Pharmacology* 25, 179-182.

World Health Organization (WHO), 2003. Polychlorinated biphenyls: Human health aspects. Concise International Chemical Assessment Document 55. ISBN 92 4 153055 3. Available at: http://www.inchem.org/documents/cicads/cicads/cicad55.htm.

Eel fisheries regulation and consumption advice got the attention of the media.

Chapter 4

Volatile organic compounds in yellow eel

Patrick Roose[1], Gerlinde Van Thuyne[2], Claude Belpaire[2], Mark Raemaekers[3] and Udo Brinkman[4]

1 - Management Unit Mathematical Models of the North Sea, Royal Belgian Institute for Natural Sciences, 3e and 23e Linieregimentsplein, B-8400 Oostende, Belgium

2 - Institute for Forestry and Game Management, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium

3 **-** Sea Fisheries Department (CLO Gent), Ankerstraat 1, B-8400 Oostende, Belgium

4 - Free University, Department of Analytical Chemistry and Applied Spectroscopy, de Boelelaan, 1081 HV Amsterdam, The Netherlands

This chapter is published as:

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium).
Journal of Environmental Monitoring 5, 876-884

Summary

Twenty eel from various inland water bodies in Flanders (Belgium) were analysed for a total of 52 VOCs. The most prominent VOCs are the BTEX and a number of chlorinated compounds such as chloroform and tetrachloroethene. The observed levels could be linked to the major emission sources and the present study gives new evidence that combustion of fossil fuels is a major source of BTEX in the environment.

The concentration levels in eel seem to be a reflection of the actual concentrations in their environment. For fish from the same location similar patterns and concentrations were observed, and the concentrations agree with what can be expected from those of the water column. Generally speaking, the observed concentrations do not seem to pose a threat for organisms. More definite statements will, however, require a larger dataset.

The study suggests that yellow eel can possibly be used as a biomonitor or sentinel organism for VOCs.

Introduction

Volatile organic compounds (VOCs) are well-known atmospheric contaminants that are frequently determined in air, drinking water, fresh water, effluents and soils (Anderson *et al.*, 1991, Dewulf *et al.*, 1998; Sweet and Vermette, 1992). Most representatives of the group are important industrial compounds with a high annual production (OECD, 2001) which can be anywhere in the range from several hundred thousand tonnes for e.g. tetrachloromethane, to more than 10 billion tonnes for benzene (Howard, 1990; WHO, 1993). In Belgium, the emissions of the chlorinated hydrocarbons (CHCs) chloroform, 1,1,1-trichloroethane, and tri- and tetrachloroethene, exceed those of *e.g.* lead, lindane and atrazine (Anon, 1995). Moreover, benzene, toluene, ethylbenzene and the xylenes (BTEX) are important additives to unleaded gasoline and are present in crude oil. Several international organizations therefore regard VOCs as compounds with a high research priority (Ministerial Declaration of the 3rd International Conference on the Protection of the North Sea, 1990; Ministerial Declaration of the 4th International Conference on the Protection of the North Sea, 1995).

The low values of the logarithm of the octanol-water partition coefficients (log K_{ow}) of the VOCs, typically, 1-2, led to the general belief that bioconcentration should be considered insignificant (Howard, 1989; Howard, 1990). As a result, the presence of VOCs in organisms was studied by a limited number of research groups only and there are few recent findings in the literature (Roose and Brinkman, 2001). The considerable analytical problems associated with the determination of these compounds in environmental matrices, specifically in biota, can be regarded as another reason for the lack of information. It was somewhat surprising, therefore, that recent studies showed the general presence of a number of important VOCs in the tissue of marine organisms from different levels of the food chain (Roose and Brinkman, 2001). It was also found that the concentration levels in marine organisms were up to a thousand times higher than those in the surrounding water. The bioconcentration factors calculated from these data were generally higher than those reported in the literature. A possible explanation is the continuous exposure of organisms to low or even undetectable levels of these compounds in the water column. Determination in the water column alone is, therefore, insufficient.

Aquatic organisms can, and have been, used successfully to monitor contaminants in various ecosystems, especially when the concentrations of these compounds in the water column are extremely low (de Boer and Hagel, 1994). For an organism to become a potential biomonitor or sentinel organism, several criteria should be fulfilled. First and foremost, the organism should reflect the actual condition of the surrounding water column. This implies that it should show little or no migratory behaviour and that the species should commonly occur in the area under investigation. The yellow eel, *Anguilla anguilla* L.*,* appears to be a most adequate indicator organism for the pollution status of freshwater environments. Eels are benthic fish which have a widespread geographical distribution. They are carnivorous organisms that predate mainly on insect larvae, worms, crustaceae, snails, mussels and fish, in particular small bottom-dwelling species. Moreover, yellow eel has a high proportion of lipids in its body, which facilitates the accumulation of lipophilic contaminants. The accumulation is further promoted by the fact that no spawning occurs during the eels' stay in inland waters. Eel is also essentially sedentary and normally does not migrate (de Boer and Hagel, 1994). The same authors showed that yellow eel reflects rapid changes in the concentrations of organic contaminants in the surrounding water.

In this study, a limited number of eel, which have been sampled as part of a routine monitoring programme, were analysed by means of a previously developed method (Roose and Brinkman, 1998a) for their VOC content. The study is intended as a screening exercise to get an impression of the concentration levels of VOCs in yellow eel, the potential environmental hazard and the possibility of the future use of yellow eel as an indicator organism.

Materials and methods

Samples and sampling

Eels were sampled by means of either electrofishing along river banks, fyke fishing or seine netting. Samples were initially collected in the framework of the fish stock assessment programme of the Institute for Forestry and Game Management which aims at monitoring fish and the biotic integrity of riverine and lacustrine waters all over Flanders. The samples were subsequently analysed for their PCB, organochlorine pesticide and heavy metal content, in the framework of this study, for the presence of VOCs. An overview of the seventeen inland water stations is given in Table 4.1. The stations can be characterized as rivers (>10 m width, 3 stations), brooks (< 10 m width, 2 stations), canals (6 stations) and enclosed water bodies such as ponds (6 stations). They are located in rural as well as in densely populated industrial areas (Figure 4.1). Twenty eels were selected from the 30-70 cm size range (Table 4.1).

Samples were wrapped in aluminium foil and stored at -28°C in an airtight freezer located in a solvent-free area.

Lipids were measured by total lipid extraction following Bligh and Dyer (1959).

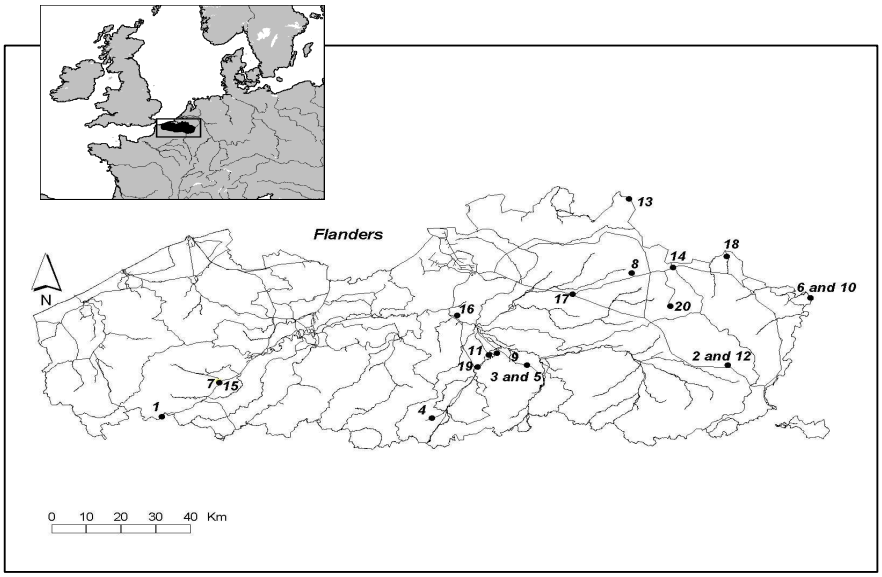


Figure 4.1. Sampling locations in the region of Flanders (Belgium) (Source: OC Gis Vlaanderen and AMINAL, Water Section; see also Table 4.1).

Table 4.1. Overview of sampling stations[1] and sampled eel.

No	Location	Type of water	Surroundings	River basin	Length (cm)	Weight (g)	Lipid content (%)
1	Leie, Menen	River	Industrial	Leie	65	467	33
2	Albertkanaal, Langerlo	Canal	Industrial	Demer	67	616	31
3	Kanaal Leuven-Dijle, Tildonk	Canal	Industrial	Dijle-Zenne	57	390	30
4	Groot Zuunbekken, St.-Pieters-Leeuw	Pond	Industrial	Dijle-Zenne	55	321	9
5	Kanaal Leuven-Dijle, Tildonk	Canal	Industrial	Dijle-Zenne	50	251	33
6	Grensmaas, Molensteen	River	Rural	Maas	67	601	26
7	Oude Leie Ooigem	Pond	Rural	Leie	62	411	24
8	Witte Nete, Dessel	River	Rural	Nete	52	281	16
9	Pond at Rijksdomein, Hofstade	Pond	Rural	Dijle-Zenne	65	625	29
10	Grensmaas, Molensteen	River	Rural	Maas	57	365	23
11	Zandwinningsput, Weerde	Pond	Industrial	Dijle-Zenne	60	385	25
12	Albertkanaal, Langerlo	Canal	Industrial	Demer	37	539	33
13	A, Poppel	Brook	Rural	Maas	45	177	16
14	Kanaal Bocholt-Herentals, Blekerheide	Canal	Industrial	Maas	51	262	30
15	Oude Leie, Wevelgem	Pond	Industrial	Leie	57	307	25
16	Putten van Niel, Niel	Pond	Industrial	Benedenschelde	45	181	20
17	Kanaal Bocholt-Herentals, Sluis Herentals	Canal	Industrial	Nete	50	262	24
18	Warmbeek, Achel	Brook	Rural	Maas	53	277	16
19	Darse, Vilvoorde	Canal	Industrial	Dijle-Zenne	47	191	31
20	Kanaal Beverlo, Leopoldsburg	Canal	Industrial	Nete	59	321	21

[1] Also see Figure 4.1

Analytical methodology

A detailed description of the analytical methodology is given elsewhere (Roose and Brinkman, 1998a; Roose and Brinkman, 1998b). Briefly, biological tissue is first homogenised at 0℃ in an ultra-turrax blender and transferred to a 40-ml vial. After addition of 25 ml of water and the internal standard (1,1,1-trifluorotoluene), the homogenate is treated for 20 min at 0℃ in an ultra sonic bath to further disrupt the tissue. The glass vessel is then connected to a Tekmar (Cincinnati, OH, USA) LSC 2000 purge-and-trap apparatus coupled to a Finnigan Magnum (Finnigan, San José, CA, USA) gas chromatograph-mass spectrometer (GC-MS). The volatiles are forced out of the tissue by purging with a stream of helium while heating at 70℃, and trapped onto a Vocarb 4000 sorbent trap (Supelco, Bellefonte, PA, USA). After purging, the trap is backflushed while being rapidly heated to 250℃, the analytes are desorbed and, next, trapped in a cryofocusing module (-120℃) connected to the GC column (J&W, Folsom, CA, USA, DB-VRX, 60 m, 0.25 mm id, 1.4 µm film).

The analytes were injected into the column by rapidly heating the module from -120℃ to 200℃ in 0.75 min. Temperature progr amming of the GC and data acquisition were started simultaneously. The temperature of the GC oven was held at 35℃ for 6 min and then linearly increased to 200℃ at 4℃/min. This temperature was then held for 4 min. Helium with an inlet pressure of 16 psi was used as the carrier gas. The ion-trap detector was operated in the electron ionisation (EI) mode with the multiplier voltage set at 2400 V, the axial modulation (A/M) amplitude at 3.5 V and the emission current at 12 µA. The manifold temperature was set at 220℃. The mass range was 50-250 amu and the scan rate, 1000 ms. The filament delay was

180 s, and a mass defect of 50 mmass / 100 amu and a background mass of 55 amu were selected.

VOC concentrations are expressed on a wet weight basis throughout the paper.

Results and discussion

VOC concentrations in eel

The twenty eel from the various inland water bodies were analysed for a total of 52 VOCs which are listed in Table 4.2. Compounds were identified on the basis of their mass spectrum and their concentrations were calculated by using at least two selected ion masses (exceptions: benzene and toluene). As an illustration, a full scan GC-MS chromatogram and a selected ion chromatogram for tetrachloroethene in eel sample No. 5 are shown in Figure 4.2. Detection limits (LODs) in the selected-ion mode for 40 g samples were calculated on the basis of a signal-to-noise ratio of 3 or 3 times the standard deviation of the blank. They varied between 0.01 ng/g wet weight (1,2-dichoroethane, 1,1-dichloroethane and tetrachloromethane) and 6 ng/g wet weight (trichlorobenzene) depending on the background levels and the amount of sample.

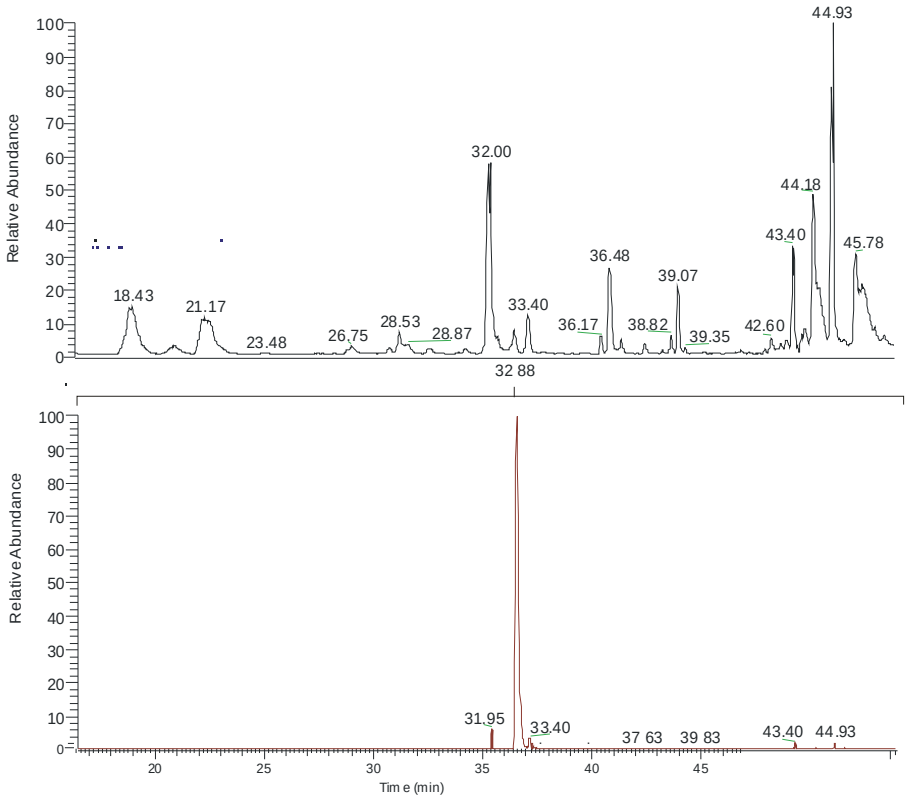


Figure 4.2. Total ion count GC-MS chromatogram for eel sample No. 5 and extracted ion chromatogram (*m/z* 164+165) for tetrachloroethene (bottom).

Table 4.2. Set of 52 VOCs studied and relevant analytical information

Sequence number	Compound	Masses[1] (*m/z*)	Retention time (min)	LOD[2] (ng/g)
1	*trans*-1,2-Dichloroethene	61/96/98	2:24	0.1
2	1,1-Dichloroethane	63/83/97	3:26	0.1
3	*cis*-1,2-Dichloroethene	61/96/98	6:04	0.1
4	2,2-Dichloropropane	77/79/97	7:14	0.1
5	Bromochloromethane	130/128/49	6:56	0.1
6	Chloroform	83/85	7:17	0.3
7	1,1,1-Trichloroethane	97/61/99	11:40	0.05
8	Tetrachloromethane	117/119	14:24	0.1
9	Dichloropropene	39/110/77	13:20	0.2
10	Benzene	78	15:04	0.2
11	1,2-Dichloroethane	62/64	11:12	0.01
12	Trichloroethene	130/95/60	20:34	0.5
13	1,2-Dichloropropane	62/63/76	19:45	0.2
14	Dibromomethane	174/172/93	19:57	0.5
15	Bromodichloromethane	83/85/47	20:53	0.4
I.S.	Trifluorotoluene[3]	146/127/96	23:00	-
16	*cis*-1,3-Dichloropropene	75/110/39	25:14	0.05
17	Toluene	91	29:22	0.4
18	*trans*-1,3-Dichloropropene	75/110/39	27:49	0.1
19	1,1,2-Trichloroethane	97/61/99	28:21	0.01
20	Tetrachloroethene	166/129/94	32:33	0.1
21	1,3-Dichloropropane	76/78/41	29:38	0.05
22	Dibromochloromethane	129/127/48	30:28	0.05
23	1,2-Dibromoethane	107/109/27	31:34	0.05
24	Chlorobenzene	112/114/77	35:30	0.1
25	1,1,1,2-T-etrachloroethane	131/133/95/122	35:14	0.02
26	Ethylbenzene	91/105/106	36:36	0.1
27	*m*-Xylene	91/105/106	37:30	0.2
28	*p*-Xylene	91/105/106	37:30	0.2
29	*o*-Xylene	91/105/106	39:02	0.2
30	Styrene	103/78/51	38:48	0.05
31	Bromoform	173/171/175	37:22	0.05
32	Isopropylbenzene	105/120/77	40:34	0.1
33	1,1,2,2-Tetrachloroethane	83/101/131	38:57	0.1
34	Bromobenzene	158/156/77	41:11	0.1
35	1,2,3-Trichloropropane	75/110/39	39:32	0.3
36	*n*-Propylbenzene	91/105/120	42:18	0.3
37	2-Chlorotoluene	91/126	42:28	0.1
38	1,3,5-Trimethylbenzene	105/120/77	43:35	0.1
39	4-Chlorotoluene	91/126	42:49	0.05
40	*tert*-Butylbenzene	91/119	44:31	0.05
41	1,2,4-Trimethylbenzene	105/77/120	45:01	0.3
42	*sec*-Butylbenzene	134/105	45:21	0.2
43	1,3-Dichlorobenzene	146/111/75	45:25	0.2
44	*p*-Isopropyltoluene	119/91/39	46:10	0.1
45	1,4-Dichlorobenzene	146/111/75	45:41	0.1
46	*n*-Butylbenzene	91/134	47:42	- [4]
47	1,2-Dichlorobenzene	146/111/75	46:58	0.05
48	1,2-Dibromo-3-chloropropane	157/75/57	48:44	0.05
49	1,2,4-Trichlorobenzene	180/145/109	53:55	6
50	Hexachlorobutadiene	260/225/190	55:10	0.4
51	Naphthalene	128/102	54:48	4
52	1,2,3-Trichlorobenzene	180/145/109	55:33	6

[1] In order of relative abundance, [2] For a 40-g sample with extracted ions, [3] Internal standard, [4] not determined

All relevant data are presented in Table 4.3. The results show that about half of the target VOCs, *i.e.* 25 out of 52, were detected in one or more eel samples. A detailed breakdown of the results is presented in Figure 4.3 which shows the percentage of samples that was positive for a given VOC. One striking observation is that the BTEX compounds were present in all samples. A further five compounds, chlorobenzene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, naphthalene and chloroform, were present in 70-90% of all samples, and a 35-60% positive score was obtained for nine VOCs, 1,3,5-trimethylbenzene, isopropylbenzene, tetrachloroethene, 1,2,4-trimethylbenzene, 1,2-dichlorobenzene, hexachlorobutadiene, 1,2-dichloroethane, *p*-isopropyltoluene and 1,2,3 trichlorobenzene. The other VOCs were found in 20% of the samples or less.

Table 4.3. Concentrations (ng/g WW) of VOCs detected in freshwater eel [1]

VOC	Sampling stations[2]																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Chloroform	15	9.4	17	96	30	2.9	3.9	-	-	11	9.7	7.4	1.0	-	-	10	16	-	13	23
1,1,1-Trichloroethane	2.2	-	-	-	-	-	-	-	-	-	-	0.5	-	1.5	-	-	0.7	-	-	-
Benzene	2.6	2.2	7.0	19	10	3.1	2.7	11	4.9	6.9	4.8	3.5	1.2	8.9	6.0	3.9	4.2	1.7	4.2	6.6
1,2-Dichloroethane	-	1.8	-	-	-	-	-	-	2.5	3.3	3.5	2.4	-	4.9	-	1.4	2.0	-	2.0	-
Toluene	10	5.2	33	73	47	7.4	6.7	41	13	20	13	12	1.9	22	11	11	11	3.7	8.5	30
Tetrachloroethene	64	11	42	1.5	89	2.0	-	-	-	3.6	-	18	-	31	6.2	-	-	-	-	-
Chlorobenzene	0.3	0.3	0.5	-	1.3	0.3	0.2	0.3	0.6	1.1	0.9	0.5	0.1	0.6	0.8	0.6	0.9	-	0.7	1.1
Ethylbenzene	5.7	5.7	13	21	36	7.9	4.9	10	15	30	20	14	1.2	18	12	12	24	5.8	13	29
m&p-Xylene	7.8	3.1	8.9	35	18	4.0	3.0	8.6	7.8	13	8.2	7.1	0.7	11	6.9	6.2	9.7	2.4	5.5	15
o-Xylene	5.9	2.2	6.6	40	12	2.9	2.1	9.2	4.3	7.1	4.5	4.8	0.6	8.3	4.7	4.1	5.8	1.6	3.6	11
Isopropylbenzene	0.5	0.2	0.5	1.2	0.5	0.2	-	-	-	-	-	-	-	0.7	0.5	0.8	0.5	-	0.4	-
n-Propylbenzene	-	-	-	5.0	-	-	-	-	-	-	-	-	-	-	-	1.0	2.8	-	-	-
1,3,5-Trimethylbenzene	7.9	5.4	9.3	13	-	1.2	-	-	-	-	-	-	-	3.6	6.9	1.7	2.5	0.7	1.6	3.9
1,2,4-Trimethylbenzene	8.8	3.4	4.6	74	-	-	-	-	-	-	-	-	-	7.1	14	6.7	9.0	3.3	5.4	-
1,3-Dichlorobenzene	5.1	7.7	18	-	-	1.2	7.9	17	11	11	8.3	8.4	3.9	18	18	17	10	5.8	8.4	21
p-Isopropyltoluene	-	-	-	-	-	-	-	-	-	-	1.7	1.7	1.0	-	2.5	0.9	2.7	1.5	1.7	36
1,4-Dichlorobenzene	6.9	3.7	4.6	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5	-	-	-
1,2-Dichlorobenzene	41	7.7	1.6	-	-	-	0.4	0.9	-	-	-	-	-	-	85	11	1.1	0.2	0.4	-
1,2-Dibromo-3-chloropropane	-	706	265	-	-	23	-	30	-	-	-	-	-	-	-	-	-	-	-	-
1,2,4-Trichlorobenzene	4.0	8.3	2.9	-	-	1.0	0.5	1.6	0.7	-	0.7	0.5	0.2	5.1	31	11	11	24	14	3.6
Hexachlorobutadiene	-	0.3	0.3	-	-	-	-	-	-	0.2	-	-	-	3.8	12	1.6	5.4	6.9	1.5	0.4
Naphthalene	1.9	3.5	2.9	-	63	1.6	-	3.3	1.9	4.0	2.0	-	-	3.1	2.7	1.7	2.3	1.5	1.9	2.0
1,2,3-Trichlorobenzene	-	3.3	-	-	-	-	-	-	-	-	-	-	-	-	6.2	5.8	1.7	5.4	10	2.3

[1] Values below LOD, as given in Table 4.2, are reported as "-".
[2] For locations, see Table 4.1.

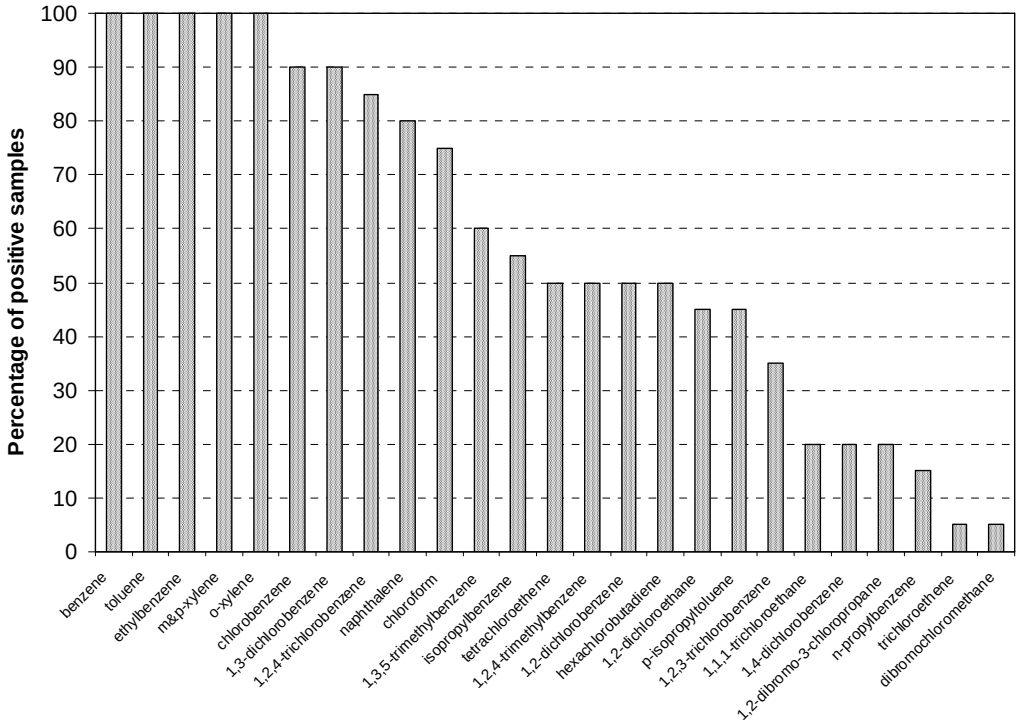


Figure 4.3. Percentage of positive samples for the detected VOCs in order of abundance.

The concentrations of the VOCs that were detected varied considerably, as is graphically illustrated by the box and whisker plot of Figure 4.4. The median concentrations typically were 1-10 ng/g, ranging from 0.5 ng/g for isopropylbenzene to 14 ng/g wet weight for tetrachloroethene. High concentrations of over 30 ng/g were found for twelve of the VOCs, with a staggering 700 ng/g wet weight for 1,2-dibromo-3-chloropropane in eel from the Albertkanaal, Langerlo, as the maximum. Extensive statistical testing, such as principal component analysis, seemed inappropriate because of the limited number of statistical cases. Nonetheless, a correlation analysis was performed for the concentrations of the reported VOCs. While no significant correlation was found for any of the other VOCs, the BTEX compounds were found to correlate extremely well with each other, with correlation coefficients of 0.77 and better (Table 4.4).

Table 4.4. Correlation matrix for BTEX compounds[1].

	Benzene	Toluene	Ethylbenzene	*m&p*-Xylene	*o*-Xylene
Benzene	1.00	0.96	0.77	0.90	0.92
Toluene	0.96	1.00	0.77	0.92	0.95
Ethylbenzene	0.77	0.77	1.00	0.90	0.80
m&p-Xylene	0.90	0.92	0.90	1.00	0.98
o-Xylene	0.92	0.95	0.80	0.98	1.00

[1] Reported coefficients are significant at $p<0.05$ (n=20)

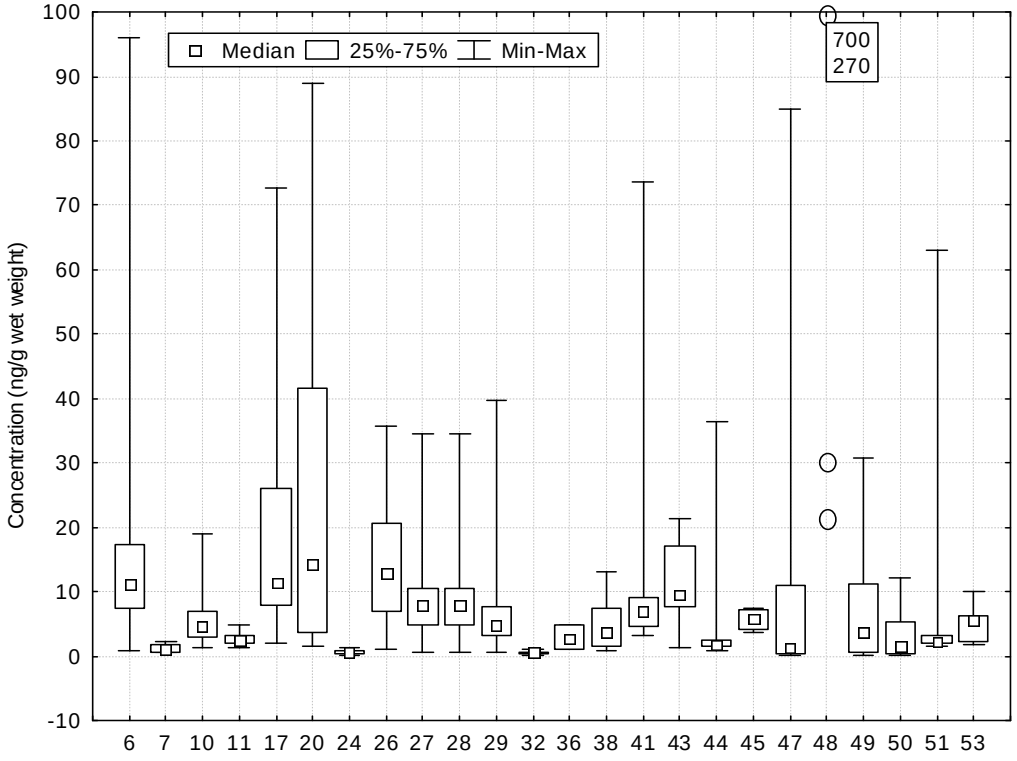


Figure 4.4. Box and whisker plot of the detected VOCs for all eel samples, with from left to right: (6) chloroform; (7) 1,1,1-trichloroethane; (10) benzene; (11) 1,2-dichloroethane; (17) toluene; (20) tetrachloroethene; (24) chlorobenzene; (26) ethylbenzene; (27) *m*-xylene; (28) *p*-xylene; (29) *o*-xylene; (32) isopropylbenzene; (36) *n*-propylbenzene; (38) 1,3,5-trimethylbenzene; (41) 1,2,4-trimethylbenzene; (43) 1,3-dichlorobenzene; (44) *p*-isopropyltoluene; (45) 1,4-dichlorobenzene; (47) 1,2-dichlorobenzene; (48) 1,2-dibromo-3-chloropropane; (49) 1,2,4-trichlorobenzene; (50) hexachlorobutadiene; (51) naphthalene; (53) 1,2,3-trichlorobenzene.

The fairly high concentrations found in this study do not come as a complete surprise: the general picture agrees with earlier observations which, actually, triggered this work. The earlier studies showed that various VOCs were present in both marine organisms and in eel from the Scheldt estuary (Roose and Brinkman, 1998a; Roose and Brinkman, 2001). In Figure 4.5 the concentration levels of a number of priority VOCs in marine organisms from the Belgian coastal water are compared with the results of this study. The concentrations of the chlorinated hydrocarbons (CHCs) are seen to be generally significantly lower in marine fish than in eel from inland waters. This is the case even for lipid-rich tissues such as the liver. Literature data on CHCs in eel are very limited. An exception is the overview by Howard (1989) which reports tetrachloroethene concentrations in American eel of 105–250 ng/g that are at least an order of magnitude higher than in marine organisms. This is similar to what is observed here. Especially for this analyte, the observed median concentrations are a lot higher in eel than in marine fish. Tetrachloroethene has a limited bioconcentration capacity and accumulation occurs

in the lipid-rich tissues of both man and animals (WHO, 1984). The higher observed levels in eel are therefore more than likely the result of a higher exposure of freshwater organisms to this compound. The same also seems to apply to the other CHCs, although to a lesser extent. The difference is probably related to differences in uptake and metabolisation rates and the lower bioconcentration capacity of the other CHCs.

In contrast to the CHCs, median concentration levels of BTEX in eel are more or less the same as those found in the liver of marine fish, with the exception of, perhaps, toluene. In contrast to CHCs, BTEX emissions are not solely related to industrial processes, i.e. local sources. BTEX were indeed found at all sampling locations and the variability of the data is somewhat less than for the other VOCs (Figure 4.4). BTEX are common constituents of diesel oil and many petrochemical products, and are emitted in the exhaust gases of combustion engines (Crookes *et al*., 1993; Howard, 1989; Howard, 1990). This fits well with the observed correlation between the BTEX compounds and is in line with our earlier observations on VOCs in marine organisms (Roose and Brinkman, 2001). In that study, the observed correlation for these compounds was related to this common source and it was suggested that the principal source of BTEX in marine organisms is the use of fossil fuel. Dewulf *et al.* (1998) observed higher levels of MAHs (monoaromatic hydrocarbons) than of CHCs in water and air samples from the same region and attributed this also to anthropogenic emissions from marine traffic in this coastal area. The same group also carried out an extended study of VOCs in the water column of the estuary of the Scheldt River and found similar results for BTEX in the water column (Dewulf *et al.*, 1998). These authors observed significant correlations between the various BTEX and a more uniform distribution of the concentrations throughout the estuary compared to CHCs. BTEX concentrations in this study were also of the same order of magnitude as in the marine environment, which was not the case for CHCs. These observations support the hypothesis that contamination by BTEX is of a rather diffuse nature which, in its turn, supports the conclusion that the use of fossil fuel in, e.g. traffic, is the major source of BTEX.

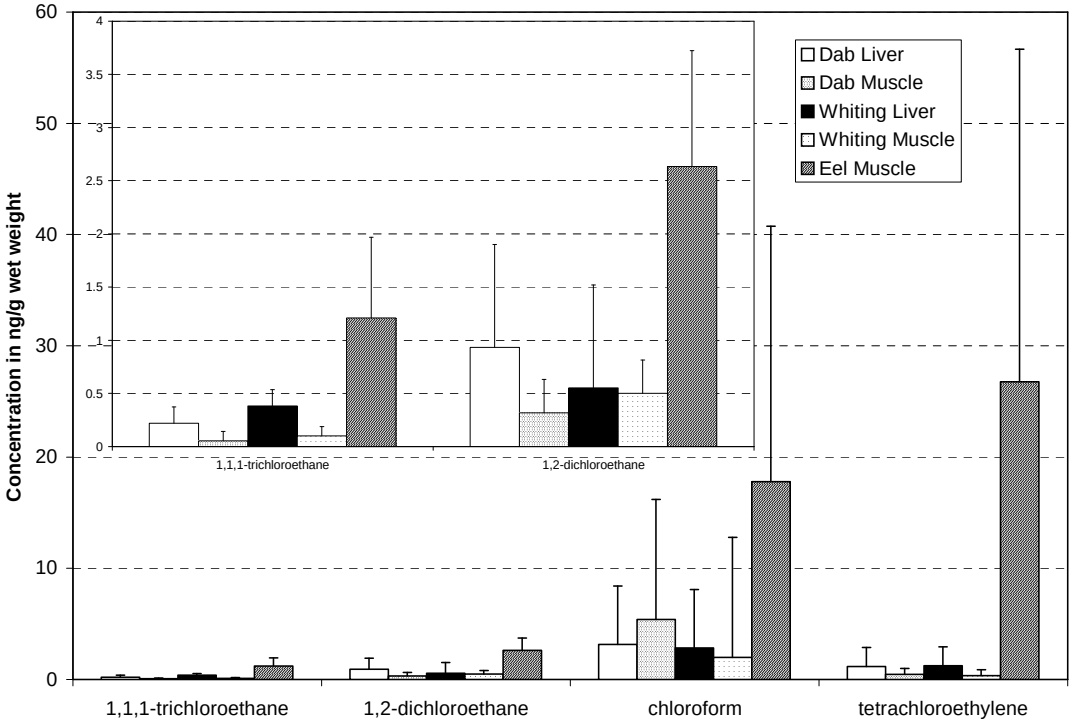


Figure 4.5a. Comparison of the concentrations of selected CHCs in tissues of marine species and freshwater eel.

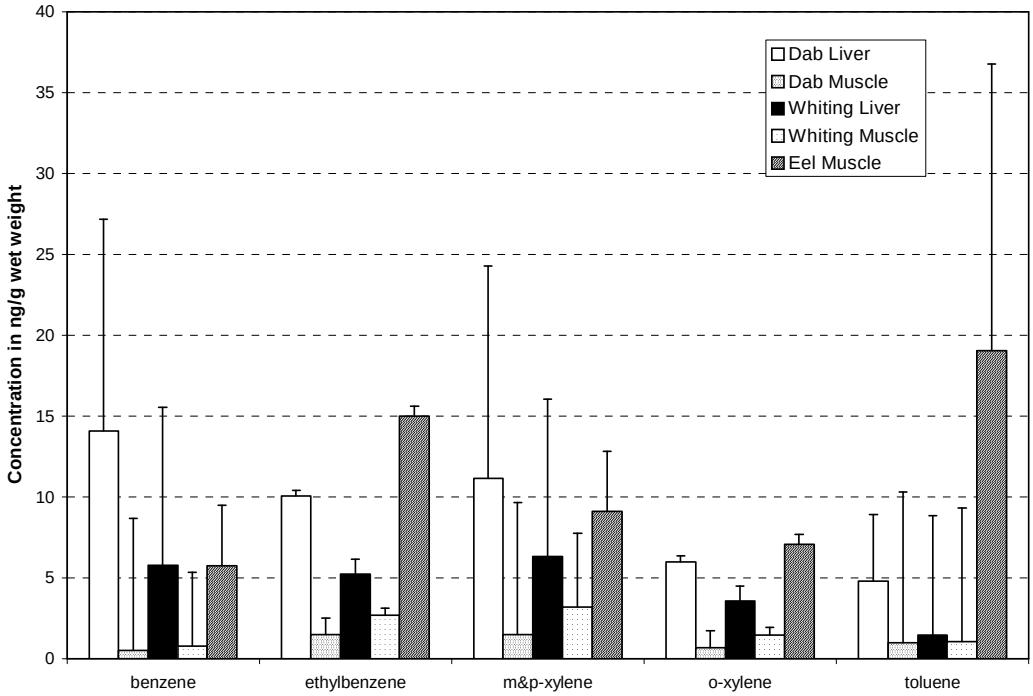


Figure 4.5b. Comparison of the concentrations of selected BTEX in tissues of marine species and freshwater eel.

Spatial distribution of VOCs and eel as a biomonitor

The current database is too limited to allow an analysis of the spatial distribution for all VOCs included in this study. Such a comparison is justified only for the most prominent VOCs. That is, the comparison was limited to chloroform and tetrachloroethene, and the BTEX compounds. The latter are considered as a group based on the correlation discussed above (Table 4.4), and are represented by their sum. Figure 4.6 gives an overview for the selected VOCs per sampling station and river basin. The patterns for eel collected at the same locations (Albertkanaal, Grensmaas, Kanaal Leuven-Dijle) are closely similar both with regard to the concentrations and their ratios. As regards the different river basins, the VOC concentrations in eel from highly industrialized and populated regions (Dijle-Zenne and Nete basins) are higher. This is especially true for BTEX. The high concentrations observed at the Groot-Zuunbekken station can possibly be explained by the fact that this is a pond in a densely populated and industrialized area, which is in the vicinity of a tributary of the Zenne River, the Zuunbeek, which is biologically dead. Probably, water form the brook entering the pond explains the observed results. Since there is little exchange with surrounding water masses, VOCs are lost probably only as a result of evaporation. As this is a dynamic process, it would indicate a constant high level of input into that water body. In marked contrast, eels from rural locations, such as the A at Poppel, have a significantly lower body burden.

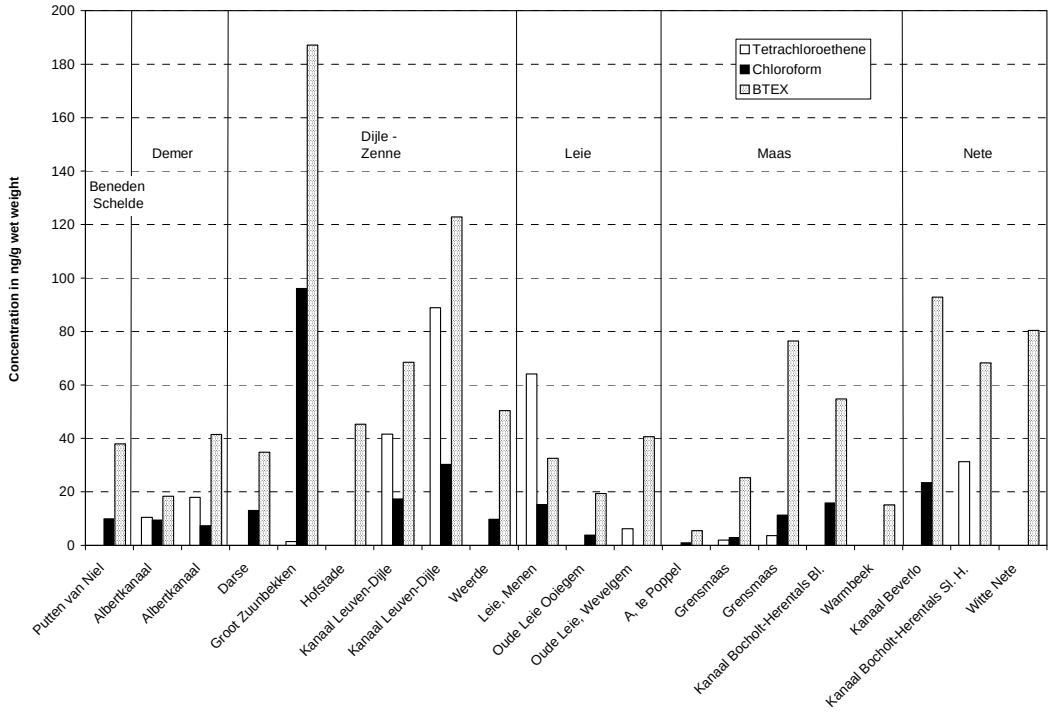


Figure 4.6. Comparison of the concentrations of tetrachloroethene, chloroform and ΣBTEX for the various sampling stations.

Recent data for the concentrations of the same VOCs as were studied here in the water columns of Flemish rivers show that these are generally below the LODs of the analytical techniques used, i.e. 0.05–2 µg/l. That is, they are below the current water-quality criteria of the Flemish government, which are set at a median value of 2 µg/l for total VOCs and 1 µg/l for each individual VOC (VMM, 2001; VMM, unpublished results). Not surprisingly, the VOCs that were detected in the water columns, are the same as the most prominent ones in this study and the highest concentrations are also found in the Dijle-Zenne basin. Taking into account that the bioconcentration factor (BCF), *viz.* the ratio of the concentrations of an analyte in the organism and the water, is between 1 and 90 for most VOCs (Freitag *et al.*, 1985; Howard, 1990; Isnard and Lambert, 1988), the concentration levels found in eel are not surprising. For instance, if the LODs of the BTEX compounds in water are taken as the actual concentrations (0.2-0.4 µg/l), concentrations of 20-40 ng/g would be expected in eel if an estimated log BCF of 2 is used (Howard, 1990). As can be observed from Figure 4.4, median values of approx. 10 ng/g were found for the various sampling stations in our study. This allows the conclusion that concentrations in eel indeed reflect the concentrations in the water column. Moreover, the – admittedly, limited – information presented above shows that eel samples from the same location have similar patterns and VOC concentration levels. There is evidence to assume that once contaminants are stored in the lipid, they will not be metabolised and become resident. Also because eel do not spawn during their stay in inland waters, the observed concentrations are valuable for time-trend analysis, and, because eel is essentially sedentary and normally does not migrate, concentration

data should allow the comparison of different river systems. An additional advantage is that yellow eel are known to reflect rapid changes in the concentrations of organic contaminants in the surrounding water (de Boer and Hagel, 1994). In summary, the yellow eel *Anguilla anguilla* L. can be considered as a potential biomonitor or sentinel organism for VOCs.

Hazard assessment

In a previous study, the observed concentration levels in the marine environment were compared with proposed safety levels. The approach used was based on quantitative structure–activity relationships (QSARs), extrapolation of toxicity data and equilibrium partitioning for the assessment of the effects of narcotic industrial pollutants (Van Leeuwen *et al.*, 1992). The extrapolation of toxicity data generated by QSARs was used to derive safe levels for water, sediment and biota. The model allows the calculation of internal toxic concentrations (ITCs) in fish tissue, which is useful for the interpretation of biomonitoring data. The safety level was arbitrarily set at 95%. This implies that a threshold concentration, the hazardous concentration HC5, is calculated which is unlikely to cause harm to more than 5% of the aquatic community. However, the usefulness of the model hinges on the applicability of the equilibrium-partitioning theory and its relation with octanol–water partitioning. The latter seemed certainly the case for marine species and there are no indications why it should not be true here. The observed levels were therefore tentatively compared with HC5 values calculated during the previous study.

Table 4.5 shows the HC5 values for some selected VOCs and their concentrations measured at the various sampling stations. The results show that in no case the HC5 is exceeded. Moreover, the experimentally determined concentrations are several orders of magnitude lower than the HC5. One may therefore assume that, in all likelihood, this is also true for those VOCs for which no HC5 data are available. On the other hand, one should note that the hazard assessment does not take into account synergistic and, thus, more damaging effects. To quote an example, the eel from Groot Zuunbekken, with the highest concentrations of VOCs, did have an abnormally low lipid content, *viz.* 9% compared to an average of 25%. Nevertheless, more definite statements regarding long-term effects cannot, as yet, be made because the dataset is far too small and the calculation of the HC5 is only one approach amongst several and needs to be further evaluated. That is, additional research, especially with regard to the long-term consequences of small doses of VOCs is urgently required and the use of eel as sentinel organisms for VOCs should be studied in more detail.

Table 4.5. Comparison between observed VOC concentrations (ng/g) and HC5 values (ng/g) calculated according to van Leeuwen *et al*. (1992)

Location	Concentrations (ng/g)							
	benzene	toluene	*p*-xylene	*o*-xylene	chloroform	tetra-chloro-ethene	1,2-di-chloro-ethane	1,1,1-tri-chloro-ethane
HC5:	**5200**	**5900**	**6400**	**6500**	**8100**	**9700**	**6700**	**8800**
Leie, Menen (1)	3	11	8	6	15	64	-	2
Albertkanaal, Langerlo (2)	2	5	3	2	9	11	2	-
Kanaal Leuven-Dijle, Tildonk (3)	7	33	9	7	17	42	-	-
Groot Zuunbekken, St.-Pieters-Leeuw (4)	19	73	35	40	96	2	-	-
Kanaal Leuven-Dijle, Tildonk (5)	10	47	18	12	30	89	-	-
Grensmaas, Molensteen (6)	3	7	4	3	3	2	-	-
Oude Leie Ooigem (7)	3	7	3	2	4	-	-	-
Witte Nete, Dessel (8)	11	41	9	9	-	-	-	-
Pond at Rijksdomein, Hofstade (9)	5	14	8	4	-	-	3	-
Grensmaas, Molensteen (10)	7	20	13	7	11	4	3	-
Zandwinningsput, Weerde (11)	5	13	8	5	10	-	4	-
Albertkanaal, Langerlo (12)	4	12	7	5	7	18	3	1
A, te Poppel (13)	1	2	1	1	1	-	-	-
Kanaal Bocholt-Herentals, Blekerheide (14)	9	22	11	8	-	31	5	2
Oude Leie, Wevelgem (15)	6	11	7	5	-	6	-	-
Putten van Niel, Niel (16)	4	11	6	4	10	-	1	-
Kanaal Bocholt-Herentals, Sluis Herentals (17)	4	11	10	6	16	-	2	1
Warmbeek, Achel (18)	2	4	2	2	-	-	-	-
Darse, Vilvoorde (19)	4	9	6	4	13	-	2	-
Kanaal Beverlo, Leopoldsburg (20)	7	30	15	12	23	-	-	-

"-" values below LOD (see Table 4.2).

Conclusions

A number of important VOCs are present in eel from Flemish inland waters. The most abundant VOCs are BTEX and the chlorinated VOCs, chloroform and tetrachloroethene. In general, the concentrations of the chlorinated VOCs are higher in eel than in the lipid tissue of marine fish. However, this is not true for the BTEX, for which the levels are comparable to marine fish; this can be explained by the much more diffuse nature of the sources for BTEX.

The present exercise indicates that the VOC concentrations in eel reflect the actual concentrations in their environment. Also, if the BCFs and the concentrations in the water column are taken into account, the observed levels are well in line with expectations. In other words, eel is a potential biomonitor or sentinel organism for VOCs and further study is justified. This should include extended sampling at given locations and a more in-depth study of the behaviour of VOCs in the organism. For the rest, a follow-up study should be sufficiently wide-ranging to allow evaluation of the long-term consequences of small doses of VOCs and their synergistic effects.

Acknowledgements

The authors gratefully acknowledge the support of the Vlaamse Milieumaatschappij and specifically Ingrid Temmerman for their data and valuable comments.

References

Anderson, T.A., Beauchamp, J.J.,Walton, B.T., 1991. Degradation of a mixture of volatile and semivolatile organic chemicals in soil. *Journal of Environmental Quality* 20, 420-424.

Anon, 1995. Stofstromen naar de Noordzee (In Dutch/French). Brussels, Technical commission North Sea, Ministry of Public Health and Environment, Management Unit Mathematical Models of the North Sea and the Scheldt estuary.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37, 911-917.

Crookes, M.J., Dobson, S., Howe, P.D., 1993. Environmental hazard assessment: Xylenes. London, Toxic Substances Division, Department of the Environment, TSD/12, 57p.

de Boer, J., Hagel, P., 1994. Spatial differences and temporal trends of chlorobiphenyls in yellow eel (*Anguilla anguilla*) from inland waters of the Netherlands. *Science of the Total Environment* 141, 155-174.

Dewulf, J.P., Van Langenhove, H.R., Van Der Auwera, L.F., 1998. Air/water exchange dynamics of 13 volatile chlorinated C1- and C2-hydrocarbons and monocyclic aromatic hydrocarbons in the southern North Sea and the Scheldt estuary. *Environmental Science and Technology,* 32, 903-911.

Freitag, D., Ballhorn, L., Geyer, H., Korte, F., 1985. Environmental hazard profile of organic chemicals. *Chemosphere* 14, 1589-1616.

Howard, P.H., 1989. Handbook of Environmental Fate and Exposure data for Organic Chemicals: Volume I Large production and priority pollutants. Chelsea, USA, Lewis Publishers, 574p.

Howard, P.H., 1990. Handbook of Environmental Fate and Exposure data for Organic Chemicals: Volume II Solvents. Chelsea, USA, Lewis Publishers, 546p.

Isnard, P., Lambert, S., 1988. Estimating bioconcentration factors from octanol-. water partition coefficient and aqueous solubility. *Chemosphere* 17, 21-34.

Ministerial Declaration of the 3rd International Conference on the Protection of the North Sea, 7-8 March 1990, The Hague, The Netherlands, 198p.

Ministerial Declaration of the 4th International Conference on the Protection of the North Sea, 8-9 June 1995, Esjberg, Denmark 72p.

OECD, 2001. The 2000 OECD List of High Production Volume Chemicals. Paris, Organisation for Economic Co-operation and Development.

Roose, P., Brinkman, U.A.Th., 1998a. Improved determination of VOCs in marine biota by using on-line purge and trap-gas chromatography-mass spectrometry. *Analyst* 123, 2167-2173.

Roose, P., Brinkman, U.A.Th., 1998b. Determination of volatile organic compounds in marine biota. *Journal of Chromatogaphy A* 799, 233-248.

Roose, P., Brinkman, U.A.Th., 2001. Volatile organic compounds in various marine organisms from the southern north sea. *Marine Pollution Bulletin* 40, 1167-1177.

Sweet, C.W., Vermette, S.J., 1992. Toxic volatile organic compounds in urban air in. Illinois. *Environmental Science and Technology* 264, 165-173.

van Leeuwen, C.J., Van Der Zandt, P.T.J., Aldenberg, T., Verhaar, H.J.M., Hermens, J.L.M., 1992. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I: Narcotic industrial pollutants*. Environmental Toxicology and Chemistry* 11, 267-282.

VMM, 2001. Waterkwaliteit Lozingen in het water 2000 (in Dutch). Erembodegem, Belgium, Vlaamse Milieumaatschappij.

WHO, 1984. Tetrachloroethene, Environmental Health Criteria, 31. Geneva, World Health Organisation, 48p.

WHO, 1993. Benzene, Environmental Health Criteria nr. 150. Switzerland, World Health Organisation Geneva, 156p.

Part III

Effects of contaminants on the eel

After a fire in the storehouse of the Basel chemical company Sandoz on November 1, 1986, the Upper Rhine River in Switzerland turned red. Approximately 30 metric tons of pesticides and dyes entered the Rhine. As a consequence 150 000 eels and countless other fish and small animals were estimated to have died.

Photo: Deutsche Presse Agentur

Chapter 5

Effects of contaminants on the eel: a review

Caroline Geeraerts and Claude Belpaire

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

This chapter is presented as an unpublished manuscript.

Summary

European eel (*Anguilla anguilla* (L.)) stocks are in decline in most of their distribution area and their status is considered below safe biological limits. There are numerous possible causes for this decline. Recently, there is an increasing awareness that spawner quality might be an essential element in the decline of the species. Pollution by chemical substances may have a large impact on the reproduction success of the eel. This study gives a literature overview of the consequences of these contaminants on the biology and fitness of the European eel in order to document the role of pollution, within the decline and to support the eel management and restoration plans.

Introduction

The stocks of the European eel have declined in most of their distribution area and they are considered below safe biological limits (Dekker, 2002). There is evidence that anthropogenic factors (e.g. fisheries, pollution, habitat deterioration (such as migration obstruction) and transfer of parasites and diseases) as well as natural processes (e.g. global change and predation),have contributed to this decline (ICES, 2002). There is a growing awareness that spawner quality might be an essential element in the decline of the species. The quality of the silver eels, starting migration for reproduction, might be seriously impaired by pollution, diseases and parasites. Due to specific ecological and physiological traits, eels are particularly sensitive to bioaccumulation of lipophilic contaminants. From the INBO Eel Pollutant Monitoring Network database (network covering Flanders, northern part of Belgium), we know that a great variety exists in specificity and levels of contamination in eel, with, at specific sampling sites, extremely high values of specific substances (Goemans *et al*., 2003; Belpaire and Goemans, 2007a; Maes *et al*., 2008). Some of these contaminants accumulate in the fat-tissue of eels during their feeding stage (yellow eels), even to levels that make them far unsuited for consumption (Harrad and Smith, 1999; Bilau *et al*., 2007). Robinet and Feunteun (2002) reviewed possible toxic effects on eel. A considerable decrease in muscle fat levels in yellow eel has been reported, and contaminants influence energy storage and affect lipid metabolism through various mechanisms (Belpaire *et al.,* submitted). A high body burden of contaminants could disable normal reproduction or disturb larval development (Larsson *et al*., 1990; van den Thillart *et al*., 2005; Palstra *et al*., 2006; 2007) as during the transoceanic migration lipids and the lipophilic contaminants are mobilized, particularly towards the gonads, where they impair their quality, compromising reproduction and normal development of the early embryonic stages. It has been reported that not only the adult eels are affected by high PCB levels, but also the fertilization (Spies *et al*., 1988), the hatching (Hose *et al*., 1982; Von Westernhagen *et al*., 1987) and the vitality of the larvae (Von Westernhagen *et al*., 1981) will be harmed. It is unknown to what extent levels of PCBs rise in blood plasma, fat and gonads in the migrating silver eels during their journey to the Sargasso sea, but they may well reach toxic levels (van den Thillart *et al.*, 2005). Maes *et al*. (2005) showed a significant negative relationship between heavy metal bioaccumulation, condition and genetic variability. A contaminant-induced decrease in condition and lipid energy stores might be responsible for failed migration and/or impairment of successful reproduction (Belpaire and Goemans, 2007b).

Therefore it is important to obtain insight in the harmful effects of (bioaccumulating) contaminants. Within the eel, reviews of the possible effects of various contaminants on the reproductive biology and physiology, have been elaborated by Bruslé (1991), Knights (1997), and Robinet and Feunteun (2002). Also for other teleosts, contamination levels and pollution pathways have been studied (Kinter *et al.*, 1972; Edwards *et al*., 1999; Andres *et al*., 2000; Bordajandi *et al*., 2003; Durrieu *et al*., 2005; Ruangsomboon and Wongrat, 2006; Labandeira *et al*., 2007; Roosens *et al*., 2008) Since the last update, quality issues on the eel benefit of increasing attention in the framework of the international conservation measures for restoring eel stocks. The Joint EIFAC/ICES Working Group on Eel (WG Eel, 2006) recommended to identify areas producing high quality spawners with low contaminant burdens in order to maximize protection for these areas. Attention should be paid to pollution monitoring within the Eel Management Plans and within the evaluation of the chemical status under implementation of the Water Framework Directive. In 2007, an European

Eel Quality Database (EEQD) was set up to collate information on contamination in eels over Europe (Belpaire *et al*., in prep.). WG Eel (2007) recommended to develop and maintain this database and to initiate harmonized monitoring strategies for eel within member countries. As a consequence of the increased international concern about the decline of the stocks, also research actions have paid increasing attention to analyze contaminants in the eel and to investigate the effects of these substances in the eel. As a result a large and growing number of information became available. The objective of this paper is to summarize and review these new insights on the effects of contamination in the European eel, in order to document the role of pollution within the decline and to support the eel management and restoration plans.

Effects of pollution exposure on fish

Residues of hazardous chemicals in river bed sediment and aquatic biota are an environmental concern for wildlife and human health. Because of their toxicity and persistence in the environment, many of them have been banned in Europe in the 1970s. Yet, more than 30 years later, residues of DDT and other organochlorine pesticides continue to be detected in air, rain, soil, surface water, river bed sediment, and aquatic as well as terrestrial biota. Moreover, recent research suggests that low levels of some contaminants have the potential to affect the development, reproduction, and behaviour of fish and wildlife, and possibly of humans as well (Nowell *et al*. 1999). Adverse impacts of contaminants have been described in several fish species in many fresh water habitats and the effects of contaminants on fish and fisheries are relatively well studied (for reviews see e.g. Lawrence and Hemingway, 2003, Nowell *et al*., 1999). Pollution impacts include effects on a variety of levels of biological organisation, from the subcellular and molecular level, through organism to population and community levels and subsequently to socio-economic consequences. Impacts are highly determined by the type of contaminant, and eventually by synergetic processes associated through the combination of a mixture of chemicals. The type of response will also depend on the developmental stage of the fish and will be influenced by other environmental factors (e.g. temperature, salinity, oxygen, pH) (Lawrence and Hemingway, 2003).

Many studies have examined the impact of a wide variety of xenobiotics on various aspects of fish biochemistry, physiology and population structure. In some cases of acute pollution, direct effects are clearly visible as fish may be moribund or dying. But contaminant exposure can lead to a decrease in growth or a lowered or deficient immunological system, causing an increased sensitivity to infectious diseases and parasites. But in most cases, these effects have been induced by effects on molecular and subcellular level. The last 20 years, an increasing number of reports deal with studying causality between pressure of xenobiotics and response at the subcellular level.

Cajaraville *et al*. (2003) reviewed molecular and cellular impacts of pollution, including genetic damage. A growing number of biomarkers are being developed indicating specific subcellular responses to a contaminant exposure (metallothioneins, stress proteins, …), and also enzymes are now included in current studies. Direct genetic damage may occur by mutagenic chemicals or radiation, and may affect a wide range of cellular functions. There is however, limited quantitative knowledge about molecular and genetic damage, and their consequences on individual health, fecundity and population productivity and viability.

The effects of contamination on the reproductive endocrine system of fish are well documented (Kime, 1995). Field studies have found reproductive impairment associated with high concentrations of chemical contaminants (Slooff and de Zwart,

1983; Stott *et al*., 1983; Johnson *et al*., 1992). Life cycle tests with chemical stressors have shown that intersexual interaction and development can be impaired at concentrations that do not affect embryonic development, hatching, or growth (Folmar, 1993). Reproductive hormones and vitellogenin may be suppressed in fish exposed to xenobiotic chemicals in the field or laboratory (Folmar, 1993). Endocrine disruption in freshwater fish presenting intersex individuals with ovotestes, has now been reported from many places and in many freshwater and marine fish species (Jobling *et al*., 1998). Indirectly, endocrine disruption might also affect fat storage due to specific chemicals, some of them mimicking the steroid hormone estrogen (Turner and Sharpe, 1997), which may be particularly harmful for long distance migrating species, such as the eel.

Ecotoxicological effects of contaminants on eels

Eels accumulate lipophilic xenobiotics in the fat. They often reside in contaminated sediments accumulating high levels of lipophilic compounds through gills, skin and contaminated foods (van Leeuwen *et al*., 2002). These accumulating contaminants may attain a very high level, even to a considerable higher degree than in other fish species. Eel is semelparous, carnivorous in its yellow stage, benthic, and often burrowed in the sediment. The eel, in its yellow stage, seems quite resistant, surviving in poor water conditions, and not seldom living in habitats polluted by diverse contaminants. Eel in the yellow stage are subadults, and hence do not reproduce in freshwater. Therefore, body burdens are not seasonally affected by a reproduction cycle neither by associated changes in lipid metabolism. Unlike iteroparous species, there is no loss of contaminants, specifically associated with annual reproductive processes (fat metabolisation and production and release of gametes). They can stay for a prolonged period in freshwater (on average 5.9 years for males and 8.7 years for females (Vøllestad, 1992; Figure 5.1)), continuing to bioaccumulate xenobiotics, and increasing their levels with age, reaching a maximum prior to silvering and emigration. They generally show life-long accumulation and low depuration rates (Larsson *et al*., 1991; Tulonen and Vuorinen, 1996; Knights, 1997; Daverat *et al*., 2006).

Many authors have reported (high) levels of a variety of xenobiotics in eel, which is used as a bioindicator (Belpaire and Goemans, 2007a, Belpaire *et al*., 2008) for those compounds in several countries. Belpaire and Goemans (2007b) compiled an overview of recent reports describing bioaccumulation of various chemicals in eel within EC countries. The most studied persistent organic compounds in eels are polychlorinated biphenyls (PCBs), pesticides and heavy metals. However, also dioxins and furans, brominated flame retardants (BFRs), volatile organic compounds (VOCs), perfluorooctanesulfonic acid (PFOS), polyaromatic hydrocarbons (PAHs) and metallothioneins (as response to elevated levels of metal exposure), were reported. In Belgium, lipophilic compounds in feral eels have been measured in concentrations of an uncommonly high level. Levels of the sum of the seven indicator PCBs of eels from the Canal Congovaart for example peaked at 62,608 ng g^{-1} lipid weight (mean of n= 21, 10/08/2001), whereas 9,255 ng g^{-1} lipid weight lindane was measured in eels from the Kemmelbeek (mean of n= 3, 07/05/2002) and 8,136 ng g^{-1} lipid weight *p,p'*-DDE in eels from the Oude Schelde (at Meilegem) (mean of n= 5, 21/10/2000). It was reported before (Belpaire *et al*., 2003; Figure 5.1) that eels from River Scheldt at Oudenaarde showed in 2000 extremely high concentrations of PBDEs and HBCD, respectively 31,639 and 33,000 ng g^{-1} lipid weight.

Considering the amounts of hazardous compounds accumulating in eel, it is reasonable to assume that toxic effects in the eel will be more obvious compared to

other species. This was discussed before by Robinet and Feunteun (2002). They reviewed sublethal toxicity of accumulating xenobiotics and extrapolated these finding to the population level, hypothesizing that the quality of future spawners, the silver eels leaving freshwaters is a prime factor for the conservation of the eel.

Lawrence and Elliott (2003) presented a conceptual model to illustrate recognized and potential links between pollution pressure and effects on fish at the individual or population level. The model shows possible mechanistic relations between the various hierarchical levels of biological response to pollution, from molecular to population, and even socio-economic level (fisheries). We simplified this model and adapted it towards eel (Figure 5.1). It is a simplified model and it is important to remember that there are many other factors, both biotic as abiotic, which affect eel throughout its life. Here we liked to restrict the model to identify the potential mechanistic links between pollution pressure and the responses on various levels and ultimately on population structure. However, as suggested by Lawrence and Elliott (2003), it could potentially provide the framework around which a mathematical model can be developed with predictive capability. Through the text references to the conceptual model are given.

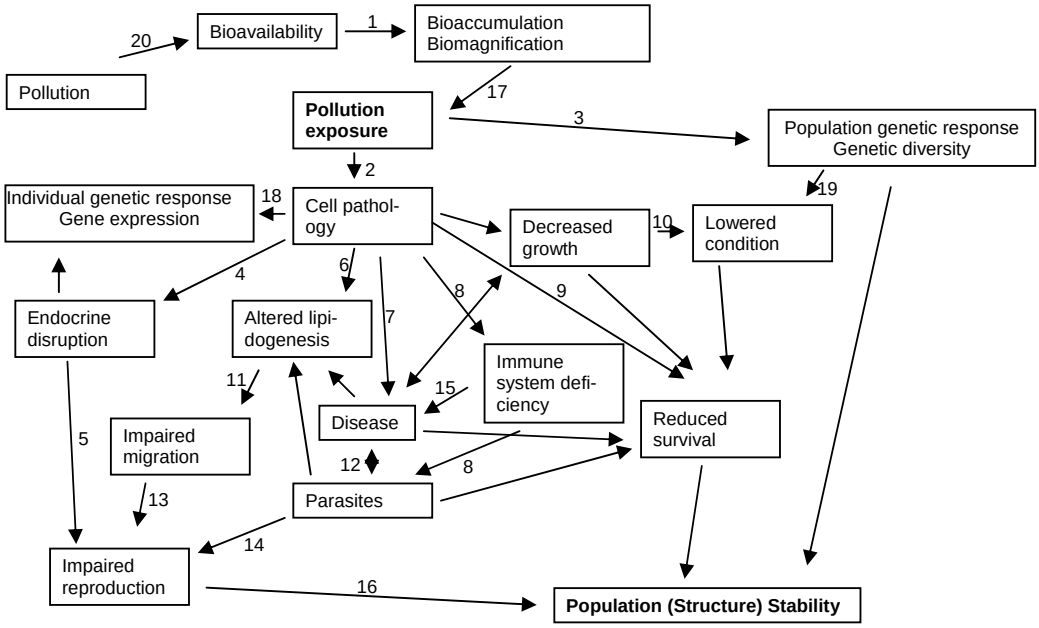


Figure 5.1. A simplified conceptual model of the effects of pollution exposure on the population structure of the European eel, *A. anguilla*. Adapted from Lawrence and Elliott (2003). Numbers refer to references: (1) Vollestad, 1992; (2) Tuurula & Soivio, 1982; Svobodova *et al*., 1994; Azzalis *et al*., 1995; Stohs & Bagghi, 1995; Sanch *et al*., 1997; Ibuki and Goto, 2002; Pacheco and Santos, 2002; (3) Nigro *et al*., 2002; Jha, 2004; Maes *et al*., 2005; Nogueira *et al*., 2006; (4) McKinney and Waller, 1994; Versonnen *et al*., 2004; (5) Jobling *et al*., 2002b; (6) Jimenez and Burtis, 1989; Ceron *et al*., 1996; Sancho *et al*., 1998; Fernandez-Vega *et al*., 1999; Robinet and Feunteun, 2002; Hu *et al*., 2003; Pierron *et al*., 2007a; (7) Roche *et al*., 2002; (8) Sures and Knopf, 2004; Sures, 2006; (9) Sancho *et al*., 1997; (10) Gony, 1987; (11) Ceron *et al*., 2003; van den Thillart *et al*., 2005; (12) Van Ginneken *et al*., 2005; (13) Johnson *et al*., 1998; Palstra *et al*., 2007; (14) Sures, 2006; (15) Van Ginneken *et al*., 2005; (16) Corsi *et al*., 2003; (17) Van Campenhout *et al*., 2008; (18) Ahmad *et al*., 2006; Maria *et al*., 2006; (19) Jha, 2004; Maes *et al*., 2005; (20) Belpaire *et al*., 2003.

Evidence has been presented that different kinds of chemical compounds such as e.g. PCBs, pesticides, heavy metals and PFOS have an impact on the health of the European eel. These studies may thus describe processes on various levels of the conceptual model presented in Figure 5.1. As stated before, impact of pollution is highly dependent of the type of chemical compound. The possible effects of various contaminants on the reproduction biology and physiology in eel is revised separately for each group of compounds.

Polychlorinated biphenyls (PCBs)

PCBs can affect different physiological aspects of the eel. Selected responses are discussed. Svobodová *et al*. (1994) e.g. described as long term effects the considerable histopathological changes in liver, spleen and kidney from fish exposed to PCBs both in nature and under laboratory exposure conditions. A review on the effects of polluents on reproduction of fish has been made by Kime (1995).

Detoxification

Almost all metabolic processes in a cell need enzymes in order to occur at physiological appropriate rates. Since they are extremely selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways preferentially occur in a cell. It is proven that PCBs can disturb these metabolic pathways.

Organisms dispose of a mono-oxygenase system which helps them to detoxify contaminants, to remove them from tissues and to excrete them. The greater part of the PCBs, however, cannot at all or only to an extremely small extent be detoxificated. So, the fastest way to get rid of PCBs is to store them in lipid tissue, a metabolic relatively inactive compartment (Bruijs *et al.*, 2002). The mono-oxygenase system can be partially inhibited by hormones e.g. steroid hormones but also by temperature, sex, age and food. Reproductive hormones regulate seasonal changes in mono-oxygenase activity. Before the spawning season, this activity decreases by natural factors in many fish species (Walton *et al*., 1983). The detoxification mechanism of fish is also stimulated by foreign chemicals like PCBs (Melancon *et al*., 1981; Ankley *et al.*, 1986; Kleinow *et al*., 1987). This was proven by high levels of mono-oxygenase activity in fish liver in the Rhine downstream a PCB-incinerator (Monod *et al*., 1988).

Jimenez *et al*. (1988) showed that starved fish present a lower mono-oxygenase activity than well-fed fish. As a consequence the food intake can influence the activity; Jimenez and Burtis (1989) also proved that pollutants can induce the mono-oxygenase activity in a two weeks starvation period. An eel supposed fasting during its reproductive migration will have an induced mono-oxygenase activity by the increasing PCB-concentration in his body because of release from the lipid tissue (Figure 5.1). Bruijs *et al*. (2002) state that induction of the mono-oxygenase activity during spawning can influence the breeding success of eel and lead to a decrease in numbers of young eels.

Endocrine responses

The endocrine system is a tightly regulated system comprised of a number of specialized glands which synthesize and secrete hormones under control of the hypothalamic-pituitary axis (HPI). It is instrumental in regulating physiological processes such as metabolism, growth and the homeostatic control, reproduction, energy production and osmoregulation.

PCBs are known as endocrine disrupters and effects have been shown in many fish. There is also a large body of evidence on the endocrine (hormone) disrupting properties of alkylphenols. Jobling and Sumpter (1993) used rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) hepatocytes in an in-vitro study focusing on estrogenic (capable of mimicking the action of the female hormone estrogen) chemicals (including alkylphenols) in sewage effluents discharged into UK rivers and estuaries. Disruption in gonadal development of wild roach (*Rutilus rutilus* L.) is manifest in a variety of ways, ranging from malformation of the germ cells and/or reproductive ducts to altered gamete production. Intersex fish were also found to have an altered endocrine status and an elevated concentration of plasma vitellogenin (VTG) (Jobling *et al*., 2002a; Bjerregaard *et al*., 2006). Under natural conditions, VTG is only produced by mature female fish as a yolk precursor and has therefore been widely used to detect exposure to compounds with estrogenic properties (Versonnen *et al*., 2004; Gillemot, 2003). Intersexuality also influences reproductive success. Gamete production is reduced in intersex roach. Moreover, sperm motility (percentage of motile sperm and curvilinear velocity) and the ability of sperm to successfully fertilize eggs and produce viable offspring is reduced in intersex fish compared with normal male fish. This documents a relationship between the morphological effects (e.g. intersex) of endocrine disruption and the reproductive capabilities of any wild vertebrate (Jobling *et al*., 2002b). From a monitoring program in British rivers it has been proven that steroidal estrogens play a major role in the appearance of intersex. Their appearance shows correlation with the location and severity of pollution by estrogen-like compounds (Jobling *et al*., 2006).

Versonnen *et al*. (2004) investigated plasma vitellogenin (VTG) content, measured in 142 eels sampled at 20 different locations in Belgium, in relation to the internal pollution levels (PCBs, organochlorine pesticides, metals). No correlations were found between VTG content and weight, length, condition, fat content, contaminants or date of sampling. Plasma VTG content of eels from the field study was very low, despite a very high internal load of endocrine disrupters. These results, together with previously published studies (Livingstone *et al*., 2000; Peters *et al*., 2001) of eel sampled at different locations in the UK during different seasons, suggest that immature yellow European eel might not be the best sentinel species to study the effects of estrogenic compounds on VTG levels of wild fish populations (Versonnen *et al*., 2004). The fact that yellow eel might be relatively insensitive (regarding VTG levels) to waterborne endocrine disrupters is also confirmed by Burzawa-Gerard and Dumas-Vidal (1991) and Luizi *et al*. (1997) who found that high doses of (injected) E2 (17β-estradiol; at least 5 x 0.5 µg g^{-1} w.w. during 12 days) were needed to induce VTG production in immature eels (Versonnen *et al*., 2004). The onset of maturation in the European eel only takes place during a period of prolonged swimming which might be a necessary physiological stimulus (van Ginneken *et al.*, 2007). It is therefore possible that endocrine disrupting effects of pollutants become apparent during the starvation period during migration or during the spawning itself (Versonnen *et al.,* 2004; Figure 5.1). Therefore, research under experimental conditions (swim tunnels) with silver eels is recommended.

PCBs and other toxic substances interact with a variety of endocrine control mechanisms. McKinney and Waller (1994) point that PCBs may interfere with the endocrine function by their ability to mimic natural hormones (Figure 5.1). Brouwer *et al.* (1990) point that reduced plasma retinol and thyroid hormone levels are involved in reproductive disorders and lethal viral infections in seals from the Wadden Sea, which are caused by the effect of PCB-contaminated fish. Barron *et al*. (1995) report that the acute toxicity of PCBs in birds increases with chlorination of the PCB mixture. Some of the appearing effects include reduced parental attentiveness and abnormal reproduction behaviour, endocrine disruption, immunotoxicity, and teratogenesis. Metabolism or biotransformation through the phase I (cytochrome P-450 monooxy-

genase enzymes) and phase II (conjugating enzymes) pathway is requisite for detoxification and excretion of lipophilic chemicals. Goksøyr and Förlin (1992) report that for rainbow trout, such a transformation is also responsible for the activation of foreign chemicals to the intermediates that ultimately result in toxicity, carcinogenicity, and other adverse effects.

Sures (2006) demonstrated that parasites: (i) may influence the metabolism of pollutants in infected hosts, (ii) interact with pollution in synergistic or antagonistic ways, and (iii) may induce physiological reactions in hosts which were thought to be pollutant-induced. From experimental studies we know that alterations in pollutant uptake and accumulation in different intermediate and final hosts due to parasites are very important in the field of ecotoxicology. Sures (2006) points that in addition to such alterations, there is a close interaction between the effects of pollutants and parasites which seems to be mediated at least partly by the endocrine system, which itself is closely related to the immune system in fish. Laboratory studies on eels experimentally infected with the swimbladder nematode *Anguillicola crassus* reveal that toxic chemicals such as polychlorinated biphenyls produce immunosuppressive effects which facilitate parasite infection. Similarly, an increase in serum cortisol concentration in eels due to chemical exposure and infection is correlated with decreasing levels of anti-*A. crassus* antibodies. Furthermore, parasites are able to elicit physiological changes which are attributed to chemicals with endocrine disrupting activity, e.g. the cestode *Ligula intestinalis* is known to suppress gonad development in roach (Sures, 2006; Figure 5.1).

Immune responses

Exposure of aquatic organisms to pollution pressure can change significantly innate immunity. Chemical exposure as individual environmental stressors have been shown in various fish species to alter all aspects of the immune response. Under natural conditions fish may be exposed by a multitude of stressors which can alter the immune function. Prophete *et al. (*2006) described the effects on the immune response in Japanese medaka (*Oryzias latipes*) under combined conditions of elevated temperature and nickel pollution. Immune function assays even have been shown as appropriate bioindicators for chemical stress (Rice *et al.,* 1996). Impact of pollution on eel's immune response have been described for PCBs. Eels experimentally infected with the swimbladder nematode *Anguillicola crassus* reveal that toxic chemicals such as PCBs produce immunosuppressive effects facilitating parasite infection. Sures and Knopf (2004) experimentally infected eels with the swim bladder nematode *Anguillicola crassus* and exposed them to sublethal concentrations of Cd and 3,3',4,4',5-pentachlorobiphenyl (PCB 126) A significant increase of *Anguillicola*-specific antibodies in the peripheral blood was first detected 61 days p.i., indicating that it was not the invasive larvae but the adult worms which elicit the antibody response. The exposure to PCB 126 resulted in a complete suppression of the antibody response while the presence of Cd did not appear to modulate the production of antibodies. They found a similar effect for the combined exposure of the infected eels to Cd and PCB 126. The relationship between pollution exposure and the immune system deficiency is indicated in Figure 5.1. Sures and Knopf (2004) also indicate that from the available information it appears that the Cd concentrations (21.7 ± 12.8 µg l^{-1} (mean ± S.D.)) applied in their study were not high enough to suppress the immune response of European eels. Furthermore, as eels are able to withstand environmental pollution and tend to accumulate heavy metals to a very high degree (e.g. Mason and Barak, 1990; Sures *et al*., 1994), it seems likely that this species is not sensitive enough to show alterations in its immune response at low levels of Cd pollution.

Silvering

A number of studies have shown that the silvering process, the subsequent downstream and transoceanic reproductive migration, as well as gonad maturation, can only take place if a sufficient quantity of energy is stored as lipids. Under a critical fat mass in their yellow stage (28%), silvering may not even be initiated (Thurow, 1959; Larsson *et al*., 1990). Establishing sufficient lipid energy is thus essential in the life cycle of the eel, however several authors described negative effects of PCBs (and contaminants in general) on lipid metabolism.

During the growing phase, water and proteins are progressively replaced by fat (lipids and lipoproteins, Degani *et al*., 1986). Boëtius and Boëtius (1980) estimated that the total stored lipids must exceed 20% of the body weight to cover the migratory needs of the European silver eel (female). Van den Thillart *et al*. (2004; 2005) found that European eel is able to swim 5500 km showing that energy reserves are, in principle, sufficient for migration to the Sargasso Sea. Fat consumption for a complete run would be 126.5 mg g^{-1} w.w. which corresponds to 60% of the total fat reserve of most silver eels. Calculations based on the number of oocytes and fat percentage in oocytes show that almost 40% of the total fat reserve is required for incorporation in oocytes. Thus animals with less than 13% (of the body weight) fat would not be able to swim 6000 km. An additional 7% (of the body weight) is needed for oocytes. It is hypothesized that eels with insufficient fat content (<13%; of the body weight) will not continue their migration activity but first linger downstream, only to continue their migration later. A value of 20% (of the body weight) fat is also the average for migrating silver eels implying that only half of the silver eels is capable of successful migration and reproduction (Van den Thillart *et al*., 2005).

Recently, new evidence has been provided that decreasing fat content in yellow eel may be an important factor in the stock decline (Chapter 6, Belpaire *et al.,* submitted; Figure 5.1).

The storage of sufficient lipid stores can be seriously impaired by contaminants, as reviewed by Robinet and Feunteun (2002). This relationship is shown in Figure 5.1. Contaminants may affect lipidogenesis or induce lipolysis, through various mechanisms. Chemical stress induces a higher energy demand (Calow 1991). In rainbow trout fed with PCB and mirex contaminated diets, carcass lipid content differed significantly compared to control fish, PCBs inducing an increase in lipid content, and mirex a decrease (Leatherland and Sonstegard 1980). Effects of the impact of PCBs and some specific pesticides on lipid content in feral eel is reported in Geeraerts *et al*. (2007).

Reproduction

During maturation of female European silver eels, about 60 g fat per kg eel is incorporated in the oocytes (Palstra *et al*., 2007). As a result of the impact of contaminants, fat stores might be lowered to such an extent that normal reproduction is disabled (Belpaire *et al.,* 2008). Together with the fat, however, persistent organic pollutants such as dioxin-like polychlorinated biphenyls are incorporated too. The negative impact of highly contaminated lipid reserves in eel, explaining the stock decrease in Europe, has been suggested by Larsson *et al. (*1990). While the lipid reserves are depleted during migration, contaminants are released into the blood and damage reproductive organs. Johnson *et al*. (1998) report that exposure to PACs and PCBs reduce the breeding success of female eels by which the mean weight of the eggs decrease drastically, being the result of interference with gonad development. PACs are responsible for physical and chemical genotoxicity in fish. This can lead to DNA breaks and to the formation of micronuclei in the intracytoplasmatic chromatine. Biological degradation of PACs also creates catalysts that interact with DNA (Rether

et al., 1997). Possible damage to the DNA can lead to failing of the reproduction during fertilization.

Palstra *et al*. (2007) found that the amount of fat transported to the gonads for accumulation in eggs was positively correlated to the age of the eel. This suggests an increased capacity of older eels to incorporate more fat from the muscles into the eggs. As egg quality depends heavily on incorporation of reserves, this increased capacity of older eels suggests a higher reproduction potency. Palstra *et al*. (2007) also observed a negative correlation between embryo survival time and TEQ (toxic equivalent) levels in the gonads implying TEQ-induced teratogenic effects. The disrupting effects occurred at levels below 4 pg TEQ g^{-1} w.w. gonad, which are below the EU eel consumption standard (Palstra *et al*., 2007). In addition, migrating silver eels will use at least 60 mg fat g^{-1} eel (40% of the total fat reserves) for their spawning migration (van Ginneken and van den Thillart 2000). This means that, considering a biological half-life of PCBs between 1 and 4 years (de Boer *et al*. 1994), there will be an increase in the concentration of the dioxin-like compounds of at least 40%. Hence, the TEQ values in gonads of the eels spawning in the Sargasso Sea will be even higher than those in the gonads of the artificially spawned eels (Palstra *et al*., 2007).

Van den Thillart *et al*. (2005) investigated the influence of PCBs on the physiology and gonad development of silver eel during a simulated migration. It is known that as long as the contaminants are stored in the fat reserves, toxic effects are minor. But, at the start of the migration, when the lipids are oxidized and the PCBs released, it is possible that the PCB-levels in the blood plasma shall increase till toxic levels. Van den Thillart *et al*. (2005) report a significantly 1.5-fold higher weight loss in the PCB-loaded groups which can not be ascribed to the refusal of food but may be the result of PCB effects on the intermediary metabolism. In this respect it is notable that in the PCB-loaded groups, hypoglycemia can be observed. PCB exposure, in combination with the swimming protocol, is not stressful as none of the secondary indicators of a stress response (a rise of lactic acid, an increase of potassium and an increase of glucose) increased in PCB loaded and/or swim groups (van Ginneken *et al*., 2002). From other PCB-studies it can be concluded that PCB-exposure leads to a lowering of the adrenocortical function (Van den Thillart *et al*., 2005).

Toxicants such as PCBs may also cause an immuno-suppression, which leads to a decreased resistance to diseases, viruses and parasites. In combination with swimming the EVEX virus caused hemorrhage and anemia resulting in the death of the animals after 1,000-1,500 km (van Ginneken *et al*., 2005; Figure 5.1). Possibly the world-wide decline of eel populations can have several causes or a combination of causes which can work ultimately synergistically.

Pesticides

A pesticide that enters the surface water column will redistribute itself between the water and carbon-rich compartments (such as sediment and biota) in the water column. Uptake by biota occurs via (1) direct uptake from water, (2) ingestion of contaminated food or other suspended particles, or (3) direct sorption from sediment. Pesticide accumulation by aquatic organisms varies depending of the pesticide, the organism and environmental conditions. Concentrations of a given pesticide in biota have been shown to vary with species, sex, age, body size or weight, surface-to-volume ratio, life stage or reproductive state, lipid content, trophic level, vertical distribution, physical condition, tissue or organ analyzed, migration pattern, and the season in which samples were collected. Their relative importance depends on the concentration of the pesticide in the water, the place of the species in the food web, the physical and chemical properties of the pesticide, and the possible synergetic activity with other substances or stressors as described by Nowell *et al*. (1999). As an exam-

ple of pesticide-driven events Nowell *et al*. (1999) quote the number of fish kills attributed to organochlorine and organophosphate insecticides, DDT, and pentachlorophenol. They also quote the summer die-off and decline of the striped-bass (*Morone saxatilis*) population in the Sacramento-San Joaquin Delta (California) since the mid-1970s. Unusually high chemical loads in their livers (compared to apparently healthy fish) were identified as a possible factor that has contributed to the decline of the species. Chemical residues that were detected included pollutants from industrial (such as aliphatic hydrocarbons and esters), agricultural (such as rice herbicides), and urban (such as dialkyl phthalates and petroleum-based compounds) sources. The impact of the kill is dependent of the amounts of pesticides in the water column. Fish kills on a local scale often result from inadvertent management of land users (e.g. spill) but severe fish kills often result from accidental discharge or leakage on industrial sites producing or processing pesticides or other chemical compounds. In 1986 for example incidents with atrazine were reported at Ciba Geigi-Bazel, with pesticides at BASF-Ludwigshafen, with chlorobenzol at Hoechst-Frankfurt (in the River Main), with methanol at Bayer-Leverkusen, with desinfectants at Bayer-Krefeld-Uerdingen, and with ethylene glycol at BASF. After a fire in the storehouse of the Basel chemical company Sandoz on November 1, 1986, approximately 30 metric tons of pesticides and dyes entered the Rhine. As a consequence 150,000 eels and countless other fish and small animals were estimated to have died. Following Bálint *et al.* (1997) deltamethrin (the active ingredient of the insecticide K-OTHRIN 1 ULV) contributed in the severe eel devastation that occurred in Lake Balaton in 1991 and 1995, killing respectively 300 and 30 tons of eels. It seems that when eel kills occur, it is very hard to correlate these mortalities with precise chemical factors, because of the complexity of the pollution load (including a variety of contaminants which may interact) in many polluted areas (Anonymous, 1987). In 2007, 25 ton fish were killed in the River Meuse due to the discharge of 64 kg chloropyriphos and 12 kg cypermethrin, two components to produce pesticides. Due to the publicity which goes hand in hand with disastrous ecological consequences of the fish kills, it became clear that more “accidents” with chemical substances appeared than were known.

Research has been done on the influence of toxic effects of pesticides on eel. Microscopic damage to gills, livers and spleens has been recently reported in other fish species than eel (Dutta and Meijer, 2003; Marty *et al*., 2003; Akaishi *et al*., 2004; Brown and Steinert, 2004). Pesticides are known for their ability to disrupt the structural integrity of fish gills and it may be assumed that as a result of the reduced efficiency of the damaged gills to function as respiratory organs, the tissues receive less oxygen (Sancho *et al*., 1997). Because gills are in contact with water and are exposed to dissolved contaminants and to trophic contamination, fusion of lamellae and aneurysms suggests an acute exposure to contaminants, while gill parasites in individuals from the same site mean compromised immune systems.

Ceron *et al*. (1996) studied the effects of diazinon on the acetyl cholinesterase (AChE) activity in different eel tissues (brains, plasma and eye tissue) exposed to a sublethal doses of 0.042 mg l^{-1} (0.50% of 96h LC50). Eye tissue was the only tissue with higher levels of AChE-activity (8.17 micromol min^{-1} g^{-1}) in non-exposed eels. Fernandez-Vega *et al*. (1999) exposed eels to a sublethal concentration (1/60 LC50-96h = 0.22 mg l^{-1}) of the herbicide thiobencarb (S-chlorobenzyldiethylthiocarbamate). The results showed that thiobencarb is significantly limiting the plasma AChE-activity from the first contact with the poison.

Sancho *et al*. (1997; Figure 5.1) investigated the sublethal effects of the organophosphate insecticide fenitrothion (*O,O*-dimethyl-*O*-3-methyl-4-nitrofenyl phosphorothioate) which is extensively used in agriculture for crop protection. A constant sublethal concentration of 0.02 mg l^{-1} of fenitrothion in the surrounding water for 96h appeared to be physiologically stressful to the European eel. A consistent hyperglycemia was seen and a spectacular increase occurred in blood, liver, and gill lactate

levels, while protein levels decreased significantly. Sancho *et al*. (1997) explain that the development of such internal hypoxic conditions may be ultimately responsible for the shift to the less efficient anaerobic metabolism, indicated by changes in lactic acid contents observed in blood, liver, and gill tissue. Ferrando and Andreu (1991a) and Gimeno *et al*. (1994) found the same results. Ferrando and Andreu (1991b) studied the effects of 96h exposure of lindane on European eel and observed a decrease in liver and muscle glycogen content and an increase in blood glucose levels. Sancho *et al*. (1997) also found that the fenitrothion-treated eels exhibited no significant change in liver glycogen levels after 5 days of exposure, but protein content decreased significantly and hepatomegaly was observed. Holmberg *et al*. (1972) found similar results in eel exposed to PCP (pentachlorophenol) for 8 days. He also found an increased hepato somatic index which can be explained by an enlargement of the liver as a result of the pesticide action. The decrease in protein content of fenitrothion-intoxicated fish also indicates the physiological adaptability of the fish to compensate for pesticide stress. To overcome the stress the animals require high energy. This energy demand might have led to the stimulation of protein catabolism (Sancho *et al*., 1997).

Pesticides (Azzalis *et al*., 1995), heavy metals (Stohs and Bagghi, 1995), polycyclic aromatic hydrocarbons (Ibuki and Goto, 2002) are associated with increased free radical concentrations within the cytosol. These oxidative forms may increase programmed cell death or disturb cell homeostasis and cellular necrosis. Also, prenecrotic areas suggest another necrosis event where the invasion of blood cells in the tissue is an evidence of cell injury. Individuals with high incidence of necrosis also displayed prenecrosis, strongly suggesting a continuous exposure to the related xenobiotic compounds present in the environment (Oliveira Ribeiro *et al*., 2005).

Sancho *et al*. (1998) report that eels exposed to fenitrothion (an organ phosphorus insecticide 0.02 en 0.04 mg l^{-1}) had significant lower fat reserves than before exposure. Shailaja and D'Silva (2003) report that PAH induce the formation of mixed function oxygenase (MFO) in fish liver together with side effects caused by the formation of highly carcinogenic transitional products. Roche *et al*. (2002) indicate that tumors in liver and spleen of eels result from long-lasting exposure to a combination of potentially carcinogenic pollutants. This relationship between pollutant exposure and cancer is indicated in Figure 5.1.

Several fish studies have reported benzo[a]pyrene (BaP; Wolkers *et al*., 1996; Pacheco and Santos, 1997), DHAA (dihydroabietic acid) and BKPME (bleached kraft pulp mill effluent; Martel *et al*., 1994; Pacheco and Santos, 1999) as EROD (ethoxyresorufine-*O*-deethylase) inducers. Pacheco and Santos (2002) studied the biotransformation response of eel on the toxicity of these compounds. The results were unexpected because nor BaP (0.22; 0.45 and 0.9 µM) nor BKPME (3.12; 6.25; 12.5%) exhibited considerable total EROD induction. The response to shorter DHAA (0.07; 0.15; 0.3 µM) exposures also did not corroborate their previous results, despite the unequivocal total EROD induction exhibited by 180-day DHAA exposed fish. Pacheco and Santos (2002) explain the discrepancy between results either by differences between fish lots or by a relative lack of sensitivity of EROD methodology concerning the measurement on the whole body. They also discovered that 30-day DHAA treated fish have epidermis exfoliation probably due to the abrasive effect of resin acids. Previous studies (Bushnell *et al*., 1985; Toivola and Isomaa, 1991) point to the fact that an eventual detergent-like action and consequent cell breakdown directly affecting the eel's body surface also has to be considered. Resin acids are important components of pulp mill effluents; therefore, DHAA may also be partially responsible for the same histological alteration observed in BKPME-treated fish said Pacheco and Santos (2002). Santos *et al*. (1990) reported skin disruption in adult eel due to BKPME and Howard *et al*. (1971) reported a favourable fish adaptation capacity in their study with pulp mill effluents. The lack of parasites or parasitic lesions in

the skin of DHAA-treated animals indicates that the above reported abrasive action of DHAA also may be adverse to parasite fixation, preventing this kind of infestation (Pacheco and Santos, 2002). DHAA also cause structural changes in the gills (Tuurala and Soivio, 1982; Pacheco and Santos, 2002). These histopathological alterations may have important adverse consequences on fish health, particularly due to the obstruction of oxygen diffusion across the gills and the impairment of the osmoregulatory function (Pacheco and Santos, 2002). The splenic hemosiderosis points to erythrocytic catabolism which may result in a decrease in the number of mature erythrocytes in the circulating blood (Hibiya, 1982). Also glomerular injury was observed impairing glomerular filtration, just as histological alterations in the renal tubules of BKPME-exposed eels (Santos *et al*., 1990).

A study by Maria *et al*. (2006) and Teles *et al*. (2007) showed that agricultural chemicals such as fertilizers and pesticides, domestic sewage, as well as heavy metals from electroplating industries, resulting in increased water pollution, have endocrine, metabolic and genotoxic responses on eel caged for 48h at sites, differing in their distances to the main known pollution source. The results revealed increased plasma cortisol and glucose concentrations at all exposure sites, displaying a similar response pattern (Teles *et al*., 2007). The field study demonstrated that the three exposure sites close to the pollution source, are polluted by pro and/or genotoxic compounds. The genotoxic effects induced in eel suggest a different contamination of the exposure sites in genotoxic chemicals (Maria *et al*., 2006).

Lipid accumulation in eel was disturbed directly by inhibition of the acetylcholinesterase activity due to pesticide exposure (Ceron *et al*. 1996; Fernandez-Vega *et al*. 1999). Under laboratory conditions, eels show an increased fat consumption in the presence of cadmium (Pierron *et al*., 2007a) or the insecticide fenitrothion (Sancho *et al*., 1998) and thus lower efficiency of lipid storage.

Already in 1971 it was accepted that in both vertebrates and invertebrates the insecticide DDT exerts a direct toxic effect on the nervous system. Janicki and Kinter (1971) report that DDT impairs fluid absorption in intestinal sacs from eels adapted to seawater. Furthermore, this functional impairment has an enzymatic basis; DDT also inhibits the (Na^+ and K^+) activated, Mg^{2+}-dependent adenosine triphosphatase (ATP) in homogenates of the intestinal mucosa and gill filaments (Kinter *et al*., 1972). Thus, the extreme sensitivity of teleosts to organochlorine pollutants may involve the disruption of osmoregulatory transport mechanisms. Moreover, other organochlorine insecticides, including endrin also inhibit Na K-ATP-ase from fish brain and endrin has been observed to disrupt osmoregulation in both marine and freshwater teleosts (Kinter *et al*., 1972).

Corsi *et al*. (2003) showed abnormal ovarian and oocyte development coupled with inhibition of AChE, resulting in oocyte deformities and smaller diameters probably due to OPs and CBs exposure during the early phase of gonad recrudescence, so lipidogenesis and subsequent migration efficiency are reduced (Figure 5.1).

Indications of impact of some specific pesticides on lipid content in feral eel is reported in Geeraerts *et al*. (2007).

Heavy metals

Copper

Copper is an essential element to life, but in higher concentrations toxic effects clearly have been demonstrated. Sublethal effects of copper on behaviour, growth, migration, and metabolism has been described in several fish species. Copper exerts a wide range of physiological effects in fishes, including increased metallothionein synthesis in hepatocytes, altered blood chemistry, and histopathology of gills and skin. It may affect reproduction success of fish through disruption of hatch

coordination with food availability and through adverse effects of larval fishes. Sublethal exposure suppresses resistance to viral and bacterial pathogens. Rate and extent of copper accumulation in fish tissues are extremely variable between species and are further modified by abiotic and biological variables (Eisler, 1998).

Rødsæther *et al*. (1977) exposed eels to copper-contaminated freshwater (30–60 ρg Cu l^{-1}) and saw that they died with signs of vibriosis (*Vibrio anguillarum*). Eels kept in non-contaminated freshwater (< 6ρg Cu l^{-1}) remained healthy. Rødsæther *et al*. (1977) suggest that *V. anguillarum* is a common inhabitant of eels and copper can change a commensal association between fish and bacterium to one of pathogenicity. Probably this illustrated a decrease in immune response induced by Cu.

Grosell *et al*. (1996) on the other hand examined the effect of pre-exposure to copper (8 and 64 g Cu l^{-1} for 6 and 28 days) in European eels with respect to uptake and distribution of ^{64}Cu among tissues. The Cu accumulation rate in muscle tissue was inversely related to body weight. Although the accumulation rates in liver and muscle tissues were significantly reduced, the accumulation rates in the gills were not affected by pre-exposure.

A similar experiment by Grosell *et al*. (1998) to measure the metabolism and elimination of copper (12 and 94 g Cu l^{-1}) uptake in both fed and starved European eels showed that the hepatic accumulation of ^{64}Cu was similar in fed and starved eels (0.55 g Cu g $liver^{-1}$ h^{-1}) at both Cu concentrations during the 28 days of exposure.

Both the redox cycling of heavy metals as well as their interaction with organic pollutants are a major contributor to the oxidative stress resulting from aquatic pollution. Ahmad *et al*. (2005) studied the oxidative stress response of European eel for 24h to copper exposure (Cu; 1 microM, 2.5 microM) and to beta-naphtoflavone (BNF; 2.7 microM) with or without pre-exposure to BNF (2.7 microM). Eel gill and kidney oxidative stress biomarker responses are lipid peroxidation (LPO), glutathione peroxidase (GPX) and catalase (CAT). Exposure to copper or BNF induces nor in the kidneys neither in the gills LPO. Double BNF exposures potentiated the risk of peroxidative damage occurrence in both organs. BNF/Cu interference on antioxidant responses differs between the studied organs. In gill, antagonistic effects were denoted with probable reflex in terms of peroxidative damage increase. In kidney, BNF preexposure prevented CAT and GPX inhibition by copper; though, no advantage of this effect was perceptible as defense against LPO generation (Ahmad *et al*., 2005).

Oliveira *et al*. (2008) indicated that Cu environmentally realistic levels may pose a serious ecological risk to fish. After 7 days European eel exposed to Cu 0.2 μmol L^{-1} revealed a significant methallothionein (MT) induction response in liver, and the erythrocytic nuclear abnormalities (ENA) frequency significantly increased in Cu exposed group. However, MT induction was insufficient to prevent endocrine and metabolic alterations as well as genotoxicity/clastogenicity in blood. Methallothionein is generally considered as a storage and supply site for essential metals such as Zn and Cu which are utilized in protein synthesis, nucleic acid metabolism and other metabolic processes (Langston *et al*., 2002). In addition to this regulatory function, MT may also play a role in metal detoxification. Langston *et al*. (2002) found that MT levels in eels are a direct function of metal concentration in surrounding sediment or water. This has recently been further studied by Van Campenhout *et al*. (2008) in Flemish eels. They studied the effect of metal exposure on the accumulation and cytosolic speciation of metals in livers of European eel measuring metallothioneins (MT) induction. Four sampling sites in Flanders with different degrees of heavy metal contamination (Cd, Cu, Ni, Pb and Zn) were selected for this purpose. The cytosolic concentration of Cd, Ni and Pb increased proportionally with the total liver levels. However, the cytosolic concentrations of Cu and Zn only increased above a certain liver tissue threshold level. Cd, Cu and Zn, but not Pb and Ni, were largely associated with the MT pool in correspondence with the environmental exposure and liver tissue con-

centrations. Most of the Pb and Ni and a considerable fraction of Cu and Zn, but not Cd, were associated to High Molecular Weight (HMW) fractions. It was concluded that the metals, rather than other stress factors, are the major factor determining MT induction.

Results from a study by Gravato *et al*. (2006) showed that the oxidative stress and genotoxic effects induced by Cu (exposed during 24h to 0, 1 and 2.5 microM) in eels pre-exposed to BNF (during 24h to 2.7 microM) are potentiated by previous exposure to BNF. BNF pre-exposure promoted a significant increase in liver ethoxyresorufin *O*-deethylase (EROD) activity, but did not change the other responses investigated in eels. Liver total glutathione, reduced glutathione (GSH) and GSH/oxidized glutathione levels were slightly decreased, liver glutathione reductase and catalase activities were significantly inhibited, and liver DNA integrity decreased by 1 and 2.5 microM Cu in eels pre-exposed to BNF.

Mercury

In his review about the effects of heavy metals on eel Bruslé (1987) mentions that the toxic level of mercuric chloride ($HgCl_2$) on young Japanese eel (*A. japonica*) is 0.02 ppm (highest tolerated concentration for 50h at 20-22℃). For European eel 1 ppm Hg and 50 ppm Cd are lethal. The lethal effect of $HgCl_2$ is due to a disturbance of the NaCl-balance in eel: in the gills mercury interacts with the movements of active ions and the osmoregulatory processes. The stagnation of the osmoregulatory mechanism seems to dependent from the specific pollutant on the Na-pump and is correlated with the membrane permeability and the disturbance of the enzyme systems (Bruslé, 1987).

Cadmium

Gony (1987) studied the effects of Cd on yellow eel. After two hours exposure of 5 µg l^{-1}, structural changes appeared in the gills like swelling of the primary and secondary lamellae caused by epithelium hypertrophy and accumulation of secondary lamellae. At the same moment melanism appears in the gill blood vessels. Dependent of the individual response on cadmium exposure also other injuries can appear like exfoliation of the epithelium and the collapse and merging of lamellae. Also liver tissue is influenced by cadmium exposure (Figure 5.1).

Cadmium also has a dose-dependent inhibition on in vitro activities of Na^+-K^+-ATPase and carbonic anhydrase (CA) on the intestines and gills of eels. Lionetto *et al*. (1998) experienced that the activities were inhibited by increasing cadmium concentrations (0.5-50 µM, one hour of incubation) with a maximum inhibition (± 80%) at 5 µM and 50 µM $CdCl_2$ for gill and intestines Na^+-K^+-ATPase. Carbonic anhydrase activities, measured in gill homogenate and in cytosolic and brush border membrane fractions isolated from intestinal mucosa, were significantly inhibited by pre-incubation (1h) with $CdCl_2$. Maximal inhibition (about 80%) of branchial CA was noted at approximately 60 M; higher concentrations evoked no further significant inhibition. Intestinal CA isoforms, cytosolic and membrane-bound, exhibited lower sensitivity to the heavy metal with respect to the branchial CA activity, since the highest concentration of $CdCl_2$ tested (600 M) produced an inhibition of about 30% and 50% respectively. These results suggest that cadmium, by inhibiting the activity of CA and Na+-K+-ATPase enzymes in intestine and gills, could alter both acid-base balance and osmoregulation in teleostean fish (Lionetto *et al*., 1998).

Pierron *et al*. (2007a) investigated the possible impact of cadmium on the lipid storage efficiency of yellow eels in order to evaluate the possible contribution of this pollutant to the reported decline of European eel populations. After a one month exposure to 0 and 5 µg l^{-1} Cd, Cd toxicity was examined by studying the activity and

expression level of several enzymes involved in liver lipolysis and lipogenesis and by determining lipid content in eel muscle. The observations suggest an increased fat consumption in presence of cadmium, which could compromise successful reproduction. Pierron *et al*. (2007b) also investigated the expression level of various genes involved in the mitochondrial respiratory chain, in the cellular response to metal and oxidative stresses of glass eels. Their results showed that hypoxia enhances ventilation of the post larval stage and Cd accumulation in gills only at the lowest metal water concentration tested (2 µg Cd l^{-1}). At the gene level, Cd exposure mimics the effect of hypoxia since they observed a decrease in expression of genes involved in the respiratory chain and in the defense against oxidative stress.

Fabbri *et al*. (2003) used isolated hepatocytes of the European eel as experimental model to characterize the effects of Cd^{2+} and Hg^{2+} on either basal or epinephrine-stimulated glucose release. Results from their experiment indicate that Cd^{2+} and Hg^{2+} may impair a crucial intracellular transduction pathway involved in the adrenergic control of glucose metabolism, but also in several other routes of hormonal regulation of liver functions. Micromolar concentrations of both heavy metals significantly reduced the epinephrine-modulated cAMP levels in isolated eel hepatocytes, in good agreement with the reduction of glucose output.

Lead

Santos and Hall (1990) studied the influence of inorganic lead on the biochemical composition of eel blood by exposing eels (mean weight 50 g) for 30 days to 300 µg Pb l^{-1}. A counting of the white blood cells showed an increased number of lymphocytes in lead-treated eels. There was no difference between lead-treated and control eel in either haemoglobin or red blood cells. Biochemical analyses like glucose, total plasma protein, total plasma cholesterol, sodium and potassium plasma did not show significant differences between both groups. The plasma lactate levels increased in lead-treated fish.

The effect of salinity and the mode of application (oral versus aqueous) on the lead accumulation in different eel tissues and its parasites *Anguillicola crassus* (Nematoda) and *Paratenuisentis ambiguous* (Acanthocephala) was investigated by Zimmermann *et al*. (1999). Waterborne as well as dietary lead exposure causes an increase in the metal levels of different eel tissues and its parasites. The mode of lead application had a significant influence on the distribution of lead in the fish tissues, and the resulting metal concentrations were approximately 20 to 2,000 times higher in *P. ambiguus* than in *A. crassus*. These differences may be due to the different microhabitats and nutrient uptake mechanisms of both parasite species (Zimmermann *et al*., 1999).

Sanchez-Galan *et al*. (2001) found that both Cd and Hg, two genotoxic metals, induced micronuclei expression in eels when injected, the concentration tested being 1.7 µg metal g^{-1} body weight and the micronuclei induction being 2.64 and 2.35 micronuclei per 1000 cells for cadmium and mercury respectively. It is known that cadmium also induces micronuclei formation on other fish species such as Tilapia (Manna and Sadhukan, 1986) or brown trout (*Salmo trutta trutta* L.) (Sanchez-Galan *et al*., 1999). Contradictory effects of mercury in fish have been reported previously. Sanchez-Galan *et al*. (1999) found that the frequency of micronuclei in minnows injected with mercury nitrate had not significant increased.

Chromium

A study by Oliveira *et al*. (2003) on the effects of chromium on liver organ culture after 24 hours demonstrated a serum's protective effect against chromium EROD inhibition in liver organ culture.

Teles *et al*. (2005) studied the sequential exposure to PAHs and heavy metals by exposing eel for 24h to chromium (Cr - 100 µM and 1 mM, 24h) or copper (Cu - 1 and 2.5 µM), with or without a 24-h pre-exposure to β-naphthoflavone (BNF - 2.7 µM). The interference of BNF pre-exposure on Cr effects was observed as a significant plasma glucose increase. BNF pre-exposure prevented plasma cortisol and lactate increases; however, a greater T4 decrease was observed in eels exposed to 2.5 µM Cu. Moreover, this pre-treatment was crucial for genotoxicity expression because only BNF+2.5 µM Cu-exposed fish exhibited significant induction of erythrocytic nuclear abnormalities. Single exposures to Cr, decreased plasma T4 in eels, and to Cu resulted in elevated plasma cortisol and glucose (2.5 µM), as well as plasma lactate (1 µM), whereas a T4 (free thyroxine) decrease was found for both concentrations. In general, plasma T4 was the most affected hormone, as it responded to all Cr and Cu exposure conditions (Teles *et al*., 2005).

Ahmad *et al*. (2006) did a similar experiment to examine the oxidative stress and genotoxic effects in gill and kidney of eels. They discovered that in gills, GSH (reduced glutathione) played a crucial role over genotoxicity and that sporadic induction of antioxidant enzymes was not effective in the protection against genotoxicity. A different mechanism occurred in kidney, since the loss of DNA integrity detected for all exposed groups was not accompanied by alterations in antioxidant levels. The interference of BNF pre-exposure with the response of organs to Cr showed a marked dependence on the Cr concentration. The lowest Cr concentration induced an increase on LPO and GPX (glutathione peroxidase) as well as on catalase and GSH decrease in gills, and an LPO increase and GSH decrease in kidney. For the highest concentration, an additive effect on decrease of DNA integrity and an antagonistic effect on the increase of GPX were observed in gills, as well as a catalase and GST decrease in kidney. In contrast, an antagonistic action was observed on DNA integrity loss for both Cr concentrations (Ahmad *et al*., 2006).

Under natural conditions, Maes *et al*. (2005) found a correlation between the level of bioaccumulation of heavy metals and a reduced condition within Belgian yellow eels. They observed a significant negative correlation between heavy metal (Hg, Cd, Pb, Cu, Zn, Ni, Cr, As and Se) pollution load and condition, suggesting an impact of pollution on the health of sub-adult eels. In general, they observed a reduced genetic variability in strongly polluted eels, as well as a negative correlation between bioaccumulation level and allozymatic multi-locus heterozygosity. No pollution related differences were shown for microsatellites, suggesting a differential response at metabolic enzymes and possibly direct overdominance of heterozygous individuals. Species with a high effective population size (mostly marine) generally exhibit high levels of heterozygosity and are expected to be more resistant to pollution; multilocus heterozygotes often show an increased fitness over homozygotes (Nevo *et al*., 1986).

Perfluorooctane sulfonic acid (PFOS)

A significantly and positively related hepatic PFOS concentration with the serum alanine aminotransferase activity was proved by Hoff *et al*. (2005), just as a negative correlation with the serum protein content in eel and carp (*Cyprinius carpio* L.). The hepatic PFOS concentration in carp and eel correlated significantly and positively with the serum ALT activity, a marker for hepatic damage, showing that PFOS may induce liver damage. A decrease of the total serum protein content and an increase of hematocrit levels were suggested to be PFOS mediated. Hu *et al*. (2003) report that perfluorinated compounds are known to affect lipid metabolism, through alterations in cell membrane properties in fish.

Polyaromatic hydrocarbons (PAHs)

Benzo[a]pyrene (BaP) is a very carcinogenic compound which toxic potential is already demonstrated. Maria *et al*. (2002) described a decrease in blood and liver DNA integrity and an increase in the frequency of erythrocytic nuclear abnormalities, CYP1A protein levels, EROD activity and PAH metabolites in bile. Nigro *et al*. (2002) and Nogueira *et al*. (2006) observed an elevated DNA damage and a significant induction of apoptosis after exposure to BaP (50 µg g^{-1} w.w.). Jha (2004) concludes that BaP induced DNA strand breaks could lead to induction of chromosomal aberrations which are also associated with initiation and promotion of cancer. Induction of heritable mutations in germ cells could have long term detrimental effects on population survival (Jha, 2004).

Conclusions

It is obvious that eel is not as resistant as generally has been suggested. Due to its apparent robustness in the face of fluctuations in temperature, salinity, food availability, oxygen and temporary emersion, it is considered as a resistant species but recent studies indicate the contrary. Eel is, due to its characteristic lifecycle, very sensitive to bioaccumulating contaminants, although effects are difficult to measure in the continental immature phase. Due to the international concern about the stock decline many studies have been undertaken to study the effects of pollution on the eel, resulting in an increasing quantity of available information demonstrating the negative impact of pollution on eel at various levels, especially subcellular. However, the direct link between the reported effects at this subcellular level, and the response on population level is yet to be demonstrated. The development of good biomarkers with a great sensivity for both the concentration and length of exposure is necessary. Aubry *et al*. (2007a and b) point that the quantification of the CYP1A1 mRNA levels by real-time PCR can be used as a reliable and sensitive biomarker of exposure of the eel diverse pollution pressures.

Robinet and Feunteun (2002) already point to the importance not only of studying the cause-effect relationships on individuals but also to understand them at the population community and ecosystem levels. The toxic effects can occur at different moments in its lifecycle: during growing, silvering, migration, the development of reproductive cells, and larval stage. During the growing, yellow eel phase the effects count less because contaminants are stored in lipid tissue. Their influence starts during silvering when morphological and physiological changes take place, influenced by hormones. Silver eels migrating to the Sargasso Sea, stop feeding and live on their fat stores. Thus, a good physiological condition is necessary for a successful migration and reproduction. The energy stores must be sufficient to cover the 6000 km long journey and to produce enough good quality eggs. Van Ginneken and van den Thillart (2000) calculated that eels use 60% of the energy reserves during their journey which means that a part of the accumulated contaminants becomes available. A continuous fat burning means a continuous availability of contaminants and a large extent of toxicity in the eel. This toxicity causes disturbance of the immune system, the reproduction system, the nervous system and the endocrine system. So the toxification leads to physiological disturbance, diminished resistance to infections of viruses and parasites, leading to a disturbed reproduction and finally even death of the eel. Contaminants, thus are an important issue in understanding the reasons of the decline of the species.

Whilst the population has decreased to now historical low levels, a large number of environmentally important chemicals has still not been investigated with respect to eel toxicology. It is clear that more extensive research is necessary in or-

der to evaluate how pollutants are ecologically detrimental to eel populations (and fish populations in general).

Acknowledgements

This research was supported by the Flemish Environment Agency and the authors gratefully acknowledge the support of B. Peeters, L. Bervoets, G.Maes and K. Vlietinck. They also want to thank F. Ollevier, F. Volkaert and G. van den Thillart for their comments on the manuscript.

References

Ahmad, I., Oliveira, M., Pacheco, M., Santos, M.A., 2005. *Anguilla anguilla* L. oxidative stress biomarkers responses to copper exposure with or without beta-naphthoflavone pre-exposure. *Chemospere* 61 (2), 267-275.

Ahmad, I., Oliveira, M.V.L., Pacheco, M., Santos, M.A., 2006. Oxidative stress and genotoxic effects in gill and kidney of *Anguilla anguilla* L. exposed to chromium with or without pre-exposure to beta-naphthoflavone. *Mutation Research* 608 (1), 16-28.

Akaishi, F.M., Silva de Assis, H.C., Jakobi, S.C.G., St. Jean, S., Couternay, S.C., Lima, E., Wagner, A.L.R., Scofield, A., Oliveira Ribeiro, C.A., 2004. Morphological and neurotoxicological findings in tropical freshwater fish (*Astyanax* sp.) after waterborne and acute exposure to water soluble fraction (WSF) of crude oil. *Archives of Environmental Contamination and Toxicology* 46, 244–253.

Andres, S., Ribeyre, F., Tourencq, J.-N., Boudou, A., 2000. Interspecific comparison of cadmium and zinc contamination in the organs of four fish species along a pollymetallic pollution gradient (Lot River, France). *The Science of the Total Environment* 248 (1), 11-25.

Ankley, G.T., Blazer, V.S., Reinert, R.E., Agosin, M., 1986. Effects of Aroclor 1254 on cytochrome P450-dependent monooxygenase, glutathione-S-transferase, glucuronosyltransferase activities in channel catfish liver. *Aquatic Toxicology* 9, 91-103.

Anonymous, 1987. Rapport du comité d'experts sur la pollution transfrontière du Rhin. La Gazette Officielle de la Pêche, Nos 920-921 (27p) and 925-926 (55p).

Aubry, E., Cagnon, C., Lalanne, Y., Mouchès, C., 2007a. Assessment of young yellow European eel *Anguilla anguilla* L. exposure to a CYP1A1 inducer by the quantification of increase in hepatic CYP1A1 mRNA using real-time RT-PCR. *Journal of Fish Biology* 71 (Suppl.C), 470-477.

Aubry, E., Cagnon, C., Lalanne, Y., Mouchès, C., 2007b. Quantification of Increase in Hepatic CYP1A1 mRNA by Real-Time RT-PCR after Exposure of the European Eel (*Anguilla anguilla*) to a Diesel Oil Water Soluble Fraction. *Environmental Bioindicators* 2 (1), 47-51.

Azzalis, L.A., Junqueira, V.B.C., Simon, K., 1995. Prooxidant and antioxidant hepatic factors in rats chronically fed an ethanol regimen and treated with an acute dose of lindane. *Free Radical Biology and Medicin* 19, 147–159.

Bálint, T., Ferenczy, J., Kátai, F., Kiss, I., Kráczer, L., Kufcsák, O; Láng, G., Polyhos, C., Szabó, I., Szegletes, T., Nemcsók, J., 1997. Similarities and differences between the massive eel (*Anguilla anguilla* L.) devastations that occurred in Lake Balaton in 1991 and 1995. *Ecotoxicology and Environmental Safety* 37, 17-23.

Barron, M.G., Galbraith, H., Beltman, D., 1995. Comparative reproductive and developmental toxicology of PCBs in birds. *Comparative Biochemistry and Physiology* 112, 1-14.

Belpaire, C., Goemans, G., de Boer, J., Van Hooste, H., 2003. Verspreiding van gebromeerde vlamvertragers. In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, p. 387-395.

Belpaire, C., Goemans, G., 2007a. Eels: contaminant cocktails pinpointing environmental contamination. *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? *Vie et Milieu - Life and Environment* 57 (4), 235-252.

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,* in press.

Belpaire, C., Goemans,' G., Geeraerts,' C., Quataert,' P., Parmentier, K., Hagel, P. and De Boer, J., submitted. Decreasing eel stocks: The Survival of the Fattest? *Ecology of Freshwater Fish*

Belpaire, C., Geeraerts, C., Evans, D., Ciccotti, E., Poole, R., in prep. The European Eel Quality Database: towards a pan-European monitoring of eel quality.

Bilau, M., Sioen, I., Matthys, C., De Vocht, A., Goemans, G., Belpaire, C., Willems J.H., De Henauw, S., 2007. Polychlorinated biphenyl (PCB) exposure through eel consumption in recreation fishermens as compared to the general population, using a probabilistic approach. *Food Additives & Contaminants*, 24 (12), 1386-1393.

Bjerregaard L.B., Korsgaard, B., Bjerregaard, P., 2006. Intersex in wild roach (*Rutilus rutilus*) from Danish sewage effluent-receiving streams. *Ecotoxicology and Environmental Safety* 64, 321-328.

Boëtius, J., Boëtius, I., 1980. Experimental maturation of female silver eels, *Anguilla anguilla*. Estimates of fecundity and energy reserves for migration and spawning. *Dana* 1, 1-28.

Bordajandi, L.R., Gomez, G., Fernandez, M.A., Abad, E., Rivera, J., Gonzalez, M.J., 2003. Study on PCBs, PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the River Turia (Spain). *Chemosphere* 53, 163–171.

Brouwer, A., Murk, A.J., Koeman, J.H., 1990. Biochemical and physiological approaches in ecotoxicology. *Functional Ecology* 4, 275-281.

Brown, J.S., Steinert, S.A., 2004. DNA damage and biliary PAH metabolites in flatfish from Southern California bays and harbors, and the Channel Islands. *Ecological Indicators.* 3, 263–274.

Bruijs, M., Lock R., van Ginneken, V., 2002. Bedreigen PCB's de levenscyclus van de paling? *Aquacultuur* 1, 16-20.

Bruslé, J., 1987. Eel and pollutants: a general review of the effects of heavy metals. European Inland Fishery Advisory Commission (FAO), Eel Working Group, Bristol, April 1987.

Bruslé, J., 1991. The eel (*Anguilla* sp) and organic chemical pollutants. *The Science of the Total Environment* 102, 1-19.

Burzawa-Gerard, E., Dumas-Vidal, A., 1991. Effects of 17β-estradiol and carp gonadotropin on vitellogenesis in normal and hypophysectomized European silver female eel (*Anguilla anguilla* L.) employing a homologous radioimmunoassay for vitellogenin. *General and Comparative Endocrinology* 84(2), 264–276.

Bushnell, P.G., Nikinma, M., Oikari, M., 1985. Metabolic effects of dehydroabietic acid on rainbow trout erythrocytes. *Comparative Biochemistry and Physiology* C81, 391-394.

Cajaraville, M.P., Hauser, L., Carvalho, G., Hylland, K ., Olabarietta, I., Lawrence, A.J., Lowe, D., Goksøyr, A., 2003. Genetic damage and the molecular/cellular response to pollution. In: Lawrence, A., Hemingway, K. Eds. Effects of pollution on fish: molecular effects and population responses. Oxford, U.K., Blackwell Science Ltd, 14-82.

Calow, P., 1991. Physiological costs of combating chemical toxicants: ecological implications. *Comparative Biochemistry and Physiology* 100C(1/2), 3-6.

Ceron, J.J., Ferrando, M.D., Sancho, E., Gutierrez-Panizo, C., Andreu-Moliner, E., 1996. Effects of diazinon exposure on cholinesterase activity in different tissues of European eel (*Anguilla anguilla*). *Ecotoxicology and Environmental Safety* 35, 222-225.

Corsi, I., Mariottini, M., Sensini, C., Lancini, L., Focardi, S., 2003. Cytochrome P450. Acetylcholinesterase and gonadal histology for evaluating contaminant exposure levels in fishes from a highly eutrophic brackish ecosystem: the Orbetello Lagoon, Italy. *Marine Pollution Bulletin* 46, 203–212.

Daverat, F., Limburg, K.E., Thibault, I., Shiao, J.C., Dodson, J.J., Caron, F., Tzeng, W.N., Iizuka, Y., Wickstrom, H., 2006. Phenotypic plasticity of habitat use by three temperate eel species *Anguilla anguilla*, *A. japonica* and *A. rostrata*. Marine Ecology Progress Series 308, 255-269.

de Boer, J., van de Valk, F., Kerkhoff, M.A.T., Hagel P., Brinkman, U.A.Th., 1994. 8-Year study on the elimination of PCBs and other organochlorine compounds from eel (*An-*

guilla anguilla) under natural conditions. *Environmental Science and Technology* 28(13), 2242-2248.

Degani, G., Hahamu, H., Levanin, D., 1986. The relationship of eel *Anguilla anguilla* (L.) body size, lipid, protein, glucose, ash, moisture composition and enzyme activity (aldolase). *Comparative Biochemistry and Physiology* 86(A4), 739-745.

Dekker, W., 2002. Monitoring of glass eel recruitment. Netherlands Institute of Fisheries Research, IJmuiden, the Netherlands, report C007/02-WD, 256 pp.

Durrieu, G., Maury-Brachet, R., Girardin, M., Rochard, E., Boudou, A., 2005. Contamination by heavy metals (Cd, Zn, Cu, and Hg) of eight fish species in the Gironde Estuary (France). *Estuaries* 28 (4), 581–591.

Dutta, H.M., Meijer, H.J.M., 2003. Sublethal effects of diawinon on the structure of the testis of bluegill. *Lepomis macrochirus*: a microscopic analyses. *Environmental Pollution* 125, 355–360.

Edwards, S.C., McLeod, C.L., Lester, J.N., 1999. Mercury contamination of the eel (*Anguilla* Anguilla) and roach (*Rutilus* rutilus) in East-Anglia, UK. *Environmental Monitoring and Assessment* 55, 371-387.

Eisler, R., 1998. Copper hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002.

Fabbri, E., Caselli, F., Piano, A., Sartor, G., Capuzzo, A., 2003. Cd^{2+} and Hg^{2+} affect glucose release and cAMP-dependent transduction pathway in isolated eel hepatocytes. *Aquatic Toxicology* 62(1), 55-65.

Fernandez-Vega, C., Sancho, E., Ferrando, M.D., Andreu-Moliner, E., 1999. Thiobencarb toxicity and plasma AchE inhibition in the European eel. *Journal of Environmental Science and Health* Part B 34(1), 61-73.

Ferrando, M.D., Andreu, E., 1991a. The effect of time on physiological changes in eel *Anguilla anguilla*, induced by lindane. *Comparative Biochemistry and Physiology* 100C, 95-98.

Ferrando, M.D., Andreu, E., 1991b. Effects of lindane on fish carbohydrate metabolism. *Ecotoxicological and Environmental Safety* 22, 17-23.

Folmar, L.C., 1993. Effects of chemical contaminants on blood chemistry of teleost fish: A bibliography and synopsis of selected effects – Review. *Envrionmental Toxicology and Chemistry* 12(2), 337-375.

Geeraerts, C., Goemans, G., Quataert P., Belpaire, C., 2007. Ecologische en ecotoxicologische betekenis van verontreinigende stoffen in paling. Studie uitgevoerd in opdracht van de Vlaamse Milieumaatschappij, MIRA, MIRA/ 2007/05, INBO/R/2007/40. Instituut voor Natuur- en Bosonderzoek. p.207.

Gillemot, P., 2003. Evaluatie van PFOS vervuiling en xeno-oestrogene effecten in endogene vispopulaties in Vlaanderen. Eindverhandeling UA, Lic. Biomedische Wetenschappen, 44p.

Gimeno, L., Ferrando, M.D., Sanchez, S., Andreu, E., 1994. Endosulfan effects on liver and blood of the eel, *Anguilla anguilla*. *Comparative Biochemistry and Physiology* 108C, 343-348.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. Rapport van het Instituut voor Bosbouw en Wildbeheer, IBW.Wb.V.R.2003.99. 169 p.

Goksøyr, A., Frölin, L., 1992. The cytochrome P450 system in fish, aquatic toxicology and environmental monitoring. *Aquatic Toxicology* 22, 287-312.

Gony, S., 1987. Experimental research on the effects of cadmium on young yellow-eels: a histological and SEM structural approach. Eel Working Group, Bristol, April 1987, p.1-7.

Gravato, C, Teles, M., Oliveira, M., Santos, M.A., 2006. Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla* L.--the influence of pre-exposure to beta-naphthoflavone. *Chemosphere* 65(10), 1821-1830.

Grosell, M., Boëtius, I., Hansen, J.H.J.M., Rosenkilde, P., 1996. Influence of preexposure to sublethal levels of copper on ^{64}Cu uptake and distribution among tissues of the European eel (*Anguilla anguilla*). *Comparative Biochemistry and Physiology* C 114 (3), 229-235.

Grosell, M., Hansen, H.J.M., Rosenkilde, P., 1998. Cu uptake, metabolism and elimination in fed and starved European eels (*Anguilla anguilla*) during adaptation to water-borne Cu exposure. *Comparative Biochemistry and Physiology* C 120, 295–305.

Harrad, S., Smith, D., 1999. Eel consumption as a pathway of human exposure to PCBs. *International Journal of Environmental Health Research* 9, 31 – 37.

Hibiya, T., 1982. An Atlas of Fish Histology – Normal and Pathological Features. Kodansha, Tokyo.

Hoff, P.T., Van Campenhout, K., Van de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., Goemans, G., Belpaire, C., Blust R., De Coen, W., 2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Environmental Pollution* 137, 324-333.

Holmberg, B., Jensen, S., Larsson, A., Lewander, R., Olsson, M., 1972. Metabolic effects of technical pentachlorophenol (PCP) on the eel *Anguilla anguilla*. *Comparative Biochemistry and Physiology* 43B, 171-183.

Hose, J.E., Hannah, J.B., DiJulio, D., Landolt, M.L., Miller, B.S., Iwaoka W.T., Felton, S.P. 1982. Effects of bezo[a]pyrene on early development of flatfish. *Archives Environmental Contamination and Toxicology* 11, 167-171.

Howard, T.E., McLeay, Walden, C.C., 1971. Sublethal effects of bleached kraft mill effluents to fish. CPAR Project Report 9-2, Pulp and Paper Pollution Abatement, Ottawa, Canada, 63 pp.

Hu, W., Jones, P.D., De Coen, W., King, L., Fraker, P., Newsted, J., 2003. Alterations in cell membrane properties caused by perfluorinated compounds. *Comparative Biochemistry and Physiology C Toxicological Pharmacology* 135, 77-88.

Ibuki, Y., Goto, R., 2002. Photo toxicity of benzo[a]pyrene by ultraviolet A irradiation: induction of apoptosis in Jurkat cells. *Environmental Toxicology & Pharmacology* 11, 101–109.

ICES, 2002. International Council for the Exploration of the Sea. Report of the ICES/EIFAC Working Group on Eels. ICES C.M. 2002/ACFM:03.

Janicki, R.H., Kinter, W.B., 1971. DDT: Disrupted osmoregulatory events in the intestine of the eel *Anguilla rostrata* adapted to seawater. *Science* 173 (4002), 1146-1148.

Jha, A.N., 2004. Genotoxicological studies in aquatic organisms: an overview. *Mutation Research* 552, 1-17.

Jimenez, B.D., Burtis, L.S., 1989. Influence of environmental variables on the hepatic mixed) function oxidase system in the bluegill sunfish (*Lepomis macrochirus*). *Comparative Biochemistry and Physiology* 93, 11-21.

Jimenez, B.D., Burtis, L.S., Ezell, G.H., Egan, B.Z., Lee, N.E., Beauchamp, J.J., McCarthy, J. F., 1988. The mixed-function oxidase system of bluegill sunfish *Lepomis macrochirus*: correlation of activities in experimental and wild fish. *Environmental Toxicology and Chemistry* 7, 623-634.

Jobling, S., Sumpter, J.P., 1993. Detergent components in sewage effluent are weakly estrogenic to fish - An in-vitro study using rainbow-trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic Toxicology*, 27(3-4), 361-372.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. *Environmental Science & Technology* 32(17), 2498–2506.

Jobling, S., Beresforda, N., Nolanb, M., Rodgers-Grayc, T., Brightyd, G.C., Sumpter, J.P., Tyler, C.R., 2002a. Altered sexual maturation and gamete production in wild roach (R*utilus rutilus*) living in rivers that receive treated sewage effluents. *Biology of Reproduction* 66, 272-281.

Jobling S., Coey, S., Whitmore, J.G., Kime, D.E., Van Look, K.J.W., McAllister, B.G. , Beresforda, N., Henshaw, A.C., Brighty, G., Tyler, C.R., Sumpter J.P., 2002b. Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Biology of Reproduction* 67, 515-524.

Jobling, S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nolan, M., Tyler, C. R., van Aerle, R., Santos, E., Brighty, G. (2006). Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations. *Environmental Health Perspectives* 114 (1), 32-39.

Johnson, L.L., C.M. Stehr, O.P. Olson, M.S. Myer, S.M. Pierce, C.A., Wigren, B.B., McCain, B.B., Varanasi, U., 1992. Bioindicators of contaminant exposure, liver pathology, and reproductive development in prespawning female winter flounder (*Pleuronectes americanus*) from urban and nonurban estuaries on the northeast Atlantic coast: U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northwest Fisheries Science Centre, NOAA Technical Memornadum NMFS-NWFSC series no.1, p.76.

Johnson, L.L., Misitano, D., Sol, S.Y., Nelson, G.M., French, B., Ylitalo G.M., Hom, T., 1998. Contaminants effects on ovarian development and spawning success in rock sole from Puget Sound, Washington. *Transactions of the American Fisheries Society* 127(3), 375-392.

Kime, D.E., 1995. The effects of pollution on reproduction in fish. *Reviews in Fish Biology and Fisheries* 5, 52-96.

Kinter, W.B., Merkens, L.S., Janicki, R.H., Guarino, A.M., 1972. Studies on the mechanism of toxicity of DDT and polychiorinated biphenyls (PCBs): disruption of osmoregulation in marine fish. *Environmental Health Perspectives* 1, 169-173.

Kleinow, K.M., Melancon, M.J. Lech, J.J., 1987. Biotransformation and induction: implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. *Environmental Health Perspectives* 71, 105-119.

Knights, B., 1997. Risk assessment and management of contamination of eels (*Anguilla* spp.) by persistent xenobiotic organochlorine compounds. *Chemistry and Ecology* 13, 171-212.

Labandeira, A., Eljarrat, E., Barcelo, D. 2007. Congener distribution of polybrominated diphenyl ethers in feral carp (*Cyprinus carpio*) from the Llobregat River, Spain. *Environmental Pollution* 146, 188-195.

Langston, W.J., Chesman, B.S., Burt, G.R., Pope, N.D., McEvoy, J., 2002. Metallothionein in liver of eels *Anguilla anguilla* from the Thames Estuary: an indicator of environmental quality? *Marine Environmental Research* 53, 263-293.

Larsson, P., Hamrin, S., Okla, L., 1990. Fat content as a factor inducing migratory behavior in the eel (*Anguilla anguilla* L.) to the Sargasso Sea. *Naturwissenschaften* 77, 488–490.

Larsson, P., Hamrin, S., Okla, L., 1991. Factors determining the uptake of persistent pollutants in an eel population (*Anguilla anguilla* L.). *Environmental Pollution* 69, 39-50.

Lawrence, A., Hemingway, K., 2003. Effects of pollution on fish: molecular effects and population responses. Oxford, U.K., Blackwell Science Ltd, 342 p.

Lawrence, A.J., Elliot, M., 2003. Introduction and conceptual model. In: Lawrence, A., Hemingway, K. Eds. *Effects of pollution on fish: molecular effects and population responses*. Oxford, U.K., Blackwell Science Ltd, 1-13.

Leatherhand, J.F., Sonstegard, R.A., 1980. Effect of dietary Mirex and PCBs in combination with food deprivation and testosterone administration on thyroid activity and bioaccumulation of or-ganochlorines in rainbow trout, Salmo garidneri Richardson. *Journal of Fish Diseases* 3(2), 115-124.

Lionetto, M.G., Maffia, M., Cappello, M.S., Giordano, M.E., Storelli, C., Schettino, T., 1998. Effect of cadmium on carbonic anhydrase and Na^+-K^+-ATPase in eel, *Anguilla anguilla*, intestine and gills. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, Volume 120, Number 1, 89-91(3).

Livingstone, D.R., Mitchelmore, C.L., Peters, L.D., O'Hara, S.C.M., Shaw, J.P., Chesman, B.S., Doyotte, A., McEvoy, J., Ronisz, D., Larsson, D.G.J., Förlin, L., 2000. Development of hepatic CYP1A and blood vitellogenin in eel (*Anguilla anguilla*) for use as biomarkers in the Thames Estuary, UK. *Marine Environmental Research* 50(1–5), 367–371.

Luizi, F., Korsgaard, B., Petersen, I.M., 1997. Plasma lipoproteins in European eels (*Anguilla anguilla*): effects of estradiol. *Fish Physiology and Biochemistry* 16(4), 273–280.

Maes, G.E., Raymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert,, F.A.M., 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: Relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology*, 73, 99-114.

Manna, G.K., Sadhukan, A., 1986. Use of cells of gill and kidney of tilapia fish in micronucleus test (MNT). *Current Science* 55, 498-501.

Maria, V.L., Correia, A.C., Santos, M.A., 2002. *Anguilla anguilla* L. bochemical and genotoxic responses to benzo[a]pyrene. *Ecotoxicology and Environmental Safety* 53, 86-92.

Maria, V.L., Pacheco, M., Santos, M.A., 2006. *Anguilla anguilla* L. Genotoxic responses after in situ exposure to freshwater wetland (Pateira de Fermentelos, Portugal). *Environment international* 32(4), 510-515.

Martel, P.H., Kovacs, T.G., O'Connor, B.I., Voss, R.H., 1994. A survey of pulp and paper mill effluents for their potential to induce mixed function oxidase enzyme activity in fish. *Water Research* 28, 1835-1844.

Marty, G.D., Hoffmann, A., Okihiro, M.S., Hepler, K., Hanes, D., 2003. Retrospective analyses: bile hydrocarbons and histopathology of demersal rockfish in Prince William sound, Alaska, after the Exxon Valdez oil spill. *Marine Environmental Research* 56, 569–584.

Mason, C.F., Barak, N.A.E., 1990. A catchment survey for heavy metals using the eel (*Anguilla anguilla*). *Chemosphere* 21, 695 – 699.

McKinney, J.D., Waller, C.L., 1994. Polychlorinated biphenyls as hormonally active structural analogues. *Environmental Health Perspectives* 102, 290-297.

Melancon, M.J., Elcombe, C.R., Vodicnik, M.J., Lech, J.J., 1981. Induction of cytochromes P-450 and mixed-function oxidase activity by polychlorinated biphenyls and β-naphthoflavone in carp (*Cyprinius carpio*). *Comparative Biochemistry and Physiology* 69C, 219-226.

Monod, G., Gevaux, A., Rivière, J.L., 1988. Effects of chemical pollution on the activities of hepatic xenbiotic metabolising enzymes in fish from the river Rhone France. *Science of the Total Environment* 73, 189-202.

Nevo, E., Noy, R., Lavie, B., Beiles, A., Muchtar, S., 1986. Genetic diversity and resistance to marine pollution. *Biological Journal of the Linnean Society* 29, 139–144.

Nigro, M., Frenzilli, G., Scarcelli, V., Gorbi, S., Regoli, F., 2002. Induction of DNA strand breaks and apoptosis in the eel *Anguilla Anguilla*. *Marine Environmental Research* 54, 517-520.

Nogueira, P.R., Lourenço, J., Mendo, S., Rotchell, J.M., 2006. Mutation analysis of ras gene in the liver of European eel (*Anguilla anguilla* L.) exposed to benzo[a]pyrene. *Marine Pollution Bulletin* 52, 1611-1616.

Nowell, L.H., Capel, P.D., Dileanis, P.D., 1999. Pesticides in Stream Sediment and Aquatic Biota. CRC Press, 1001 p.

Oliveira, M., Santos, M.A., Gravato, C., Pacheco, M., 2003. Chromium effects on *Anguilla anguilla* liver organ culture. *Fresenius Environmental Bulletin* 12 (4), 349-352.

Oliveira Ribeiro, C.A., Vollaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. *Aquatic Toxicology* 74, 53–69.

Oliveira, M., Serafim, A., Bebianno, M.J., Pacheco, M., Santos, M.A., 2008. European eel (*Anguilla anguilla* L.) metallothionein, endocrine, metabolic and genotoxic responses to copper exposure. *Ecotoxicology and Environmental Safety* 11,1819-2012.

Pacheco, M., Santos, M.A., 1997. Induction of EROD activity and genotoxic effects by polycyclic aromatic hydrocarbons and resin acids on the juvenile eel (*Anguilla anguilla* L.). *Ecotoxicology and Environmental Safety* 38, 252-259.

Pacheco, M., Santos, M.A., 1999. Biochemical and genetic responses of adult eel (*Anguilla anguilla* L.) to resin acids and pulp mill effluent: laboratory and field experiments. *Ecotoxicology and Environmental Safety* 42, 81-93.

Pacheco, M., Santos, M.A., 2002. Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). *Ecotoxicology and Environmental Safety* 53, 331-347.

Palstra, A.P., Curiel, D., Fekkes, M., de Bakker, M., Székely, C., van Ginneken, V.J.T., van den Thillart, G.E.E.J.M., 2007. Swimming stimulates oocyte development of European eel (*Anguilla anguilla* L.). *Journal of Fish Biology,* in press.

Palstra, A.P., Van Ginneken, V.J.T., Murk, A.J., Van Den Thillart, G.E.E.J.M., 2006. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93 (3), 145-148.

Peters, L.D., Doyotte, A., Mitchelmore, C.L., McEvoy, J., Livingstone, D.R., 2001. Seasonal variation and estradiol-dependent elevation of Thames estuary eel *Anguilla anguilla* plasma vitellogenin levels and comparisons with other estuaries. *The Science of the Total Environment* 279 (1–3), 137–150.

Pierron, F., Baudrimont, M., Bossy, A., Bourdineaud, J.-P., Brèthes, D., Elie, P., Massabuau, J.-C., 2007a. Impairment of lipid storage by cadmium in the European eel (*Anguilla anguilla*). *Aquatic Toxicology* 81(3), 304-311.

Pierron, F., Baudrimont, M., Gonzalez, P., Bourdineaud, J.-P., Elie, P., Massabuau, J.-C., 2007b. Common pattern of gene expression in response to hypoxia or cadmium in the gills of the European glass eel (*Anguilla anguilla*). *Environmental Science and Technology* 41 (8), 3005-3011.

Prophete, C., Carlson, E.A., Li, Y., Duffy, J., Steinetz, B., Lasano, S., Zelikoff, J.T., 2006. Effects of elevated temperature and nickel pollution on the immune status of Japanese medaka. *Fish & Shellfish Immunology* 21(3), 325-334.

Rether, B., Masfaraud, J.-F., Keith, G., Devaux, A., Monod, G., 1997. Biomarqueurs de génotoxicité chez les végétaux et les animaux. In Lagadic *et al.* (eds) Biomarqueurs en écotoxicologie, aspects fondamentaux. Paris: Masson, p. 185-208.

Rice, C.D., Kergosien, D.H., Adams, S.M., 1996. Innate immune function as a bioindicator of pollution stress in fish. *Ecotoxicology and Environmental Safety* 33(2), 186-192.

Robinet, T.T., Feunteun, E.E., 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265–277.

Roche H., Buet, A., Ramade, F., 2002. Accumulation of lipophilic microcontaminants and biochemical responses in eels from the Camargue Biosphere Reserve. *Ecotoxicology* 11, 155-164.

Rødsæther, M.C., Olafsen, J., Raa, J., Myhre, K., Steen, J.B., 1977, Copper as an initiating factor of vibriosis (*Vibrio anguillarum*) in eel (*Anguilla anguilla*). *Journal of Fish Biology* 10, 17-21.

Roosens, L., Dirtu, A., Goemans, G., Belpaire, C., Gheorghe, A., Neels, H., Blust, R., Covaci, A., 2008. Brominated flame retardants and polychlorinated biphenyls in fish from the River Scheldt, Belgium. *Environment International,* in press.

Ruangsomboon, S., and Wongrat, L., 2006. Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (*Chlorella vulgaris*), zooplankton (*Moina macrocopa*), and the predatory catfish *Clarias macrocephalus*×*C. gariepinus*. *Aquatic Toxicology* 78,15–20.

Sanchez-Galan, S., Linde, A.R., Garcia-Vazquez, E., 1999. Brown trout and European minnow as target species for genotoxicity tests: Differential sensitivity to heavy metals. *Ecotoxicology and Environmental Safety* 43, 301-304.

Sanchez-Galan, S., Linde, A.R., Ayllon, F., Garcia-Vazquez, E., 2001. Induction of micronuclei in eel (*Anguilla anguilla* L.) by heavy metals. *Ecotoxicology and Environmental Safety* 49(2), 139-143.

Sancho, E., Ferrando, M.D., Andreu, A., 1997. Sublethal effects of an organophosphate insecticide on the European eel, *Anguilla anguilla*. *Ecotoxicology and Environmental Safety* 36, 57-65.

Sancho, E., Ferrando, M.D., Andreu, E., 1998. Effects on sublethal exposure to a pesticide on levels of energetic compounds in *Anguilla anguilla*. *Journal of Environmental Science* Part B33 (4), 411-424.

Santos, M.A., Hall, A., 1990. Influence of inorganic lead on the biochemical blood composition of the eel, *Anguilla anguilla* L. *Ecotoxicology and Environmental Safety* 20(1), 7-9.

Santos, M.A., Pires, F., Hall, A., 1990. Metabolic effects of kraft mill effluents on the eel, *Anguilla anguilla* L., *Ecotoxicology and Environmental Safety* 20, 10-19.

Shailaja, M.S., D'Silva, C., 2003. Evaluation of impact of PAH on a tropical fish. Oreochromis mossambicus using multiple biomarkers. *Chemosphere* 53, 835–841.

Slooff, W., De Zwart, D., 1983. The growth, fecundity, and mortality of bream (*Abramis brama)* from polluted and less polluted surface waters in the Netherlands. *The Science of the Total Environment* 27, 149-162.

Spies, R.B., Rice, S.W.Jr., 1988. Effects of organic contaminants on reproduction of the starry flounder *Platichtys stallatus* in San Francisco Bay: 1. Hepatic contamination and mixed-function oxidases (MFO) activity during the reproductive season. *Marine Biology* 98, 191-200.

Stohs, S.J., Bagghi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med.* 18, 321–336.

Stott, G.G., Haensly, W., Neff, J., Sharp, J., 1983. Histopathologic surveys of ovaries of plaice, *Pleuronectes platessa* L., from Aberwrac'h and Aber Benoit, Brittany, France: long-term effects of the *Amoco Cadiz* crude oil spills. *Journal of Fish Disease* 6(5), 429-437.

Sures, B., Taraschewski, H., Jackwerth, E., 1994. Lead content of *Paratenuisentis ambiguus* (Acanthocephala), *Anguillicola crassus* (Nematodes) and their host *Anguilla anguilla*. *Diseases of Aquatic Organisms* 19, 105–107.

Sures, B., Knopf, K., 2004. Individual and combined effects of cadmium and 3,3k,4,4k,5-pentachlorobiphenyl (PCB126) on the humoral immune response in European eel

(*Anguilla anguilla*) experimentally infected with larvae of *Anguillicola crassus* (Nematoda). *Parasitology* 128, 445-454.

Sures, B., 2006. How parasitism and pollution affect the physiological homeostasis of aquatic hosts. *Journal of Helminthology* 80(2), 151-157.

Svobodová, Z., Vykusová, B., Máchová, J., Hrbková, M., Groch, L., 1994. The long-term effects of PCBs on fish. In: Sublethal and chronic effects of pollutants on freshwater fish. Müller and Lloyd (Eds), FAO, Fishing News Books, Oxford, p. 88-98.

Teles, M. , Pacheco, M., Santos, M.A., 2005. Physiological and genetic responses of European eel (*Anguilla anguilla* L.) to short-term chromium or copper exposure - Influence of preexposure to a PAH-like compound. *Environmental Toxicology* 20 (1), 92 – 99.

Teles, M., Pacheco, M. Santos, M.A., 2007. Endocrine and metabolic responses of *Anguilla anguilla* L. caged in a freshwater-wetland (Pateira de Fermentelos--Portugal). *The Science of the total environment* 372(2-3), 562-570.

Thurow, F., 1959. Über Fängerfrage und Wachstum des Aales in der westlichen Ostsee. *Zeitschrift für Fisherei und deren Hilfswissenschaften* 8, 597–626.

Toivola, D.M., Isomaa, B., 1991. Effects of dehydroabietic acid on the erytrocyte membrane. *Chemical – Biological Interactions* 79, 65-78.

Tulonen, J., Vuorinen, P.J., 1996. Concentrations of PCBs and other organochlorine compounds in eels (*Anguilla anguilla*, L) of the Vanajavesi watercourse in southern Finland, 1990-1993. *The science of the Total Environment* 187,11-18.

Turner, K.J., Sharpe, R.M., 1997. Environmental oestrogens – present understanding. *Reviews in Reproduction* 2, 69-73.

Tuurala, H., Soivio, A., 1982. Structural and circulatory changes in the secondary lamellae of *Salmo gairdneri* gills after sublethal exposure to hydroabietic acid and zinc. *Aquatic toxicology* 2, 21-29.

Van Campenhout, K., Goenaga Infante, H., Goemans, G., Belpaire, C., Adams, F., Blust, R., Bervoets, L., 2008 in press. A field survey of metal binding to metallothionein and other cytosolic ligands in liver of eels using an on-line isotope dilution method in combination with size exclusion (SE) high pressure liquid chromatography (HPLC) coupled to Inductively Coupled Plasma time-of-flight Mass Spectrometry (ICP-TOFMS). The Science of the Total Environment, 2008 in press

van den Thillart, G., van Ginneken, V., Körner, F., Heijmans, R., van der Linden, R., Gluvers, A., 2004. Endurance swimming of European eel. *Journal of Fish Biology* 65, 1-7.

van den Thillart, G.E.E.J.M., Dufour, S., Ellie, P., Volkaert, F., Sebert, P., Rankin, C., Szekely, C., van Rijsingen, J., 2005. Estimation of the reproduction capacity of European eel: EELREP Final Report, 272 p.

van Ginneken, V.J.T. Van den Thillart, G.E.E.J.M., 2000. Eel fat stores enough to reach the Sargasso. *Nature* 403, 156-157.

van Ginneken, V.J.T., Balm, B., Sommandas, V., Onderwater, M., van den Thillart, G.E.E.J.M., 2002. Acute stress syndrome of the yellow European eel (*Anguilla anguilla* Linnaeus) when exposed to a graded swimming-load*. Netherlands Journal of Zoology* 52, 29-42.

van Ginneken, V.J.T., Ballieux, B. Willemze, R., Coldenhoff, K., Lentjes, E., Antonissen, E., Haenen, O., van den Thillart, G.E.E.J.M., 2005. Hematology patterns of migrating European eels and the role of EVEX virus. Comparative Biochemistry and Physiology C *Toxicology and Pharmacology* 140(1), 97-102.

van Ginneken, V.J.T., Dufour, S., Sbaihi, M., Balm, P., Noorlander, K., de Bakker, M., Doornbos, J., Palstra, A., Antonissen, E., Mayer, I., van den Thillart, G. 2007. Does a 5,500-km swim trial stimulate early sexual maturation in the European eel (*Anguilla anguilla* L.)? *Comparative Biochemistry and Physiology*, in press, doi: 10.1016/j.cbpa.2007.03.021.

van Leeuwen, S.P.J., Traag, W.A., Hoogenboom, L.A.P., de Boer, J., 2002. Dioxins, furans and dioxin-like PCBs in wild, farmed, imported and smoked eel from the Netherlands. RIVO Rapport C034/02.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363-371.

Vøllestad, L. A., 1992. Geographic variation in age and length at metamorphosis of maturing European eel: environmental effects and phenotypic plasticity. *Journal of Animal Ecology 61,* 41–48.

Von Westernhagen, H., Rosenthal, H., Dethlefsen, V., Ernst, W., Harms, U.L., Hanse, P.D., Cameron, P., Janssen, D., 1981. Bioaccumulating substances and reproductive success in Baltic flounder *Platichthys flesus*. *Aquatic Toxicology* 1, 85-99.

Von Westernhagen, H., Dethlefsen, V., Cameron, P., Janssen, D., 1987. Chlorinated hydrocarbon residues in gonads of marine fish and effects on reproduction. *Sarsia* 72, 419-422.

Walton, D.G., Fancey, L.L. Green, J.M. Kiceniuk, J.W. Penrose, W.R., 1983. Seasonal changes in aryl hydrocarbon hydroxylase activity of a marine fish *Tautogolabrus adspersus* (Walbaum) with and without petroleum exposure. *Comparative Biochemistry and Physiology* C76, 247-253.

Wolkers, J., Jørgensen, E.H., Nijmeijer, S.M., Witkamp, R.F., 1996. Time-dependent induction of two distinct hapatic cytochrome P450A catalytic activities at low temperatures in Arctic char (*Salvelinus alpinus*) after oral exposure to benzo[a]pyrene. *Aquatic Toxicology* 35, 127-138.

WG Eel, 2006. Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels. FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Rome, 23–27 January 2006. EIFAC Occasional Paper. No. 38, ICES CM 2006/ACFM:16. Rome, FAO/Copenhagen, ICES, 2006. 352 p. Available from http://www.ices.dk/reports/ACFM/2006/WGEEL/WGEEL06.pdf

WG Eel, 2007. Report of the 2007 Session of the Joint EIFAC/ICES, Working Group on Eels, FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Bordeaux, 3–7 September 2007, EIFAC Occasional Paper No. 38, ICES CM 2007/ACFM:23, Draft, 524 p. Available from www.ices.dk/reports/ACFM/2007/WGEEL/WGEEL_07_draft.pdf

Zimmermann, S., Sures, B., Taraschewski, H., 1999. Experimental studies on lead accumulation in the eel specific endoparasites *Anguillicola crassus* (Nematoda) and *Paratenuisentis ambiguus* (Acanthocephala) as compared with their host, *Anguilla anguilla*. *Archives of Environmental Contamination and Toxicology* 37, 190-195.

A silver eel. Fat stores in eels seem to be affected by pollution. Are the energy reserves in silver eels still sufficient to reach the spawning places in the Sargasso Sea and have normal reproduction? Seeking a cause for the decline of the eel stock is one of the major questions challenging the scientific community.

Photo: INBO (Vilda – Rollin Verlinde)

Chapter 6

Decreasing eel stocks: Survival of the Fattest?

Claude Belpaire[1], Geert Goemans[1], Caroline Geeraerts[1], Paul Quataert[1], Koen Parmentier[2], Paul Hagel[3] and Jacob de Boer[4]

1 - Research Institute for Nature and Forest, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium
2 - Institute for Agricultural and Fisheries Research (ILVO Fischeries), Ankerstraat 1, B-8400 Oostend, Belgium
3 - Netherlands Institute for Fisheries Research*, P.O. Box 68, NL-1970 AB Ijmuiden, The Netherlands
4 - Institute for Environmental Studies (IVM), VU University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

* Present name : Institute for Marine Resources and Ecosystem Studies (IMARES)

This chapter is submitted as:

Belpaire, C., Goemans, G., Geeraerts, C., Quataert, P., Parmentier, K., Hagel, P., de Boer, J., submitted.
Decreasing eel stocks: Survival of the Fattest?
Ecology of Freshwater Fish, submitted.

Summary

Since the 1980s the European eel *Anguilla anguilla* stock is in steep decline. Lipid reserves are essential to cover energetic requirements for migration and reproduction. Two large and independent data sets from Belgium and The Netherlands show a one-third decrease in fat contents of yellow eels over the past 15 years. Also the condition decreased. On the basis of the somatic energy reserves, reproductive potential of female eels from various latitudes were estimated, indicating the poor status of eels throughout Europe. Only large individuals, females as well as males, with high lipid content seem to be able to contribute to the spawning stock. The decrease in fat content may be a key element in the stock decline and raises serious concerns about the chances of the stock to recover.

Introduction

Stocks of the Atlantic eel species are in steep decline. Since the 1980s the population of the European eel *Anguilla anguilla* (L.) has waned throughout almost its entire habitat. The stock is considered outside safe biological limits and current fisheries are not sustainable (ICES, 2001; WG Eel, 2007). From the spawning area in the Sargasso Sea the eel larvae reach continental waters where they grow up in fresh water and coastal habitats during their sedentary yellow eel phase. Adults leave as mature silver eels for the spawning grounds in the ocean. Since the early 1980s glass eel recruitment has dropped over the whole distribution area to about 1% of the levels encountered in the seventies (Dekker, 2003a). In June 2007 the European eel was added to the UN CITES Appendix II list and rated "critically endangered" on the Red List of species compiled by the World Conservation Union (CITES, 2007). The Council of the European Union established a framework and measures for the recovery and sustainable use of the stock of European eel and requires the preparation of national eel management plans in September 2007 (European Commission, 2007). The population crash happened over the whole European continent without a single, obvious cause (Dekker, 2003a). Fisheries yields have decreased in most European countries (Dekker, 2003b). Anthropogenic factors (e.g. exploitation, habitat loss, migration barriers (turbines and pumps), pollution, reduced eutrophication and transfer of parasites and diseases), as well as natural processes (e.g. climate and ocean change, and predation) may have contributed to the decline (WG Eel, 2006). Detrimental effects of pollution on fitness and fecundity have been suggested earlier on (Larsson *et al.*, 1990), but recently, there are indications that poor quality of the spawners, namely the silver eels migrating to the oceanic spawning grounds, might be a key factor in explaining the decline. Palstra *et al.* (2006a) argued that gonadal levels of dioxin-like contaminants, including PCBs, in eels from most European locations impair embryonic development. Pollution might also impact reproductive success through effects on genotype: a significant negative correlation between heavy metal pollution and eel genetic variability was reported by Maes *et al.* (2005). Insufficient fitness (condition and energy resources (Svedäng and Wickström, 1997)), high bioaccumulation of persistent organic pollutants (especially polychlorinated biphenyls - PCBs) (Larsson *et al.*, 1990; Robinet and Feunteun, 2002; Palstra *et al.*, 2006a) and pathological agents (Palstra *et al.*, 2007) have been reported as potential restrictive factors, disabling long distance migration and successful reproduction with prime quality gametes. It has been proposed by several authors that the lipid content of silver eel is crucial for reproduction. Under a critical fat mass in their yellow stage (28%), silvering may not even be initiated (Thurow, 1959; Larsson *et al.*, 1990). Quite diverging data upon minimum energy requirements (in lipid weight % of muscle) for the completion of their migration and successful reproduction have been proposed (Boëtius and Boëtius, 1980: 20%; Palstra *et al.*, 2007: 13.5% fat; van den Thillart *et al.*, 2007: 20.7%). Where spawner quality is poor and lipid content low, silver eels may not contribute to the overall spawning and recruitment of the European stock. In order to trace changes in lipid contents in eel over time we analysed two independent data sets of muscle lipid content in yellow eel.

Methods and study area

Samples and sampling

In Belgium (BE) and The Netherlands (NL) networks are functioning to monitor the quality of the European eel in its yellow sedentary phase. They monitor hazardous substances like PCBs, organochlorine pesticides and heavy metals in eel muscle and provide evidence of their presence in the aquatic environment and of the risks for human consumption (de Boer and Hagel, 1994; Maes *et al.*, 2008; Bilau *et al.*, 2007). Most sampling sites (Figure 6.1) are located in the basins of the rivers Scheldt, Meuse and Rhine.

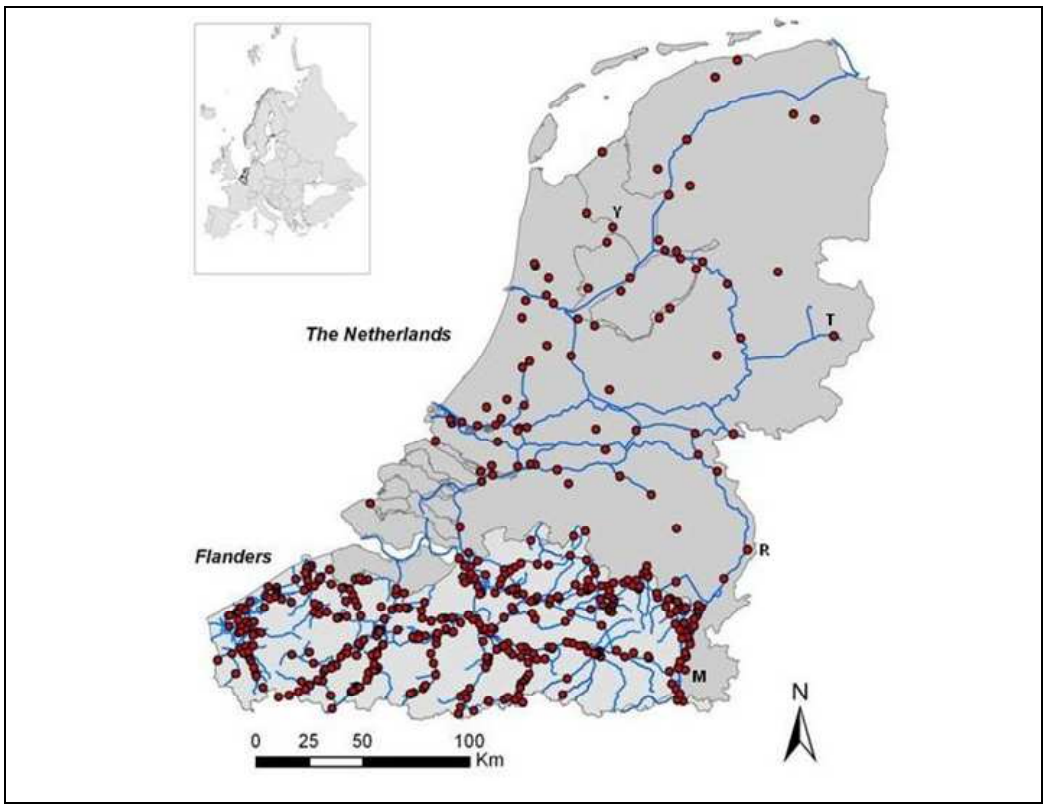


Figure 6.1. Sampling locations for measuring the fat contents in yellow eel. Map of Flanders (Belgium) and The Netherlands with locations of monitoring sites in both networks. Locations Y, M, R and T refer to Lake IJsselmeer, Rivers Meuse and Roer and the Canal Twentekanaal respectively.

In Belgium, the network is confined to Flanders (the northern region) and has been operating since 1994; data are available until 2006. It consists of 359 sites, of which 38% have been monitored more than once. In The Netherlands, the network has been running from 1977 and annual data are available until 2004. The network

consists of 92 sites; each year on average 20 sites are sampled. In both countries, eels were sampled by electro- and fyke-fishing. In Belgium, usually five eels were analysed individually from each site, and this study is based on the individual analysis of 2,467 yellow eels with a selected length between 30 and 60 cm. In The Netherlands, analysis is carried out on 560 pooled yellow eel samples (25 eels per pool), eels being selected from the length class 30 - 40 cm. The condition factor was calculated for the Belgian eels only, following Le Cren's relative condition factor (Le Cren, 1951). The sex of the eels was not determined, with the exception of one year at four sites in The Netherlands.

Four water bodies of different typology were selected from the Dutch network (Lake IJsselmeer, Rivers Meuse and Roer and the Canal Twentekanaal) to illustrate temporal trend at specific sites (Figure 6.1). The IJsselmeer is a large, shallow freshwater lake (1,136 km^2). The River Meuse is a major European river (total length 925 km), originating in France and flowing through Belgium and The Netherlands to the North Sea. The site at Eijsden is situated near the Belgian border at 300 km from the river mouth. The River Roer is a tributary (170 km) originating in Germany and flowing through The Netherlands into the River Meuse. It has been historically polluted by PCBs, tetrachlorobenzyltoluenes, and some brominated flame retardants (de Boer and Hagel, 1994). The Twentekanaal is a 65 km long canal in the north-east of The Netherlands within the Rhine River basin.

Eels were skinned and filleted, and the same part of the muscle was used for analysis throughout the full period (mid part of the body for Belgian eels, and dorsal part, posterior to the head for eels in The Netherlands). In Belgium, lipid was extracted from the muscle tissue and quantified using the Bligh and Dyer (1959) method. Quality was assured by participation in QUASIMEME interlaboratory proficiency testing schemes (www.quasimeme.org). Z-scores rarely exceeded 0.6 in absolute value, whereas Z-scores below 2 are satisfactory. In the Dutch eels, the fat contents were determined after Soxhlet extractions with pentane/dichloromethane (1:1, v/v). As the fat in eels consists for more than 95% of triglycerides, results of this Soxhlet method could easily be compared with the Bligh and Dyer results (de Boer, 1988). The quality of the Soxhlet lipid determination was underpinned by analysing in-house eel reference material with each series of samples, by an official accreditation (RvA, L097) and by successful participation, twice a year, in the QUASIMEME proficiency-testing scheme. The fat content is measured as the lipid concentration in muscle and is expressed in % of muscle wet weight (w.w.).

Statistical analysis

Both datasets were analysed using a regression model. The Belgian data (condition factor and fat content of every single eel) were averaged per site per year. To study the time (*period*) effect on the fat percentage, the following regression model was used: FpctA ~ Period. FpctA is the angular transformation of the fat percentage to normalize the data (Sokal and Rohlf, 1995). *Period* is a three-level factor indicating the periods divided in year groups (1994-1998, 1999-2003, 2004-2006 for the Belgian data and 1977-1981, 1982-1986, 1987-1991, 1992-1996, 1997-2001, 2002-2004 for the data from The Netherlands). Grouping was done on a five years basis, but was different for both countries in order to ensure a sufficient number of data and to guarantee representativeness (sufficient variety of sites with respect to typology). To take into account that some data originate from the same location, the intercept was modelled as random. Thus a linear mixed model was constructed (Pinheiro and Bates, 2000). This regression model was validated with a residual analysis. The Tukey test was used to test if mean length and mean weight are significantly different between periods. Similarly significant differences between fat percentages and

condition factor and periods was tested. Statistical analyses were performed with the statistical program S-PLUS 6.2 Professional.

Results

Mean total length and weight of the Belgian eels over the three year groups are represented in Figure 6.2. Mean total length over the whole dataset was 41.7 cm ± 6.6 s.d. There is a slight but significant variation in mean total length (1994-1998: 44.5 cm (min 30 - max 60); 1999-2003: 41.2 cm (min 30 - max 60); 2004-2006: 42.5 cm (min 30.2 - max 59.6)). Mean weight of all the eels is 137.4 g ± 80.1 s.d. The weight of the eels in the first year group is larger than in the other two groups (1994-1998: 180.1 g (min 48 - max 667.5); 1999-2003: 133.3 g (min 33.7 - max 550.3); 2004-2006: 138.4 g (min 36.7 - max 432.8)). Individual lengths or weights of the eels from The Netherlands were not available, but eels over the whole period were selected within the 30-40 cm range.

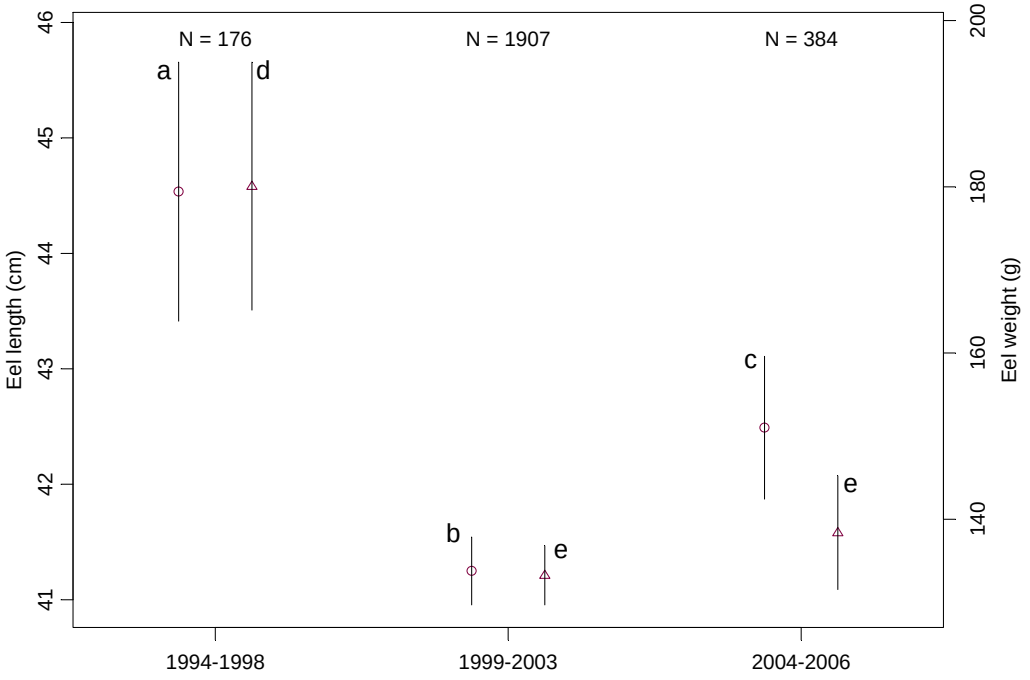


Figure 6.2. Morphological parameters of the yellow eels of the three year groups in Belgium. Mean lengths (○) and weights (Δ) of the yellow eels from Belgium, over the three year groups between 1994 and 2006, analysed for muscle fat content. Bars indicate standard errors. The number of eels is indicated. Means of periods with the same letter are not significantly different from each other (Tukey test, 95% simultaneous confidence intervals).

Fat content in yellow eel varies considerably between sites, both in Belgium and in The Netherlands. In Belgium the mean lipid content per site for 2004 varied

between 2.0 and 23.4% (25 sites, mean 12.7%), while in The Netherlands analysis of pooled samples of 22 sites in 2004 varied between 4.2 and 22.6% (mean 14.1%).

Total-lipid contents of Belgian eels from the different year groups were compared (Figure 6.3a). A significant decrease of 7.7% in lipid content on a w.w. basis over a 13 year period was observed in Belgian eels (1994-1998: 20.0% (min 1.5 - max 34.6); 1999-2003: 14.8% (min 1.7 - max 36.8); 2004-2006: 12.3% (min 2.0 - max 27.5)). Condition factors decreased significantly (1994-1998: 1.06 (min 0.83 - max 1.53); 1999-2003: 1.01 (min 0.65 - max 1.57); 2004-2006: 0.95 (min 0.76 - max 1.19)). *Period* was highly significant in the linear mixed model both for lipid content (ANOVA $p < 0.0001$) and condition (ANOVA $p < 0.0001$). All periods were significantly different from each other, indicating a monotone negative trend, both for fat ($p < 0.0001$) and condition ($p < 0.0001$). No systematic patterns in the residuals were found.

The time trend of the mean lipid content in pooled yellow eel samples from 92 locations in The Netherlands between 1977 and 2004 is presented in Figure 6.4a. Whereas before 1990 the mean fat content was generally superior to 20%, a clear and significant decrease occurred after 1990 (1977-1981: 20.8% (min 5.3 – max 30.1); 1982-1986: 20.9% (min 9.6 – 32.6); 1987-1991: 19.5% (min 6.3 – max 34.2); 1992-1996: 16.9% (min 6.1 – max 29.7); 1997-2001: 14.8% (min 3.7 - max 29.2); 2002-2004: 13.1% (min 3.5 – max 23.4)). Statistical analysis confirmed that *Period* was highly significant in the linear mixed model for lipid content ($p < 0.0001$). While the analysis indicated a monotone negative trend for lipid contents ($p < 0.0001$), not all consecutive groups were significantly different from each other. The decrease in lipid content was evident and amounts to 7.5% on a w.w. basis over a 15 year period, as shown by the mean lipid content measured at sites sampled before 1991 (1977-1990: 20.6% ± 5.6 s.d., n = 217) compared with later years (2002-2004: 13.1% ± 5.7 s.d., n = 66). In Figures 6.3b and 6.4b lipid content distribution within consecutive year classes is presented, respectively for BE and NL.

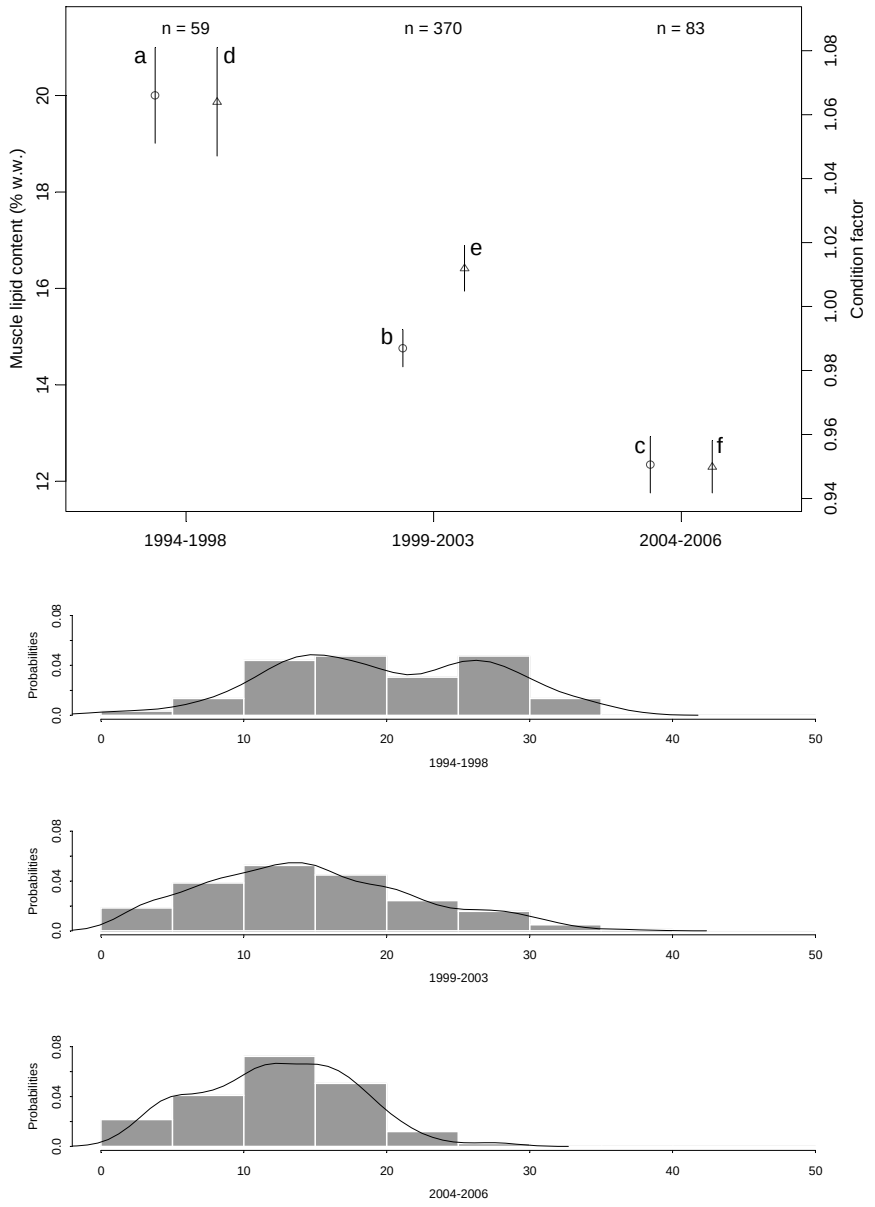


Figure 6.3. Temporal trend in fat contents of yellow eels in Belgium. **a**, Decreasing fat contents (○) and condition factor (Δ) (means, bars indicating standard errors) in yellow eels in Belgium between 1994 and 2006. Secondary Y-axis is the relative condition factor. The number of sites is indicated. The means of the different periods are significantly different from each other, both for fat content and condition (Tukey test, 95% simultaneous confidence intervals). **b,** Frequency distribution of lipid content in yellow eel in Belgium from the three periods.

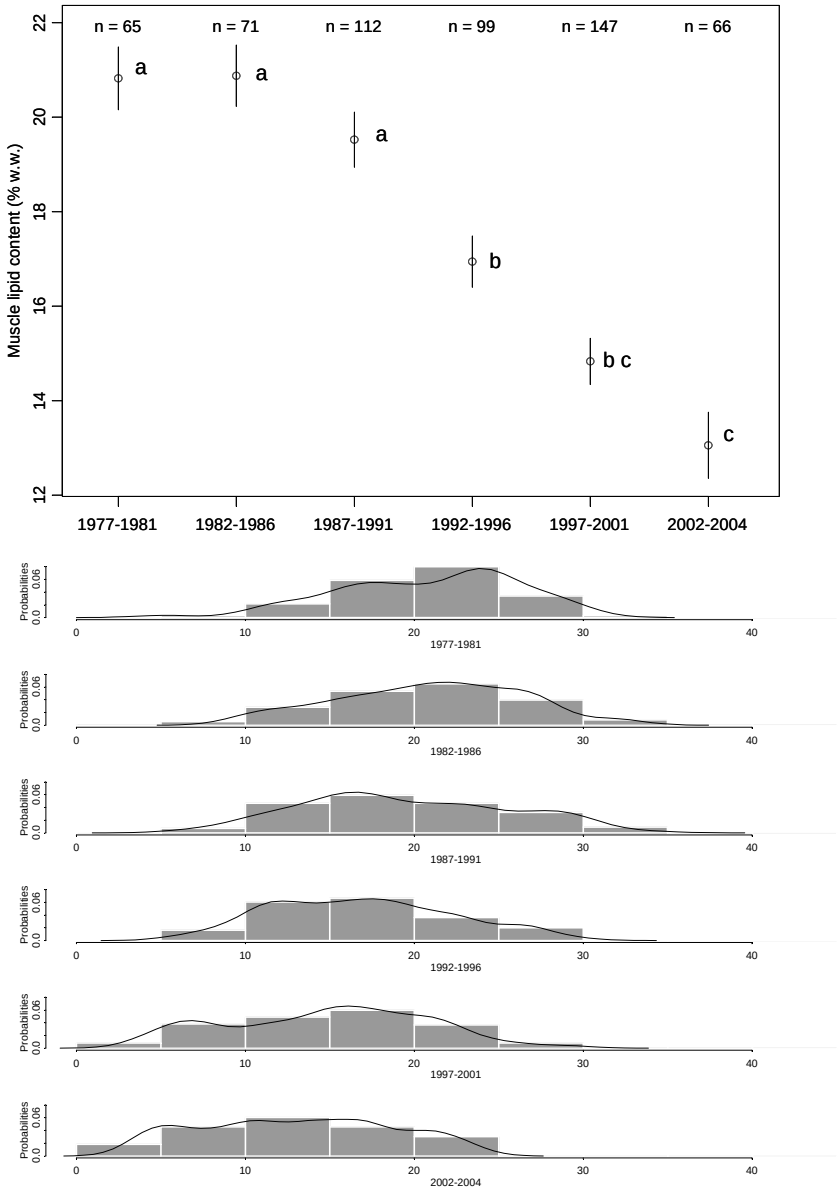


Figure 6.4. Temporal trend in fat contents of yellow eels in The Netherlands. a, Decreasing fat contents (means, bars indicating standard errors) in yellow eels in The Netherlands between 1977 and 2004. The number of sites is indicated. Means of periods with the same letter are not significantly different from each other (Tukey test, 95% simultaneous confidence intervals). **b,** Frequency distribution of lipid content in yellow eel from the Netherlands during the six periods.

The four water bodies of different typology with a long time series selected from the Dutch data illustrate this decrease at specific sites (Figure 6.5). The negative trend in fat contents was consistently present in eels from different sites and different typology. All eels in The Netherlands and Belgium seem affected by this phenomenon. There is large variation in lipid contents in eels from different water bodies : eels from Lake IJsselmeer (22.6% in 2004) are considerably fatter than from River Meuse (7.1% in 2004).

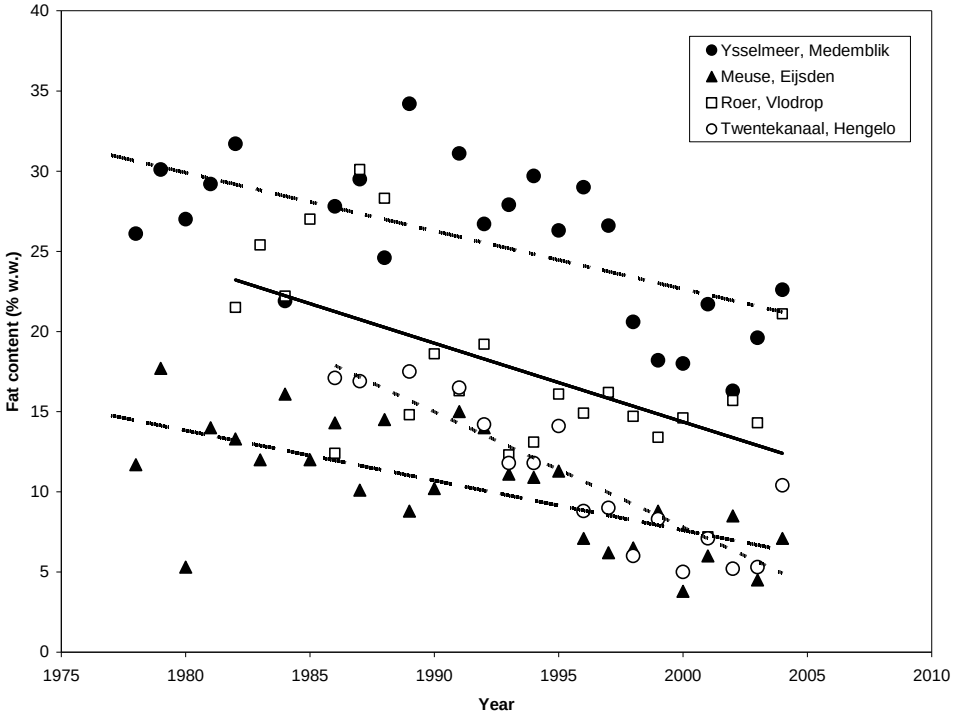


Figure 6.5. Temporal trends in fat contents in yellow eels from four water bodies of different typology. Time trend of the fat content in muscle tissue (pooled samples) from yellow eels in a lake (IJsselmeer at Medemblik (Y)), a large river (Meuse at Eijsden (M), a small river (Roer at Vlodrop (R)) and a canal (Twentekanaal at Hengelo (T)) in The Netherlands. Y, M, R and T refer to locations presented in Figure 6.1. Regression curves IJsselmeer (dash-dot line): $y = -721{,}24 \ln(x) + 5504$, $R^2 = 0{,}38$; Twentekanaal (dotted line): $y = -1435{,}4 \ln(x) + 10918$, $R^2 = 0{,}78$; Roer (solid line): $y = -979{,}6 \ln(x) + 7460$, $R^2 = 0{,}34$; Meuse (dashed line): $y = -619{,}14 \ln(x) + 4713$, $R^2 = 0{,}43$.

In Table 6.1 a hypothetical model is presented calculating the remaining energy (ER) in eels at arrival at the spawning grounds, based on different scenarios combining body weight (300, 500 and 1000 g), geographical variation in their freshwater habitat (Sweden, Belgium and Portugal) and the temporal decrease in fat (as measured in yellow eel from The Netherlands). River systems from Scandinavian countries are situated quite further from eel's spawning area than those of the west coast of the Iberian peninsula. Silver eels from the Swedish Lake Malaren have to swim at least 7500 km from Stockholm to the Sargasso Sea whereas eels from the River Tagus leaving Lissabon have to swim 5000 km to reach their spawning ground (distances calculated to Sargasso Sea at location 61°00'W and 26°30'N, the centre of the area described in van Ginneken and Maes (2005)). Several assumptions have been made: (1) yellow eel fat stores are representative for the silver eel energy budget, (2) silver eels with lowered fat stores do start their migration, (3) total net lipid was calculated on the basis of the muscle lipid weight (assuming lipids are predominantly stored in white muscle (Lewander *et al.*, 1974) and assuming muscle lipid concentration is indicative for the whole body), (4) a fixed value for energy requirement for migration (g fat/km) was taken regardless of the length of the eel. This value was deduced from van Ginneken *et al.* (2005) who measured the energy requirements for migration of 73 cm long eels kept in swimming tunnels for 173 days and covering a swimming distance of 5500 km. This was carried out through two different methods (oxygen consumption and bomb-calorimetry). Measurements of the cost of transport (COT) resulted in 0.42 $kJ.km^{-1}.kg^{-1}$ for the oxygen consumption method and 0.62 $kJ.km^{-1}.kg^{-1}$ for the bomb-calorimetry. If we use the mean value of both methods (0.51 $kJ.km^{-1}.kg^{-1}$), these eels (weighing 860 g) metabolize 66.6 g fat during a 6000 km journey to their spawning ground or 11.1 mg $fat.km^{-1}$. COT for eels of that size to complete their journey to the Sargasso Sea can thus be estimated as 55 g fat, 67 g fat and 83 g fat for eels originating from River Tagus (Lissabon), River IJzer (Nieuwpoort) and Lake Malaren (Stockholm), respectively. Comparing net fat quantities with COT, the ER can be deduced. From this we can conclude that in the period 2002-2004 female eels of a weight of 300 g and a muscle lipid content of 13.1% will not reach their spawning grounds, regardless their freshwater origin. Northern eels of 500 g with the same muscle lipid content will probably not be able to reach the Sargasso Sea, while individuals of more southern fresh water habitats could succeed to reach their spawning site, but considering the energy left (10.5 g fat for River Tagus eels) will probably not be able to contribute to the spawning stock or only have a negligible contribution. Large females (1000 g) with this reduced muscle lipid content will be able to reach their spawning ground, and still have some energy reserves for spawning and reproduction (48 g fat for Swedish eels and 76 g fat for River Tagus eels). However this net remaining energy reserve is less than 50% of the amount of energy remaining in similar sized eels during 1982-1986 (126 g fat for Swedish eels and 154 g fat for River Tagus eels).

The reproductive potential (RP) was calculated as the biomass of eggs which can be produced from the remaining energy in females which succeeded to reach their spawning grounds. We assumed energy for activities associated to mating and spawning as zero, and all remaining energy was converted to egg production. van Ginneken and van den Thillart (2000) used a conversion of 1.72 g eggs.g-1 fat, and the same calculation was used in our model (Table 6.1), suggesting that (1) only large female eels (>500 g) are able to contribute to reproduction and (2) even for these large female eels the reproductive potential is very limited. Estimates of egg production for 1 kg eels at the current mean muscle lipid level vary between 131 (Portugal), 110 (Belgium) and 83 g eggs (Sweden), dependent of the latitude.

Table 6.1. Different scenarios of hypothetical calculations of the energy remaining for reproduction (ER) and reproductive potential (RP) in female eels by arrival at their spawning ground. Calculations were made for eels from three origins in Europe, with a weight of 300, 500 and 1000 g, and with fat contents conform the means in lipid fat content measured in eels from The Netherlands over the last 25 years. The cost of transport (COT), being the energy cost (in g fat) for migration to the spawning ground, of 11.1 mg fat/km was used (deduced from van Ginneken *et al.* (2005) for 73 cm long eels, see text). Assumption was made of an equal net energy requirement for migration in eels of 300 and 1000 g. Migration distance from Lissabon, Nieuwpoort or Lake Malaren to spawning location (61°W and 26°30'N) was estimated as 5000, 6000 and 7500 km respectively. Net fat content was calculated assuming all fat is muscle fat. RP was calculated as the mass of eggs which could be produced by using all remaining energy through a conversion factor of 1.72 g eggs.g^{-1} fat (as used in van Ginneken and van den Thillart, 2000). †: eels do not reach spawning grounds due to lack of energy.

Eel weight	Year	Mean muscle lipid content	Net fat	River Tagus, Lissabon (COT=55 g fat)		River IJzer, Nieuwpoort (COT=67 g fat)		Lake Malaren, Stockholm (COT=83 g fat)	
(g)		(% w.w.)	(g)	ER (g fat)	RP (g eggs)	ER (g fat)	RP (g eggs)	ER (g fat)	RP (g eggs)
300	1982-1986	20,9	62,7	7,7	13	†	0	†	0
300	1987-1991	19,5	58,5	3,5	6	†	0	†	0
300	1992-1996	16,9	50,7	†	0	†	0	†	0
300	1997-2001	14,8	44,4	†	0	†	0	†	0
300	2002-2004	13,1	39,3	†	0	†	0	†	0
500	1982-1986	20,9	104,5	49,5	85	37,5	65	21,5	37
500	1987-1991	19,5	97,5	42,5	73	30,5	52	14,5	25
500	1992-1996	16,9	84,5	29,5	51	17,5	30	1,5	3
500	1997-2001	14,8	74	19	33	7	12	†	0
500	2002-2004	13,1	65,5	10,5	18	†	0	†	0
1000	1982-1986	20,9	209	154	265	142	244	126	217
1000	1987-1991	19,5	195	140	241	128	220	112	193
1000	1992-1996	16,9	169	114	196	102	175	86	148
1000	1997-2001	14,8	148	93	160	81	139	65	112
1000	2002-2004	13,1	131	76	131	64	110	48	83

Discussion

The two large data sets of lipid contents in yellow eels from Belgium and The Netherlands were collected independently; monitoring design and analytic methodologies differed considerably between both countries. The number of stations and periodicity were quite different, and samples were analysed individually (BE) or pooled (NL).

Large geographical variations in fat contents between yellow eels have been described earlier on (Piatek, 1970, Svedäng and Wickström, 1997). The phenomenon might be linked to variations in environmental conditions, e.g. temperature and

salinity (Andersson *et al.*, 1991), fish assemblages, eel density (Svedäng and Wickström, 1997), water typology (Piatek, 1970), or trophic status (Svedäng *et al.*, 1996). Notwithstanding the differences in both network concepts, and large variation in lipid contents of eels from various water bodies, similar trends were obvious in Belgium and The Netherlands: a drop in lipid contents over the past 15 years by about one-third (from ca 20% to 13%).

Muscle lipid contents in yellow eels increase with length, both under culture and natural conditions. Eels accumulate lipids during development from the elver to silver stage (Boëtius and Boëtius, 1985). Andersson *et al.* (1991) reported gradually increasing fat contents in stocked yellow eels sampled in 1986 from a Swedish thermal effluent area at the Baltic from 30 to about 65 cm. Here they tended to reach an upper limit at 35-40%, whereas Larsson *et al.* (1990) reported a linear increase from 5 to 28% up to a weight of ca 350 g (55 cm) in eels from an eutrophic lake in southern Scandinavia in 1988. Also in eel farms, the fat content in the eel body notably increased in relation with size (Garcia-Gallego and Akharbach, 1998). Due to difficulties in sampling eels within a narrow size class, Belgian eels were selected in the 30-60 cm size range. Because mean length in the Belgian eels was 3.3 cm smaller in group 1999-2003 compared to 1994-1998, we can not rule out that this length difference had an effect on lipid content measured. Back-calculating the data of lipid measurements in 39 yellow eels in the 70-345 g weight range presented in Larsson *et al.* (1990) a size difference of 3.3 cm would correspond to a decrease in 3.0% fat on a w.w. basis. The recorded decrease amounts to 5.2%. In the subsequent period (2004-2006), we expected an increase in fat content, as mean eel length was again 1.3 cm larger. However, the actual fat content decreased with another 2.5%. Hence the observed decrease in fat in the Belgian eels cannot be explained by differences in the size of the eels over the years. As eels from The Netherlands were selected from the same, narrow size class (30-40 cm) during 28 years, it seems unlikely that size differences in Dutch eels can have biased the results.

Possible causes

Possible causes for the observed decrease in fat stores are multiple and not easy to pinpoint. Accumulation of energy through lipid storage may be affected by environmental factors such as pollution pressure (and – more specifically – endocrine disrupting substances), disease agents, changes in food availability, other global changes in the environment and even life-history characteristics like e.g. restocking.

Pollution pressure

Evidence has been reported that contaminants may play a major role. The impact of contaminants on metabolic functions and on behaviour is broad (Robinet and Feunteun, 2002). It may affect lipidogenesis or induce lipolysis through various mechanisms. Chemical stress induces a higher energy demand (Calow, 1991). PCBs are known to disrupt thyroid hormone action in humans (Zoeller, 2001). Fat accumulation may be disabled through disturbed thyroid function in fish (Leatherland and Sonstegard, 1979; Singh, 1989). In rainbow trout (*Oncorhynchus mykiss*) fed with PCB and mirex contaminated diets, carcass lipid content differed significantly compared to control fish, with PCBs inducing an increase in lipid content, and mirex a decrease (Leatherland and Sonstegard, 1979). However Narbonne *et al.* (1988) found no change in carcass lipid content in mullet (*Chelon labrosus*) after feeding a PCB enriched diet. Lipid accumulation in eel was disturbed directly by inhibition of the acetylcholinesterase activity due to pesticide exposure (Ceron *et al.*, 1996;

Fernandez-Vega *et al*., 1999). Under laboratory conditions, eels show an increased fat consumption in the presence of cadmium (Pierron *et al.*, 2007) or the insecticide fenitrothion (Sancho *et al.*, 1998). Under natural conditions, Maes *et al.* (2005) found a strong correlation between heavy metals and a reduced condition factor in Belgian yellow eels. Also new substances, like perfluorinated compounds, are known to affect lipid metabolism, through alterations in cell membrane properties in fish (Hu *et al.*, 2003). Indications of impact of PCBs and some pesticides on lipid content in natural eel from Belgium were reported by Geeraerts *et al.* (2007). Contaminant levels in Belgium and The Netherlands are relatively high in comparison with elsewhere in Europe (de Boer and Hagel, 1994). The contamination in eels from Belgium and The Netherlands is in line with these observations. Many lipophilic contaminants in wild yellow eel in both countries are very high (de Boer and Hagel, 1994; Maes *et al.*, 2008). Eels are particularly prone to the bioaccumulation of lipophilic contaminants. The PCB concentrations (measured as the sum of the seven indicators PCBs) in Belgian feral eel (n = 2524) had an average of 605 $ng.g^{-1}$ wet weight (min 3 – max 12455) (Maes *et al.*, 2008), a 200-fold of the concentrations measured in marine fish (mean 3.1 $ng.g^{-1}$ wet weight (min 0.5 – max 25) (33 individuals from five marine species from the Belgian market) (Baeyens *et al.*, 2007). PCBs, several organochlorine pesticides and some heavy metals (e.g. lead) in yellow eels show a decreasing trend (Maes *et al.*, 2008). However, an extensive series of emerging and less known contaminants are believed to pose new threats to our environments. BTEX (benzene, toluene, ethylene and xylene), chloroform and tetrachloroethene are present in feral yellow eel in Belgium (Roose *et al.*, 2003). Brominated flame retardants (BFRs), like polybrominated diphenylethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A appeared to be present in fishes and marine mammals (de Boer *et al.*, 1998), and peaking concentrations have been found in Belgian eels from industrial locations along the River Scheldt in 1999 (Morris *et al.*, 2004). Perfluorinated compounds (e.g. perfluorinated octylsulfonate) have been detected in marine mammals, fish and birds (Kannan *et al.*, 2002). Many of these substances show increasing concentrations. PBDE analysis in a sediment core from Norway showed increasing concentrations in the environment since the beginning of the industrial production of PBDEs, e.g. the decabrominated diphenylethers become apparent in the late 1970s to increase gradually in the 1980s and 1990s (Zegers *et al.*, 2003).

Indirectly, fat storage might be affected by endocrine disruption, due to specific chemicals, some of them having biological effects similar of those of the steroid hormone estrogen (Turner and Sharpe, 1997). Sexual disruption and development of ovotestes have been reported in freshwater and marine fish in Europe (Jobling *et al.*, 1998). Female yellow eels have lower fat contents compared to males (de Boer and Hagel, 1994). Therefore, endocrine disruption could be one of the indirect causes of the lower fat contents, due to a higher number of feminized eels. However, apparently there is currently no evidence for endocrine disruption in yellow eels. Plasma vitellogenin content in yellow eels are relatively low compared with other fish species exposed to high concentrations of estrogens. Research in Belgium (Versonnen *et al.*, 2004) and in the U.K. on the River Thames (Livingstone *et al.*, 2000; Peters *et al.*, 2001) indicated that - despite the high exposure to and uptake of pollutants - European yellow eel under natural conditions are not sensitive to the effects of (xeno-)estrogens, as measured by the vitellogenin induction. The onset of maturation in the European eel only takes place during a period of prolonged swimming which might be a physiological stimulus necessary (van Ginneken *et al.*, 2007). It is therefore possible that endocrine disrupting effects of pollutants become apparent during the starvation period during migration or during the spawning itself (Versonnen *et al.*, 2004).

Diseases

Another possible cause of the reduction of fat contents in eels could be infections by specific diseases. Eels are prone to new diseases (parasites, bacteria, viruses), which recently invaded the population through anthropogenic impacts. A well-known example is the parasitic nematode *Anguillicola crassus*, which invaded the European eel population in the early 1980s, that damages the swim-bladder (De Charleroy *et al.*, 1990) and may be responsible for reduced swimming capacities (Sprengel and Luchtenberg, 1991; Nimeth *et al.*, 2000; Palstra *et al.*, 2007). The nematode is known to induce stress in eels and to increase cortisol plasma levels (Sures *et al.*, 2001), which leads to increases in energy metabolism and adversely affects energy accumulation (Robinet and Feunteun, 2002). It was also shown (Palstra *et al.*, 2007) that heavily infected eels and eels with a damaged swim-bladder had impaired swimming performance and spend more energy for migration, and increase overall energy consumption.

Global environmental changes

Global environmental changes (such as climate change and decreasing eutrophication) and overfishing, through complex interactions on the aquatic ecosystems and their communities, might be responsible for a lower fat content, although specific mechanisms remain unknown. Factors like food availability, water temperature, sex ratio, and others may be implicated. Eels collect energy from available food and they store this as lipids in muscles and internal organs. In some species, like herring, fat stores indicate the feeding conditions experienced by the fish, being high when there is plenty of food available and low when food is scarce (Wood, 1958). Significant decreases in fat levels have been reported in Baltic herring (*Clupea harengus membras*) since the late 1970s until 2000 (Ådjers *et al.*, 2000). They were thought to be linked to large scale oceanographic changes, especially a decrease in availability of the energy-rich marine copepods. Bottom-up processes mediated via changes in mesozooplankton species composition have also induced a longer-term failure in feeding success and a decline in fat content and herring growth (Flinkman *et al.*, 1988). Whether food availability in eel affects lipid content in eel is poorly understood: it was reported that in eels under culture conditions, lipid content can be influenced by the energy content of the food provided (Garcia-Gallego and Akharbach, 1998). However male silver eels did not show any decrease in lipid content when kept for two years under starvation conditions (Boëtius and Boëtius, 1985). In many water bodies over Belgium and The Netherlands water quality parameters have fluctuated considerably over the last 50 years. Processes like organic pollution and eutrophication, and subsequent water purification efforts have resulted in changing environmental conditions inevitably influencing diversity and quantity of food organisms. Scientific basis is far too fragmentary to ascertain if and to what extent the decrease in lipid content could be related to suboptimal feeding conditions. In contrast, it could be argued that the low recruitment observed since the last 25 years resulting in lower eel densities and a lower level of intraspecific competition for food, and an overall gradual increase in water quality seem to indicate better feeding conditions for the eel.

Impact of global change on fat reserves might be sex thriven, as the gender of an eel influences its lipid reserve, female yellow eels having lower fat than males (de Boer and Hagel, 1994). The sex of developing gonads is labile; eel is a gonochorist where gender is determined principally by environmental factors like population density, recruitment, and catchment characteristics. Davey and Jellyman (2005) described sex determination in eels as primarily metagamic whereby individual

growth rate during the early part of the freshwater phase is the key mechanism by which environmental conditions affect the gender of developing elvers. Causal relationships between feeding conditions and/or temperature and sex differentiation in European eel have been suggested (Lammens and Visser, 1990; Holmgren, 1996; Beullens *et al.*, 1997). In the French river Frémur, Lafaille *et al.* (2006) observed over a nine year study (1996-2004) a gradual shift of silver eel sex ratio from male to female. They suggest a possible relationship between the observed increase in the size of silver eels and change in the sex ratio, with growth conditions resulting from an increase in the trophic status and water temperature. But also low recruitment and consequent lower densities could be a determining factor, as high densities lead to more males whereas females are predominant in low density habitats (Parsons *et al.*, 1977).

High temperatures have been proposed to favour development as males (Beullens *et al.*, 1997). Northern and southern eel stocks are characterised by a clear shift in sex ratio, northern regions producing mostly large females (Vøllestad, 1992), where in southern stocks males greatly outnumber females (Lobón-Cervia and Carrascal, 1992). If temperature is considered as one of the determining factor in sex determination, which is still under debate (Davey and Jellyman, 2005), the general increase in water temperature recorded in European rivers during last century (Eisenreich *et al.*, 2005) would result in an increasing proportion of males. However our observations do not endorse this, as in this case we would rather expect increasing fat levels.

Stock management measures

Observations of low lipid content in silver eels in a freshwater lake on the island of Gotland (Baltic Sea) have been related to stocking practices. It has been debated that in some water bodies where eels have been stocked, after silvering these eels increase motoric activity triggered by their migratory instinct, but due to a lack of imprinting they lack orientation to their spawning grounds, and thus begin to loose fat and weight (Westin, 2003). Limburg *et al.* (2003) found a tendency towards a higher fat content in silver eels from wild versus stocked origin eels, but concluded that stocked eels nevertheless are able to migrate and show potential to contribute to the spawning stock. Our data could not support nor reject the Westin hypothesis as our lipid analysis concerns only yellow eel. However from the Belgian data it has been deduced that lipid content in yellow eels collected from closed waters (such as lakes and oxbow lakes) are generally lower than in rivers or canals (Geeraerts *et al.*, 2007). Considering that in Belgium, eels in closed waters exclusively originate from restocking with glass eel, this could illustrate that also in yellow eels from restocking lipid content is lower than normal. However, this could also be the effect of typology or a result of high restocking rates as most Belgian closed water bodies are small and are restocked at high rates which could have lead to suboptimal feeding conditions.

Effects of low energy stores

Jonsson and Jonsson (2005) showed that especially in fish species with long distance migrations, storage of somatic reserve energy is essential in fulfilling their life cycle. As energy stores are known to be essential within the reproduction migration, effects of lowered fat content will be most acute within the silver(ing) eel, affecting migration and reproduction. The data of lipid content presented here were obtained through a monitoring study for contamination in sedentary eel with the objective to follow pollution pressure on the sampling locations. Consequently, measurements were carried out on eels in their yellow phase. So great care must be

taken when extrapolating observations on yellow eel fat contents to conclusions on silver phased eels. In the absence of long time monitoring series in lipid content in silver eels, and lacking quantitative models for lipid metabolism between yellow and silver eels, we are confident that the yellow eel data can be used as a valuable proxy for the lipid status in silver eel. We believe that the decrease in lipid content as observed in yellow eels is indicative of a similar proportional decrease of energy stores in the silver eel, but data to prove this are lacking. We therefore stress, that following considerations on effect on migration and reproduction, are the outcome of a hypothetical model based on the available information. Comparative studies of lipid content and lipid metabolism in yellow versus silver eels are urgently needed.

Minimum lipid content as condition for silvering

In 1959, Thurow reported that an obtainment of 'breeding livery' depends on some physiological changes, on annual increase of condition factors and on fat accumulation. He mentioned 28% fat as a critical limit. Piatek (1970) stated that the content of fat in meat tissue 'is one of the characteristics in silver eel, which stimulates it for spawning migrations'. While silver eels usually contain on average 30% of fat (Boëtius and Boëtius, 1985), large individual variation in fat content in silver eels were reported in eels from a lake in Norway: they contained between 12.5 and 41.9% fat (Bergersen and Klemetsen, 1988). Also in Sweden, fat analysis in female silver eels from 9 different localities revealed diverging results, with means <10% to 28%, the proportion of eels with muscle fat content <20% was varying from 4 to 100% (Svedäng and Wickström, 1997). In both countries these lower fat stores have been reported in descending silver eels, indicating that also low fat silver eels start their migration. Other authors (Larsson *et al.*, 1990) made the assumption that silver eels only start to migrate once their fat content reached a minimal value (28%), sharing the view of Thurow (1959). It was suggested that, when fat content in the muscle reaches a level of saturation at 28%, lipid levels in the blood start to increase, triggering the production of hormones responsible for metamorphosis and sexual maturation (Larsson *et al.*, 1990). This idea that a critical fat mass must be reached before silvering has been generally accepted as the cue to initiate silvering (Lokman *et al.*, 2003). If the silvering process is independent on the fat content in the yellow eel prior to silvering (Svedäng and Wickström, 1997), these low fat silver eels most probably will be unsuccessful as the fat contents will be too low to permit a successful migration, a normal maturation and spawning (Bergersen and Klemetsen, 1988), or migration will be delayed as these low fat silver eels will try to compensate the lack of fat by eating more until they have reached the desired fat contents for their journey back to the Sargasso Sea (Svedäng and Wickström, 1997). In case the silvering is dependent on a minimum fat content in their yellow stage (Larsson *et al.*, 1990), then silvering may not even take place or only to a limited extent. Anyway, in most scenarios a negative effect of the decrease in fat on the reproduction success is to be expected.

Insufficient energy for migration

Several authors described the requirements of energy for spawners to migrate and reproduce, in terms of percentage of lipids in muscle wet weight, or on body weight basis, which is commonly assumed as equal. Boëtius and Boëtius (1980) estimated that 18% of the energy available was used for development of the gonads, 27% was lost to routine metabolism and to metabolic activities related to maturation processes, 30% was available for migration and 25% was the residual energy after spawning. They calculated that a minimum of 20% of total lipid on body weight basis is required for successful migration and reproduction. More recently, through

experiments with eels in swimming tunnels, the energy required for migration was estimated as 7.7% (van Ginneken and van den Thillart, 2000), 12.6% (van den Thillart *et al.*, 2004), 7.8% (Palstra *et al.*, 2006a) and 6% fat (van den Thillart *et al.*, 2007). Palstra *et al.* (2006b) reported that besides 7.8% fat for migration, 5.7% is required for incorporation in oocytes, and a total of 13.5% fat is the estimated requirement for healthy migrating silver eels (Palstra *et al.*, 2007). van den Thillart *et al.* (2007) concluded that with eels having around 20% fat, there is more than enough left after reaching the spawning site for gonad development and spawning behaviour. However, they further discuss that at least 13% is necessary for swimming (independently of size) and on average 7.7% is incorporated in eggs indicating that silver eels should have a fat percentage of 20.7% to be able to migrate and reproduce successfully.

If we assume 20% as the minimum limit for a normal migration and reproduction, we can compare this benchmark to our data. From Figures 6.3b and 6.4b the increase in the proportion of sites with (yellow) eels having fat contents below 20.0% is evident (BE 1994-1998: 54.2%, 2004-2006: 92.8% and NL 1977-1981: 41.5%, 2002-2004: 84.8%). The magnitude of the decrease in fat contents described above with a 7.7% drop over 13 years in Belgium and a 7.5% drop over 15 years in The Netherlands, with fat content dropping to 12.3% and to 13.4% respectively, is believed to be sufficient to compromise reproduction.

The study area is situated in the centre of the latitudinal distribution of the European eel and by that may be representative for the whole population. It could be argued that local environmental conditions (e.g. high pollution pressure in Belgium and The Netherlands) might be responsible for a lower fat content in eels from Belgium and The Netherlands compared to the rest of the population in other countries. Unfortunately, there are no other long time series on lipid content in yellow or silver eel available. If we make the assumption that the reported decrease extends beyond Belgium and The Netherlands and is general over the distribution area of the eel, and considering energy stores being a restrictive factor for successful migration and reproduction as debated here, there is a differentiation in reproductive success of silver eels dependent of the latitude of the river system where the eels originated (Table 6.1). Southern eels need less net energy for their spawning migration compared to northern ones. That would mean that at an equal lipid level, southern female silver eels could be more successful in fulfilling their migration and still have enough energy for successful reproduction. The general accepted idea that especially northern areas are the main contributors to the spawning stocks as they produce a high proportion of large highly productive females, may be somewhat counteracted by this hypothesis. However, female silver eels from the south are only available in low quantities (e.g Lobón-Cervia and Carrascal, 1992).

In addition, it cannot be precluded, that also males may have considerable difficulties in reaching their spawning grounds. It may be assumed that male eels once arrived at their spawning ground, do not need as much remaining energy for reproduction as females, but as male silver eels are small sized and seem to get leaner, fulfilling their migration successfully could be problematic. Male silver eels in River Frémur emigrating between 1999 and 2004 measured between 27.0 and 44.2 cm length (Lafaille *et al.*, 2006), with a mean length of 37.2 cm. Male silver eels usually do not exceed 150 g and the decreasing trend in muscle fat content might also affect males in their successful reproduction migration. A male silver eel of 37.2 cm has an estimated weight of 91 g and with a 13.1% muscle fat content has only 11.9 g fat available. Measurements of energy requirement of eels of 43 cm swimming in tunnel trials resulted in a COT of 0.68 $kJ.km^{-1}.kg^{-1}$ (van Ginneken and van den Thillart, 2005). On this basis we calculated that these eels need 13.3, 16.0 and 20.0 g fat for completing their journey from Lissabon, Nieuwpoort or Stockholm to the Sargasso Sea. From these calculations it seems that currently, many male eels are

not able to reach their spawning grounds. Only individuals with higher net lipid content will be able to complete their journey, but the question arises if the remaining lipid energy in these individuals is sufficient to guarantee all activities required for successful mating.

Low fecundity

Lipid energy is essential for reproduction, mobilization of lipids fuels the ovarian growth and the production of good quality eggs. Female herrings (*Clupea harengus membras*) with a higher condition factor or muscle fat content produced eggs which suffered less from early mortality and also had better total survival and hatching success (Laine and Rajasilta, 1999). It has been shown that in the northern Baltic Sea, condition and fat content in herring vary seasonally and annually (Rajasilta, 1992) and there are temporal differences in the diameter of spawned eggs, and in the fat content of the ovaries, which may influence the development and mortality of herring eggs and contribute to seasonal or annual variations in the production of larvae (Laine and Rajasilta, 1999). In case fat reserves are low, poor fecundity is to be expected. Decreases in the lipid content of fish at the onset of the spawning season are common in many species. Lipid content in sockeye salmon (*Oncorhynchus nerka*) decreases from 9.7 to 1.8% during spawning migration from the sea to the river (Thurston and Newman, 1962). In Pacific herring (*Clupea harengus pallasi*) a decrease of muscle fat content (w.w. basis) of 10.8% in non-spawning herring versus 2.4% in spawning herring was reported (Huyngh *et al.*, 2007), indicating that the amount of energy required for reproduction approaches 8.5% of muscle lipid content.

It was reported before that larger eels have more fully developed ovaries (larger oocytes) than smaller eels (Kohnenko and Bezdzenyezhnykh, 1973), but as a consequence of decreased lipid energy it seems that - on average - only the large female eels contribute to reproduction, and this contribution is poor (Table 6.1). Belgian 1 kg female silver eels with a mean lipid content of 13.1% can produce 110 g of eggs, or ca. 310 000 eggs using the conversion factor described in van den Thillart *et al.* (2007), which is very low compared to the quantity of eggs (0.93-2.10 millions) recorded after experimental maturation in female silver eels between 800 and 1200 g (Boëtius and Boëtius, 1980).

In addition, these large females are rare. Size (and age) at the silver eel stage varies considerably within as well as between sampling sites (Svedäng *et al.*, 1996). Bergersen and Klemetsen (1988) reported that descending silver eels from a Norwegian coastal lake (Skogsfjordvatn) in 1983 mostly fall in the 300-400 g weight class, and the largest eels being in the 700 g weight class. In River Frémur (France) emigrating female silver eels are between 36.6 and 111.2 cm length, but length >70 cm are scarce (Lafaille *et al.*, 2006) and the emigrating eel population is dominated by males (Feunteun *et al.*, 2000). In exploited habitats, like lake IJsselmeer, large sized females are even scarcer and mostly completely depleted by fisheries (Dekker, 2000). In southern areas eel stocks are characterised by a dominant proportion of males (Lobón-Cervia and Carrascal, 1992) and females are scarce. These data suggest that the proportion and quantity of large sized female eels over the whole stock may be limited, but emphasise the importance of these individuals as spawners and give further basis to recommend special protection measures for this part of the population.

One has to bear in mind that this assumptive approach is based on mean values of lipid content. Considering the large variation in lipid content between eels (within and between sites) (see also Figures 6.3b and 6.4b), it is clear that a much better view could be acquired when integrating frequency data of lipid content in this analysis. Several assumptions have been made which need to be assessed in more

detail. Further research on the energetic requirements for migration (and reproduction) of the male and female silver eels of various size classes, combined with a better assessment of the variation in lipid content and the demographic characteristics (length, weight, sex ratio) of the silver eels over Europe will be needed to analyse reproductive potential and predict reproduction success of the migrating stock in function of latitudinal differentiation.

Decreasing energy stores in yellow eel and stock decline

In general fat contents in fish are considered as an indication of good health of both individual basis and stock basis. If we consider the fat content of eel populations *as such* as a general indicator for the health of the population, current observations of *poor lipid health* consolidate the view that the population is in a vulnerable shape. To our knowledge this is the first reporting of decreasing fat contents in a waning population. This health indicator is in line with other, well-known stock indicators such as low recruitment and decreasing fisheries yields. Figure 6.6 compares periods of decrease in glass eel recruitment of the stock, and the period of the decrease in mean muscle lipids in yellow eels from the data of The Netherlands. Glass eel recruitment dropped at the beginning of the 1980s after the high levels of the late 1970s, and the trend kept downward since then (WG Eel, 2007). The drop in lipid stores, as can be deduced from data from The Netherlands, seems to start some ten years later, beginning of the 1990s. Although we believe that the decrease in fat stores of the yellow eels has a negative impact on the migration and reproduction capacity in the silver eels and thus results in decreased recruitment, the timing of the decrease for both time series does not seem to endorse a causal relationship between decrease in fat content and lowered recruitment in the 1980s. However, this can not be excluded, as unfortunately, to the best of our knowledge, there are no time series for fat content in eels dating back earlier than 1977, and still it could be possible that muscle lipid content of yellow eels prior to 1977 would have been higher than the ca 20% in the eels of The Netherlands from the end of the 1970s. Piatek (1970) found in narrow-headed eels sampled in 1961 in various habitats from Polish waters an average fat percentage of 25.1% (n = 25). Bergersen and Klemetsen (1988) reported mean muscle fat content of 21.2% (s.d. 5.1 n = 13) in yellow eels from the Norwegian coastal lake Skogsfjordvatn in 1983 which is similar to the mean values from The Netherlands in this period. Yellow eels between 70 and 350 g (~35-60 cm size range), sampled in 1988 in a southern Scandinavian eutrophic lake, had a mean fat content of ca 21.8% (s.d. 7.2, min 5, max 35, n = 39) as deduced from a figure from Larsson *et al.* (1990). However, great care must be given when comparing literature data on eel fat levels between authors, as methodological and analytical issues might vary to some extent and description is often missing.

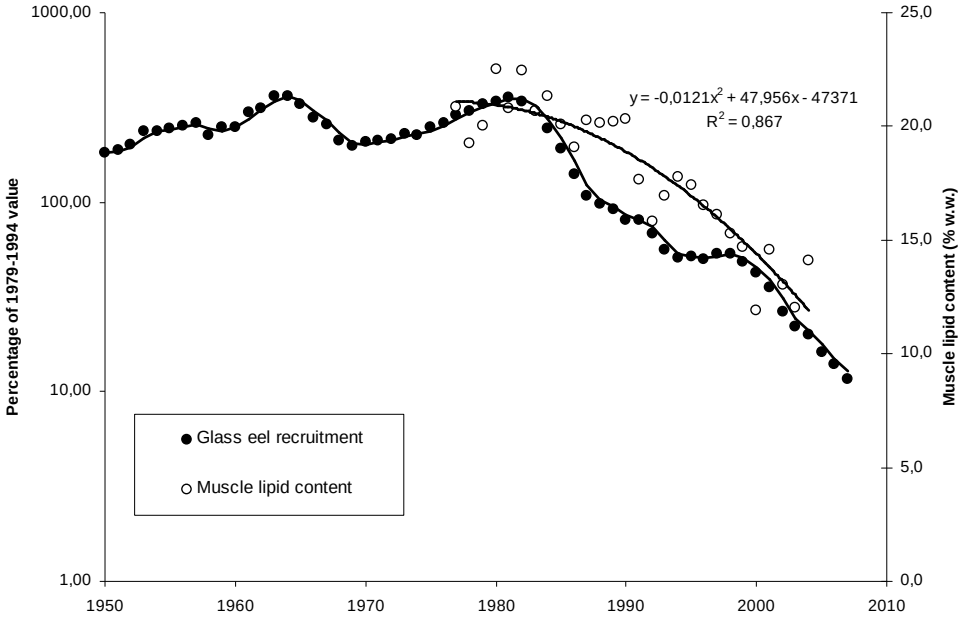


Figure 6.6. Time-series of glass eel recruitment in Europe (WG Eel, 2007) and of muscle lipid contents in yellow eels from The Netherlands. Data of the time-series of glass eel recruitment are geometric means of monitoring data of recruiting biomasses in 21 European rivers, each series being scaled to its 1979–1994 average. Data of muscle lipid contents are means of pooled yellow eel samples from The Netherlands between 1977 and 2004. Trend line for the lipid content: $y = -0{,}0121x^2 + 47{,}956x - 47371$, $R^2 = 0{,}867$.

The initial decline in recruitment at the start of the 1980s and the subsequent decrease in lipid content in the 1990s could be the result of the same cause: the emergence and continuing release of toxic substances in the environment. We hypothesize the following idea as a possible key mechanism for the decline of the species: new contaminants, being produced and released into the environment during the 1970s, bioaccumulate in the fat deposits in eel with steadily increasing concentrations. These contaminants attain critical levels at the end of the 1970s, and are being metabolized (together with fat metabolization) into the migrating silver eel during starvation. Blood concentrations of the contaminants reach toxic levels and cause detrimental impact on the silver eels or the quality of their gonads. As a result recruitment levels drop at the start of the 1980s. Simultaneously, these contaminants have negative impact on lipidogenesis or can induce lipolysis, so fat contents in yellow eels start to decrease during the 1990s. Lean eels still silver and do start their migration but, due to insufficient energy stores migration and/or reproduction are not successful, and recruitment further goes down. Considering the further decrease of fat stores it is likely that also recruitment still will continue to decrease. To date, there is not enough evidence to hypothesize which specific contaminants could be responsible, either as single compounds or collectively. Endocrine disrupting chemicals may be the most important ones in this respect. There are an increasing number of studies reporting on effects of some new compounds on biota (like e.g. brominated flame retardants), and their presence in aquatic organisms, and specifically in anguillid eels over the world (e.g. Ashley *et al.*, 2007, Fromme *et al.*, 1999, Belpaire and Goemans, 2007a, for a review in *A. anguilla* see Belpaire and

Goemans, 2007b). For some of those compounds time series of their presence in the environment are available and their time trend coincide with the trend in stock decline. Decabrominated diphenylethers appeared in the late 1970s in Western-Europe, and increased gradually in the 1980s to peak in the 1990s (Zegers et al., 2003). But in relation to the huge number of chemical substances produced world wide and released in the environment, ecotoxicologic information is only available for a few substances. Possibly, the decline is not caused by one contaminant, but may be the result of contaminant cocktails, combining several (newer or older) substances with synergetic effects. In this view it is expected that the actual low quantities of recruiting glass eel could be the direct progeny of silver eels brought up in the cleaner, remote, fresh water habitats, where contaminant pressure is low. Within the national and international eel restoration plans, it makes sense to give high priority to special protection measures for eel stocks of these areas, to ensure a maximal migration of good quality spawners, including specific protection of large sized females. But for restoring the population, it is evident that substantial solutions can only be gained if the production and release of chemicals with ecotoxic properties is stopped, and further research is needed in this field. The Water Framework Directive recently (European Commission, 2006b) proposed to monitor a selection of priority substances to achieve good chemical status of European water bodies, there is however serious concern if its objective, namely the protection of aquatic life and human health, can be met, as the list of substances is very limited and monitoring strategies, measuring lipophilic compounds in water, are not adapted to avoid bioaccumulation in biota (Belpaire and Goemans, 2007b). The European REACH program (European Commission, 2006a), regulating the registration, evaluation and authorisation of chemicals, could be a more effective instrument to prevent the release of toxic compounds into the environment. The more or less simultaneous decreases in recruitment in the Northern-Hemisphere *Anguilla* species, like in *A. rostrata* (Richkus and Whalen, 2000; Casselman, 2003) and in *A. japonica* (Tatsukawa, 2003), during the last 30 years, is an additional argument endorsing the idea that some new contaminants quickly spreading over the industrialized world, are key elements in the decline. Programs to prevent these compounds to enter our aquatic ecosystems should therefore not be restricted to Europe alone.

Further recommendations

These EU eel recovery plan (European Commission, 2007) concentrates on increasing the quantity of silver eels leaving their catchment. National eel management plans will focus on a reduction of anthropogenic mortalities within river basin districts, and aim to allow an escapement to the ocean of at least 40% of the biomass of silver eel, defined as the best estimate of the theoretical escapement if the stock had been completely free of anthropogenic influences. It was advised in 2005 (Dekker, 2005) and 2006 (WG Eel, 2006) to take into account fat content and *Anguillicola crassus* as additional parameters to be monitored within the eel restoration plans and the EC - Data Collection Regulation of the common fisheries policy. This study underlines the importance to include quality targets (such as lipid content, contamination and infection rate) within management targets and monitoring. A first step is the recent initiative taken by WG Eel (2007) to set up a database (the European Eel Quality Database) to compile all information on quality elements, including lipid content, in the European eel over its distribution area). Our observations of the declining fat content give new insight into the decline of the stock and raises serious concerns over the ability of the stock to recover. Therefore, we emphasize the need to include further studies on both fat contents and condition

factors in eel, particularly silver eel, in the proposed stock-wide eel recovery plan. In addition, we recommend studying the relation between fat content and sex of individual eels, the effects of specific contaminants and parasites on fat metabolism and a possible relation between the decreasing fat contents in eel and environmental variables such as changing temperature, decreasing eutrophication, food availability and trophic status.

Acknowledgements

This research was supported by the Flemish government, the Flemish Environment Agency and the Dutch Ministry of Agriculture, Nature and Food Quality. We explicitly thank all people involved in eel sampling, analysing and data processing in The Netherlands and Belgium. We also wish to thank I. Lambeens, Y. Maes, K. Peirsman and P. Verschelde for their help in the preparation of the paper. We acknowledge J. Maes, R. Poole, F. Volckaert and H. Wickström for critical comments on the manuscript.

References

Ådjers, K., Sandström, O., Bignert, A., 2000. Fisk och fiske - fisketrycket för hårt (Fishery in trouble with English summary). In: Tidlund A, Brenner,U (eds) Östersjö '99 *Årsrapport från miljöövervakningen i egentliga Östersjön (Swedish Marine Monitoring in the Baltic Proper).* Stockholm Marine Research Center, 35–36.

Andersson, J., Sandström, O., Hansen, H.J.M., 1991. Elver (*Anguilla anguilla* L.) stockings in a Swedish thermal effluent – recaptures, growth and body condition. *Journal of Applied Ichthyology* 7, 78-89.

Ashley, J.T.F., Libero, D., Halscheid, E., Zaoudeh, L., Stapleton, H.M., 2007. Polybrominated diphenyl ethers in American eels (*Anguilla rostrata*) from the Delaware River, USA. *Bulletin of Environmental Contamination and Toxicology* 79, 99-103.

Baeyens, W., Leermakers, M., Elskens, M., Van Larebeke, N., De Bont, R., Vanderperren, H., Fontaine, A., Degroodt, J.-M., Goeyens, L., Hanot, V., Windal, I., 2007. PCBs and PCDD/FS in fish and fish products and their impact on the human body burden in Belgium. *Archives of Environmental Contamination and Toxicology* 52, 563–571 (2007). DOI: 10.1007/s00244-005-0266-6.

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.

Bergersen, R., Klemetsen, A., 1988. Freshwater eel *Anguilla anguilla* (L.) from north Norway, with emphasis on occurrence, food, age, and downstream migration. *Nordic Journal of Freshwater Research* 64, 54-66.

Beullens, K., Eding, E.H., Gilson, P., Ollevier, F., Komen, J., Richter, C.J.J., 1997. Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguilla* L.) maintained in captivity. *Aquaculture* 153, 135-150.

Bilau, M., Sioen, I., Matthys, C., De Vocht, A., Goemans, G., Belpaire, C., Willems, J.L., De Henauw, S., 2007. Polychlorinated biphenyl (PCB) exposure through eel consumption in recreational fishermen as compared to the general population, using a probabilistic approach. *Food Additives and Contaminants* 24 (12), 1386-1393.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37, 911-917.

Böetius, I., Böetius, J., 1980. Experimental maturation of female silver eels, *Anguilla anguilla*. Estimates of fecundity and energy reserves for migration and spawning. *Dana* 1, 1-28.

Böetius, I., Böetius, J., 1985. Lipid and protein content in *Anguilla anguilla* during growth and starvation. *Dana* 4, 1-17.

Calow, P., 1991. Physiological costs of combatting chemical toxicants: ecological implications. *Comparative Biochemistry and Physiology* 100C(1/2), 3-6.

Casselman, J.M., 2003. Dynamics of resources of the American eel, *Anguilla rostrata*: declining abundance in the 1990s. In: *Aida*, K., Tsukamoto, K., Yamauchi, K., eds. Eel biology. Tokyo: Springer-Verlag, pp. 255-274.

Ceron, J.J., Ferrando, M.D., Sancho, E., Gutierrez-Panizo, C., Andreu-Moliner, E., 1996. Effects of diazinon exposure on cholinesterase activity in different tissues of European eel (*Anguilla anguilla*). *Ecotoxicology and Environmental Safety* 35, 222-225.

CITES, 2007. Convention on international trade in endangered species of wild fauna and flora. Consideration of proposals for amendment of appendices I and II. Fourteenth meeting of the Conference of the Parties The Hague (Netherlands), 3-15 June 2007. 39 pp. Available on http://www.cites.org/common/cop/14/raw_props/E-DE04-Anguilla%20anguilla.pdf

Davey, A.J.H., Jellyman, D.J., 2005. Sex determination in freshwater eels and management options for manipulation of sex. *Reviews in fish biology and fisheries* 15(1-2), 37-52.

de Boer, J., 1988. Chlorobiphenyls in bound and non-bound lipids of fishes; comparison of different extraction methods. *Chemosphere* 17, 1803-1810.

de Boer, J., Hagel, P., 1994. Spatial differences and temporal trends of chlorobiphenyls in yellow eel (*Anguilla anguilla*) from inland water of The Netherlands*. Science of the Total Environment* 141, 155-174.

de Boer, J., Wester, P.G., Klamer, J.C., Lewis, W.E., Boon, J.P., 1998. Brominated flame retardants in sperm whales and other marine mammals - a new threat to ocean life? *Nature* 394, 28-29.

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C., Ollevier, F., 1990. The life cycle of *Anguillicola crassus*. *Diseases of Aquatic Organisms* 8, 77-84.

Dekker, W., 2000. Impact of yellow eel exploitation on spawner production in Lake IJsselmeer, the Netherlands. *Dana* 12, 17-32.

Dekker, W., 2003a. Eel stocks dangerously close to collapse. ICES International Council for the Exploration of the Sea. http://www.ices.dk/marineworld/eel.asp

Dekker, W., 2003b. Did lack of spawners cause the collapse of the European eel, *Anguilla anguilla*? *Fisheries Management and Ecology* 10, 365-376.

Dekker, W., 2005. Report of the Workshop on National Data Collection for the European Eel, Sånga Säby (Stockholm, Sweden), 6 – 8 September 2005. Report to STECF.

Eisenreich S. J., *et al.,* 2005. Climate Change and the European Water Dimension, European Commission - Joint Research Centre, Ispra, Italy, EUR 21553, 253 pp. Available at

http://ccu.jrc.it/Pubblications/Climate_Change_and_the_European_Water_Dimension_2005.pdf

European Commission, 2006a. Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No. 793/93 and Commission Regulation (EC) No. 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal of the European Union L396, 1-849.

European Commission, 2006b. 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).

European Commission, 2007. Council Regulation (EC) No 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. Official Journal of the European Union 22.9.2007 L 248/17-23.

Fernandez-Vega, C., Sancho, E., Ferrando, M.D., Andreu-Moliner, E., 1999. Thiobencarb toxicity and plasma AchE inhibition in the European eel. *Journal of Environmental Science and Health* Part B – Pesticides. *Food Contaminants and Agricultural Wastes* 34(1), 61-73.

Feunteun, E., Acou, A., Laffaille, P., Legault, A., 2000. European eel (*Anguilla anguilla*): prediction of spawner escapement from continental population parameters. *Canadian Journal of Fisheries and Aquatic Sciences* 57, 1627-1635.

Flinkman, J., Aro, E., Vuorinen, I., Viitasalo, M., 1998. Changes in northern Baltic zooplankton and herring nutrition from 1980s to 1990s: top-down and bottom-up processes at work. Marine *Ecology Progress Series* 165, 127-136.

Fromme, H., Otto, T., Pilz, K., Neugebauer, F., 1999. Levels of synthetic musks; bromocyclene and PCBs in eel (*Anguilla anguilla*) and PCBs in sediment samples from some waters of Berlin/Germany. *Chemosphere* 39, 1723-1735.

Garcia-Gallego, M., Akharbach, H., 1998. Evolution of body composition of European eels during their growth phase in a fish farm, with special emphasis on the lipid component. *Aquaculture International* 6, 345-356.

Geeraerts, C., Goemans, G., Quataert, P., Belpaire, C., 2007. Ecologische en ecotoxicologische betekenis van verontreinigende stoffen in paling. Studie uitgevoerd in opdracht van de Vlaamse Milieumaatschappij, MIRA, MIRA/2007/05, INBO/R/2007/40. Groenendaal-Hoeilaart: Instituut voor Natuur- en Bosonderzoek. 207 pp.

Holmgren, K., 1996. Effect of water temperature and growth variation on the sex ratio of experimentally reared eels. *Ecology of Freshwater Fish* 5, 203–212.

Hu, W.Y., Jones, P.D., De Coen, W., King, L., Fraker, P., Newsted, J., Giesy, J.P., 2003. Alterations in cell membrane properties caused by perfluorinated compounds. *Comparative Biochemistry and Physiology* C 135, 77-88.

Huyngh, M.D., Kitts, D.M., Hu, C., Trites, A.W., 2007. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. *Comparative Biochemistry and Physiology* Part B 146, 504-511.

ICES, 2001. Report of the EIFAC/ICES Working Group on Eels. ICES CM 2001/ACFM:03. 87 pp.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. *Environmental Science & Technology* 32(17), 2498–2506.

Jonsson, B., Jonsson, N., 2005. Lipid energy reserves influence life-history decision of Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in fresh water. *Ecology of Freshwater Fish* 14, 296–301.

Kannan, K., Corsolini, S., Falandysz, J., Oheme, G., Focardi, S., Giesy, J.P., 2002. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean seas. *Environmental Science & Technology* 36, 3210–3216.

Kohnenko, S.W., Bezdzenyezhnykh, V.A., 1973. Development of the ovocytes of the eel as a function of its size (Beloruss.). Vyestsi Akad. Nauk BSSR. *Ser. Byal. Nauk*., 2, 90-94.

Laffaille, P., Acou, A., Guillouët, J., Mounaix, B., Legault, A., 2006. Patterns of silver eel (*Anguilla anguilla* L.) sex ratio in a catchment. *Ecology of Freshwater Fish* 15(4), 583–588.

Laine, P., Rajasilta, M., 1999. The hatching success of Baltic herring eggs and its relation to female condition. *Journal of Experimental Marine Biology and Ecology* 237, 61-73

Lammens, E.H.R.R., Visser, J.T., 1990. Variability in mouth width in European eel, Anguilla anguilla, in relation to varying feeding conditions in three Dutch lakes. *Environmental Biology of Fishes* 6, 63–75.

Larsson, P., Hamrin, S., Okla, L., 1990. Fat content as a factor inducing migratory behavior in the eel (*Anguilla anguilla* L.) to the Sargasso Sea. Naturwissenschaften 77, 488–490.

Le Cren, E.D., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch *Perca fluviatilis. Journal of Animal Ecology* 20, 201-219.

Leatherland, J.F., Sonstegard, R.A., 1979. Effect of dietary mirex and PCB (Aroclor 1254) on thyroid activity and lipid reserves in rainbow trout *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 2 (1), 43–48.

Lewander, K., Dave, G., Johansson, M.-L., Larsson, A., Lidman, U., 1974. Metabolic and haematological studies on the yellow and silver phases of the European eel, *Anguilla anguilla* L.-I. carbohydrate, lipid, protein and inorganic ion metabolism. *Comparative Biochemistry and Physiology* 47B, 571-581.

Limburg, K.E., Wickström, H., Svedäng, H., Elfman, M., Kristansson, P., 2003. Do stocked freshwater eels migrate? Evidence from the Baltic suggests “Yes”. In: Dixon, *D.A., ed. Biology, management and protection of catadromous eels*. Bethesda, Maryland, USA: American Fisheries Society, 275-284.

Livingstone, D.R., Mitchelmore, C.L., Peters, L.D., O’Hara, S.C.M., Shaw, J.P., Chesman, B.S., Doyotte, A., McEvoy, J., Ronisz, D., Larsson, D.G.J., Förlin, L., 2000. Development of hepatic CYP1A and blood vitellogenin in eel (*Anguilla anguilla*) for use as biomarkers in the Thames Estuary, UK. *Marine Environmental Research* 50(1-5), 367-371.

Lobón-Cervia, J., Carrascal, M., 1992. Seasonal timing of silver eels (*Anguilla anguilla* L.) in a cantabrian stream (north Spain). *Archiv für Hydrobiologie* 125(1), 121-126

Lokman, P.M., Rohr, D.H., Davie, P.S., Young, G., 2003. The physiology of silvering in anguillid eels: androgens and control of metamorphosis from the yellow to silver stage. In: *Aida*, K., Tsukamoto, K. and Yamauchi, K., eds. Eel biology. Tokyo: Springer-Verlag, 331-349.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology : Relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology* 73, 99-114.

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.

Morris, S., Allchin, C.R., Zegers, B.N., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P.E.G., Leeuwen, S.P.J. van, Boer, J. de, 2004. Distributon and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environmental Science & Technology*, 38(21), 5497-5504.

Narbonne, J.F., Suteau, P.M., Grolier, P.A., Daubèze, M.C., 1988. Metabolic effects of a polychlorinated biphenyl (Phenoclor DP6) on mullets, *Chelon labrosus*. *Bulletin of Environmental Contamination and Toxicology* 40(1), 8-12.

Nimeth, K., Zwerger, P., Wurtz, J., Salvenmoser, W., Pelster, B., 2000. Infection of the glass-eel swimbladder with the nematode *Anguillicola crassus*. *Parasitology* 121(1), 75-83.

Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., van den Thillart, G.E.E.J.M., 2006a. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93, 145-148.

Palstra, A.P., Antonissen, E., Clavero, M.E., Nieveen, M., Niemantsverdriet, P., van Ginneken, V.J.T., van den Thillart, G.E.E.J.M., 2006b. The fate of fat in silver eels: lipid requirements for spawning migration. In: Palstra, A.P. Energetic requirements and environmental constraints of reproductive migration and maturation of European silver eel (*Anguilla anguilla* L.). PhD dissertation. Leiden, The Netherlands: University of Leiden. 184 pp.

Palstra, A.P., Heppener, D.F.M., van Ginneken, V.J.T., Székely, C., van den Thillart, G.E.E.J.M., 2007. Swimming performance of silver eels is severely impaired by the swim-bladder parasite *Anguillicola crassus*. *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe., 2007. In press.

Parsons, J., Vickers, K., Warden, Y., 1977. Relationship between elver recruitment and changes in the sex ratio of silver eels *Anguilla anguilla* L., migrating from Lough Neagh, Northern Ireland. *Journal of Fish Biology* 10, 211-229

Peters, L.D., Doyotte, A., Mitchelmore, C.L., McEvoy, J., Livingstone, D.R., 2001. Seasonal variation and estradiol-dependent elevation of Thames estuary eel *Anguilla anguilla* plasma vitellogenin levels and comparisons with other estuaries. *Science of the Total Environment* 279(1–3), 137–150.

Piatek, M., 1970. Age, growth and changes of weight proportions and of chemical composition of eel during its life in Polish waters. *Acta Ichthyologica et Piscatoria* 1, 73-96.

Pierron, F., Baudrimont, M., Bossy, A., Bourdineaud, J.-P., Brèthes, D., Elie, P., Massabuau, J.-C., 2007. Impairment of lipid storage by cadmium in the European eel (*Anguilla anguilla*). *Aquatic Toxicology* 81, 304–311.

Pinheiro, J.C., Bates, D.M., 2000. Mixed-Effects Models in S and S-PLUS. New York: Springer. 528 pp.

Rajasilta, M., 1992. relationship between food, fat, sexual maturation, and spawning time of Baltic herring (*Clupea harengus membras*) in the Archipelo Sea. *Canadian Journal of Fisheries and Aquatic Sciences* 49, 644-654.

Richkus, W.A., Whalen, K., 2000. Evidence for a decline in the abundance of the American eel, *Anguilla rostrata* (LeSueur), in North America since the early 1980s. *Dana* 12, 83-97.

Robinet, T., Feunteun, E., 2002., Sublethal effects of exposure to chemical compounds : a cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265-277.

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *Journal of Environmental Monitoring* 5, 876-884.

Sancho, E., Ferrando, M.D., Andreu, E., 1998. Effects of sublethal exposure to a pesticide on levels of energetic compounds in *Anguilla anguilla. Journal of Environmental Science and Health Part B – Pesticides Food Contaminants and Agricultural Wastes* 33(4), 411-424.

Singh, H., 1989. Interaction of xenobiotics with reproductive endocrine functions in a teleost, *Monopterus albus. Marine Environmental Research* 28 : 285-289.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. New York: Freeman and Company, 887 pp.

Sprengel, G., Luchtenberg, H., 1991. Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla anguilla*. *Diseases of Aquatic Organisms* 11, 31-35.

Sures, B., Knopf, K., Kloas, W., 2001. Induction of stress by the swimbladder nematode *Anguillicola crassus* in European eels, *Anguilla anguilla*, after repeated experimental infection. *Parasitology* 123, 179-184.

Svedäng, H., Neuman, E., Wickström, H., 1996. Maturation patterns in female European eel: age and size at the silver eel stage. *Journal of Fish Biology* 48(3), 342–351.

Svedäng, H., Wickström, H., 1997. Low fat contents in female silver eels: indications of insufficient energetic stores for migration and gonadal development. *Journal of Fish Biology* 50 (3), 475–486.

Tatsukawa, K., 2003. Eel resources in East Asia. In: *Aida*, K., Tsukamoto, K., Yamauchi, K., eds. Eel biology. Tokyo: Springer-Verlag, pp. 293-298.

Turner, K.J., Sharpe, R.M., 1997. Environmental oestrogens – present understanding. *Reviews of Reproduction* 2, 69–73.

Thurow, F., 1959. Über Fängerfrage und Wachstum des Aales in der westlichen Ostsee. *Zeitschrift für Fisherei und deren Hilfswissenschaften* 8, 597–626.

Thurston, C.E., Newman, H.W., 1962. Proximate composition changes in sockeye salmon (*Oncorhynchus nerka*) during spawning migration. *Fishing Industry Research* 2, 15.

van Ginneken, V.J.T., van den Thillart, G.E.E.J.M., 2000. Eel fat stores are enough to reach the Sargasso. *Nature* 403, 156-157.

van Ginneken, V.J.T., Maes, G.E., 2005. The European eel (*Anguilla anguilla*, Linnaeus), its lifecycle, evolution and reproduction: a literature review. Reviews in *Fish Biology and Fisheries* 15, 367–398.

van Ginneken, V., Dufour, S., Sbaihi, M., Balm, P., Noorlander, K., de Bakker, M., Doornbos, J., Palstra, A., Antonissen, E., Mayer, I., van den Thillart, G. 2007. Does a 5,500-km swim trial stimulate early sexual maturation in the European eel (*Anguilla anguilla* L.)? *Comparative Biochemistry and Physiology*, in press, doi: 10.1016/j.cbpa.2007.03.021

van den Thillart, G.E.E.J.M., van Ginneken, V., Körner, F., Heijmans, R., Linden, R. van der Gluvers, A., 2004. Endurance swimming of European eel. *Journal of Fish Biology* 65, 312-318.

van den Thillart, G.E.E.J.M., Palstra, A.P., van Ginneken, V.J.T., 2007. Simulated migration of European silver eel: swim capacity and cost of transport. *Journal of Marine Science and Technology* 15 (special issue), 1-16.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363-371.

Vøllestad, L. A., 1992. Geographic variation in age and length at metamorphosis of maturing European eel: environmental effects and phenotypic plasticity. *Journal of Animal Ecology 61,* 41–48.

Westin, L., 2003. Migration failure in stocked eels *Anguilla anguilla. Marine Ecology Progress Series* 254, 307–311.

WG Eel 2006. Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels. FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Rome, 23–27 January 2006. EIFAC Occasional Paper. No. 38, ICES CM 2006/ACFM:16. Rome, FAO/Copenhagen, ICES, 2006. 352 p. Available from http://www.ices.dk/reports/ACFM/2006/WGEEL/WGEEL06.pdf

WG Eel 2007. Report of the 2007 Session of the Joint EIFAC/ICES, Working Group on Eels, FAO European Inland Exploration of the Sea, Bordeaux, 3–7 September 2007, EIFAC Occasional Paper No. 38, ICES CM 2007/ACFM:23, Draft, 524p. Available from www.ices.dk/reports/ACFM/2007/WGEEL/WGEEL_07_draft.pdf

Wood, R.J., 1958. Fat cycles of North Sea herring. *ICES Journal of Marine Science* 23, 390-398.

Zegers, B., Lewis, W.E., Booij, K., Smittenberg, R.H., Boer, W., de Boer, J., Boon, J.P., 2003. Levels of polybrominated diphenylether (PBDE) flame retardants in sediment cores from western Europe. *Environmental Science & Technology 37*, 3803-3807.

Zoeller, R.T., 2001. Polychlorinated biphenyls as disruptors of thyroid hormone action. In: Robertson, L.H., Hansen, L.W., eds. PCBs: recent advances in environmental toxicology and health effects. Kentucky: University Press, 265-272.

Outbreak of a bacterial disease in eels from the Boudewijnkanaal (Belgium) in September 2000. Diseased eels were only found in the vicinity of the water purification unit and disease is believed to be triggered by local heavy metal contamination (Pb and Cd).

Photo: INBO

Chapter 7

Pollution, condition and genetic variability

Gregory Maes[1], Joost Raeymaekers[1], Christophe Pampoulie[1], Adriaan Seynaeve[1], Geert Goemans[2], Claude Belpaire[2] and Filip Volckaert[1]

1 - Katholieke Universiteit Leuven, Laboratory of Aquatic Ecology, Ch. de Bériotstraat, 32, B-3000 Leuven, Belgium

2 - Institute for Forestry and Game Management, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium

This chapter was published as:

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005.
The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology : Relationship between heavy metal bioaccumulation, condition and genetic variability.
Aquatic Toxicology 73, 99-114.

Summary

Understanding the effects of pollutants on the genome is of crucial importance to preserve the evolutionary potential of endangered natural populations. The highly vagile European eel (*Anguilla anguilla* L.) has suffered a dramatic decline in recruitment since two decades, urging for a better understanding of the genetic impact of pollution. Its catadromous life history constitutes a model to assess local selection of pollutants on condition and genetic variability, as juveniles recruit in European rivers without appreciable pollution load or interfering genetic background. Because of its high fat content and local benthic feeding behaviour, the feeding stage is considered extremely prone to the bioaccumulation of pollutants. We studied the relationship between heavy metal bioaccumulation, fitness (condition) and genetic variability in the European eel. The muscle tissues of 78 sub-adult eels, originating from three Belgian river basins (Scheldt, Meuse and Yser), were examined for nine heavy metal pollutants (Hg, Cd, Pb, Cu, Zn, Ni, Cr, As and Se), while in total 123 individuals were genotyped at 12 allozyme and 8 microsatellite loci. A significant negative correlation between heavy metal pollution load and condition was observed, suggesting an impact of pollution on the health of sub-adult eels. In general, we observed a reduced genetic variability in strongly polluted eels, as well as a negative correlation between level of bioaccumulation and allozymatic multi-locus heterozygosity (MLH). Microsatellite genetic variability did not show any pollution related differences, suggesting a differential response at metabolic enzymes and possibly direct overdominance of heterozygous individuals.

Introduction

Evidence from animal and plant populations indicates that allozymatic polymorphism and heterozygosity might be linked to environmental heterogeneity and stress (Nevo *et al.*, 1986; Ben-Shlomo and Nevo, 1988; Chagnon and Guttman, 1989; Gillespie and Guttman, 1989; Stanton *et al.*, 2000; Nevo, 2001). Understanding the effects of pollutants on the genome is of crucial importance to preserve the evolutionary potential of endangered natural populations, as a high genetic diversity provides a population the potential to adapt to selective forces (Gillespie and Guttman, 1989). Under natural conditions (e.g. absence of anthropogenic influences), allelic frequencies within a population fluctuate with time according to stochastic processes (drift), migration and/or environmental selection pressures (such as climate or habitat changes), while maintaining polymorphism. However, severe perturbations on a short temporal scale, such as man-induced pollution and harvesting, may lower the condition and genetic variability, reducing the viability (fitness) of natural populations. Hence the susceptibility to additional environmental stress increases, weakening the survival of the species (Thorpe *et al.*, 1981; Leary *et al.*, 1987; Stanton *et al.*, 2000). The importance of genetic variation to survive anthropogenic environmental changes relates to factors such as resistance to heat stress (mummichog, *Fundulus heteroclitus*; Powers *et al.*, 1991), oil pollution (mussels, *Mytilus edulis*; Fevolden and Garner, 1986) and radiation (central stoneroller, *Campostoma anomalum*; Gillespie and Guttman, 1989).

There are four ways in which toxicants may affect the genetic variability in a population (Van Straalen, 1999; Van Straalen and Timmermans, 2002): (1) some toxicants are mutagenic, increasing directly the mutation rate; (2) they may indirectly affect the mutation rate by affecting DNA repair mechanisms; (3) they may favour more tolerant genotypes than others and change the genetic composition of the population towards a higher mean tolerance; and (4) they may cause bottlenecks or alter migration. The first two mechanisms will increase genetic diversity, while the two latter ones will decrease it, possibly exhausting genetic variation in natural populations. This process is referred as “genetic erosion” (Van Straalen and Timmermans, 2002).

The impact of pollutants or toxicants, such as heavy metals, pesticides or industrial waste, on the genetic diversity and structure of natural populations relates to a reduced genetic variability in polluted populations, genotype-specific survivorship and subsequent shift in the distribution of tolerant genotypes without net loss of diversity, or significant correlations between pollutants and allele frequencies (Hvilsom, 1983; Fevolden and Garner; 1986; Klerks and Weis, 1987; Patarnello and Battaglia., 1992; Posthuma and Van Straalen, 1993). Heavy metal pollutants seem to strongly affect allelic selection or allele frequency shifts at polymorphic loci (Hvilsom, 1983; Ben-Shlomo and Nevo, 1988; Chagnon and Guttman, 1989; Frati *et al.*, 1992). Most of these studies focused on well-defined populations, with low dispersal capability and reproducing locally. Organisms with a catadromous life history (i.e. spawning at sea, feeding in rivers and lakes) are expected to reflect local pollutants impact faithfully, as somatic and population genetic comparisons can be made after dispersal without worrying about different genetic background, parental influence or larval pollution load. Species with a high effective population size (mostly marine) generally exhibit high levels of heterozygosity and are expected to be more resistant to pollution; multi-locus heterozygotes often show an increased fitness over homozygotes (Nevo *et al.*, 1986; David, 1998). The question remains whether the effect of pollutants can also be measured on condition and genetic variability in highly vagile species.

There are few analyses of the relationship between the bioaccumulation of contaminants and genetic diversity in natural populations (Van Straalen, 1999). An important aspect when quantifying contaminant pressure is not only the exposure concentration but also the actual uptake of the contaminant in the body, namely the level of bioaccumulation (Van der Oost *et al.*, 2003). Concentrations of environmental pollutants do not always reflect the actual level of contamination of the individuals; lab based experimental studies often use higher concentrations than present in the natural habitat (Newman and Jagoe, 1998, but see Belfiore and Anderson, 2001). Hence, a combination of experimental and field-based studies remains ideal to encompass both molecular and population-genetic influences of environmental contaminants (Bickham *et al*., 2000).

The organism of interest in this study is the European eel (*Anguilla anguilla* L., Anguillidae, Teleostei*)*, a marine fish spending most of its lifetime in European freshwater rivers, lagoons or lakes, but spawning in the Sargasso Sea in the central North Atlantic Ocean (Tesch, 1977). Leptocephali larvae migrate along the Gulf Stream and North Atlantic Drift to reach the European continent, enter the rivers as glass eels, feed at least for 3 (males) to 6 years (females) until their spawning migration as silver eels (Tesch, 1977). Its catadromous life history constitutes a model to assess local selection of pollutants on condition and on genetic variability, as juveniles recruit without appreciable pollution load or interfering genetic background. Despite extensive spawning migrations, the feeding stage (yellow eel) seems relatively sedentary (Tesch, 1977). In fact, because of its high fat content and local benthic feeding behaviour, the sub-adult stage is considered extremely prone to the bioaccumulation of pollutants (Linde *et al.*, 1996; Roche *et al.*, 2003).

The European eel stock is declining rapidly and is now at its minimum since 1970, probably due to climate/current changes, but compounded by anthropogenic changes like habitat destruction, migration barriers, overfishing and pollution (Dekker, 2000, Feunteun, 2002). The impact of pollutants in eel is known (see Robinet and Feunteun, 2002 for a review), but it is unclear to what extent the reproductive potential is affected and whether strong differential selection may alter the genetic composition of resident freshwater populations before the spawning migration.

Although there is firm proof that higher pollution levels decrease fitness traits, the impact of genetic variability in a catadromous organism like eel to withstand environmental stress is not known. The objectives of this study were threefold: (1) we quantified the relationship between heavy metal bioaccumulation and fitness (condition) of yellow eels from three Belgian river basins, assuming that a high environmental load is reflected in the biota. (2) We tested the hypothesis of equal post settlement allozymatic and microsatellite genetic constitution among basins and among *post-hoc* defined groups exhibiting various levels of heavy metal bioaccumulation. (3) We tested whether there is a correlation between multi-locus heterozygosity (MLH) at both genetic markers, the individual level of bioaccumulation and condition. If contaminants cause selective mortality of individuals with specific genomes, then allele and genotype frequencies should differ between populations from impacted sites (lower genetic variability) and those from not or less impacted sites (Gillespie and Guttman, 1986). A positive correlation between MLH and condition indices or negative correlation with level of bioaccumulation, would suggest a higher fitness (less contamination) for more heterozygous individuals (heterosis) (Nevo *et al.*, 1986).

Materials and methods

Samples

A total of sixteen sites were sampled in three river basins (Figure 7.1). Approximately ten sub-adult freshwater eels (yellow eel stage) were collected either by electro-fishing or with fyke nets at each site during the year 2000 (n = 123). The sampling was part of an extensive eel pollution-monitoring network for Flanders in 2000 (Goemans *et al.*, 2003). To detect inter-basin differences and to improve the statistical power of the analyses (especially by avoiding low sampling bias in genetic variability estimates), we initially grouped samples by river basin. The first set of samples originated from the River Scheldt (S1, S2, S3, S4, S5, S6 and S7), the second set was sampled in the River Meuse (M1, M2, M3 and M4), while the third set was sampled in the River Yser (Y1, Y2, Y3, Y4 and Y5) (Figure 7.1). Eels were kept alive in oxygenated tanks for maximally three days and processed in the laboratory. Standard length (L), body weight (W_B) and liver weight (W_L) were determined for each individual. Samples from muscle and liver tissues were collected for ecotoxicological (heavy metal concentration) and genetic (allozymes and microsatellites) analyses.

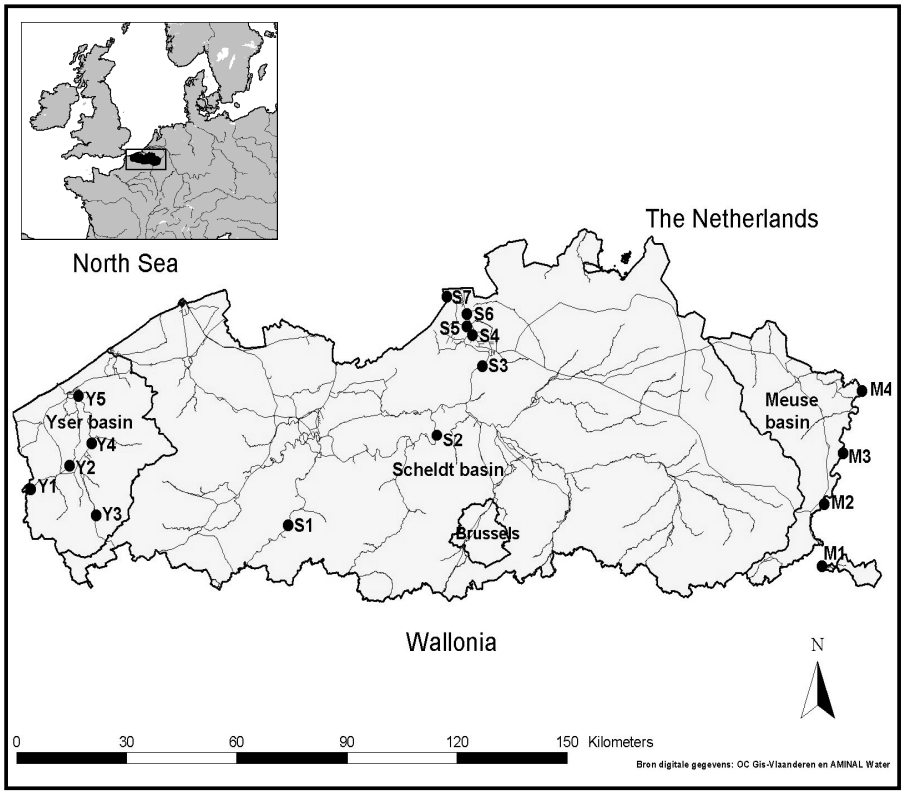


Figure 7.1. *Anguilla anguilla*: Flanders with sampling sites along three Belgian river basins; Yser: Y1, Y2, Y3, Y4, Y5; Scheldt: S1, S2, S3, S4, S5, S6, S7; Meuse: M1, M2, M3, M4.

Heavy metal load measurements and analysis

A sample of about 3-5 g of muscle tissue was removed, labelled and frozen at –20°C before analysis. A total of nine heavy metal concentrations were measured for three to six eels per sampling site (n = 78) by ICP-OES (Inductive Coupled Plasma-Optical Emission Spectrometry) for Cr, Ni, Cu, Zn, Cd and Pb. Quantification of As and Se was performed by GF-AAS (Graphite Furnace Atomic Absorption Spectrometry) according to standard procedures (Skoog, 1997, Goemans *et al.*, 2003). Concentrations were expressed in $\mu g.kg^{-1}$ (Hg, Cd, Pb, Ni, Cr, As and Se) or $mg.kg^{-1}$ (Cu and Zn) wet weight.

Allozyme electrophoresis

A total of 123 individuals (including the 78 individuals characterised for pollutants) were genotyped using Cellulose Acetate Gel Electrophoresis (CAGE, Harris and Hopkinson, 1976; Richardson, Baverstock and Adams, 1986). Electrophoresis and staining procedures followed Maes and Volckaert (2002). The most common allele was called '100' and other alleles were classified according to their relative mobility to allele '100' for the locus under study. The nomenclature used for enzymes followed Shaklee *et al.* (1990). Buffers used in the electrophoretic analyses were Tris-Glycine (TG) and Tris-Maleate (TM); both liver (L) and muscle (M) tissue were used. The following nine enzyme systems (coding for 16 loci) were scored: Alcohol dehydrogenase (*ADH-1**, *ADH-2**, EC 1.1.1.1, TG, L), Aspartate aminotransferase (*AAT-1**, *AAT-2**, EC 2.6.1.1, TM, L), Glucose-6-phosphate isomerase (*GPI-1**, *GPI-2**, EC 5.3.1.9, TG, M), Isocitrate dehydrogenase (*IDH-1**, *IDH-2**, EC 1.1.1.42, TM, L), L-lactate dehydrogenase (*LDH-A**, *LDH-B**, EC 1.1.1.27, TM, M), Malate dehydrogenase (*MDH-1**, *MDH-2**, EC 1.1.1.37, TM, L), Malic enzyme (*MEP-1**, *MEP-2**, EC 1.1.1.40, TM, L), Mannose-6-phosphate isomerase (*MPI*-1*, EC 5.3.1.8, TG, L) and Phospho-glucomutase (*PGM-1**, EC 5.4.2.2, TG, M). Twelve presumed polymorphic loci were scored to examine genetic diversity and genotype distribution.

DNA extraction and microsatellite amplification

Minute sections of tissue from ethanol preserved yellow eel fins (same individuals as allozymes, n = 123) were digested in a lysis buffer containing 200 µl 5% Chelex 100 solution (BioRad), 7 µl of 1M DTT (Dithiothreitol) solution pH 5.2 (diluted in 0.08M NaAc) and 10 µl Protein K solution (10 $mg.ml^{-1}$) for at least 4 h at 56°C. After incubation at 100°C for 10 min, the sam ples were centrifuged at 13,000 rpm (10,000 g) for another 10 min; the supernatant was stored at –20°C for later analysis. Genotypes were examined at 8 dinucleotide repeat microsatellite loci: *AAN 01*, *AAN 02*, *AAN 05* (Daemen *et al.*, 2001); *ARO 095*, *ARO 054*, *ANG 151*, *ANG 114* and *ARO 121* (Wirth and Bernatchez, 2001). PCR reaction conditions were as follows: denaturation at 95°C for 3 min followed by a cycle of denaturation at 95°C for 35 s, annealing at 61°C (*AAN 01*, *AAN 02*) or 57°C (*AAN 05*) for 30 s and finally elongation at 72°C for 40 s. This cycle was repeate d 30 (*AAN 01*, *AAN 02*) or 25 (*AAN 05*) times, after which an additional elongation of 10 min at 72°C was performed. Single PCR reactions consisted of 1 X PCR buffer (supplied with polymerase), $MgCl_2$ at a concentration of 1 mM (*AAN 02*, *AAN 05*) or 1.5 mM (*AAN 01*), 200 µM of dNTP, 0.4 µM of labeled forward and non-labeled reverse primer, 0.5 U of Goldstar *Taq* polymerase (Eurogentec, Seraing, Belgium) and 10-100 ng of gDNA. Double distilled water was added up to 10 µl. Loci *ARO 095*, *ARO 054*, *ANG 151*, *ANG 114* and *ARO* 121 were run in a multiplex with the following PCR

conditions: denaturation at 95℃ for 5 min followed by a cycle of denaturation at 95℃ for 30 s, annealing at 57℃ for 30 s and a final el ongation at 72℃ for 30 s. This cycle was repeated 25 times, after which an additional elongation of 8 min at 72℃ was performed. Multiplex PCR reactions consisted of 1X PCR buffer (supplied with polymerase), $MgCl_2$ at a concentration of 1.5 mM, 80 µM of dNTP, on average 0.4 µM of fluorochrome labeled (IRD700 or 800, Westburg, The Netherlands) forward and non-labeled reverse primer, 0.5 U of Goldstar *Taq* polymerase and 10-100 ηg of gDNA. Double dH_2O was added up to 25 µl. PCR products were run on a 5.5% acrylamide 7 M urea sequencing gel using an automated sequencer (LICOR 4200). Along with the PCR products, a molecular ladder (Westburg) was run in order to quantify the allele sizes.

Analyses of condition and heavy metal data

To assess the relative condition of individuals under pollutant stress, two condition factors were used. (1) Ricker's (1975) condition index (CI) was calculated as $1000(W_B / L^b)$, where respectively *L* and *W* relate to standard length in millimetres and body weight in milligrams (King, 1995). (2) The hepato-somatic index (HSI) was calculated as HSI = $(W_L / W_B).100$, where W_L and W_B represent wet liver weight and wet body weight, respectively. The coefficient *b* in (1) was calculated as the slope from the Log W_B -Log *L* regression analysis for all three basins, as allometric growth was detected. We then assessed the relative condition of each individual from each basin using the formula (1). ANOVA tests were performed to compare the mean relative condition between basins. Since the liver is the major detoxification and lipid storage reserve organ, changes in weight of this organ will relate to detoxification and energy storage. Weight effects on HSI were removed from (2), followed by an ANOVA on the residuals of the weight-HSI regression for group comparison.

We used a Multivariate ANOVA on a set of seven heavy metals and univariate ANOVA's per metal followed by Tukey tests to detect the influence of basin on heavy metal load. We calculated a relative bioaccumulation index by dividing (standardizing) the individual concentration of heavy metal i (C_i) by the maximum observed concentration (C_{imax}) and averaging over all metals, to relate heavy metal bioaccumulation to condition and genetic variability. Thus, the individual mean (multi-metal) bioaccumulation index (IMBI) was defined as:

$$\text{IMBI} = \left[\sum_{i=1}^{n} \left(C_i / C_{i\max} \right) \right] / n$$

with

n = total number of metals,

C_i = individual concentration of heavy metal i,

$C_{i\max}$ = maximal observed concentration of heavy metal *i* and 0 < IMBI < 1.

To compare heavy metal bioaccumulation among basins, an ANOVA analysis was performed on the IMBI values, followed by post-hoc analyses (Tukey tests). We calculated Pearson's correlation coefficients between individual IMBI values and condition indices (CI and HSI) to assess pollutant impact on condition. All analyses were performed in STATISTICA version 6.0 (StatSoft, 2001).

Genetic data analyses

Allozymatic and microsatellite genetic diversity was evaluated based on genotype and allele frequencies, the level of polymorphism (P), observed and expected heterozygosity (H_O and H_E), total number of alleles and mean number of alleles per locus (MNA). Multi-locus heterozygosity (MLH) was calculated as the percentage heterozygous loci per individual (corrected for non scored loci). Homogeneity of allele frequencies among samples was tested with the program GENEPOP version 3.1d (Raymond and Rousset, 1995). Departures from Hardy-Weinberg (H&W) equilibrium were calculated as $D = (H_O - H_E) / H_E$ with GENEPOP version 3.1d (Raymond and Rousset, 1995) using the Markov chain method. The standard deviation of each value was estimated by the jack-knife method over loci as implemented in GENETIX version 4.02 (Belkhir *et al.,* 1999) and the linkage disequilibrium between loci was calculated using the LINKDIS procedure implemented in GENETIX (Belkhir *et al.,* 1999). Population structure was characterised using hierarchical F-statistics (theta) and G_{ST}-values as implemented in the GENETIX 4.02 software package (Belkhir *et al.,* 1999). Due to the subtle differentiation and the high number of rare alleles, we chose to estimate the fixation index ($F_{ST(RB)}$) following Robertson and Hill (1984) after correction by Raufaste and Bonhomme (2000). Significance of multi-locus F_{ST} was assessed with permutation tests (1000 replicates). Genetic diversity indices (H_E, H_O, MNA, MLH and P) were compared between individuals, river basins and *post-hoc* defined LOW-HIGH pollution groups (LOW = IMBI < 0.22 and HIGH = IMBI > 0.25, values of 0.22 < IMBI < 0.25 were removed to avoid overlap between both groups). Because of the absence of reproductively isolated populations within each river basin (Tesch, 1977; Maes and Volckaert, 2002), the proposed division by pollution load is justified. In all cases significance levels were corrected for multiple comparisons using a sequential Bonferroni correction (Rice, 1989). Locus-by-locus heterozygosities (H_O), Allelic richness (AR) were compared using a pairwise t-test for dependant samples, while individual MLH values of both pollution groups were compared using an ANOVA. Bivariate regression analyses helped us to assess the relationship between condition and genetic estimators. Subsequent multiple regression analysis (Sokal and Rolf, 1997) was performed to test the overall contribution of MLH (allozymes and microsatellites) and condition (CI and HSI) on the level of bioaccumulation (IMBI). Analyses were performed in STATISTICA version 6.0 (StatSoft, 2001).

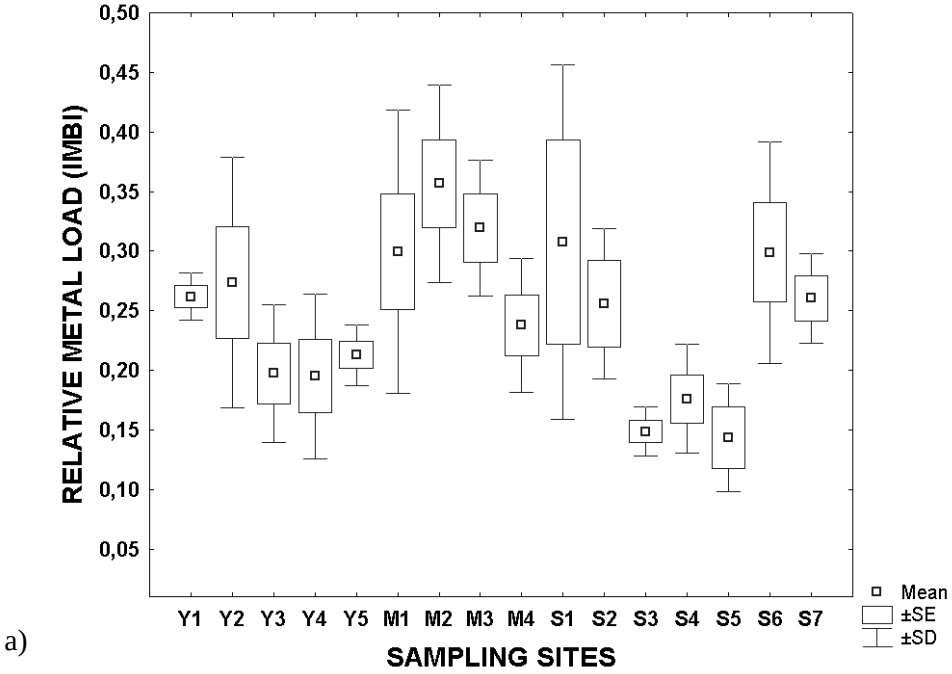
Results

Heavy metal bioaccumulation

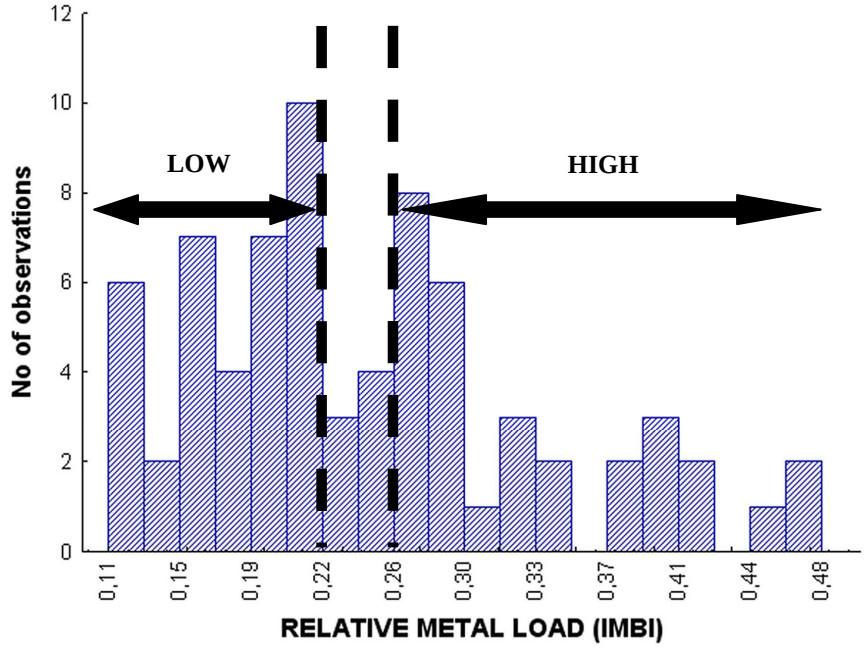
Comparisons of heavy metal pollution between river basins pointed to a strong heterogeneity in pollution load between sites (MANOVA, $F_{14,138} = 5.044$, $p < 0.0001$, $n = 78$, (see Appendix 1 and Table 7.1). The metals As and Se were not included in the statistical treatment because of the low number of analyses performed (1-5 individuals per river basin). The Meuse basin exhibited the highest concentrations for six out of nine heavy metal measurements (when including As and Se), but only two out of seven heavy metal (Hg, Cd) concentrations differed significantly between river basins (ANOVA, Table 7.1). The Scheldt showed the lowest Hg concentration, while the Meuse had the highest level of Cd. The distribution of heavy metal concentrations was heterogeneous between sites within river basin (Appendix 1), as shown in Figure 7.2a. The distribution of the IMBI values (based on seven metals) ranged from 0.113 to 0.479 and showed a roughly bimodal pattern of lowly and highly polluted individuals (Figure 7.2b). Later on this separation was used in the genetic analyses to define the "HIGH" and "LOW" pollution groups. An ANOVA of the IMBI values followed by a Tukey test indicated the Meuse basin as being significantly stronger polluted than the other two rivers ($F_{2,75} = 6.834$, $p < 0.01$, $n = 78$, Figure 7.3). Considering the possible relationship between size and pollution load (length is only weakly correlated with age in yellow eels), we found no significant correlation between length and pollutant concentration for any heavy metal (data not shown).

Table 7.1. Average heavy metal concentration per river basin of *Anguilla anguilla* L. Multivariate and univariate ANOVA's for equal heavy metal bioaccumulation in eel tissue originating from the Yser, Meuse and Scheldt basin. Values for Hg, Cd, Pb, Ni, Cr, As and Se are expressed in $\mu g.kg^{-1}$. Values for Cu and Zn are expressed in $mg.kg^{-1}$. The highest values are listed in bold.

Basin	n	Hg	Cd	Pb	Cu	Zn	Ni	Cr	As	Se	All metals
Yser	25	150.32	2.448	41.68	0.518	23.88	46.52	295.68	135	329	W-value = 0.438
Meuse	20	**173.6**	**19.485**	37.6	0.493	**26.31**	**65.7**	**361.5**	**371.25**	663.5	F = 5.044
Scheldt	33	93.6	2.993	**52.78**	**0.643**	25.14	46.54	174.36	308.67	**1022.8**	df = 14
p-value		0.0006	0.0000	0.4600	0.5222	0.5152	0.1482	0.3818	/	/	0.0000



a)



b)

Figure 7.2. Level of heavy metal pollution (IMBI) in *Anguilla anguilla*. a) Per sampling site within river basin; b) Histogram: the "HIGH" and "LOW" group are defined from the bimodal distribution of IMBI values.

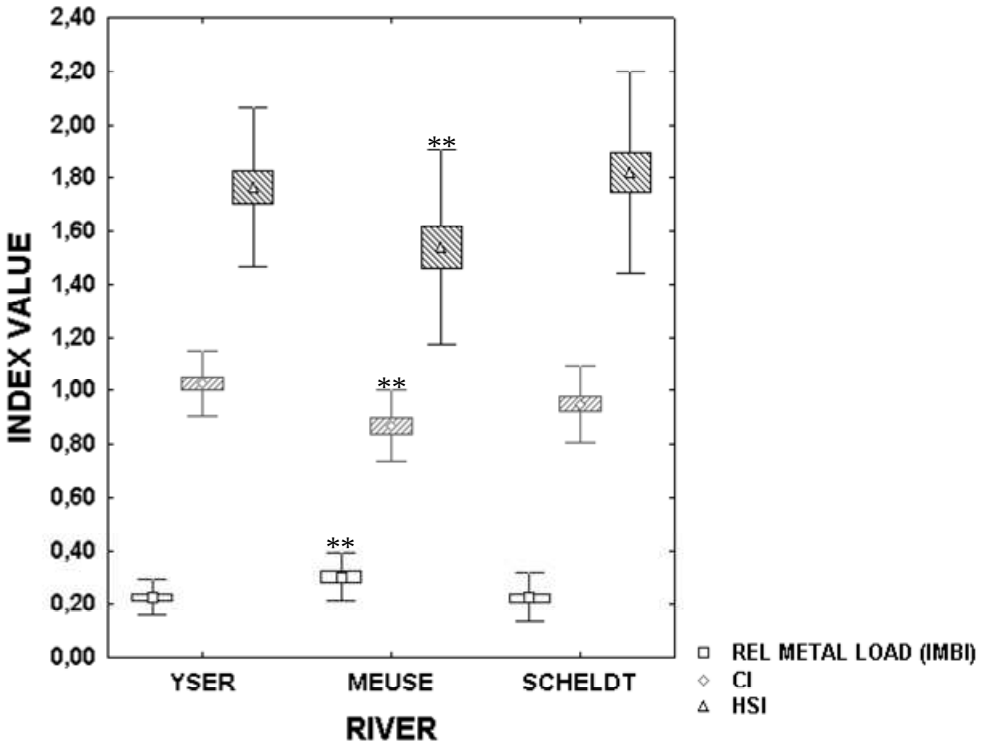
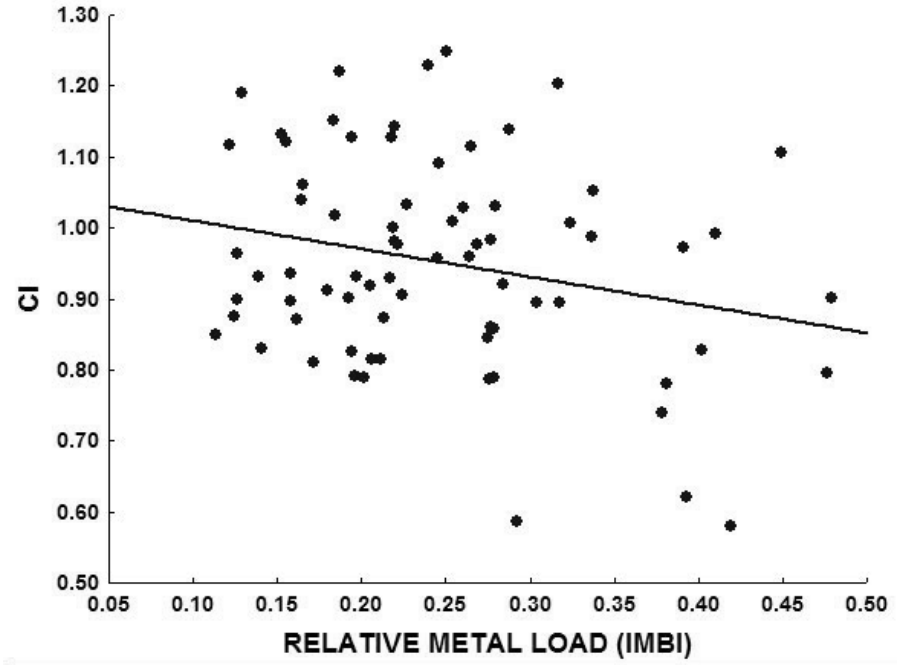


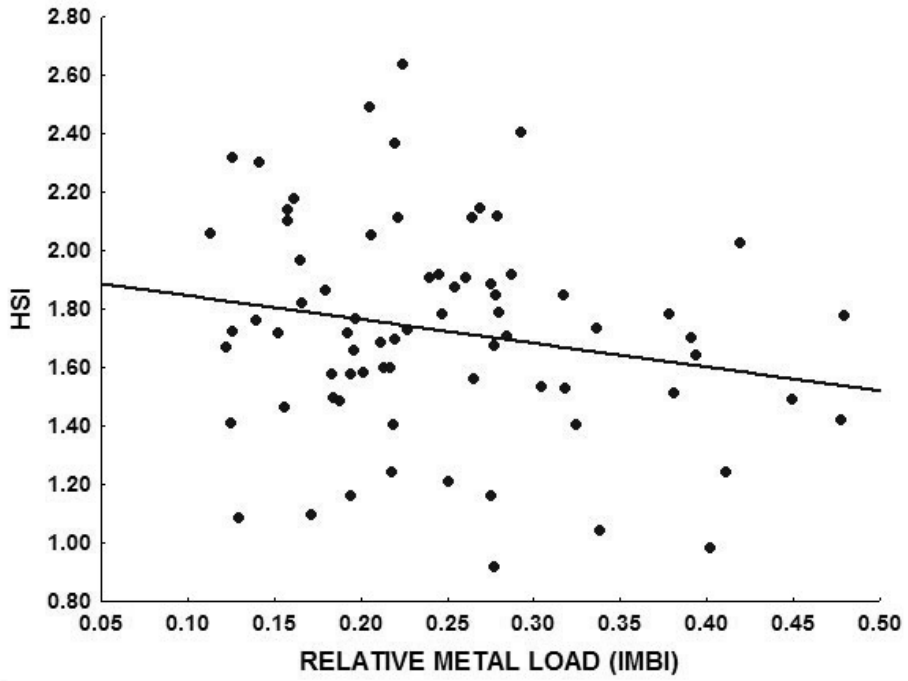
Figure 7.3. Boxplots representing relative condition index (CI ± SD), hepatosomatic index (HSI ± SD) and level of heavy metal pollution (IMBI ± SD) of *Anguilla anguilla* for each river basin. ** = $p < 0.01$

Condition (CI) and Hepatosomatic (HSI) indices

The regression equation between length and weight was log (*W*) = 3.155 log (*L*) - 3.032 (r = 0.9746, n = 123, $p < 0.001$). The relative condition index (CI) was calculated as $1000(W/L^{3.155})$ and varied significantly among basins (ANOVA, $F_{2, 120} = 10.565$, $p < 0.001$), with the Meuse showing the lowest condition (Figure 7.3). HSI values varied from 0.917 to 2.639 among basins. The correlation between Weight and HSI was r = -0.27; $p < 0.01$. The relative hepato-somatic index, measured as the residuals of the former regression, differed significantly among basins (ANOVA, $F_{2, 120} = 5.897$, $p < 0.01$), pointing to the Meuse river as exhibiting the lowest values (Tukey test). Finally, there was a significant negative correlation between heavy metal bioaccumulation (IMBI) and condition (r = -0.24; $p < 0.05$, Figure 7.4a) and a negative relationship between IMBI and HSI (r = -0.20; p = 0.09) (Figure 7.4b). No correlation was observed between CI and HSI (data not shown).



a)



b)

Figure 7.4. Correlation between heavy metal bioaccumulation (IMBI) of *A. anguilla* and a) condition index (CI) with R = - 0.24; p = 0.039; b) hepatosomatic index (HSI) with R = - 0.20; p = 0.09 for all individuals (n = 73).

Intra- and inter-basin genetic variability

A total of 12 enzymatic loci were scored. The total number of alleles per locus ranged from 1 to 6 and from 2.3 to 2.6 per sample over all loci. Observed (H_O) and expected (H_E) heterozygosities per sample ranged from 0.103 to 0.119 and from 0.122 to 0.129 respectively (Table 7.2; Appendix 2). An overall probability test of H&W equilibrium based on 1000 iterations was highly significant ($p < 0.001$), pointing to the non-random distribution of alleles within some samples. A global test based on 1000 iterations with H_1 = heterozygote deficiency was only highly significant for the Meuse river (F_{IS}: 0.21; $p < 0.001$, Table 7.2), mainly due to loci *GPI-1**, *GPI-2**, *MDH-2** and *ADH-1**. A more detailed analysis of heterozygosities within river basin showed that the Meuse exhibited the highest expected heterozygosity (H_E = 0.129), the highest mean number of alleles (MNA = 2.58) and the highest level of polymorphism ($P_{(0.95)}$ = 0.50). In contrast, this population exhibited the lowest observed heterozygosity (H_O = 0.107) (Table 7.2). No linkage disequilibrium was observed in the three populations.

The microsatellite loci revealed higher levels of variability than the allozymes as the total number of alleles per locus ranged from 12 (*AAN 05*) to 40 (*ANG 114*) and heterozygosity values (H_E) per locus ranged from 0.735 to 0.939 (Appendix 2). The mean number of alleles per locus by population varied between 14.4 (Scheldt) and 16.3 (Meuse). Observed and expected heterozygosity (H_O and H_E) per population were highly variable, ranging from 0.792 to 0.822 and from 0.850 to 0.869, respectively (Table 7.2). Exact tests assuming H1 = heterozygote deficiency, revealed significant departures from the null hypothesis of H&W equilibrium in all samples (Table 7.2). The deficits could be attributed to a particular locus, namely *AAN 02* which exhibited the strongest inbreeding coefficients (F_{IS} = 0.22, $p < 0.001$), most likely due to null alleles. Detailed analysis of population specific genetic variability defines the Meuse population as the most variable, with the highest mean number of alleles (MNA = 16.3), expected heterozygosity (H_E = 0.869) and observed heterozygosity (H_O = 0.822) (Table 7.2). No linkage disequilibrium was observed in the three populations.

Table 7.2. Allozymatic and microsatellite genetic variability of *Anguilla anguilla* L. in the three river basins and in the LOW and HIGH pollution group. Expected (H_E) and observed (H_O) heterozygosity per sample/*post-hoc* group over all loci, level of polymorphism (P) and mean number of alleles (MNA) per sample/ *post-hoc* group over all loci. *N* : number of individuals; S.E.: standard error; $P_{(0.95)}$ or $P_{(0.99)}$: 95% or 99% polymorphism criterion respectively. ** = p < 0.01.

	SAMPLE	N	H_E ±S.E.	H_O ±S.E.	$P_{(0.95)}$	$P_{(0.99)}$	M.N.A.	F_{IS}
ALLOZYMES	Yser	41	0.122 ± 0.154	0.111 ± 0.119	0.417	0.750	2.333	0.104
	Meuse	41	0.129 ± 0.147	0.103 ± 0.107	0.500	0.750	2.583	0.212**
	Scheldt	41	0.125 ± 0.165	0.119 ± 0.171	0.417	0.583	2.417	0.063
	LOW	35	0.140 ± 0.159	0.131 ± 0.151	0.500	0.750	2.833	0.075
	HIGH	32	0.109 ± 0.140	0.088 ± 0.094	0.417	0.667	2.333	0.212**
MICROSATELLITES	Yser	41	0.850 ± 0.073	0.792 ± 0.089	1.0	1.0	14.875	0.082**
	Meuse	41	0.869 ± 0.068	0.822 ± 0.087	1.0	1.0	16.250	0.069**
	Scheldt	41	0.851 ± 0.078	0.802 ± 0.054	1.0	1.0	14.375	0.072**
	LOW	35	0.863 ± 0.073	0.803 ± 0.077	1.0	1.0	15.875	0.087**
	HIGH	32	0.856 ± 0.080	0.817 ± 0.049	1.0	1.0	15.250	0.062**

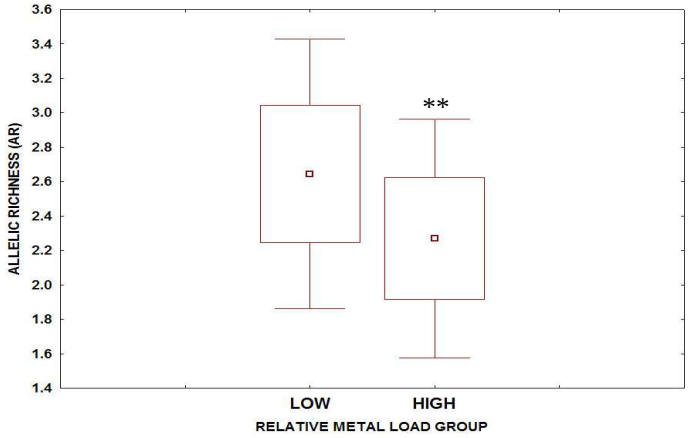
Micro-scale genetic structure

Overall genetic differentiation was significant ($p < 0.05$), but the multi-locus unbiased differentiation estimators were very low for allozymes ($F_{ST(RB)} = 0.007$, $G_{ST} = 0.001$) and for microsatellites ($F_{ST(RB)} = 0.018$, $G_{ST} = 0.003$). Pairwise genetic differentiation shows discrepancies between both markers. The microsatellite genotypes of the Meuse basin are most distinct from the Yser ($F_{ST(RB)} = 0.025$, $p < 0.01$), while the allozyme genotypes differentiate Yser and Scheldt the most ($F_{ST(RB)} = 0.017$, $p < 0.05$).

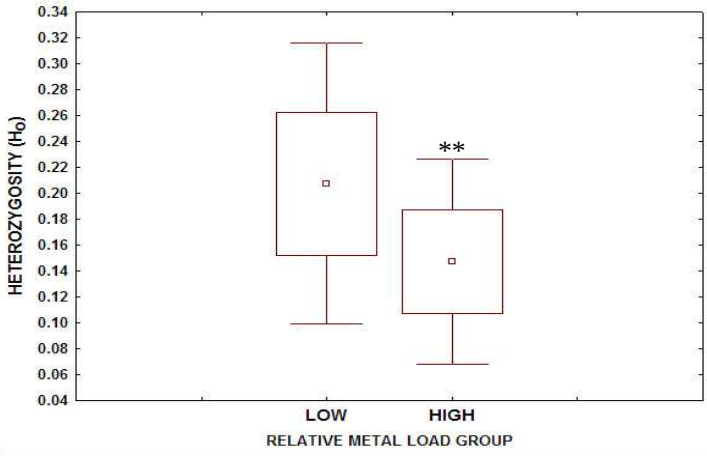
Genetic composition of the "HIGH" and "LOW" pollution group

The bimodal distribution of the IMBI values allowed us to define two groups ranked by their magnitude of relative metal load (Figure 7.2b). A total of 67 individuals were ultimately selected, with 35 and 32 individuals in "HIGH" and "LOW" polluted condition respectively. To exclude redundancy, we tested for the independence between basin and HIGH-LOW pollution groups (Chi-square = 7.33; df= 5; $p > 0.05$). The "HIGH" pollution group clearly exhibited a lower allozymatic genetic variability (H_E, H_O, level of polymorphism and MNA) than the "LOW" pollution group (Table 7.2, Appendix 2). The proportional difference between both groups ($H_{LOW} - H_{HIGH} / H_{LOW}$) amounted to 21.5% H_E and 34.6% H_O between both pollution groups. Locus-by-locus heterozygosity (H_O) and allelic richness (AR) analysis points to a significantly lower AR (t-test, $p = 0.01$, Figure 7.5a) and lower H_O (t-test, $p = 0.03$, Figure 7.5b) for polluted individuals. Finally, the number of multi-locus genotypes (28 vs. 22) was higher in the "LOW" pollution group. In contrast, microsatellite variability (multi - and locus-by-locus analyses) showed no appreciable difference in expected or observed heterozygosity, allelic richness or number of alleles between both groups (data not shown). When individual MLH were compared for allozymes and microsatellites, we observed marginally significant lower allozyme MLH values for HIGH polluted individuals (ANOVA, $F_{1, 65} = 3.898$, $p = 0.05$), while for microsatellites no differences could be detected ($p > 0.05$) (Figure 7.5c)

a)



b)



c)

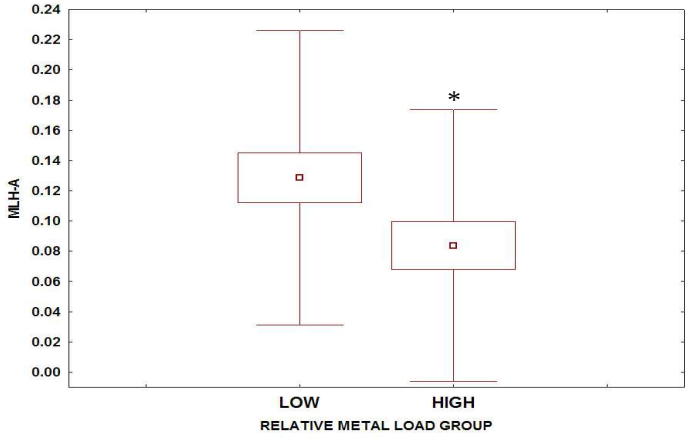


Figure 7.5. Allozymatic genetic variability of *A. anguilla*. Boxplots representing mean (± SE) a) allelic richness (AR, t-test; p = 0.01), b) observed heterozygosity ($H_{O;}$ t-test; p = 0.03) locus-by- locus and c) Multi-locus Heterozygosity (MLH, ANOVA, p = 0.05) comparisons between "HIGH" and "LOW" heavy metal pollution groups. * = p = 0.05, ** = p < 0.05

MLH, condition and heavy metal load regression

Regression analysis between MLH (allozymes and microsatellites) and condition indices (CI and HSI) yielded a negative trend, but no significant correlation (n = 123, data not shown). Regression between individual allozyme MLH and IMBI values yielded a significant negative correlation (R = -0.28; p = 0.016), while microsatellite MLH was not correlated with IMBI (R = 0.07, p > 0.05) (Figure 7.6). Similarly, when performing a regression analysis using the mean IMBI and MLH values per sampling site (n = 16), a marginally significant negative correlation (R = -0.48, p = 0.05) is observed (data not shown), indicating that similar results can be obtained if comparing individual sampling sites. Multiple regression analysis of IMBI as dependent variable versus MLH allozymes, MLH microsatellites, CI and HSI resulted in a better model to explain variation in bioaccumulation ($F_{4,\ 68}$ = 5.776, p < 0.001; adjusted R^2 = 0.21) than each variable alone (see Table 7.3, Figure 7.6). All variables, except the MLH of microsatellites, were correlated with IMBI values. MLH of allozymes contributed the most (β-weights) to the variation in IMBI, followed by CI, HSI and MLH of microsatellites (Table 7.3).

Table 7.3. Multiple regression analysis relating individual heavy metal bioaccumulation (IMBI) to condition (CI and HSI) and multi-locus heterozygosity (allozymes; MLH-A and microsatellites; MLH-M). The relative contribution of each variable is reported as beta-weight (standardized regression coefficient). Standard errors are given in parentheses.

	Parameter (s.e.)	DF	t	p-value	Beta weights (s.e.)
Intercept	0.535 (0.100)	1	5.352	0.000	/
CI	-0.210 (0.066)	1	-3.185	0.002	-0.343 (0.108)
HSI	-0.070 (0.026)	1	-2.653	0.010	-0.285 (0.107)
MLH- A	-0.350 (0.100)	1	-3.486	0.001	-0.376 (0.108)
MLH- M	0.086 (0.071)	1	1.209	0.231	0.129 (0.107)
Error	/	68	/	/	/

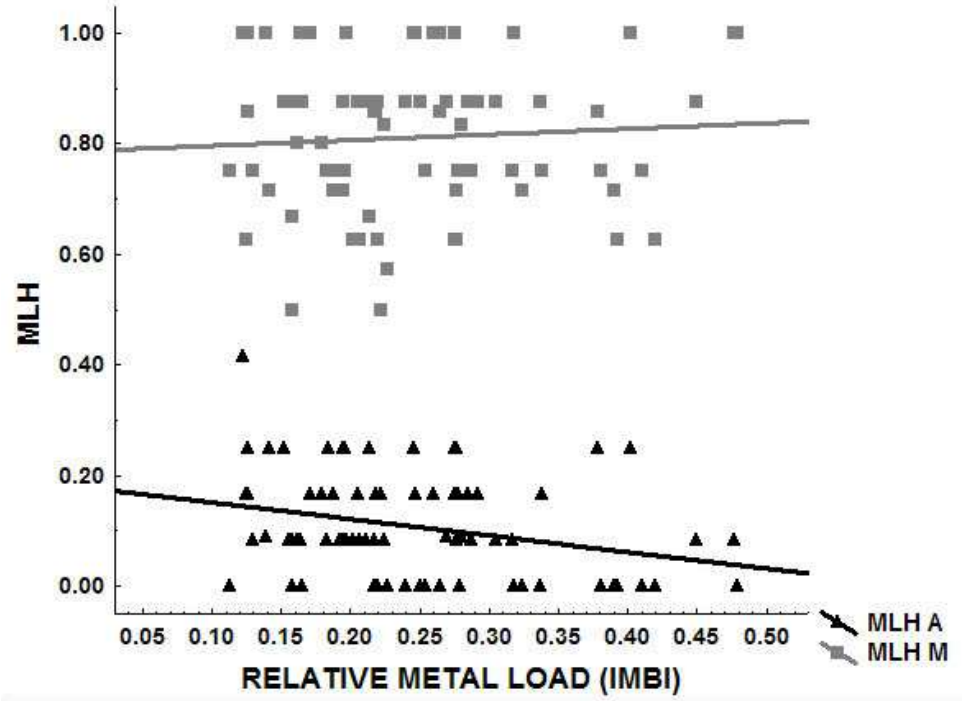


Figure 7.6. Correlation between relative heavy metal bioaccumulation (IMBI) and multi-locus heterozygosity (MLH) in *A. anguilla* for allozymes (MLH A) with R = -0.28; p = 0.016 and microsatellites (MLH M) with R = 0.07; p = 0.56.

Discussion

Although the impact of pollution on genetic variability has been assessed previously (Bickham *et al.*, 2000; Belfiore and Anderson, 2001 for a review), this study is novel in several aspects. First, our study focused on the level of bioaccumulation in a species extremely prone to pollution due to its high fat content, reflecting the actual pollution stress in the organism (Collings *et al.*, 1996). Its catadromous life history enables the detection of local pollutant influences on somatic and genetic characteristics, as juveniles enter rivers with much less pollution load or differential genetic background than locally reproducing and genetically distinct freshwater species. Their level of bioaccumulation after a few years spent in the rivers can be considered as indicative of their fitness, because strongly polluted eels detoxify less efficiently, have a lower condition and might be less successful spawners (Feunteun, 2002). Secondly, it has been suggested that several genetic markers should be used to discriminate between the influence of selection and other factors that might be marker specific (Belfiore and Anderson, 2001). In this study we compared patterns from strictly neutral genetic markers (microsatellite DNA) with enzymatic markers (allozymes), for which the assumption of selective neutrality has often been challenged (Eanes, 1999). The significance of assessing biometric

(weight, condition, growth) responses has also been underlined as a measure of pollutant impact on the organism (Van Straalen and Timmermans, 2002). Finally, the study of highly vagile organisms with a catadromous life-history like eel remains underrepresented, due to the difficulty of defining biologically relevant populations. Earlier studies used reproductively isolated populations, enabling straightforward population comparisons in the light of the "genetic erosion" hypothesis (Van Straalen and Timmermans, 2002). Here, we explain this issue in two ways, namely (1) by assessing the impact of pollutants on genetic variability ("Genetic Erosion" hypothesis) and (2) by considering individual genetic variability as an advantage to cope with pollution ("Heterosis" or "overdominance" hypothesis). Nevertheless, due to the catadromous life-history of eel and its failure to breed in captivity, no strong conclusions about evolutionary consequences can be drawn from our observations.

Spatial heterogeneity in pollution and condition

Although the European eel is a highly vagile fish species (Tesch, 1977), the feeding stage inhabiting the freshwater environment is remarkably sedentary and pollutants are expected to have a local influence. Our results confirm this knowledge; the accumulation of heavy metals is strongly heterogeneous between and within basins (Table 7.1, Figure 7.2). We found significant differences between individuals originating from other river basins, pointing to locally highly and less polluted sites. Despite intra-riverine variability, the Meuse basin was the strongest polluted river (Table 7.1), in line with current perception (Maeckelberghe, 2003; Cellule Etat de l'Environnement Wallon, 2003). The bioaccumulation of heavy metals, defined as a relative index (IMBI), confirmed single metal predictions, namely pointing to the Meuse eels as significantly stronger polluted than the eels of other basins. Earlier studies on European eel have confirmed the heterogeneous distribution of pollutants in rivers and lakes (Linde *et al.*, 1996; Belpaire *et al.*, 2002; Goemans *et al.*, 2003). The individual level of bioaccumulation might provide both an estimate of the environmental quality of the sediment (eels are benthic feeders) and a measure of health condition (fitness) of the organism (Bervoets and Blust, 2003). As no correlation was found between eel length and pollutants, the capacity of detoxification of individuals seemed unrelated to their size.

Life-history traits, such as condition, growth and fecundity, reflect the environmental quality and the organism's historic experience (Meffe, 1991; Ridley, 1996). We expected an impact of the level of bioaccumulation on the condition of European eel, due to the excess energy required for detoxification. We clearly showed a relationship between an increased heavy metal content and a lower condition in eel. The Meuse population exhibited a significantly lower condition than the other two river basins. Regression analysis revealed a strong negative correlation between individual bioaccumulation and condition indices (Figure 7.4a and b), which confirms the literature. For example, in the Sydney rock oyster (*Saccostrea commercialis*), bioaccumulation strongly correlated with condition (Avery *et al.*, 1996). Hence, we have strong indications that the bioaccumulation of heavy metals is a predictor of the condition in European eel and that pollution might significantly affect individual fitness. Due to the mobilisation of fat reserves during the spawning migration, it is expected that highly polluted individuals will have a lower reproductive success during spawning (van Ginneken & van den Thillart, 2001; Robinet and Feunteun, 2002).

Bioaccumulation vs. intra river and post-hoc genetic variability

Because of the absence of reproductively isolated groups in Belgium and Europe (Wirth and Bernatchez, 2001; Maes and Volckaert, 2002), it remains difficult to sustain the concept of "populations" in a river basin. Analyses performed at the population level are mostly testing for Hardy-Weinberg equilibrium, which can also be interpreted as randomness in genotypic distribution within rivers instead of random mating amongst individuals. Our proposal to analyse on the one hand natural populations (river basin) and on the other hand phenotypic traits (pollution charge), aims first at analysing the influence of a geographically divergent pollution level (local pattern of genetic variability) and subsequently mainly at comparing the genetic variability based on pollutant concentration in "general", where an individual's heterozygosity determines its response to pollutants. The level of bioaccumulation does not necessarily reflect the environmental pollution but also the individual capacity for detoxification. Hence, we argue that individual bioaccumulation is also determined by the genetic make-up.

Considering genetic variability within a river basin, we showed that the Meuse, despite exhibiting the highest expected variability, was in strong H&W disequilibrium, pointing to a non-random distribution of genotypes and possibly differential selection. Such results may have several causes, like population substructure, null alleles, inbreeding and selection (Hartl and Clarck, 1997). Because of the absence of reproductively active populations and the lack of similar results on microsatellites (excluding locus AAN 02), selection seems the most plausible explanation for the genotypic shift. The most strongly polluted population was the least heterozygous (H_O) at allozymes, possibly attributing weaker detoxification ability to more homozygous individuals and/or shifts towards certain homozygote classes.

Due to the heterogeneous distribution of metals and the absence of "biological" populations in rivers, we ranked individuals in *post-hoc* groups according to their level of bioaccumulation. The pattern exhibited here was much more unambiguous, namely a lower overall genetic allozymatic variability in strongly polluted individuals and again a strong H&W disequilibrium. A similar decrease in genetic variability has been demonstrated in various other freshwater, marine and terrestrial organisms under natural and laboratory conditions (Hvilsom, 1983; Fevolden and Garner, 1986; Klerks and Weis, 1987; Patarnello *et al.*, 1989; Posthuma and Van Straalen, 1993). Changes in diversity were mostly attributed to the selective advantage of certain genotypes or a reduction in population size (N_e). Remarkably, in most studies either only a few enzymatic loci were screened or an impact was observed at few loci (Chagnon and Guttman, 1989; Gillespie and Guttman, 1989; Paternello and Battaglia, 1992; Newman and Jagoe, 1998). In the present study, we observed a multi-locus response on pollution, namely at seven out of nine enzymatic loci. Only locus *GPI-1** remained constant, while *MPI-1** even exhibited a higher variability in strongly polluted individuals, possibly pointing to a heavy metal tolerant allele. We observed a lower number of genotypes in the highly polluted group, which fits the expectations (Ben-Shlomo and Nevo, 1988; Chagnon and Guttman, 1989; Diamond *et al.*, 1991), and suggests differential mortality or genotype shifts. Interestingly, 74% of Meuse individuals belong to the HIGH pollution group compared to 40% and 35% for the Yser and Scheldt respectively. This confirms the lower observed variability (H_O) in the Meuse, while the remaining low polluted individuals from this river may have raised the MNA and hence the expected heterozygosity by carrying rare alleles. The genetic variability at strictly neutral markers did not show any pollution related differences, despite the high number of alleles and the higher resolution expected from this marker (Hedrick, 1999). Nevertheless, other studies using similar markers have found a strong correlation between a decrease in neutral genetic variation and the level of pollution in natural

aquatic (Nadig *et al.*, 1998; Krane *et al.*, 1999; Ma *et al.*, 2000; Matson *et al.*, 2000) and terrestrial (Theodokaris *et al.*, 2001) habitats. This result was somewhat expected as the only selection possibly influencing the genetic pattern of eel is direct selection on metabolically important enzymes, as microsatellites evolve strictly neutrally, mainly enabling the detection of post-reproductive selection (Bickham *et al*., 2000; Belfiore and Anderson, 2001).

Bioaccumulation vs. individual genetic variability

A comparison between individual-based pollution characteristics and population summary statistics (H_E, P, MNA, H&W equilibrium) holds several difficulties for the interpretation, due to the assumption of "population" in genetic estimators. Therefore, we chose to analyse the relationships between all variables using individual based regression analyses. A negative correlation was observed between IMBI and condition indices, as well as between IMBI and allozymatic MLH. This suggests that strongly polluted individuals need more energy for detoxification and are on average in a worse condition, while more heterozygous individuals may accumulate less (Van Straalen and Kammenga, 1998). Increased fitness with heterozygosity has been empirically demonstrated in a large number of plants and animal species (see David, 1998 for a review), as heterozygotes are better buffered against environmental fluctuations, are superior due to their multimeric enzymes (Nevo *et al.*, 1986) and have a lower energetic demand, favouring such individuals in strongly polluted conditions (heterosis). Due to the multi-locus response or cause of the correlation, an overall metabolic gain in efficiency may be proposed as cause for the correlation (Eanes, 1999); most allozymes studied belong to the glycolysis or citric acid cycle. No Heterozygosity Fitness Correlation (HFC) was found at microsatellites, results concordant with recent findings in farmed eel where growth rate was correlated to allozymatic but not to microsatellite MLH (Pujolar *et al*., 2005). The relative importance of condition and genetic variability to explain differences in heavy metal bioaccumulation as assessed by multiple regression analysis, pointed to allozymatic MLH, followed by the condition index as the main factors influencing bioaccumulation. Hence, an individual's enzymatic heterozygosity (and not necessarily its genome-wide heterozygosity) seems to play an important role in the potential to counteract pollutant bioaccumulation.

Conclusions

We clearly showed a strong correlation between the level of bioaccumulation and a reduced condition within resident eel populations. We also found an obvious link between pollution and a lower allozymatic genetic variability at the individual level and in two *post-hoc* defined groups of different pollution levels. Microsatellite variability did not reflect any pollution or condition related trend, and no individual HFC pattern. We hypothesize that enzymatic genetic variability (MLH) is a key issue to explain differences in the bioaccumulation of toxicants (or detoxification success), in other words to retain fitness. Hence, direct overdominance seems the most likely explanation for the observed pattern in eel and thus not associative overdominance or genetic erosion (only detectable after reproduction). Complementary sampling and experimental studies should increase our confidence about the strength of ecological consequences in catadromous organisms, as well as about the heterosis effect (HFC) detected in this study. Conditional is the optimisation of artificial breeding before evolutionary inferences can be made experimentally. Our results also underline the complexity of evolutionary toxicology research in diadromous species, which switch

between habitats. The knowledge of the genetic make-up is crucial to infer evolutionary consequences of pollutants in such species, which is only possible when assessing the interaction between ecology and genetics.

Acknowledgements

This research was funded by The Ministry of the Flemish Community (AMINAL). Many thanks go to K. Peirsman for organizing the fishing and processing of the samples. We thank J.P. Nash, J.K.J. Van Houdt and two anonymous referees who helped improve the quality of this manuscript. G.E. Maes received a PhD scholarship from the IWT (Institute for the Promotion of Innovation by Science and Technology in Flanders). J.A.M. Raeymaekers received a PhD fellowship of the Fund for Scientific Research (F.W.O.-Vlaanderen). C. Pampoulie received a post-doctoral fellowship from FWO-Vlaanderen and the KULeuven.

References

Avery, E.L., Dunstan, R.H., Nell, J.A., 1996. The detection of pollutant impact in marine environments: condition index, oxidative DNA damage, and their associations with metal bioaccumulation in the sydney rock oyster *Saccostrea commercialis*. *Archives of Environmental Contamination and Toxicology* 31, 192-198.

Belfiore, N.M., Anderson, S.L., 2001. Effects of contaminants on genetic patterns in aquatic organisms: a review. *Mutation Research – Reviews in Mutation Research* 489, 97-122.

Belkhir, K., Borsa, P., Goudet, J., Bonhomme, F., 1999. Genetix: logiciel sous Windows pour la genetique des populations. version 3.0, Laboratoire Genome & Population, CNRS-UPR, Université de Montpellier II, Montpellier (France).

Belpaire C., Derwich A., Goemans G., Van Thuyne G., Cooreman K, Guns M., Ollevier F., 2002. Intra-lake spatial variations in pollution patterns of eel *A. anguilla*. In: Aida, K., *et al.* (eds.), Proceedings of the International Symposium 'Advances in eel biology'. University of Tokyo, 28-30 September 2003, p.170-174.

Benshlomo, R., Nevo, E., 1988. Isozyme polymorphism as monitoring of marine environments - the interactive effect of cadmium and mercury pollution on the shrimp, *Palaemon elegans*. *Marine Pollution Bulletin* 19, 314-317.

Bervoets, L, Blust, R., 2003. Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. *Environmental Pollution* 126 (1), 9-19.

Bickham, J.W. , Sandhu, S., Hebert, P.D.N., Chikhi, L., Athwal, R., 2000. Effects of chemical contaminants and genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutation Research – Reviews in Mutation* 463, 33-51.

Cellule Etat de l'Environnement Wallon, 2003. Tableau de bord de l'environnement wallon 2003. Ed. MRW Ð DGRNE, 144p.

Chagnon, N.L., Guttman, S.I., 1989. Differential survivorship of allozyme genotypes in mosquitofish populations exposed to copper or cadmium. *Environmental Toxicology and Chemistry* 8, 319-326.

Collings, S.E., Johnson, M.S., Leah, R.T., 1996. Metal contamination of angler-caught fish from the Mersey estuary. *Marine Environmental Research* 41, 281-297.

Daemen, E., Cross, T., Ollevier, F., Volckaert, F.A.M., 2001. Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and mtDNA markers. *Marine Biology* 139, 755-764.

David, P., 1998. Heterozygosity-fitness correlations: new perspectives on old problems. *Heredity* 80, 531-537.

Dekker, W., 2000. A procrustean assessment of the European eel stock. *ICES Journal of Marine Science* 57, 938-947.

Diamond, S.A., Newman, M.C., Mulvey, M., Guttman, S.I., 1991. Allozyme genotype and time-to-death of mosquitofish, Gambusia holbrooki, during acute inorganic mercury exposure - a comparison of populations. *Aquatic Toxicology* 21, 119-134.

Eanes, W.F., 1999. Analysis of selection on enzyme polymorphisms. *Annual Review of Ecology and Systematics* 30, 301-326.

Feunteun, E., 2002. Management and restoration of European eel population (*Anguilla anguilla*): an impossible bargain. *Ecological Engineering* 18, 575-591.

Fevolden, S.E., Garner, S.P., 1986. Population-genetics of *Mytilus edulis* from Oslofjorden, Norway, in oil-polluted and non oil-polluted water. *Sarsia* 71, 247-257.

Frati, F., Fanciulli, P.P., Posthuma, L., 1992. Allozyme variation in reference and metal-exposed natural populations of *Orchesella cincta* (insecta, collembola). *Biochemical Systematics and Ecology* 20, 297-310.

Gillespie, R.B., Guttman, S.I., 1989. Effects of contaminants on the frequencies of allozymes in populations of the central stoneroller. *Environmental Toxicology and Chemistry* 8, 309-317.

Goemans, G., Belpaire, C., Raemaekers, M., Guns M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling (report nr: IBW.Wb.V.R.2003.99). 169p.

Harris, H., Hopkinson, D.A., 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Co., Oxford.

Hartl, D.L., Clark, A.G., 1997. Principles of population genetics. Sinauer Associates, Sunderland (USA), third edition, 542p.

Hedrick, P.W., 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution*. 53, 313-318.

Hvilsom, M.M., 1983. Copper-induced differential mortality in the mussel *Mytilus edulis*. *Marine Biology* 76, 291-295.

King, M., 1995. Fisheries Biology, Assessment and Management. Fishing news books, Blackwell Science Ltd., Oxford, England. 341p.

Klerks, P.L., Weiss, J.S., 1987. Genetic adaptation to heavy metals in aquatic organisms - a review. *Environmental Pollution* 45, 173-205.

Krane, D.E., Sternberg, D.C., Burton, G.A., 1999. Randomly amplified polymorphic DNA profile-based measures of genetic diversity in crayfish correlated with environmental impacts. *Environmental Toxicology and Chemistry* 18, 504-508.

Leary, R.F., Allendorf, F.W., Knudsen, K.L., 1987. Differences in inbreeding coefficients do not explain the association between heterozygosity at allozyme loci and developmental stability in rainbow-trout. *Evolution* 41, 1413-1415.

Lewis, S.S., Klerks, P.L., Leberg, P.L., 2001. Relationship between allozyme genotype and sensitivity to stressors in the western mosquitofish *Gambusia*

affinis detected for elevated temperature but not mercury. *Aquatic Toxicology* 52 , 205-216.

Linde, A.R., Arribas, P., Sanchez Galan, S., and Garcia Vazquez, E., 1996. Eel (*Anguilla anguilla*) and brown trout (*Salmo trutta*) target species to assess the biological impact of trace metal pollution in freshwater ecosystems. *Archives of Environmental Contamination and Toxicology* 31(3), 297-302.

Ma, X.L., Cowles, D.L., Carter, R.L., 2000. Effect of pollution on genetic diversity in the bay mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. *Marine Environmental Research* 50, 559-563.

Maeckelberghe, H., 2003. De kwaliteit van het Vlaamse oppervlaktewater en de afvalwaterlozingen in 2002. *Water* 3, 1-6.

Maes G.E., Volckaert F.A.M., 2002. Clinal variation and isolation by distance within the European eel (*Anguilla anguilla* L.). *Biological Journal of the Linnean Society* 77, 509-521.

Matson, C.W., Rodgers, B.E., Chesser, R.K., Baker, R.J., 2000. Genetic diversity of Clethrionomys glareolus populations from highly contaminated sites in the Chornobyl region, Ukraine. *Environmental Toxicology and Chemistry* 19, 2130-2135.

Meffe, G.K., 1991. Life-history changes in Eastern mosquitofish (*Gambusia-holbrooki*) induced by thermal elevation. *Canadian Journal of Fisheries and Aquatic Science* 48, 60-66.

Nadig, S.G., Lee, K.L., Adams, S.M., 1998. Evaluating alterations of genetic diversity in sunfish populations exposed to contaminants using RAPD assay. *Aquatic Toxicology* 43, 163-178.

Nevo, E., 2001. Evolution of genome-phenome diversity under environmental stress. *PNAS* 98, 6233-6240.

Nevo, E., Noy, R., Lavie, B., Beiles, A., Muchtar, S., 1986. Genetic diversity and resistance to marine pollution. *Biological Journal of the Linnean Society* 29, 139-144.

Newman, M.C., Jagoe, R.H., 1998. Allozymes reflect the population-level effect of mercury: simulations of the mosquitofish (*Gambusia holbrooki girard*) GPI-2 response. *Ecotoxicology* 7, 141-150.

Patarnello, T., Battaglia, B., 1992. Glucosephosphate isomerase and fitness - effects of temperature on genotype dependent mortality and enzyme-activity in 2 species of the genus *Gammarus* (Crustacea, Amphipoda). *Evolution* 46, 1568-1573.

Posthuma, L., Vanstraalen, N.M., 1993. Heavy-metal adaptation in terrestrial invertebrates - a review of occurrence, genetics, physiology and ecological consequences. *Comparative Biochemistry and Physiology C – Pharmacology, Toxicology and Endocrinology* 106 , 11-38.

Powers, D.A., Lauerman, T., Crawford, D., Dimichele, L., 1991. Genetic mechanisms for adapting to a changing environment. *Annual Review of Genetics* 25, 629-659.

Pujolar, J.M., Maes, G.E. & Volckaert, F.A.M. 2005. Growth Rate Correlates To Individual Heterozygosity in European eel, *Anguilla anguilla* L. *Evolution* 59 (1), 189-199.

Raufaste, N., Bonhomme, F., 2000. Properties of bias and variance of two multiallelic estimators of F_{ST}. *Theoretical Population Biology* 57, 285-296.

Raymond, M., Rousset, F., 1995. Genepop (version 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86, 248-249.

Rice, W.R., 1989. Analyzing tables of statistical tests. *Evolution* 43, 223-225.

Richardson, B.J., Baverstock, P.R., Adams, M., 1986. Allozyme electrophoresis: A handbook for animal systematics and population studies. Academic Press, Inc., California, San Diego. 410p.

Ricker, W.E., 1975. Computation and interpretation of the biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* 191, 1-382

Ridley, M., 1996. Evolution. Blackwell Science, Inc., Cambridge.719p.?

Robertson, A., Hill, W.G., 1984. Deviations from Hardy-Weinberg proportions - sampling variances and use in estimation of inbreeding coefficients. *Genetics* 107, 703-718.

Robinet, T.T., Feunteun, E.E., 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265-277.

Roche, N., Buet, A., Ramade, F., 2003. Characterization and validation of ecotoxicological biomarkers in an eel population exposed to persistent organic pollutants in the Vaccares lake, French national reserve of Camargue. Rev. D Ecol. - La Terre et La Vie. 58, 127-141.

Shaklee, J.B., Allendorf, F.W., Morizot, D.C., Whitt, G.S., 1990. Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society* 119, 2-15.

Skoog, D.A., 1997. Principles of instrumental Analysis. Brooks Cole Eds., 5th edition, Florence, USA. 870p.

Sokal, R.R., Rohlf F.J. 1997. Biometry, the principles and practice of statistics in biological Research. W.H. Freeman and Company, New York. 887p.

Stanton, M.L., Roy, B.A., Thiede, D.A., 2000. Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution* 54, 93-111.

StatSoft, Inc. 2001. STATISTICA (data analysis software system), version 6. www.statsoft.com

Tesch, F.W., 1977. The Eel, biology and management of Anguillid eels. Chapman & Hall, London. 434p.

Theodorakis, C.W., Bickham, J.W., Lamb, T., Medica, P.A., Lyne, T.B., 2001. Integration of genotoxicity and population genetic analyses in kangaroo rats (*Dipodomys merriami*) exposed to radionuclide contamination at the Nevada test site, USA. *Environmental Toxicology and Chemistry* 20, 317-326.

Thorpe, J.E., Koonce, J.F., Borgeson, D., Henderson, B., Lamsa, A., Maitland, P.S., Ross, M.A., Simon, R.C., Walters, C., 1981. Assessing and managing mans impact on fish genetic resources. *Canadian Journal of Fisheries and Aquatic Science* 38, 1899-1907.

Van Ginneken, V.J.T., Van den Thillart, G.E.E.J.M., 2000. Eel fat stores are enough to reach the Sargasso. *Nature* 403 (6766), 156-157.

Van Der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13, 57-149.

Van Straalen, N.M. 1999. Genetic biodiversity in toxicant-stressed populations. Prog. Environ. Sci. 1, 195-201.

Van Straalen, N.M., Kammenga, J.E. 1998. Assessment of ecotoxicity at the population level using demographic parameters. In: Schüürmann, G., Markert, B. (eds.), Ecotoxicology. John Wiley & Sons, Inc. and Spektrum Akademischer Verlag, p.621-644.

Van Straalen, N.M., Timmermans M.J.T.N., 2002. Genetic variation in toxicant-stressed populations: an evaluation of the "genetic erosion" hypothesis. *Human and Ecological Risk Assessment* 8, 983-1002.

Wirth, T., Bernatchez, L., 2001. Genetic evidence against panmixia in the European eel. *Nature* 409, 1037-1040.

Appendix 1. Average heavy metal concentration per kg of wet *Anguilla anguilla* tissue per sampling site: n = number of individuals analysed for heavy metals. Values for As and Se correspond to one individual at the respective sampling site. Values for Hg, Cd, Pb, Ni, Cr, As and Se are expressed in $\mu g.kg^{-1}$. Values for Cu and Zn are expressed in $mg.kg^{-1}$.

River	Sampling site	n	Hg	Cd	Pb	Cu	Zn	Ni	Cr	As	Se
YSER	Y1	5	245.20	2.60	49.80	0.37	25.76	46.00	171.00	/	/
YSER	Y2	5	59.60	1.78	75.40	1.20	19.20	77.20	632.40	135.00	329.00
YSER	Y3	5	139.40	2.18	24.80	0.39	23.78	37.80	285.60	/	/
YSER	Y4	5	194.60	2.58	20.00	0.37	27.36	17.20	146.60	/	/
YSER	Y5	5	112.80	3.10	38.40	0.33	23.30	54.40	242.80	/	/
MEUSE	M1	6	152.50	23.15	10.33	0.68	27.22	55.67	823.67	263.00	1081.00
MEUSE	M2	5	215.40	22.72	39.20	0.53	30.24	94.00	138.40	168.00	743.00
MEUSE	M3	4	175.20	22.58	28.00	0.33	23.92	82.20	157.60	733.00	488.00
MEUSE	M4	5	144.20	5.16	83.60	0.36	23.84	27.00	197.20	321.00	342.00
SCHELDT	S1	3	70.33	1.67	85.67	0.83	32.50	92.00	183.67	243.00	667.00
SCHELDT	S2	3	142.00	1.50	95.33	0.70	23.83	37.33	135.33	257.00	913.00
SCHELDT	S3	5	91.20	3.86	5.00	0.58	19.74	27.00	139.00	229.00	1064.00
SCHELDT	S4	5	66.00	1.50	55.60	0.83	24.46	5.00	200.20	/	/
SCHELDT	S5	3	99.67	1.50	15.67	0.50	17.00	25.00	187.33	254.00	1166.00
SCHELDT	S6	5	66.20	6.98	85.60	0.57	28.90	90.00	174.60	/	/
SCHELDT	S7	4	127.75	1.50	73.75	0.67	24.75	61.25	181.25	704.00	1556.00

Appendix 2. Allele frequency of the 12 allozymatic and 8 microsatellite loci per sample; R : Allele range; N: number of individuals; A: number of alleles;H_E : expected heterozygosity,H_O : observed heterozygosity.

ALLOZYMES				MICROSATELLITES			
LOCUS	**YSER**	**MEUSE**	**SCHELDT**	LOCUS	**YSER**	**MEUSE**	**SCHELDT**
IDH-1*				AAN 01			
N	41	41	39	R	218-244	218-246	218-240
A	2	3	4	N	37	30	28
He	0.0705	0.0479	0.1903	A	9	10	10
Ho	0.0732	0.0488	0.2051	*He*	0.7356	0.7761	0.7353
GPI-1*				*Ho*	0.7838	0.7000	0.7143
N	40	39	40	***AAN 02***			
A	3	3	4	R	175-307	173-263	175-227
He	0.1403	0.1893	0.3231	N	34	38	36
Ho	0.1500	0.1282	0.2750	A	22	23	23
GPI-2*				*He*	0.9321	0.9224	0.9340
N	41	41	40	*Ho*	0.5588	0.6316	0.5278
A	4	4	2	***AAN 05***			
He	0.0714	0.1823	0.0247	R	177-199	177-197	177-197
Ho	0.0732	0.1463	0.0250	N	39	37	36
AAT-1*				A	9	11	9
N	41	41	38	*He*	0.7558	0.7513	0.7411
A	3	4	6	*Ho*	0.7692	0.8919	0.7500
He	0.1978	0.2023	0.1967	***ARO 095***			
Ho	0.2195	0.2195	0.1842	R	112-136	108-132	110-132
AAT-2*				N	35	29	33
N	41	41	38	A	12	13	12
A	2	2	2	*He*	0.8547	0.8740	0.8508
He	0.0241	0.0476	0.0260	*Ho*	0.8571	0.8893	0.5152
Ho	0.0244	0.0488	0.0263	***ARO 054***			
LDH-A*				R	142-170	150-172	144-166
N	41	41	39	N	39	39	41
A	1	1	1	A	14	11	11
He	0.0000	0.0000	0.0000	*He*	0.8586	0.8797	0.8507
Ho	0.0000	0.0000	0.0000	*Ho*	0.7436	0.8974	0.8780
LDH-B*				***ANG 151***			
N	41	41	39	R	164-202	160-196	158-186
A	1	1	1	N	39	32	38
He	0.0000	0.0000	0.0000	A	13	15	12
Ho	0.0000	0.0000	0.0000	*He*	0.8416	0.8936	0.8397
MPI-1*				*Ho*	0.8718	0.6875	0.8158
N	41	41	39	***ANG 114***			
A	3	3	1	R	191-263	190-351	190-233
He	0.1157	0.1579	0.0000	N	39	36	37
Ho	0.1220	0.1707	0.0000	A	19	29	17
MDH-1*				*He*	0.8958	0.9255	0.9069
N	41	41	41	*Ho*	0.7949	0.9444	0.8649
A	2	2	1	***ARO 121***			
He	0.0241	0.0241	0.0000	R	110-147	101-149	106-143
Ho	0.0244	0.0244	0.0000	N	39	31	36
MDH-2*				A	20	17	21
N	41	40	41	*He*	0.9293	0.9126	0.9317
A	3	4	4	*Ho*	0.8718	0.8065	0.8056
He	0.3067	0.1841	0.2439				
Ho	0.3171	0.1000	0.1463				
PGM-1*							
N	41	41	40				
A	1	1	1				
He	0.0000	0.0000	0.0000				
Ho	0.0000	0.0000	0.0000				
ADH-1*							
N	40	34	39				
A	3	3	2				
He	0.5116	0.5134	0.4970				

Studies on endocrine disruption in silver eel are difficult to undertake as the maturation only takes place during its oceanic migration.

Photo: INBO (Vilda – Rollin Verlinde)

Chapter 8

Vitellogenin in eel

Bram Versonnen[1], Geert Goemans[2], Claude Belpaire[2] and Colin Janssen[1]

1 - Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, J. Plateaustraat 22, B-9000 Gent, Belgium

2 - Institute for Forestry and Game Management, Duboislaan 14, B-1560 Groenendaal, Belgium

This chapter was published as:

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363–371

Summary

As part of a large-scale monitoring program of bioaccumulating contaminants in the European eel (*Anguilla anguilla*) in Flanders (Belgium), we investigated potential effects of xenoestrogens in these fish. The present paper describes the results of the plasma vitellogenin (VTG) content, measured in 142 eels sampled at 20 different locations, in relation to the internal pollution levels. To validate the blood VTG assays, a small number of eels (n = 8) was exposed to 10 µg ethinylestradiol / L (EE2) for 9 days. In this experiment, VTG was detected as a protein with a molecular weight of 214 kDa and confirmed by Western blotting. Compared to the solvent controls, significantly higher concentrations of VTG were measured in EE2 exposed eel. However, the VTG content was relatively low compared to other fish species exposed to high concentrations of estrogens. The plasma VTG content of eels from the field study was very low, despite a very high internal load of endocrine disrupters. These results, together with previously published studies, suggest that immature yellow European eel might not be the best sentinel species to study the effects of estrogenic compounds on VTG levels of wild fish populations.

Introduction

There is growing evidence that anthropogenic xenobiotics can affect the endocrine status of wildlife (Damstra *et al*., 2002). Although precise mechanisms of action are poorly understood and the causative chemicals are not always known, extensive evidence is available that sewage treatment effluents can disturb endocrine function in fish (Harries *et al*., 1999; McArdle *et al*., 2000; Damstra *et al*., 2002). Flanders (Belgium) is a very densely populated area in Western Europe with about 440 inhabitants/km². About 57% of the household sewage water is treated (De Cooman *et al*., 2002). From this, it may be suggested that compounds with estrogenic activity can occur in these surface waters in relatively high concentrations. Organochlorine pesticides for instance are detected at concentrations exceeding the environmental standards in 42% of the routinely monitored sampling stations (De Cooman *et al*., 2002). Despite the concerns, little is known about the incidence of endocrine disrupters and their effects in fish of Flemish surface waters. Eel (*Anguilla anguilla*) has proven to be a good indicator organism for measuring chlorobiphenyls and persistent organochlorine compounds (de Boer and Hagel, 1994; de Boer and Brinkman, 1994). Through carnivorous feeding behaviour - predating on insect larvae, worms, crustaceans, snails, mussels and fish - eel bioaccumulates numerous chemical residues (Tesch, 1977). Eels are widespread and can be found in most aquatic habitats. Furthermore, eels are present throughout the year, the species is not very sensitive to (handling) stress (Livingstone *et al*., 2000) and European eel is quite resistant to various forms of water pollution. In the yellow eel phase, eels are sedentary and normally do not migrate (Tesch, 1977). Measurements of residues in the tissues of the yellow eel phase therefore reflect the quality of their environment, at least with respect to organochlorines (Belpaire *et al*., 1999). Many persistent organic micropollutants have an extremely low solubility in water and are consequently not easy to measure in water. Hence, measurements of bioaccumulation in the eel for pollution monitoring has been initiated in several countries (e.g. Desjardins *et al*., 1983; Castonguay *et al*., 1989; de Boer and Brinkman, 1994; de Boer and Hagel, 1994; Hodson *et al*., 1994; Knights, 1997). Also in Flanders a monitoring network has been developed and implemented (Belpaire *et al*., 1999; Goemans *et al*., 2003). However, attempts to use eels for measuring the biological effects of pollution are scarce. Considering the high pollution pressure on eels in some locations, one might expect endocrine effects, like VTG induction, in these eel populations. Contaminants like PCB's, organochlorine pesticides and heavy metals can be found in considerable concentrations in eels living in Flemish waters (Goemans *et al*., 2003). Although a number of these compounds interact with the endocrine system, specific endocrine disrupters (e.g. synthetic or natural hormones, bisphenol A, alkylphenols, …) can be less persistent, bioaccumulative and lipophilic than those aromatic hydrocarbons (Yamamoto *et al*., 2003).

Anguilla anguilla is an undifferentiated gonochoristic fish, i.e. its gonad development occurs through an ambisexual stage in which both male and female germ cells are present (Grandi *et al.*, 2000). In natural circumstances, European eel only starts reproducing after 3-20 years of juvenile growth in continental waters. Investigations in natural European waters show that the 'continental' age is on average 8.7 years in females and 5.9 years in males (Vøllestad, 1992). At the start of their reproductive migration towards the Sargasso Sea (central North Atlantic Ocean), European eels are still immature. The lack of sexual maturation (vitellogenesis in females) is due to a deficiency in the production of pituitary gonadotropin. The circulating gonadal steroid levels are low and plasma VTG concentrations range from undetectable to 10 µg/ml (Peyon *et al.*, 1997, Luizi *et al*., 1997). To date, adult mature

European eels have never been caught, so gonadal steroid and VTG levels during natural reproduction are still unknown (Peyon *et al*., 1997). VTG induction in fish has been widely used to detect exposure to xenoestrogenic compounds. Under natural conditions, VTG is only produced by mature female fish as a yolk precursor. When male or juvenile fish are exposed to (xeno-)estrogens, they can also produce this protein (Copeland *et al*., 1986; Allner *et al*., 1999; Tyler *et al*., 1998). Therefore, VTG induction is considered to be a good biomarker of exposure to compounds with estrogenic properties. It has been demonstrated that treatment with 17β-estradiol (E2) can also induce VTG synthesis in *Anguilla* sp. (Peters *et al*., 2001).

To investigate the use of European eel as a sentinel species to monitor pollution in surface waters, and to determine the extent of possible endocrine disruption in Flemish surface waters, 650 eels from various localities with varying bioaccumulation profiles were collected in the field and the plasma VTG concentration was determined in 142 of the specimens. In this paper we discuss the results of length, weight and VTG measurements in these 142 eels. Further, the results of a laboratory exposure to waterborne EE2 to validate the VTG assays are discussed.

Materials and methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (Belgium), except where indicated differently.

The synthetic estrogen 17α-ethinylestradiol (98% pure, 17α-ethinyl-1,3,5[10]-estratriene-3,17β-diol 3-cyclopentyl ether) was dissolved in ethanol (96%, Merck-Eurolab, Belgium).

Eel exposure

Eels for the laboratory experiments were purchased from a local eel trader (Borremans, Belgium) and acclimated in aerated 200 L tanks with carbon filtered tap water (pH 7.8, hardness 350 mg $CaCO_3$/L, temperature 18.0 ± 1.5°C, 14/10 light/dark cycle) for 10 days. The same water was used in the experiments. Eight eels were exposed in a 200 L aquarium to 10 µg EE2/L for 9 days. EE2 was dissolved in EtOH and the final EtOH concentration was 0.01%. As a control, 8 fish were kept in a 200 L aquarium in 0.01% EtOH. The fish had a length of 50 ± 8 cm and a weight of 178 ± 47 g. After exposure, the fish were anaesthetized with 2-phenoxyethanol and blood was sampled with heparine-rinsed syringes. After addition of 25 µl aprotinin / ml blood, the blood was centrifuged (3500 g, 4°C, 10 minutes), plasma was shock-frozen in liquid nitrogen and stored at –80°C. The condition factor (Cf) was calculated as total weight (g) / total length³ (cm).

Sampling

In total, 650 eels with varying bioaccumulation profiles were sampled at 160 different locations. Blood of 142 eels (from 20 locations) was sampled for VTG analysis (Figure 8.1, Table 8.1). The eels were captured by electro-fishing or fyke-fishing between May and November 2000. The selected eels were kept alive in freshwater tanks until processing (0-6 days after capture). Length and weight were determined and blood samples of the fish were taken and treated as in the exposure experiment. Plasma VTG was determined with protein electrophoresis. Plasma alkali

labile phosphate (ALP) and Ca measurements were performed on a selected number of fish (n=20).

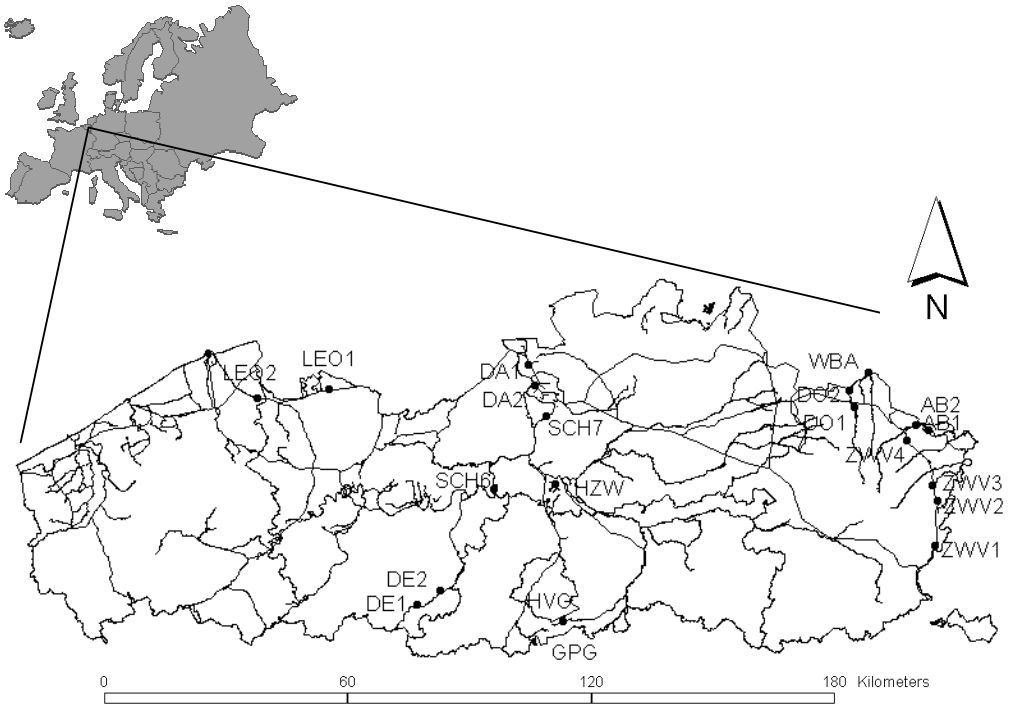


Figure 8.1. Location of the different sampling sites (the inset top left is Europe).

Table 8.1. Summary of sampling sites and date of collection, mean length (± standard deviation) of the eels and organochlorine and PCB load.

Code	Location	Water type	Date of collection	Organochlorines class [1]	PCBs class [2]	Mean length (cm)
AB1	Abeek, Bocholt	River	24/05/2000	2	3	45 ± 5
AB2	Abeek, Kinrooi	River	24/05/2000	2	3	40 ± 4
DA1	Docks of Antwerp	Canal	03/10/2000	2	4	48 ± 9
DA2	Docks of Antwerp	Canal	03/10/2000	2	4	51 ± 10
DE1	Dender, Geraardsbergen	River	13/10/2000	3	3	32 ± 6
DE2	Dender, Ninove	River	16/10/2000	3	3	56 ± 11
DO1	Dommel, Overpelt	River	23/05/2000	2	2	41 ± 8
DO2	Dommel, Neerpelt	River	23/05/2000	2	2	45 ± 8
GPG	Lake Ganzepoot, Hoeilaart	Closed water body	05/05/2000	1	2	48 ± 6
HVG	Fishing pond, Hoeilaart	Closed water body	05/05/2000	1	2	47 ± 5
HZW	Lake Hazewinkel, Willebroek	Closed water body	10/05/2000	2	2	36 ± 6
LEO1	Canal Leopold, Sint Laureins	Canal	03/10/2000	2	1	41 ± 4
LEO2	Canal Leopold, Damme	Canal	03/10/2000	2	1	33 ± 4
SCH6	Scheldt, Hamme	River	10/10/2000	3	3	52 ± 19
SCH7	Scheldt, Antwerp	River	10/10/2000	2	4	38 ± 4
WBA	Warmbeek, Hamont-Achel	River	23/05/2000	2	3	39 ± 3
ZWV1	Zuid-Willemsvaart, Maasmechelen	Canal	09/05/2000	2	4	36 ± 3
ZWV2	Zuid-Willemsvaart, Dilsen-Stokkem	Canal	09/05/2000	2	4	42 ± 9
ZWV3	Zuid-Willemsvaart, Dilsen-Stokkem	Canal	09/05/2000	2	4	39 ± 5
ZWV4	Zuid-Willemsvaart, Bree	Canal	09/05/2000	2	4	42 ± 5

[1] 1: <375 ng/g, 2: 375-950 ng/g, 3: 950-2400 ng/g, and 4: >2400 ng/g lipid weight (sum of the concentrations of hexachlorobenzene, endrin, dieldrin, α-hexachlorohexane, γ-hexachlorohexane, DDT and its metabolites)
[2] 1: <475 ng/g, 2: 475-1192.5 ng/g, 3: 1192.5-2995.5 ng/g, and 4: >2995.5 ng/g lipid weight (sum of the concentrations of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180)

Protein measurement, blood protein electrophoresis and Western blotting

Denaturing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described in Versonnen *et al*. (2003). In short, the protein concentration of the samples was determined according to Bradford (1976) and plasma samples corresponding to 10 µg of protein were loaded on the gel. Denaturing protein electrophoresis was performed with Bio Rad Protean II xi Cell electrophoresis equipment (Bio Rad, Belgium). Protein electrophoresis was performed according to Laemmli (1970). Per sample two replicates were loaded. Gels were run with a constant voltage of 200 V and stained with Coommassie blue dye which was obtained by dissolving 1.2 g of Coommassie Brilliant Blue R-250 (ICN Biomedicals, Belgium) in 500 ml of methanol and adding 200 ml of acetic acid (Merck-Eurolab, Belgium). Destaining was performed with a 40% methanol 10% acetic acid solution in water.

For the Western blotting, plasma samples were separated by SDS-PAGE. A prestained broad range molecular weight standard was used (Bio-Rad, Belgium). The proteins were subsequently transferred onto nitrocellulose membranes (Bio-Rad, Belgium) with a Mini Trans-Blot Electrophoretic Transfer Cell (1h at 100 V). The primary antibody (ND-3G2 monoclonal mouse anti-striped bass VTG) was purchased

from Biosense (Norway). The blotted membranes were shaken in 5% low-fat powdered milk phosphate buffered saline (PBS) for 1 h, prior to incubating them with the primary antibody for 1 h, under continuous agitation and at room temperature. The primary antibody was diluted 1/3000 in 5% low-fat powdered milk PBS. After incubation, the membranes were washed twice in PBST (1 ml Tween/L PBS) and once in PBS. The secondary antibody (anti-mouse IgG alkaline phosphatase conjugated) was diluted 1/1000 in low-fat powdered milk PBS and membranes were incubated under continuous agitation and at room temperature for 1 h, after which they were washed twice with PBST and once with PBS. Coloration was performed with Sigma fast BCIP/NBT tablets for 20 minutes.

Protein gels and Western blot gels were scanned with a GelDoc 2000 system and analysed with Quantity One® software (Bio-Rad, Belgium). The relative VTG content was calculated as the percentage of protein(s) with a weight of 214 kDa, relative to the total protein content. The results of each sample are the mean of at least 2 determinations.

Plasma calcium and ALP determination

Due to the high calcium and ALP concentrations present in the VTG protein, plasma Ca and ALP concentrations can be used as indirect measures of the plasma VTG concentration (Verslycke *et al*., 2002). Therefore, the plasma Ca and ALP concentrations were determined in the eels from the laboratory experiments and in 20 eels from the field sampling, as described in detail elsewhere (Verslycke *et al*., 2002). Briefly, plasma Ca was measured by atomic absorption spectrophotometry (SpectraAA-100, Varian) in 1/10 dilutions in 1% HNO_3. Plasma ALP concentrations were determined through a colorimetric measurement of acidified phosphomolibdate complexes using a commercially available kit (Sigma-Aldrich, Belgium). The Ca and ALP concentration in each plasma sample were measured at least 2 times and the results are the mean of these measurements.

Statistical analysis

Statistical analysis was performed with Statistica® software (Statsoft Inc., USA): all data were tested for homogeneity of variances and normality with Levene's test and Kolmogorov-Smirnov's test, respectively. If these assumptions were met, one way analysis of variance (ANOVA) followed by Dunnett's test was performed. Mann-Whitney U tests were performed when the homogeneity and normality assumptions were not met. The differences described were statistically significant at $p < 0.05$. Non-parametric Spearman tests were used in the correlation analyses (R^2 and P-level).

Results and Discussion

Laboratory exposure

All fish survived the experiment. No significant differences in length, weight and condition factor were detected between the control and EE2-exposed fish (Table 8.2). The plasma protein content of exposed fish was significantly different from that of the controls, and this is most probably due to the production of VTG in exposed eels. This is similar to findings with other fish species (e.g. rainbow trout) where exposure to environmentally relevant EE2 concentrations resulted in elevated plasma protein concentrations (e.g. Verslycke *et al*., 2002; Bon *et al*., 1997). Plasma

protein concentrations in control fish (55 ± 10 mg/ml) correspond well with the concentrations obtained by Luizi *et al.* (1997), who measured 50 to 60 mg/ml in the plasma of untreated eels. An average concentration of 128 ± 9 µg Ca/ml was measured in plasma of control fish. These Ca concentrations are similar to those reported by Suzuki *et al*. (1999), who measured a plasma Ca concentration of 105 ± 5 µg/ml in Japanese eel (*Anguilla japonica*). Plasma ALP concentrations in the controls (37 ± 4 µg/ml) were similar to those found in mud eel (*Amphipnous cuchia*, Srivastav *et a*l., 1998), rainbow trout (*Oncorhynchus mykiss*, Verslycke *et al*., 2002) and in crucian carp (*Carassius carassius*, Tinsley, 1985) but up to 25 times higher than those measured in European eel by Luizi *et al.* (1997). It is not clear what caused this discrepancy.

Table 8.2. Weight, length, condition factor (Cf), plasma protein content and plasma vitellogenin content (VTG, expressed as µg ALP/ml, µg Ca/ml or % of total protein content determined with protein electrophoresis) of eel exposed to 10 µg ethinylestradiol / L (EE2) compared with controls (n = 8, values in parentheses are standard deviations on the mean).

Treatment	weight (g)	length (cm)	Cf (g/cm³)	plasma protein (mg/ml)	plasma VTG content		
					ALP (µg/ml)	Ca (µg/ml)	%
Solvent control	157 (31)	49 (4)	0.0013 (0.0001)	55 (10)	37 (4)	128 (9)	1.6 (1.9)
EE2	161 (36)	50 (4)	0.0013 (0.0001)	*75 [2] (19)*	*762 [2] (145)*	*636 [2] (183)*	*28.3 [2] (10.6)*

[2] = significantly different from the control, $P < 0.05$

A differentially induced protein with a molecular weight of 214 kDa was detected in the EE2-exposed fish. Only background concentrations (less than 2%) of proteins with this molecular weight were measured in unexposed eels (Table 8.2, Figure 8.2a). Monoclonal mouse anti-striped bass (*Morone saxatilis*) VTG antibodies cross-reacted with this protein in the exposed fish, but not in unexposed fish during western blotting of the plasma samples (Figure 8.2b). Peters *et al*. (2001) and Livingstone *et al*. (2000) detected a plasma protein of 211 kDa with protein electrophoresis in E2-injected European eel, which also cross-reacted with antibodies against VTG of striped bass. Komatsu and Hayashi (1998) detected VTG in Japanese eel (*Anguilla japonica*) as a protein with a molecular weight of 196 kDa. Burzawa-Gerard and Dumas-Vidal (1991) and Hara *et al*. (1980), however, reported molecular weights of 340 and 350 kDa, respectively. We assume for several reasons that the detected 214 kDa protein is VTG: (1) the protein has a similar weight as the VTGs observed in recent studies with *A. anguilla* (Peters *et al*., 2001) and *A. japonica* (Komatsu and Hayashi, 1998); (2) the protein cross-reacted with antibodies against VTG of striped bass; (3) the 214 kDa protein is induced by EE2 and only very low to undetectable levels circulate in sexually immature control fish. A drawback of using protein electrophoresis is that proteins with similar molecular weights as VTG, might erroneously be quantified as VTG. This is confirmed by the Western blotting of the untreated eels, showing no reaction products in the controls, while the VTG content measured with protein electrophoresis was 1.6%.

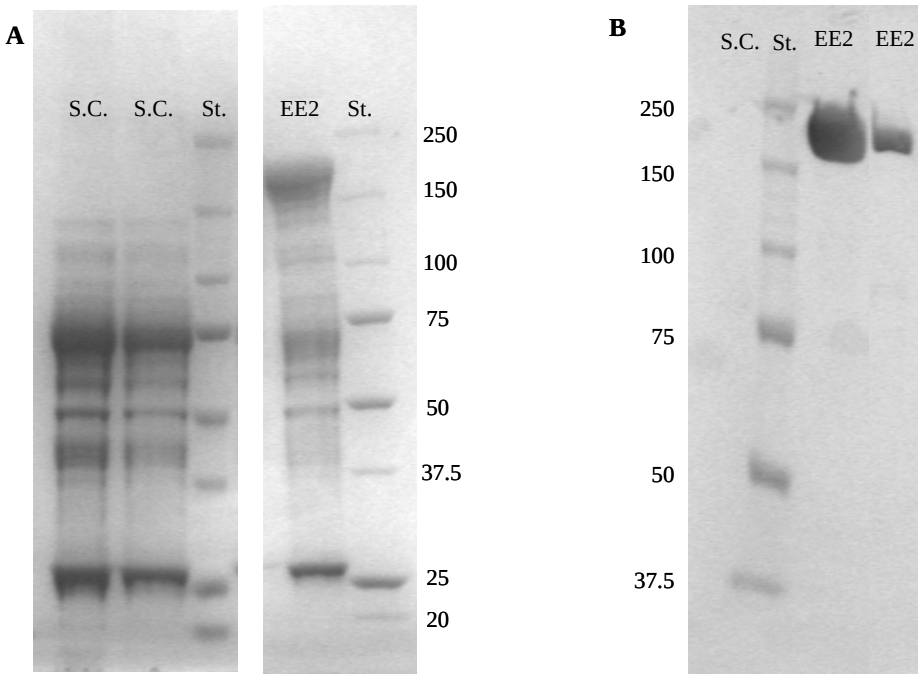


Figure 8.2. (A) A typical gel after protein electrophoresis of plasma of ethinylestradiol-exposed eel (EE2) and solvent control eel (S.C.) and (B) a typical Western blot gel. Values indicate the molecular weight of the protein standard (St.), given in kilodaltons.

Significantly elevated VTG-concentrations (as determined by Ca, ALP and relative plasma VTG content) were detected in EE2-exposed fish (Table 8.2). Although the eels were exposed to a high concentration of EE2 (10 µg/L) for 9 days, the relative VTG content was relatively low (28.3 ± 10.6%, measured with protein electrophoresis). Studies with other fish species showed that VTG concentrations in EE2- or E2-exposed fish can account for 40% or more of the total plasma proteins (Allner *et al*., 1999; Versonnen *et al*., 2003). According to Allner *et al.* (1999), a 10 day exposure of *Leuciscus idus* to 50 ng EE2/L led to an increase in plasma VTG content (measured with protein electrophoresis) from 0.07% to 32% of the total protein content. Exposure of rainbow trout to 4 ng EE2/L for 7 days increased VTG levels from 0.15% to 10%. Previous studies at our laboratory (Versonnen *et al*., 2003) showed that relative VTG levels (measured with protein electrophoresis) increased to up to more than 40% in adult zebrafish exposed to 10, 50 or 100 ng EE2/L. De Vlaming *et al*. (1980) even measured concentrations of up to 80% in estradiol-exposed goldfish (*Carassius auratus*). These data may suggest that immature eels are relatively insensitive for the effects of xenoestrogens on vitellogenesis, compared with other species: eels exposed to 10 µg EE2/L in the present research had a relative blood VTG concentration of 28%. Although nominal concentrations (10 µg EE2/L) were used in this research, one can assume that the actual concentration in the exposure tank will be far exceeding 4 to 100 ng EE2/L (used by Allner *et al*., 1999 and Versonnen *et al*., 2003). The fact that eel might be relatively insensitive to

waterborne endocrine disrupters is also confirmed by Burzawa-Gerard and Dumas-Vidal (1991) and Luizi *et al*. (1997) who found that high doses of (injected) E2 (at least 5 x 0.5 mg/kg during 12 days) were needed to induce VTG production in immature eels. Peters *et al*. (2001) and Livingstone *et al.* (2000) induced VTG in eel by intraperitoneal injection with high doses of E2 (4 x 10 mg/kg during 4 weeks and 2 x 5 mg/kg during 6 days, respectively). Plasma VTG levels were 260 000 to 750 000 times higher in exposed fish (up to 50 mg VTG / ml), compared to the controls after 4 weeks of exposure (Peters *et al*., 2001).

When comparing the different techniques for measuring VTG used in our study, we found that all techniques were capable of detecting enhanced VTG concentrations, but the highest induction factor (exposed/control fish) was obtained with the ALP assay. EE2-exposed fish had a 21 times higher ALP concentration than unexposed fish. Protein electrophoresis and Ca measurements showed an induction factor of 17.5 and 4.5, respectively. However, the coefficient of variation of 4 consecutive measurements of the same plasma samples (6 samples were measured 4 times) is less than 6% for protein electrophoresis and Ca measurement, and 22% for ALP. A more thorough comparison of these techniques can be found in Verslycke *et al*. (2002). Overall, the data show that the three techniques are suited for measuring VTG.

Field samples

A map of the sampling sites is shown in Figure 8.1. The length of the eels at the different sampling points is given in Table 8.1. Length and weight are strongly correlated ($R^2 = 0.92$, data not shown) and a similar pattern is found for the weight at the different sampling points (data not shown). All fish had a length between 25 and 68 cm and a weight between 21 and 618 g.

The pollutant load, measured in fish tissue is discussed in detail elsewhere (Goemans *et al*., 2003). Internal PCB and organochlorine pesticide concentrations are represented in classes in Table 8.1. For the PCBs, the total indicator PCB concentration (sum of concentrations of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180 on lipid basis) is given, divided in 4 classes. For the organochlorine pesticides, the sum of the concentrations of hexachlorobenzene, endrin, dieldrin, α-hexachlorohexane, γ-hexachlorohexane, DDT and its metabolites are given, classified in 4 classes (Goemans *et al*., 2003). The total indicator PCB concentrations ranged from 274 ± 176 ng/g in station LEO1 to 14400 ± 9700 ng/g in station ZWV3, the sum of organochlorine pesticides ranged from 286 ± 114 ng/g in station HVG to 2370 ± 440 ng/g in station DE1. These data indicate that a wide range of pollutants are present at sometimes extremely high concentrations in eel tissue (Goemans *et al*., 2003).

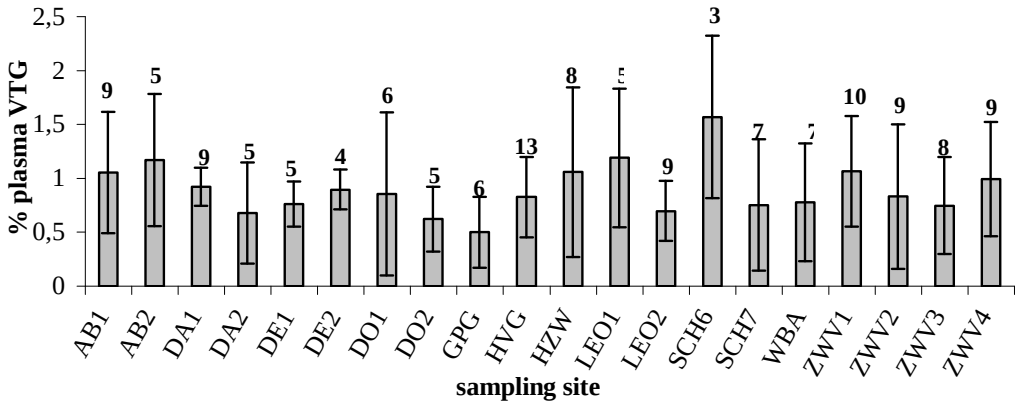


Figure 8.3. Relative plasma vitellogenin (VTG) content of the eels sampled at different locations in Flanders, represented as mean ± standard deviation. The number of fish is given for each sampling site.

Nevertheless, none of the eels collected in the field had increased plasma VTG levels, measured with all three methods: protein electrophoresis (Figure 8.3), and for 20 eels ALP or Ca (data not shown). The overall mean VTG concentration in the samples was 0.9 ± 0.5%. The highest relative plasma VTG concentration was 2.45%. No correlations were found between VTG content and weight, length, Cf, fat content, contaminants (PCBs, organochlorine pesticides, metals) or date of sampling. Our results are in agreement with findings of Livingstone *et al*. (2000) and Peters *et al*. (2001). These authors did not detect any differences in plasma VTG content of eel sampled at different locations in the UK during different seasons. Only limited data exist on the endocrine status of wild maturing eels, because to date, no adult sexually mature eels have been caught (Peyon *et al*., 1997, Lokman *et al*., 1998). However, since eel is a gonochoristic undifferentiated fish, one might expect that it can be influenced by xenoestrogens (Peters *et al*., 2001). Although high internal levels of pollutants were measured, no evidence for effects of estrogenic compounds was detected in eels caught in Flemish surface waters. Therefore, the present research provides a number of hints that continental European eel is rather insensitive to the effects of xenoestrogens. This is confirmed by our laboratory results and the studies of others mentioned above. Internal concentrations of a number of potent endocrine disrupters like natural and synthetic hormones were, however, not measured in the present research. Moreover, it must be emphasized that European eel only comes to full sexual maturation when spawning in the Sargasso Sea. Furthermore, eel stops feeding at the start of the migration (Tesch, 1997). It is therefore possible that effects of pollutants (e.g. endocrine disrupters) become apparent during the starvation period while migrating or during the spawning itself. Sexually mature eels, however, have not been caught to date.

Few reports are available on endocrine disruption in Belgian surface waters. Witters *et al.* (2001) measured the estrogenic activity in 16 Flemish rivers, effluents of municipal wastewater treatment plants and reservoirs for drinking water with a yeast estrogenic screen. The highest estrogenic potency (up to 81 ng/L E2-equivalents) was detected in rivers. It was suggested that the potencies detected in these rivers could adversely influence resident fish populations. Further, studies on field collected roach (*Rutilus rutilus*), tench (*Tinca tinca*) and rudd (*Scardinius erythrophtalmus*)

performed by our laboratory have revealed that intersex and elevated plasma VTG concentrations occur in fish sampled in highly polluted areas in Flanders (Versonnen *et al*., in prep., Van Campenhout *et al*., 2002).

The determination of VTG in plasma of sexually immature eels caught in surface waters did not confirm these findings, although very high concentrations of (possible) endocrine disrupters were sometimes present in their tissues. This high pollutant load in eel indicates that a wide range of pollutants and possible endocrine disrupters occur in Flemish surface waters and can be accumulated in fish.

Conclusions

Measuring internal concentrations of pollutants in eel has proven to be very useful as a monitoring tool for the quality of surface waters as it gives additional information which can be used in an ecological risk assessment. Recently, the very high pollutant loads detected in European eels of Flanders have lead to a catch and release obligation for anglers.

The present study and the results of previous studies do not prove that – despite the high exposure to and uptake of pollutants – European yellow eel under natural conditions are sensitive to the effects of (xeno-)estrogens, as measured by the VTG induction. Although European eel is a useful species for measuring pollutants, we did not find any indications for estrogenic effects to occur in natural freshwater eel populations in Flanders.

Acknowledgements

Bram Versonnen is the recipient of a grant from the Flemish Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT). This research was partly supported by the Flemish government, project number TWOL 2000 AMINAL/BG/IBW/2000-4. We wish to thank Barbara Deryckere and Annelies Hoebeeck for their excellent practical work and all colleagues who helped us in the collection of eel samples.

References

Allner, B., Wegener, G., Knacker, T., Stahlschmidt-Allner, P., 1999. Electrophoretic determination of estrogen-induced protein in fish exposed to synthetic and naturally occurring chemicals. *The Science of the Total Environment* 233 (1-3), 21-31.

Belpaire, C., Van Thuyne, G., Callaars, S., Roose, P., Cooreman, K., Bossier, P., 1999. Spatial and temporal variation in organochlorine pesticide and polychlorinated biphenyl pollution in fresh water aquatic ecosystems in Flanders using the European eel (*Anguilla anguilla* L.) as an indicator. EIFAC/ICES, Working group on eel, Silkeborg (DK), 20-25 September 1999.

Bon, E., Barbe, U., Rodriguez, J.N, Cuisset, B., Pelissero, C., Sumpter, J.P., Le Menn, F., 1997. Plasma vitellogenin levels during the annual reproductive cycle of the female rainbow trout (*Oncorhynchus mykiss*): establishment and validation of an ELISA. *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology* 117 (1), 75-84.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein, utilizing the principle of protein-dye landing. *Analytical Biochemistry* 72 (1-2)**,** 248-254.

Burzawa-Gerard, E., Dumas-Vidal, A., 1991. Effects of 17β-estradiol and carp gonadotropin on vitellogenesis in normal and hypophysectomized European silver female eel (*Anguilla anguilla* L.) employing a homologous radioimmunoassay for vitellogenin. *General and Comparative Endocrinology* 84 (2), 264-276.

Castonguay, M., Dutil, J.D., Desjardins, C., 1989. Distinction between American eels (*Anguilla rostrata*) of different geographic origins on the basis of their organochlorine contaminant levels. *Canadian. Journal of Fisheries and Aquatic Sciences* 46 (5), 836-843.

Copeland, P.A., Sumpter, J.P., Walker, T.K., Croft, M., 1986. Vitellogenin levels in male and female rainbow trout (*Salmo gairdneri* Richardson) at various stages of the reproductive cycle. *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology* 83 (2), 487-493.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R., Van Der Kraak, G., 2002. Global assessment of the state-of-the-science of endocrine disruptors. WHO-ILO-UNEP.

De Boer, J., Brinkman, U.A.T., 1994. The use of fish as biomonitors for the determination of contamination of the aquatic environment by persistent organochlorine compounds. *Trends in Analytical Chemistry* 13 (9), 397-404.

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 (2), 155-174.

De Cooman, W., Maeckelberghe, H., Vos, G., De Corte, S., Van Erdeghem, M., Van Wauwe, P., Vannevel, R., Belpaire, C., Breine, J., Schneiders, A., Peeters, B., 2002. Kwaliteit oppervlaktewater. In: Van Steertegem, M. (Ed.), Milieu- en natuurrapport Vlaanderen: Thema's Mira-T 2002. Garant, Antwerpen, p.289-307.

Desjardins, C., Dutil, J.-D. and Gelinas, R., 1983. Polychlorinated biphenyl contamination of eel (*Anguilla rostrata*) in the St. Lawrence River basin. *Sciences Halieutiques et Aquatiques* 144, 1-61.

De Vlaming, V.L., Wiley, H.S., Delahunty, G., Wallace, R.A., 1980. Goldfish (*Carassius auratus*) vitellogenin: induction, isolation, properties and relationship to yolk proteins. *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology* 67 (4), 613-623.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994–2001: gehalten aan polychloorbifenylen, 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.

Grandi, G., Poerio, F., Colombo, G, Chicca, M., 2000. Effects of diet supplementation with carp ovary on gonad differentiation and growth of the European eel. *Journal of Fish Biology* 57 (6), 1505-1525.

Hara, A., Yamauchi, K., Hirai, H., 1980. Studies on female-specific serum protein (vitellogenin) and egg yolk protein in Japanese eel (*Anguilla japonica*). *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology* 65 (2), 315-320.

Harries, J.E., Janbakhsh, A., Jobling, S., Matthiessen, P., Sumpter, J.P., Tyler, C.R., 1999. Estrogenic potency of effluent from sewage treatment works in the United Kingdom. *Environmental Toxicology and Chemistry* 18 (5), 932-937.

Hodson, P.V., Castonguay, M., Couillard, C.M., Desjardins, C., Pelletier, E. and McLeod, R., 1994. Spatial and temporal variations in chemical contamination of American eels, *Anguilla rostrata*) captured in the estuary of the St. Lawrence River. *Canadian Journal of Fisheries and Aquatic Sciences* 51 (2), 464-478.

Komatsu, M., Hayashi, S., 1998. Optimal conditions for estradiol-17β induced vitellogenin synthesis by cultured hepatocyte of eel *Anguilla japonica*. *Fisheries Science* 64 (4), 658-659.

Knights, B., 1997. Risk assessment and management of contamination of eels (*Anguilla* spp.) by persistent xenobiotic organochlorine compounds. *Chemistry and Ecology* 13 (3), 171-212.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227 (2)**,** 680-685.

Livingstone, D.R., Mitchelmore, C.L., Peters, L.D., O'Hara, S.C.M., Shaw, J.P., Chesman, B.S., Doyotte, A., McEvoy, J., Ronisz, D., Larsson, D.G.J., Förlin, L., 2000. Development of hepatic CYP1A and blood vitellogenin in eel (*Anguilla anguilla*) for use as biomarkers in the Thames Estuary, UK. Marine *Environmental Research* 50 (1-5), 367-371.

Lokman, P.M., Vermeulen, G.J., Lambert, J.G.D., Young, G., 1998. Gonad histology and plasma steroid profiles in wild New Zealand freshwater eels (*Anguilla dieffenbachia* and *A. australis*) before and at the onset of the natural spawning migration. I. Females. *Fish Physiology and Biochemistry* 19 (4), 325-338.

Luizi, F., Korsgaard, B., Petersen, I.M., 1997. Plasma lipoproteins in European eels (*Anguilla anguilla*): effects of estradiol. *Fish Physiology and Biochemistry* 16 (4), 273-280.

McArdle, M., Elskus, A., McElroy, A., Larsen, B., Benson, W., Schlenk, D., 2000. Estrogenic and CYP1A response of mummichogs and sunshine bass to sewage effluent. *Marine Environmental Research* 50 (1-5), 175-179.

Peters, L.D., Doyotte, A., Mitchelmore, C.L., McEvoy, J., Livingstone, D.R., 2001. Seasonal variation and estradiol-dependent elevation of Thames estuary eel *Anguilla anguilla* plasma vitellogenin levels and comparisons with other estuaries. *The Science of the Total Environment* 279 (1-3), 137-150.

Peyon, P., Baloche, S., Burzawa-Gerard, E., 1997. Investigation into the possible role of androgens in the induction of hepatic vitellogenesis in the European eel: *in vivo* and *in vitro* studies. *Fish Physiology and Biochemistry* 16 (2), 107-118.

Srivastav, A.K., Tiwari, P.R., Srivastav, S.K., Sasayama, Y., Suzuki, N., 1998. Serum calcium and phosphate levels of calcitonin treated freshwater mud eel, *Amphipnous cuchia* kept in different calcium environments. Netherlands *Journal of Zoology* 48 (2), 189-198.

Suzuki, N., Suzuki, D., Sasayama, Y., Srivastav, A.K., Kambegawa, A., Asahina, K., 1999. Plasma calcium and calcitonin levels in eels fed a high calcium solution or transferred to seawater. *General and Comparative Endocrinology* 114 (3), 324-329.

Tesch F.-W., 1977. The eel. Biology and management of anguillid eels. Chapman and Hall, London 434p.

Tinsley, D., 1985. A comparison of plasma levels of phosphoprotein, total protein and total calcium as indirect indices of exogenous vitellogenesis in the crucian carp *Carassius carassius* (L.). *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology* 80 (4), 913-916.

Tyler, C.R., Jobling, S., Sumpter, J.P., 1998. Endocrine disruption in wildlife. *Critical Reviews in Toxicology* 28 (4), 319-361.

Van Campenhout, K., De Coen, W.M., Vandenbergh, G., Denayer, B., Janssen, C.R., 2002. A screening of roach, *Rutilus rutilus*, populations for oestrogenic effects

in Flanders, Belgium. In: Collares-Pereira M.J., Cowx I.G., Coelho M.M. (eds.), Conservation of freshwater fishes: options for the future. Blackwell Science, Oxford, p.262-270

Verslycke, T., Vandenbergh, G.F., Versonnen, B.J., Arijs, K., Janssen, C.R., 2002. Induction of vitellogenesis in 17α-ethinylestradiol-exposed rainbow trout (*Oncorhynchus mykiss*): a method comparison. *Comparative Biochemistry and Physiology C Toxicology and Pharmacology* 132 (4), 483-492.

Versonnen, B.J., Arijs, K.A., Verslycke, T.V., Lema, W., Janssen, C.R., 2003. *In vitro* and *in vivo* estrogenicity and toxicity of *o*-, *m*- and *p*-dichlorobenzene. *Environmental Toxicology and Chemistry* 22 (2), 329-335.

Vøllestad, L. A., 1992. Geographic variation in age and length at metamorphosis of maturing European eel: environmental effects and phenotypic plasticity. *Journal of Animal Ecology 61,* 41–48.

Witters, H.E., Vangenechten, C., Berckmans, P., 2001. Detection of estrogenic activity in Flemish surface waters using and *in vitro* recombinant assay with yeast cells. *Water Science and Technology* 43 (2), 117-123.

Yamamoto, H., Liljestrand, H.M., Shimizu, Y., Morita, M., 2003. Effects of physical-chemical characteristics on the sorption of selected endocrine disruptors by dissolved organic matter surrogates. *Environmental Science and Technology* 37 (12), 2646-2657.

Part IV

The use of the European eel as an indicator of pollution

Eel fishing in a lake.
Photo: Peter Van Hoof

Chapter 9

Intra lake spatial variations in pollution patterns of eel

Claude Belpaire[1], Agnieszka Derwich[2], Geert Goemans[1], Gerlinde Van Thuyne[1], Kris Cooreman[3], Marc Guns[4] and Frans Ollevier[2]

1 - Institute for Forestry and Game Management, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium

2 - Catholic University of Leuven, Laboratory for Ecology and Aquaculture, De Beriotstraat 32, B-3000 Leuven, Belgium

3 - Veterinary and Agrochemical Research Centre, Leuvensesteenweg 17, B-3080 Tervuren, Belgium

4 - Centre for Agricultural Research, Sea Fisheries Department, Ankerstraat 1, B-8400 Ostend, Belgium

This chapter was published as:

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: Proceedings of the International Symposium 'Advances in Eel Biology' (Aida *et al*., Eds), University of Tokyo, 28-30 September 2001, p.170-174.

Summary

Variations in bioaccumulation load of polychlorinated biphenyls, organochlorine biocides and heavy metals in eel *Anguilla anguilla* L. from 4 areas of a 90 ha lake in central Flanders were studied. Although for most of individual pollutants no significant differences were found between eels of the different areas, there seems to be a slight shift in overall pollution pattern between the eels caught in the different areas of the lake. The study revealed significant differences in lindane concentrations in muscle tissue of eels from different areas. No evidence was found for potential causes of this pollution. This study illustrates the potential of using eel as monitoring organism for pollution by some persistant substances within lacustrine environments, even within rather small lakes.

Introduction

Spatial variations in pollution patterns in eels from different locations along a river system have been reported on several occasions (Castonguay *et al*., 1989; de Boer and Brinkman, 1994; de Boer and Hagel, 1994; Belpaire *et al.*, 1999; Steinbacher *et al*., 2000, …), demonstrating the applicability of using eels as biomonitors of polychlorinated biphenyls, biocides and heavy metals in the environment (EIFAC, 1991; Knights, 1997; Geuzens *et al.*, 1999). Spatial variations in pollution patterns of eels within lacustrine environments, however, have seldom been studied. Within one lake, pollution loads might vary as a consequence of diverse causes like diffuse pollution through run off biocides from agricultural areas or accidental spills (e.g. in lake Balaton in 1991 and 1995 causing vast eel kill in certain parts of the lake (330 tons) (Bálint *et al.*, 1997). While concentrations found in water and sediment are low and even under detection limits, high concentrations can be measured in aquatic organisms. As sediment and water analyses may not always be able to detect these spatial differences in pollution within lakes (due to the hydrophobicity of these micropollutants and analytical restrictions), analysis of these pollutants bioaccumulated in eel might be a valuable method to detect these gradients.

Materials and methods

Lake Schulen is a small artificial eutrophic lake (90 ha) situated in Flanders (Belgium) and used occasionally in flood periods as a water retaining basin within the river systems of Dijle and Demer. The lake was excavated in 1974, overall depth is 4.5-5m. In the past years a water quality gradient (with respect to some physicochemical parameters) was reported with increasing quality from east to west, being the result of an influx of polluted water in the eastern part. In the west superfluous water can leave the lake at the outlet. Differences in fish assemblages and species abundances also seem to occur within the lake (Simoens *et al.*, 2002). In the east a part of the lake (Zone D) is distinct from the major part but is connected through a funnel. In order to study the variation in pollution load in the eels, 17 specimens from 4 different zones of the lake (Figure 9.1) were sampled by electrofishing or with fyke nets in september 1999. All eels were in the yellow eel phase and their length varied between 34.0 and 43.8 cm. Eels were analysed individually for a series of toxic substances (polychlorinated biphenyls, organochlorine biocides and heavy metals, see Table 9.1).

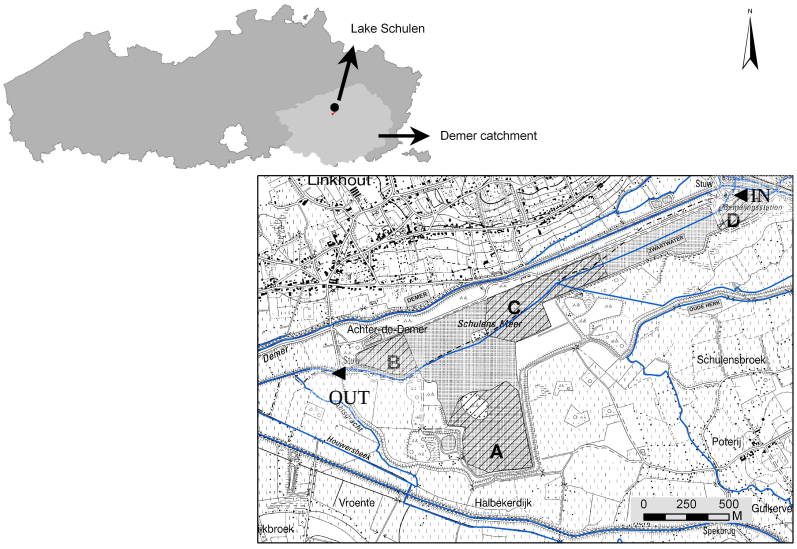


Figure 9.1. Position of Lake Schulen in Flanders with the indication of the different zones and in- and outlet of the lake.

From each eel a sample of ca 50 g of muscle tissue was removed, labeled and frozen before analysis. The analysis were performed by the Belgian Sea Fisheries Department in Ostend (PCBs and pesticides) and by the Veterinary and Agrochemical Research Centre in Tervuren (heavy metals).

Lipids were measured by total lipid extraction following Bligh & Dyer (1959). The techniques for analysis of PCBs and biocides are described in Roose *et al*. (1998). Analysis was performed on a Carlo Erba 8000 GC gas chromatograph with an electron capture detector and a 60m DB-17 and a DB-5 column, both with a film thicknes of 0.25 µm and an internal diameter of 0.25 mm. The detection limit was 0.1 ng/g fat weight.

Quality assurance consisted of the analysis of procedural blanks, reproducibility and repeatability tests, injection of standard solutions as unknowns, and analysis of a certified reference material (BCR CRM 349). The lab routinely analyses sample in the framework of the international proficiency testing scheme QUASIMEME for organochlorines in biological samples.

Analysis less than detection limit was set on 0.05 $ng.g^{-1}$ fat weight. Results were expressed as $ng.g^{-1}$ fat weight. Results were also calculated and expressed for total PCB (Sum PCB) being the sum of the means of the 10 congeners measured and for total DDT (sum of the means of the isomers and breakdown products mentioned in Table 9.1). To enable comparison with the proposed Belgian standard for PCBs in fish and derived products, PCBs were also expressed in $ng.g^{-1}$ body

weight for Sum 7 PCB (being the sum of the seven marker PCBs (PCB 28, 52, 101, 118, 138, 153 and 180).

Concentrations of heavy metals were measured by atomic absorption spectrometry and expressed as $ng.g^{-1}$ body weight.

Statistical analysis consisted in nonparametric analysis of variance with the Mann Whitney U test with the significance level set at 5% and factor analysis (biplot analysis) using Statistica version 5. The factor analysis was performed on the individual concentrations of the pollutants (for PCBs and pesticides expressed in ng/g fat weight and heavy metals in ng/g body weight). Some of the pollutants were left out of this analysis as all measurements were around the detection limit.

Table 9.1. List of pollutants measured

Heavy metals	cadmium, mercury and lead
Polychlorinated biphenyls	PCB 28/PCB 31, PCB 52, PCB 101, PCB 105, PCB 118, PCB138, PCB153, PCB 156, PCB 180
Hexachlorine cyclohexanes	α-HCH, γ-HCH (Lindane)
Cyclodienes (drins)	Dieldrin, Aldrin, Endrin
Polychlorobenzenes	Hexachlorobenzene (HCB)
Chloroethanes	*p,p'*-DDD (TDE), *p,p'*-DDT, *p,p'*-DDE, trans-nonachlor

Results and Discussion

For most of the contaminants analysed (Hg, Pb, PCBs, lindane, dieldrin, aldrin, endrin, HCB and Sum DDT), concentrations in eel from Lake Schulen are *not deviating* from reference values used in Flanders (Van Thuyne *et al.*, 2000; Belpaire *et al*., 2000b), with the exception of cadmium which is *slightly deviating* in zones A, B and C and *deviating* from the reference value in zone D. In Belgium, consumption standards only exist for the heavy metals Hg, Cd and Pb. Two eels (zone D and C) exceed the 50 ng/g BW consumption standard for cadmium (58 and 62 ng/g BW). For PCBs a stringent consumption standard for fish products of 75 ng/g body weight (Sum 7 PCBs) has been proposed recently (BS, in prep). According to this decree proposal, fixing the maximal allowed concentrations of dioxines and PCBs in fish and derived products, 82% (n = 17) of the Lake Schulen eels should be considered as unfit for human consumption. Mean of Sum 7 PCBs is 175.1 ng/g BW (min 40.4, max 591.4), which in comparison to eels from most other waters in Flanders is relatively low, some of them being as high as 8500 ng/g BW (Belpaire *et al*., 2000b).

Analysing the data of the individual contaminants in eel caught in the different zones, there seems to be no significant variation between zones (Figure 9.2), with the exception of the lindane concentrations which were significantly different between zones A and B, the latter being higher (Mann Whitney U test, $p<0.05$). In many countries the legal use of lindane (gamma HCH) has been banned. In Belgium lindane is still being used, mainly in some agrocultures (mostly cultures of beet, corn and ornamental flowers), and it has been demonstrated to be present in eels, bioaccumulating in high concentrations, especially in the Demer and Dijle basins and in the IJzer catchment (western Flanders) (Belpaire *et al.*, 2000b). Presumably these patterns are related to land use activities. In Lake Schulen the origin of the higher lindane concentrations in eels of the zones B and C could not be traced.

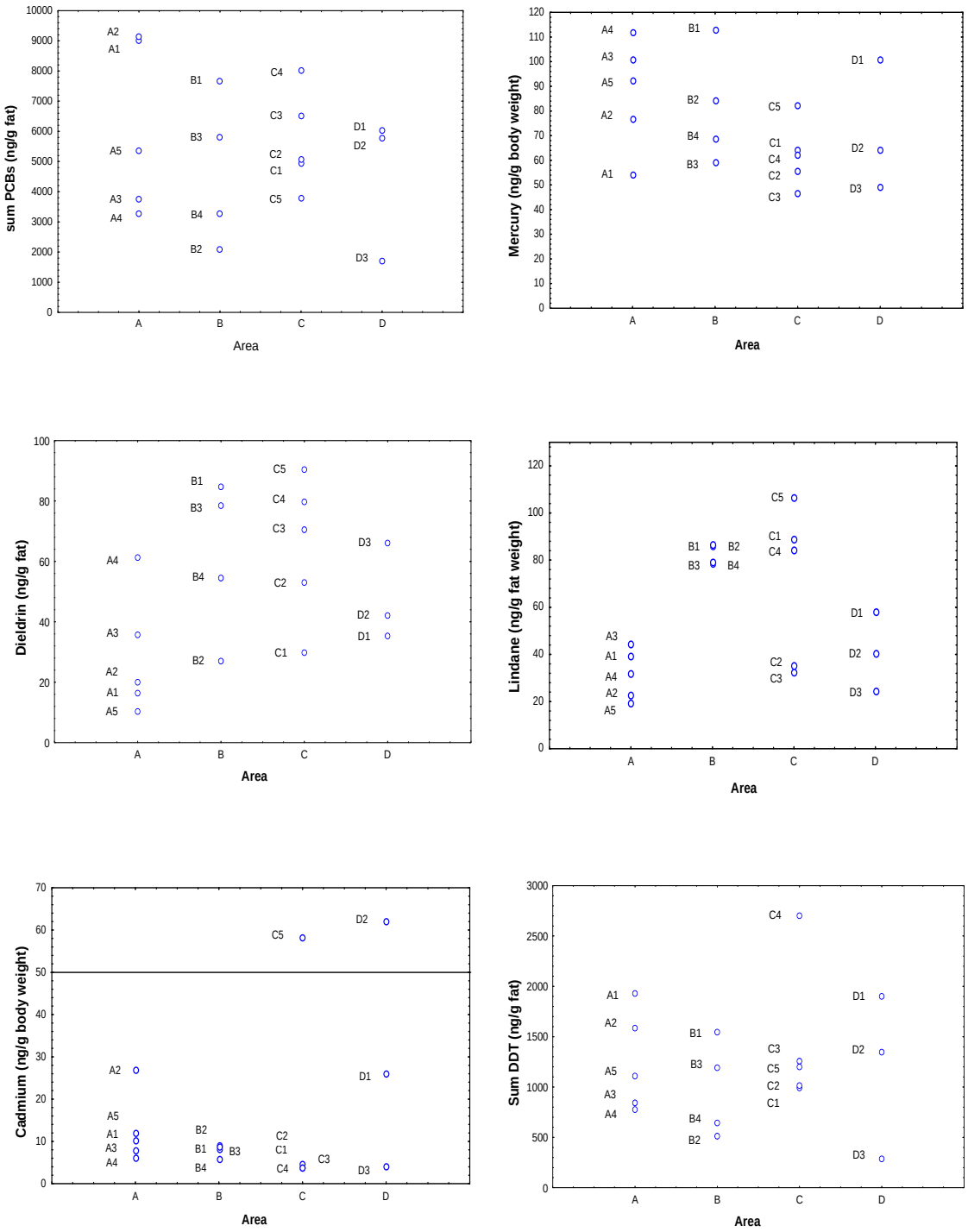


Figure 9.2. Concentrations of Sum PCBs, mercury, dieldrin, lindane, cadmium and Sum DDT in individual eels from the different zones. The concentrations are expressed in ng/g fat weight except for the heavy metals (in ng/g body weight). Horizontal line: Belgian consumption standard for cadmium.

The factor analysis of the combined data show that zone A is distinct from C, D and B (Figure 9.3). The distinct overall pollution pattern in the eels from zone A may be explained by the southern position of A, which has been less influenced by incoming (polluted) water from the eastern inlet point. The distinctive character of the A zone has been suggested earlier by Simoens *et al*. (2002) who found some significant differences in fish assemblages between some zones and calculated the flandrian Index of Biotic Integrity (Belpaire *et al*., 2000a) of the different zones showing a gradual increase in the fish based ecological quality from the zones from east to west, with zone A having the best IBI score.

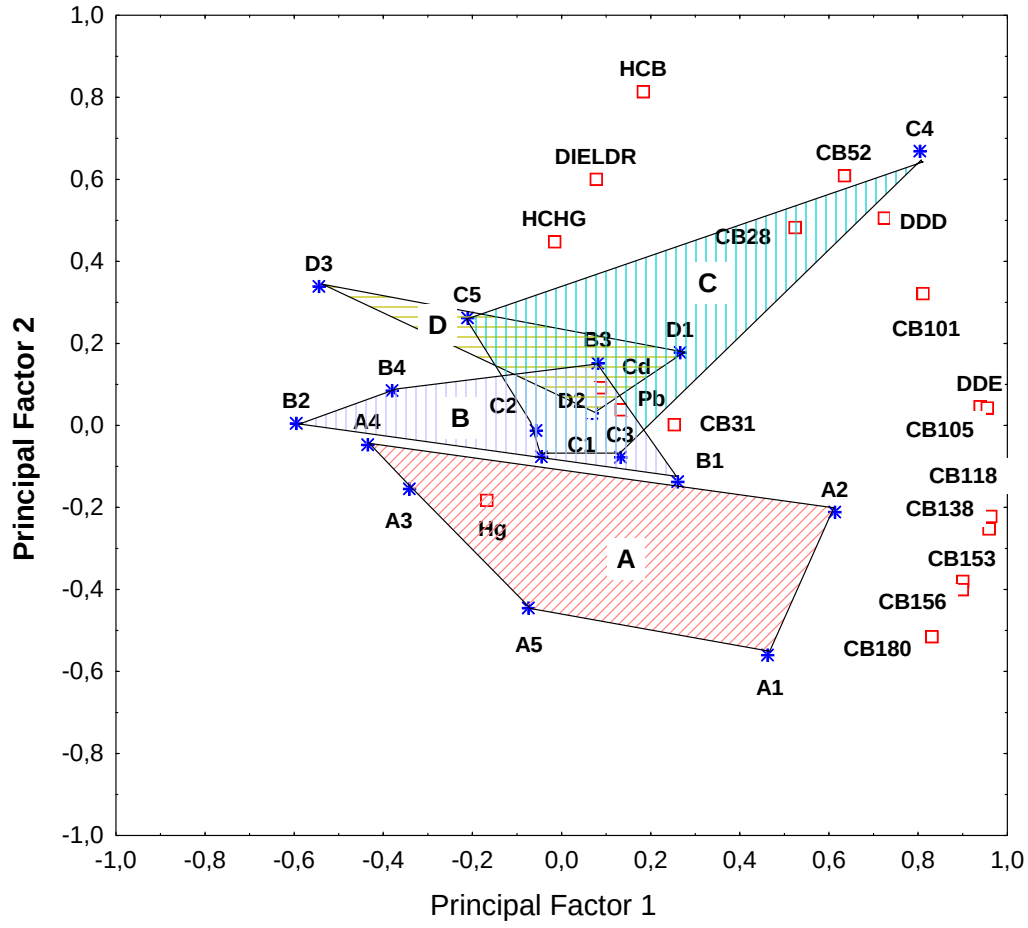


Figure 9.3. Factor analysis of contaminant concentrations in the eels of the different zones. Eels are numbered per zone (A1, A2, …). HCHG is gamma - HCH (lindane).

In riverine systems eel is known as a good bioindicator to monitor the presence of contaminants. An essential element is the narrow home range of the species during its growing phase (yellow eel phase) (Tesch, 1977). Also in a lacustrine environment, the home range of eel seems to be restricted: in Lake Schulen during recapture experiments in a recent fish assessment survey (Simoens *et al.*, 2002), 92% of the eels (n = 48) were recaptured in the same zone. This strongly supports the usefulness of eels as a biomonitor for (variations of) pollution load within lakes. Even in relatively small lakes it might be usefull to analyse concentrations of contaminants in eels in order to map the distribution of contaminants throughout the lake. Monitoring of contaminants in eel is a very accurate instrument to monitor the pollution load in aquatic ecosystems. Policy makers and water quality managers should consider the use of this indicator in their environmental reports. A comparative study of the efficiency of the various strategies for the measurement of pollutants in aquatic ecosystems is necessary in the perspective of a durable monitoring strategy.

Acknowledgements

The authors gratefully acknowledge Ilse Simoens, Jan Breine and Hugo Verreycken for collecting the samples and the Provincial Fisheries Commission of Limburg for financing part of the project.

References

Bálint, T., Ferenczy, J., Kátai, F., Kiss, I., Kráczer, L., Kufcsák, O; Láng, G., Polyhos, C., Szabó, I., Szegletes, T., Nemcsók, J., 1997. Similarities and differences between the massive eel (*Anguilla anguilla* L.) devastations that occurred in Lake Balaton in 1991 and 1995. *Ecotoxicology and Environmental Safety* 37, 17-23.

Belpaire, C, Van Thuyne, G., Callaars, S., Roose, P. Cooreman, K., Bossier, P., 1999. Spatial and temporal variation in organochlorine pesticide and polychlorinated biphenyl pollution in fresh water aquatic ecosystems in Flanders using the European eel (*Anguilla anguilla* L.) as an indicator. "EIFAC/ICES Working Group on Eel" Silkeborg (DK), 20-25 september 1999. IBW.Wb.V.C.99.30

Belpaire, C., Smolders, R., Vanden Auweele, I., Ercken, D., Breine, J., Van Thuyne, G., Ollevier, F., 2000a. An Index of Biotic Integrity characterising fish populations and ecological quality of Flandrian water bodies. *Hydrobiologia* 434, 17-33.

Belpaire, C., Van Thuyne, G., Cooreman, K., De Cooman, W., 2000b. Polluenten in paling in Vlaanderen 2. Polychloorbiphenylen en pesticiden (1994-1999). Instituut voor Bosbouw en Wildbeheer, 19p.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification for organic compounds. *Canadian Journal of Biochemistry and Physiology* 37, 911-917.

BS, in prep. Koninklijk Besluit tot wijziging van het koninklijk besluit van 19 mei 2000 tot vaststelling van maximale gehaltes aan dioxines en polygechloreerde bifenylen in sommige voedingsmiddelen - Belgian Law, Proposal, Belgisch Staatsblad

Castonguay, M., Dutil, J.D., Desjardins, C., 1989. Distinction between American eels (*Anguilla rostrata*) of different geographic origins on the basis of their

organochlorine contaminant levels. *Canadian Journal of Fisheries and Aquatic Sciences* 46, 836-843.

de Boer, J., Brinkman, U.A.T., 1994. The use of fish as biomonitors for the determination of contamination of the aquatic environment by persistent organochlorine compounds. *Trends in Analytical Chemistry* 13 (9), 397-404.

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.

European Inland Fisheries Advisory Commission, 1991. Report of the Seventh Session of the Working Party on Eel, Dublin, Ireland, 20-25 May 1991, EIFAC Occasional Paper, No. 25, Rome, FAO. 1991. 36p.

Geuzens, P., Nouwen, J., Cornelis, C., Craps, R., De Vlieger, I., Mensink, C., Schoeters, G., Witters, H., Goyvaerts, M.P., Weltens, R., Van Thuyne, G., Belpaire, C., Guns, M., Brouwers, J., 1999. Verspreiding van zware metalen. In: Milieu- en natuurrapport Vlaanderen: thema's Mira-T 1999. Vandeweerd *et al*. (eds), Vlaamse Milieumaatschappij, Leuven, 85-106.

Knights, B., 1997. Risk assessment and management of contamination of eels (*Anguilla* spp.) by persistent xenobiotic organochlorine compounds. *Chemistry and Ecology* 13, 171-212.

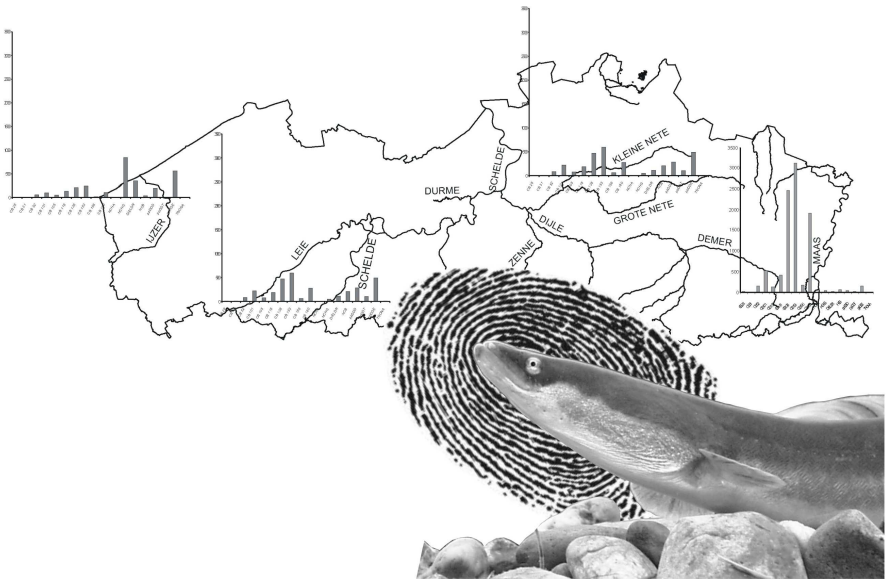
Roose, P., Cooreman, K., Vyncke, W., 1998. PCBs in cod (*Gadus morhua*), flounder (*Platichtys flesus*), blue mussel (*Mytilus edulis*) and brown shrimp (*Crangon crangon*) from the Belgian continental shelf: relation to biological parameters and trend analysis. *Chemosphere* 9-12, 2199-2210.

Simoens, I., Breine, J., Verreycken, H., Belpaire, C., 2002. Fish stock assessment of Lake Schulen, Flanders: a comparison between 1988 and 1999. In: Cowx, I., *et al.* (eds.), Management and ecology of lake and reservoir fisheries. Fishing News Books, Oxford, p.46-57.

Steinbacher, J.C., Baker, J.E., Secor, D.H., 2000. The American eel, *Anguilla rostrata*, as a sentinelspecies of environmental PCB exposure in the Hudson River. 1st Intenational Catadromous Eel Symposium, St. Louis (USA), August 2000.

Tesch, F.-W., 1977. The eel. Biology and management of anguillid eels. Chapman and Hall (London), 434p.

Van Thuyne, G., Belpaire, C., Guns, M., De Cooman, W., 2000. Polluenten in paling in Vlaanderen 1. Zware metalen (1994-1999). Instituut voor Bosbouw en Wildbeheer, 19p.



The yellow eel as a chemical bioindicator for monitoring pollution by lipophilic contaminants. Contaminant fingerprints in yellow eel reflect the pollution pressure on the sampling site.

Chapter 10

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

Claude Belpaire[1], Geert Goemans[1], Caroline Geeraerts[1], Paul Quataert[1] and Koen Parmentier[2]

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 km² 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.

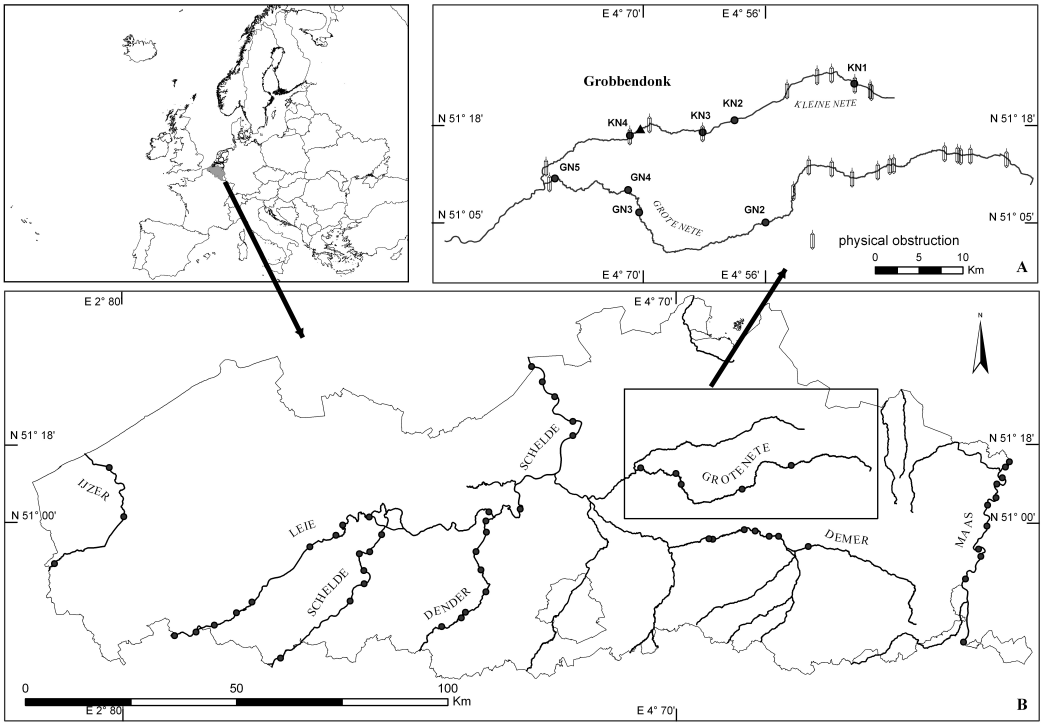


Figure 10.1. Location of sampling sites on: A. the Grote Nete and Kleine Nete in the Nete basin with indication of physical obstructions; and B. on seven rivers in Flanders (IJzer, Leie, Schelde, Dender, Grote Nete, Demer and Maas).

(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) 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 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 α and γ 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 $\mu g.kg^{-1}$ lipid weight (LW). The detection limit (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 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 ± 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 discrimated 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 ± 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 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 γ-HCH, concentrations were higher in eels from the Grote Nete, but without a consistent trend along the river (Figure 10.2F). Overall, α-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, α-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 ($\mu g.kg^{-1}$ 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.

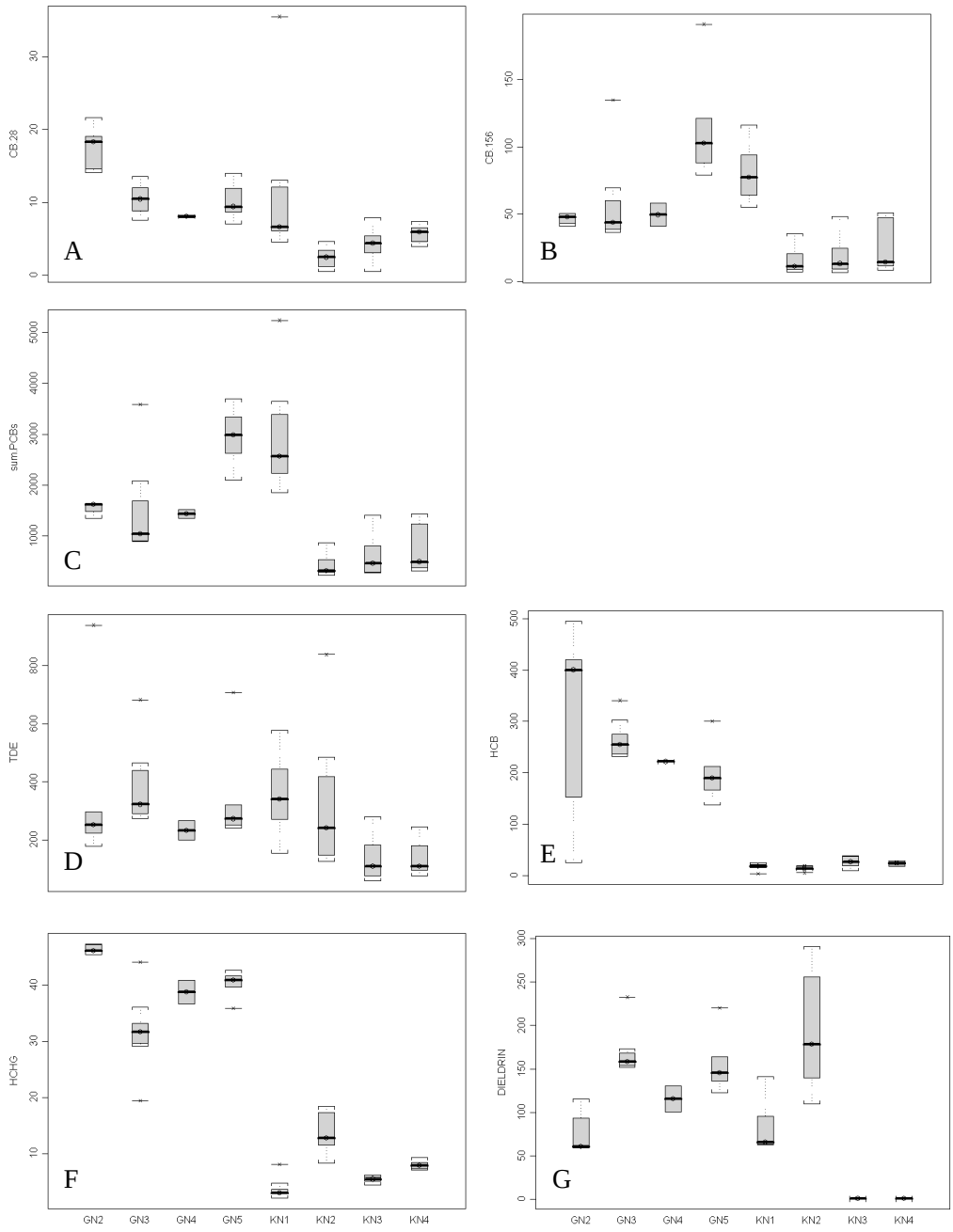


Figure 10.2. Box-and-Whisker plots (minimum, first quartile, median, second quartile, maximum and eventual outliers) for (A-C) PCB and (D-G) OCP concentrations (µg.kg^{-1} LW) in eels from eight sites on the Grote Nete and Kleine Nete: A. PCB 28; B. PCB 156; C. Sum PCBs; OCP concentrations - D. TDE (*p,p'*-DDD 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.

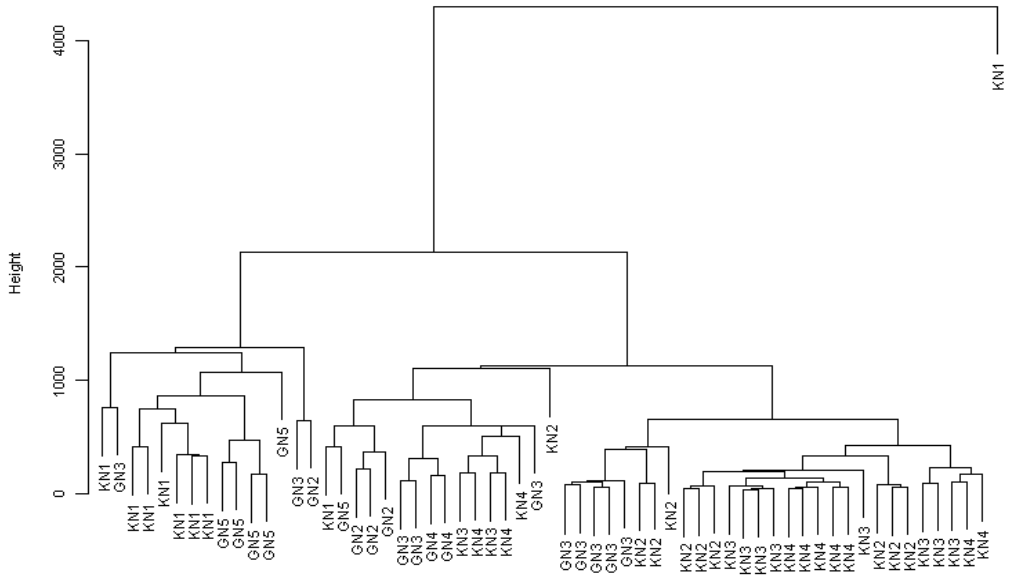


Figure 10.3. Cluster analysis of eels collected at eight sites in the Grote Nete and Kleine Nete on the basis of their PCB and OCP concentrations (n = 61).

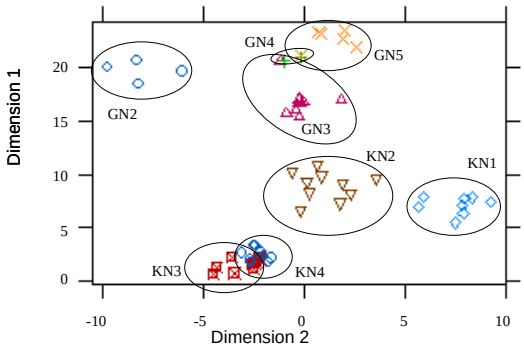


Figure 10.4. Canonical discriminant analysis of eels collected at eight sites in the Grote Nete and Kleine Nete on the basis of their PCB and OCP concentrations (N = 61). The outlier from KN1 is excluded from this analysis.

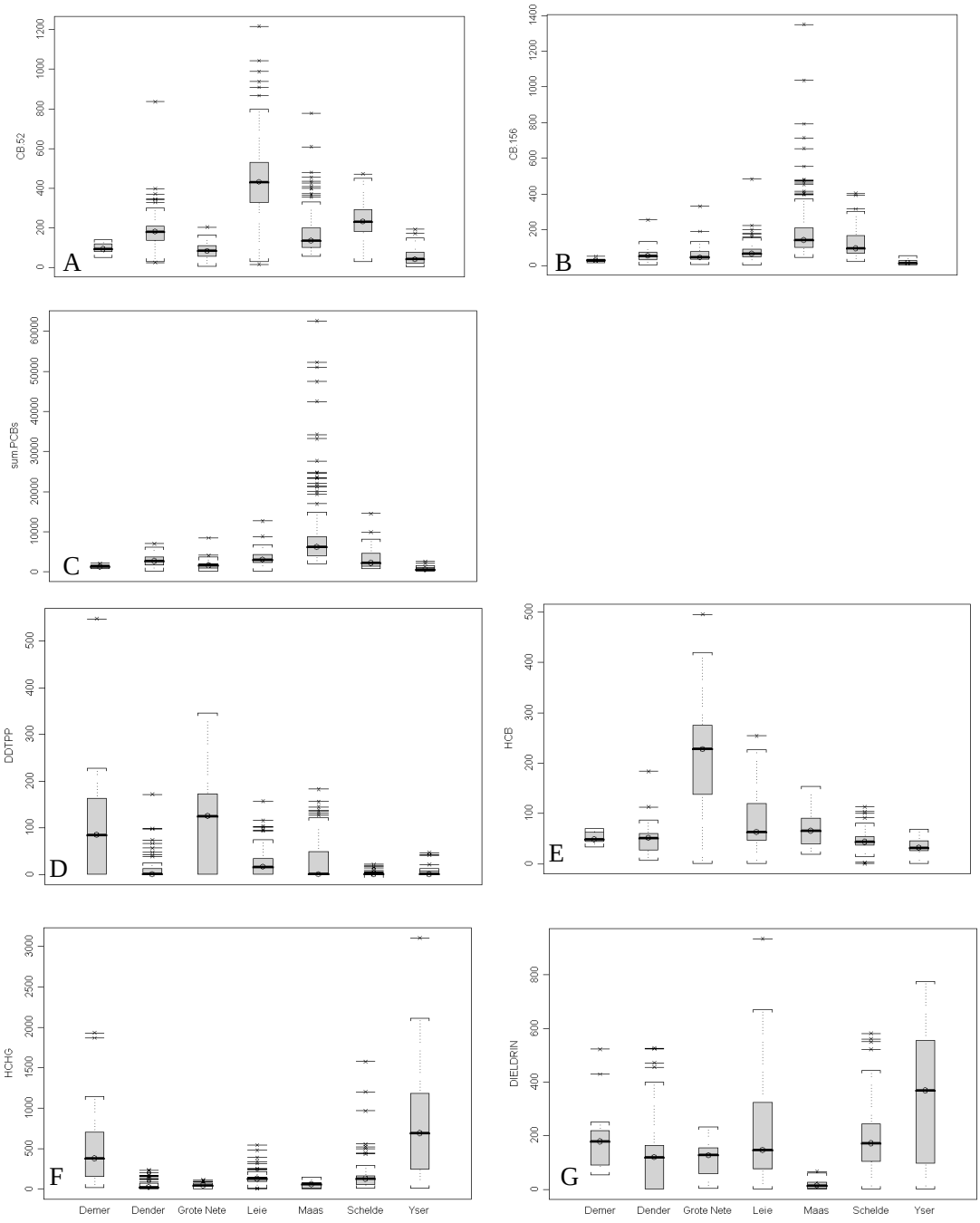


Figure 10.5. Box-and-Whisker plots (minimum, first quartile, median, second quartile, maximum and eventual outliers) for (A-C) PCB and (D-G) OCP concentrations (µg.kg^{-1} LW) in eels from seven rivers in Flanders: A. PCB 28; B. PCB 156; C. Sum PCBs; D. *p,p'*-DDT or dichlorodiphenyltrichloroethane ;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

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, wheras 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 α- and γ-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.

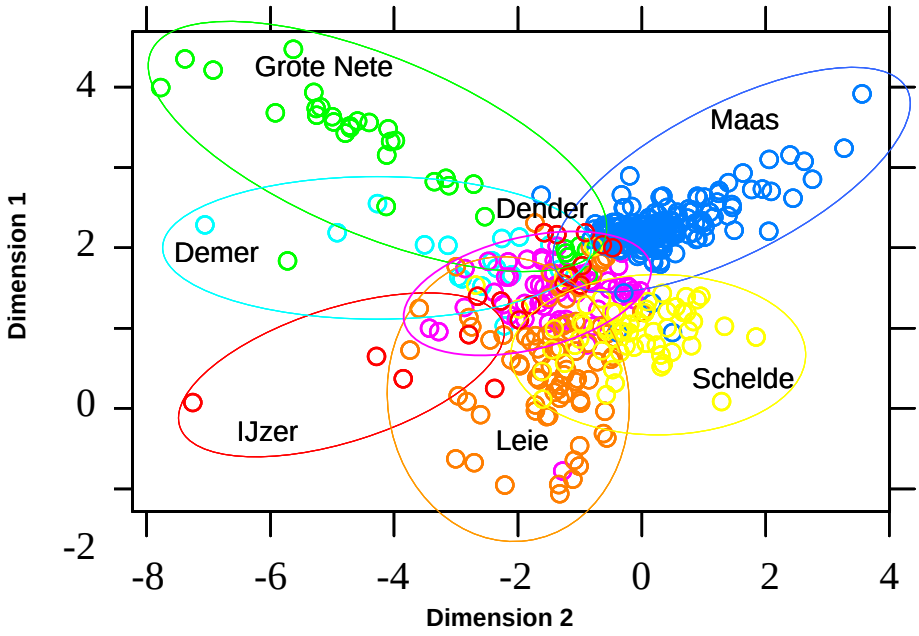


Figure 10.6. Canonical discriminant analysis of eels from seven rivers in Flanders on the basis of PCB and OCP concentrations (n = 450 eels from 56 sites).

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, 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. 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 (± SD and range in brackets; 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 (375-1258)	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)	0,23 ± 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[1]	18 ± 6 (3-26)	3.0 ± 2.2 (0.5-7.6)	359 ± 144 (155-577)	904 ± 343 (350-1524)	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)

[1] 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 ± 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 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 usefullness 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, wheras 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 Schulen (90 ha), as well as significant differences in lindane (γ-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^{-1} for water (dependent of the substance), 0.05 ng g^{-1} dry matter for sediment and 0.5 ng g^{-1} 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
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

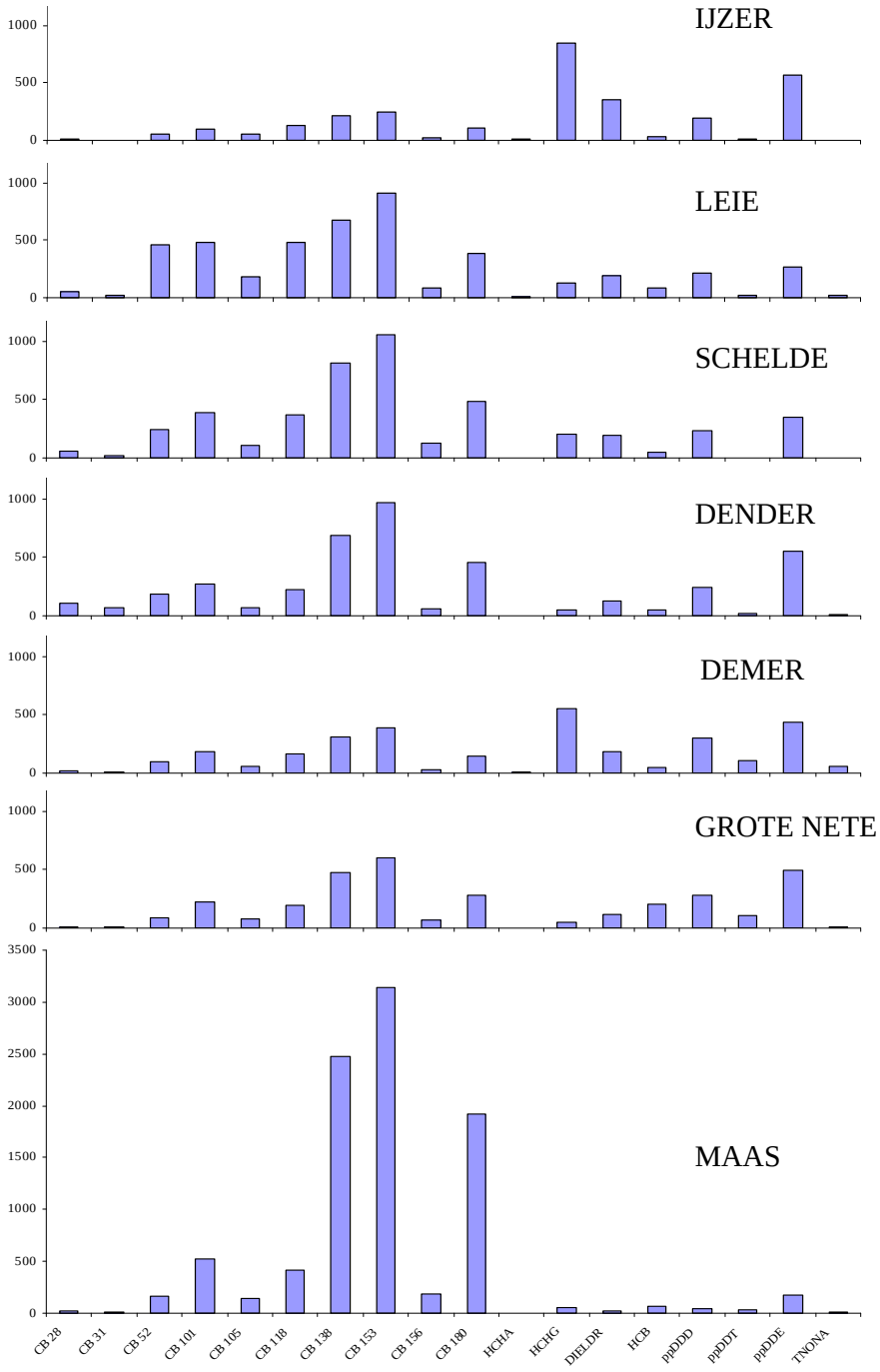


Figure 10.7. Pollution fingerprints based on means of PCB and OCP concentrations (µg.kg^{-1} LW) in eels from seven rivers in Flanders.

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 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. 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 Onckelinckx 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 anonymus 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.giuliodeleo.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 Commisssion, 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 palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, 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.

The Eel Pollutant Monitoring Network revealed that still high levels of DDT and breakdown products are present in feral eels over Flanders, despite the ban of this product in 1976. In some cases, data clearly indicated recent applications of DDT.

Photo: Ward de Cooman, VMM

Chapter 11

Congener profiles in eel as a method of tracing the origin of PCB contamination

Geert Goemans and Claude Belpaire

Institute for Forestry and Game Management, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium

This chapter was published as:

Goemans, G., Belpaire, C., 2005.
Congener profiles in European eel (*Anguilla anguilla* L.) as a method of tracing the origin of PCB contamination.
Organohalogen Compounds 67, 1304-1307

Summary

The Institute for Forestry and Game Management (IBW) analysed between 1994 and 2003 1968 individual eels collected from 325 different locations in Flanders for a series of 10 PCB congeners. In some eel samples, PCB levels as high as 7,000 ng/g BW (sum of the 7 indicator PCBs) were measured, exceeding the national PCB standard (75 ng/g BW) by nearly two orders of magnitude. Because of the serious concern about the origin of the high contamination we examined spatial and temporal variation in the congener profiles of the eels. We found that for eels from within a specific location the ratio of PCB 118 to the sum of the remaining indicator PCBs was almost constant, but this ratio varied considerably among different locations. From an analysis of PCB data in eel caught during consecutive years in a lake we noticed an increase in the relative proportion of PCB 118. We conclude that the variation in PCB profiles in eel is probably due to a combination of the commercial mixtures used and the age of the contamination. We feel that fingerprinting of contaminants such as PCBs could be a useful method to trace down the contamination source, and we would recommend combining field research with controlled laboratory tests in order to better understand the behaviour of PCB congeners in eel.

Introduction

Since 1994 the Institute for Forestry and Game Management (IBW) has built out a pollutant monitoring network for public water bodies in Flanders (Belgium) using eel (*Anguilla anguilla* L.) as a biomonitor. Eel is used for biomonitoring because it is a very fatty fish that bioaccumulates lipophilic organochlorine pesticides (OCPs) and PCBs, feeds in the benthic layer, and is sedentary during part of its life cycle (the yellow eel phase, or immature adult eels which remain in this stage in brackish or fresh waters for three to twenty years). Eels are long-living and widespread, occurring in very diverse habitats and even in polluted waters. Their position on the trophic ladder and the absence of an annual reproductive cycle, affecting lipid metabolism, are additional advantages for their use as a sentinel organism.

Contaminants analyzed were heavy metals, PCBs, OCPs, brominated flame retardants and volatile organic compounds. At present, the dataset includes results from approximately 2000 individually analyzed eels from more than 300 different localities in Flanders.

The results have been communicated to national managers as the high PCB values measured in eels from most of the locations are of great concern. Hence, immediate action has been undertaken to protect the local consumer health. A catch and release obligation for every eel caught in Flanders was set by ministerial decree. In some eel samples, PCB levels as high as 7,000 ng/g BW (measured as the sum of the 7 indicator PCBs) were measured, exceeding the national PCB standard (75 ng/g BW) by nearly two orders of magnitude.

In Flanders there exists a clear spatial variation in contamination which can be linked to human interactions and/or land use. This variation is clearly reflected by the contamination levels in eel (Goemans *et al*., 2003; Goemans and Belpaire, 2004; Morris *et al*., 2004; Belpaire *et a*l., 2003; Roose *et al*., 2003). Water managers were concerned about the origin of the high contamination. In this respect it was worthful to analyze the PCB profiles to evaluate if spatial or temporal variations exist and, if fingerprinting of contaminants such as PCBs could be a useful method to trace down the contamination source.

Materials and methods

PCB-analyses were carried out on 1968 individual eels which were collected from 325 different locations in Flanders over the period 1994-2003. The eels were collected by electrofishing, fykenets or a combination of both. Locations included rivers, canals, polder waters and closed water bodies. Some locations were sampled more than once during the study period.

Fish fillets were wrapped in aluminium paper (cleaned with hexane 99%) and stored at -20°C. All fish were analyzed individually. For this paper only the eels with a length between 30 and 50 cm are considered for standardization and comparison reasons. This final standardized dataset contained 1587 eels from 305 different locations. Chemical analyses were carried out by the Sea Fisheries Department in Ostend.

10 grams of fish fillet was extracted using the Bligh & Dyer method (Bligh and Dyer, 1959). The extract is evaporated (rotavapor) and at the most 100 mg lipid is dissolved in hexane and applied on an aluminium oxide chromatography column. After elution with hexane, the lipid free eluate is evaporated and applied on a silica gel chromatography column. Ten PCBs (PCB 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180), *p,p'*-DDE and HCB are isolated after elution with hexane.

This fraction is evaporated to 1 ml, after addition of an external standard (tetrachloronaphtalene) and separated by GC using a Rtx-5ms capillary column (60 m x 0.25 mm x 0.25 µm), with helium as a carrier gas and an electron capture detector (ECD).

Results and Discussion

Spatial variation

We observed a high similarity in the fraction that PCB 118 accounted for compared to the total of the other measured (non-dioxin like) PCBs for eel from one and the same location, but at the same time there was a striking inter-location variation. For none of the other congeners there was a pattern visible if compared to the sum of the indicator PCBs (Figure 11.1).

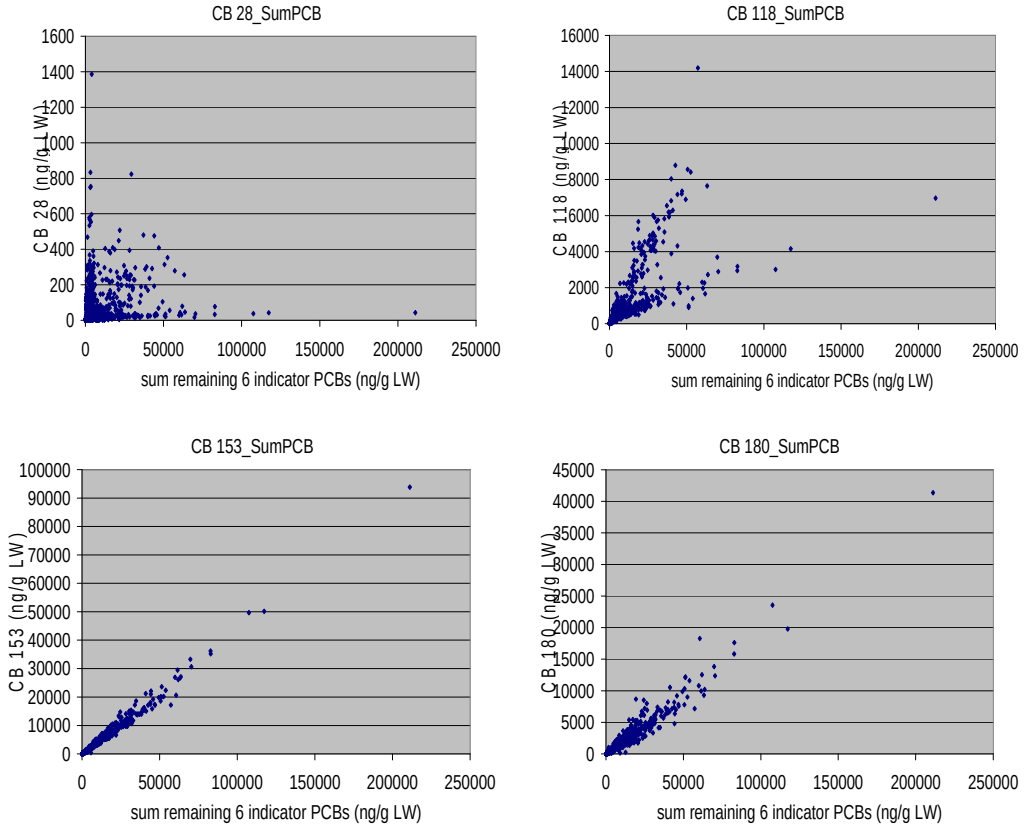


Figure 11.1: Scatter plots of different indicator PCB-congeners to the sum of the 6 remaining indicator PCBs. Only when we plotted PCB 118 we got a pattern dividing our data in more or less 2 main groups. Individual results of 1587 eels from 305 locations in Flanders, Belgium.

After analyzing these results in more detail we found that for eels from a specific location the ratio of PCB 118 to the sum of the remaining indicator PCBs was almost constant. At the same time this ratio varied considerably amongst different locations (Figure 11.2).

This variation could be depending on which commercial PCB-mixtures were used (probably related to specific industrial activities). On the other hand the PCB profile might be indicative for the "age" of the contamination. As PCB 153 is thought to be more persistent than PCB 118, high PCB 153 ratios could indicate old/older contaminations. But, probably the spatial variation is due to a combination of the commercial mixtures used and the "age" of the contamination.

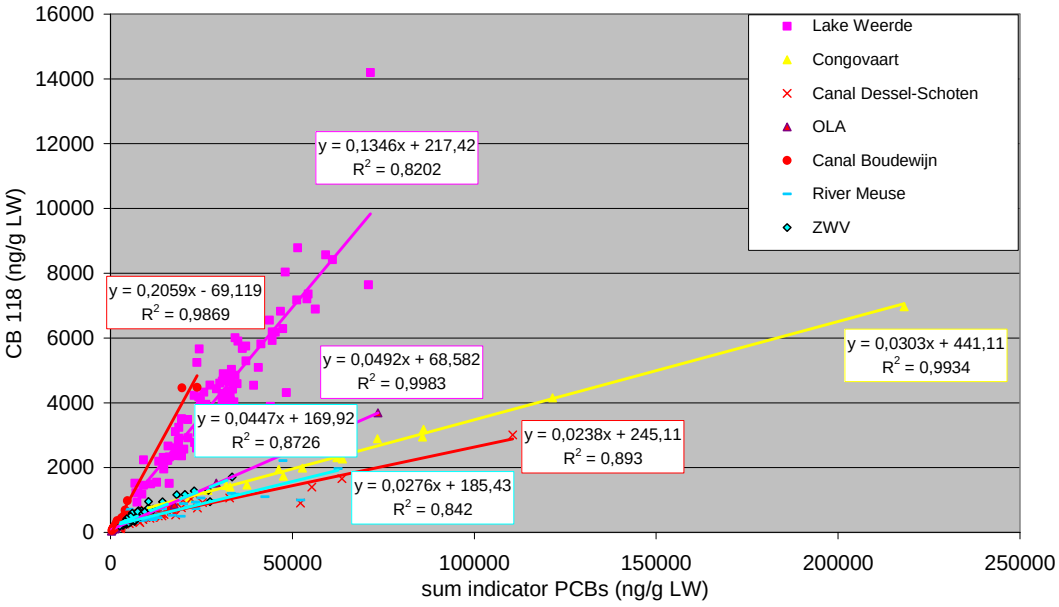


Figure 11.2: Variation in location specific ratio of PCB 118 to the sum of the indicator PCBs. Selection of 337 eels from 7 waterbodies in Flanders, Belgium (OLA - Oude Leie Astene, ZWV - Canal Zuidwillemsvaart).

Temporal trend

Repeated measurements over time are only available for a limited series of waters. For these waters, we mostly observed a shift over time for the fraction of PCB 118 compared to the total of the other measured (non-dioxin like) PCBs. In most cases there was an increase over time of the relative contribution of PCB 118 to the Sum PCB, while the fraction of PCB 153 stayed more or less stable. These findings are in contrast with the common thought of PCB 153 being more persistent than PCB 118.

In Figure 11.3 a time trend is given for lake Weerde, with exception of the year 2001 we notice a clear upwards shift in time. Before the year 2000 PCB 118 accounted for +/- 10% of the indicator PCBs, in 2001 about 15% of the concentration of indicator PCBs was PCB 118, in 2002 it accounted for almost 20% of the indicator PCB's.

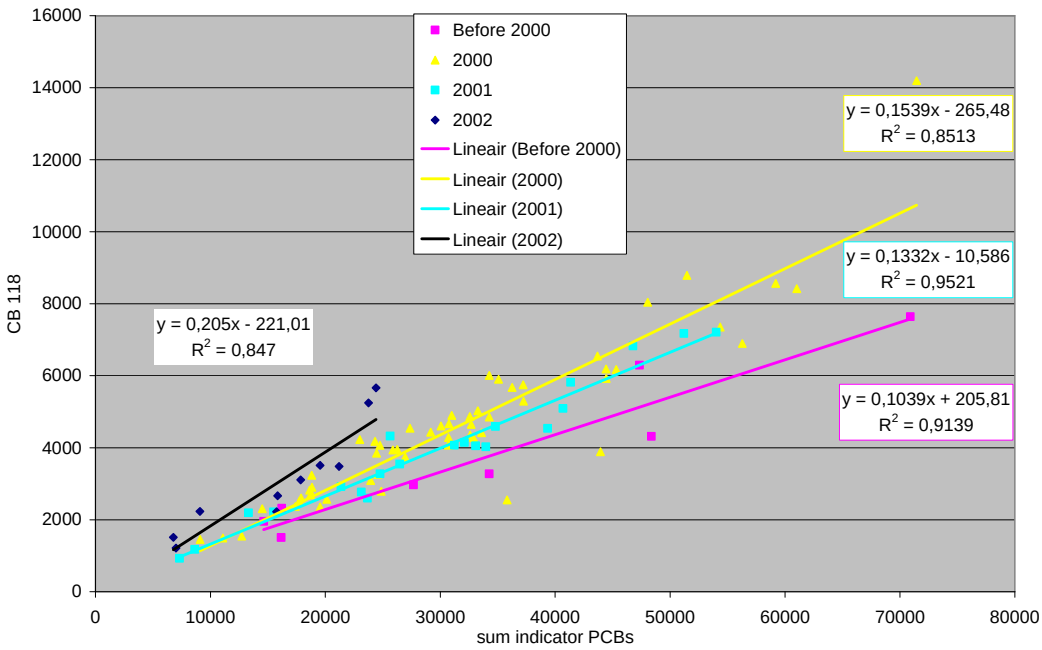


Figure 11.3: Temporal shift in relative fraction of PCB 118 compared to the sum of the indicator PCBs in individual eels from Lake Weerde. Before 2000 PCB 118 accounted for +/- 10% of the indicator PCBs, by 2002 it accounted for almost 20% of the indicator PCB's.

Conclusions

At this moment it is not very clear to us whether these spatial and temporal trends are due to the origin of the PCB-pollution or that it might be due to the highest persistence of CB 118, compared to the other indicator PCBs, in eel (biota). It would be very interesting to do some controlled laboratory tests to find out if PCB 118 indeed is more persistent in biota than PCB 153.

Acknowledgements

We would like to thank the people from the Sea Fisheries Department in Ostend for the chemical analyses and the help with the dissections of the fish and the preparation of the fish fillets.

References

Belpaire, C., Goemans, G., de Boer, J., Van Hooste, H., 2003. Distribution of Brominated Flame Retardants. In: MIRA-T 2003: Report of the Environment and Nature in Flanders. Flemish Environmental Agency and Lannoo Publishing, Heverlee, Belgium, p.387-395. (in Dutch)

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37, 911-917.

Goemans, G., Belpaire, C., 2004. The eel pollutant monitoring network in Flanders, Belgium: Results of 10 years monitoring. *Organohalogen Compounds.* 66, 1834-1840.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. The Flemish eel pollution monitoring network 1994–2001: polychlorine biphenyls, organochlorine pesticides and heavy metals in eel. Institute for Forestry and Game Management, report. IBW.Wb.V.R.2003.99. 169p. (in Dutch).

Morris, S., Allchin, C.R., Zegers, B.N., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P.E.G., Van Leeuwen, S.P.J., de Boer, J., 2004. Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environmental Science and Technology* 38, 5497-5504.

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *Journal of Environmental Monitoring* 5, 876-884.

Yellow eels.

Photo: Geert Goemans, INBO

Chapter 12

Eels: contaminant cocktails pinpointing environmental pollution

Claude Belpaire and Geert Goemans

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

This chapter is published as:

Belpaire, C., Goemans, G., 2007.
Eels: contaminant cocktails pinpointing environmental contamination.
ICES Journal of Marine Science 64, 1423-1436

Summary

There is growing concern that insufficient somatic and health conditions of silver European eels (*Anguilla anguilla)* emigrating from European waters to oceanic spawning areas might be a key causal factor in the decline of the stock. One factor that could contribute to deterioration in the status of eels is high contaminant accumulation in their body. Contaminants may affect lipid metabolism and result in lower energy stores. A high body burden of contaminants and low energy stores might be responsible for failure of migration and/or impairment of successful reproduction. During a 12-year study on a relatively small area within the river basins of IJzer, Scheldt, and Meuse (ca. 13 500 km²), 2613 eels were sampled covering a dense monitoring network of 357 stations. Eels were analysed for ca. 100 chemicals. These included PCBs, organochlorine pesticides, heavy metals, brominated flame retardants, volatile organic pollutants (VOCs), endocrine disruptors, dioxins, perfluorooctane sulphonic acids (PFOSs), metallothioneins, and polycyclic aromatic compounds. This series represents only a very small fraction (<0.5%) of the >30 000 chemicals currently marketed and used in Europe. The biomonitoring value of eels as a tool for monitoring environmental contamination is illustrated. Two major conclusions were drawn: (i) the eel is a highly suitable biomonitor for environmental contaminants, for both local and international purposes, e.g. to evaluate the chemical status for the Water Framework Directive, and (ii) dependent on the degree of pollution in their habitat, the levels of certain contaminants reported in yellow eels can be high, and might affect their potential for reproduction.

Introduction

Some recent scientific reports have posed the question whether silver eels leaving continental waters before migrating to spawning areas may be of insufficient quality[1] and that this might be a key factor explaining the overall decline of the stock (Robinet and Feunteun, 2002; Palstra *et al*., 2006). The state of eels can deteriorate through high contaminant accumulation and/or poor physiological condition (e.g. lipid weight). Contaminants are one of the elements that influence storage of energy. They may affect lipid metabolism through various mechanisms (e.g. chemical stress induces a greater energy demand, or specific contaminants can disturb thyroid function and hence fat accumulation). Poor condition and low lipid energy stores might be responsible for failed migration and/or impairment of successful reproduction. During the transoceanic migration, lipids are metabolized and the lipophilic contaminants mobilized, particularly towards the gonads where they impair the quality of gonads, compromising reproduction and normal development of the early embryonic stages. The EIFAC/ICES Working Group on Eels (WG Eel, 2006) and the Scientific, Technical and Economic Committee for Fisheries (STECF, 2006) have recommended that the Water Framework Directive (WFD; CEC, 2000) should use the eel (*Anguilla anguilla*) as a sentinel species for monitoring the chemical status of surface waters with respect to hazardous substances. The yellow eel is considered to be a good biomonitor because of its various ecological and physiological traits: eels are top carnivores, widespread, rich in lipids, resistant to pollution, and sedentary, and there is no reproduction and associated lipid metabolism in European waters.

During a 12-year study on a relatively small area within the river basins of IJzer, Scheldt, and Meuse (ca. 13 500 km²), 2613 eels were harvested over a monitoring network of 357 stations. Sampling stations were located on streams, rivers, and brooks, as well as in canals, polders, and lakes or ponds (Figure 12.1). Some 5–10 eels were sampled at each station. Each eel was analysed individually for a series of ten PCBs, nine organochlorine pesticides, and nine heavy metals. Additionally, at selected locations, a restricted number of eels was analysed for brominated flame retardants (BFRs), volatile organic pollutants (VOCs), endocrine disruptors, dioxins, perfluorooctane sulphonic acids (PFOSs), metallothioneins, and polycyclic aromatic compounds. The data have been reported in various papers (Belpaire *et al*., 2001, 2003; Goemans *et al*., 2003; Roose *et al.*, 2003; Goemans and Belpaire, 2004, 2005; Morris *et al*., 2004; Versonnen *et al.*, 2004; Hoff *et al*., 2005; Maes *et al*., 2005).

The objectives of this paper are to document the potential for pollutant monitoring using eels on both a local and international scale, using a selected set of substances. Emphasis is given to how the species meets the requirements of a good biomonitor. We also discuss the international monitoring strategy proposed in the context of the Water Framework Directive.

1 In the terms of references of the ICES/EIFAC Working Group on Eels 2006, the term 'quality of spawners' is suggested to be included in the stock management advice, describing *the capacity of silver eels to reach spawning areas and to produce viable offspring* (WG Eel, 2006). The term of reference specifically focused on quantifying the impact of pollution and parasitism.

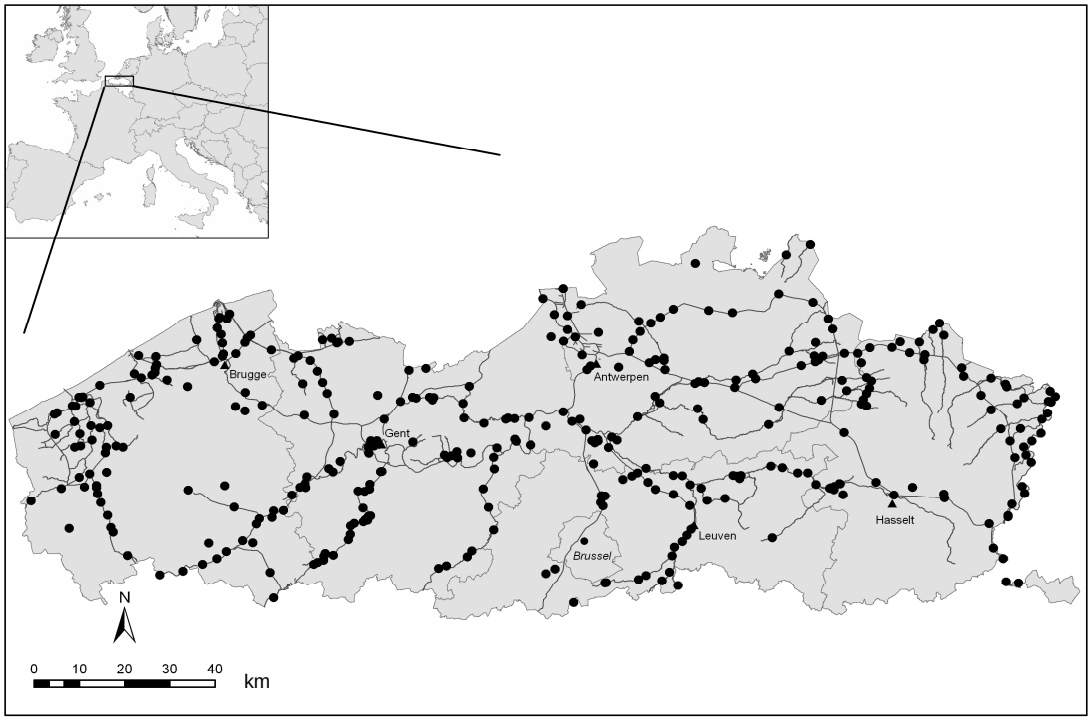


Figure 12.1. The Flemish Eel Pollutant Monitoring Network. Geographical distribution of sampling stations (n = 357).

Levels of selected chemicals in eels

Volatile organic compounds

Volatile organic compounds are atmospheric contaminants that are frequently determined in air, drinking water, fresh water, effluents, and soils. Many are substances of concern, and some are on the list of priority substances[2] proposed within the WFD (CEC, 2007). A series of 52 VOCs was analysed in eels from 20 sites, and results were reported by Roose *et al*. (2003). Only one eel was analysed from each site. The most prominent VOCs were BTEX and a number of chlorinated compounds, such as chloroform and tetrachloroethene. Here, we present data on the presence in eels of 1,2-dichlorobenzene, 1,2-dibromo-3-chloropropane, and BTEX compounds.

As reported by Roose *et al*. (2003), determination of VOCs in the water column is considered to be inadequate. Concentrations of the same VOCs as studied in Flemish eels show that these are generally below the detection limits of the analytical techniques used in the water column of Flemish rivers. VOCs detected in

2 Substances that present a significant risk to or via the aquatic environment prioritized for action on the basis of risk to or via the aquatic environment (CEC, 2000)

the water column are the same as the most prominent ones found in eels, and the highest concentrations are also found at the same sites. Further evidence supports a conclusion that concentrations in eels indeed reflect the concentrations in the water column (when detected).

1,2-dichlorobenzene (or o-dichlorobenzene)

This VOC has low water solubility (118 mg l^{-1} at 25℃) and is an intermediate for making agricultural chemicals, primarily herbicides. Other present and past uses include: use as a solvent for waxes, gums, resins, wood preservatives, and paints; as an insecticide for termites and borers; in making dyes; and as a coolant, deodorizer, or degreaser. On the basis of its volatility and the dispersive nature of its uses, it is expected that 1,2-dichlorobenzene will be released to the environment primarily in liquid effluents and atmospheric emissions from production and other facilities. It may also occur as a result of dehalogenation of more highly chlorinated chlorobenzenes (Bosma *et al.*, 1988) and can be found in emissions from incineration of organic matter containing chlorine (Young and Voorhees, 1989). 1,2-dichlorobenzene has been reported following a survey of effluents from ten Canadian textile mills conducted in 1985/86; concentrations were reported to range up to 95.5 mg l^{-1} (Environment Canada, 1989).

Analyses of this chemical in eels from 20 locations in Flanders, collected between 1996 and 1998 (Figure 12.2, drawn with data presented by Roose *et al*., 2003) show that at ten sites (50%), concentrations were below the detection limit (DL, 0.05 ng g^{-1} wet weight). However, the chemical was detectable at ten sites, and eels from two of these showed high concentrations of dichlorobenzene (Oude Leie at Wevelgem, 85 ng g^{-1} wet weight; Leie at Menen, 49 ng g^{-1} wet weight). Few studies have detailed the presence of 1,2-dichlorobenzene in other fish. In the Great Lakes in the early 1980s, the concentration of 1,2-dichlorobenzene in lake trout (*Salvelinus namaycush*) and rainbow trout (*Oncorhynchus mykiss*) averaged 0.3 ng g^{-1} and 1 ng g^{-1} wet weight, respectively (Oliver and Nicol, 1982; Oliver and Niimi, 1983).

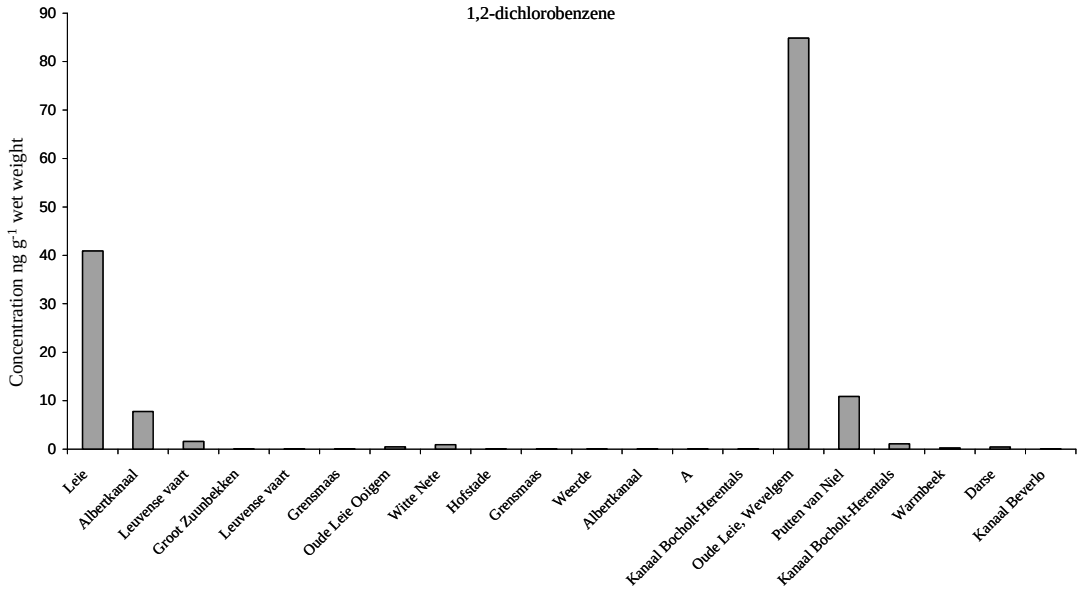


Figure 12.2. Concentrations of 1,2-dichlorobenzene in eels collected at 20 sites in Flanders (1996–1998). Values presented are the result of the analysis of one eel per location. Data from Roose *et al*. (2003).

Both sites with reported high concentrations of dichlorobenzene were situated on or in the vicinity of the River Leie, and each station was in the neighbourhood of major industrial sites. One company, located at Wevelgem, is active in the textile finishing industry, and activities conducted there include pre-treatment, dyeing, and finishing treatments, using a diverse mix of chemicals. The company is one of the largest dischargers, discharging ca. 3000 m³ water per day directly into the river. Another large manufacturing plant producing pigments used especially by the paint, ink, and plastics industries is situated at Menen, discharging ca. 3500 m³ water per day (Anon., 2003).

A network is in place for monitoring some VOCs in water at a selection of ca. 40 sites occupied monthly. From Figure 12.3 and Table 12.1, it is obvious that this compound is difficult to detect in water. In water, 95% of the measurements are below the detection limit, compared with 50% from analysis of eel tissue.

Little is known about the ecotoxicological effect of 1,2-dichlorobenzene on eels, but impairment of reproduction has been identified as the most sensitive toxicity endpoint reported for other aquatic organisms (Environment Canada, 1993). Two studies have measured LC_{50} values for rainbow trout (*Oncorhynchus mykiss*). Ahmad *et al.* (1984) reported the 96-h LC_{50} to be 1.61 mg l^{-1}. Black *et al.* (1982) studied its effects on embryos and larvae, exposing them from 20-30 min after fertilization of the egg to 4 d after hatching of the larva. The resultant LC_{50} was 3.01 mg l^{-1}, following total exposure times of 27 d.

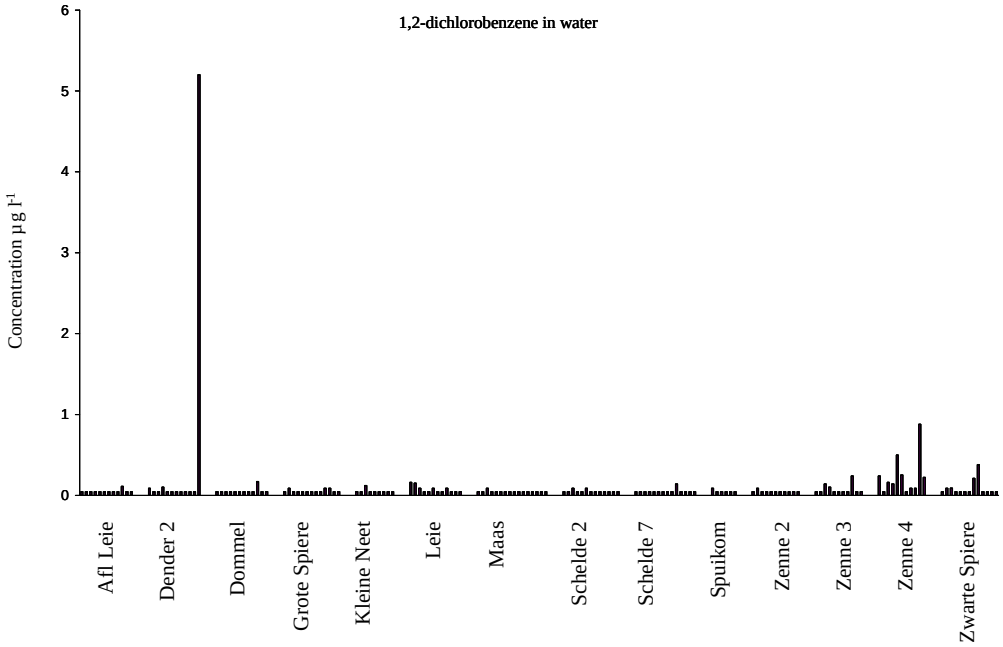


Figure 12.3. Concentrations of 1,2-dichlorobenzene in water collected monthly at 40 sites in Flanders (2005). Concentrations under the detection limit (0.044 µg $l^{-1)}$ were set at the detection limit. All measurements in the following water bodies were below the detection limit and are not shown in the graph: Demer, Dender 1, Dijle 1, Dijle 2, Dijle 3, Gaverbeek 1, Gaverbeek 2, Gent-Oostende, Gent-Terneuzen, Gete, Handzamevaart, IJzer 1, IJzer 2, Leopoldskanaal 1, Leopoldskanaal 2, Mandel, Mark, Nete, Schelde 1, Schelde 3, Schelde 4, Schelde 5, Schelde 6, Schelde-Rijnkanaal 1, Schelde-Rijnkanaal 2, and Zenne 1. Data from the Flemish Environment Agency.

Table 12.1. Concentrations of 5 VOCs in water and eel from Flanders (Belgium). Data from the Flemish Environment Agency and Roose *et al*. (2003) respectively. Values in water are expressed in µg l^{-1}, in eels in ng g^{-1} wet weight.

Substance	Water (470 measurements, 2005)			Eel (20 sites, 1996–1998)		
	Min - Max	Mean	% < DL	Min - Max	Mean	% < DL
1, 2-dichlorobenzene	0.044- 5.2	0.06	95.5	0.02-84.8	7.5	50
Benzene	0.007-2.68	0.06	83,4	1.2-18.9	5.7	0
Toluene	0.03-15	0.28	86.4	1.0-72.6	19.0	0
o-xylene	0.05-1.6	0.07	94.9	0.6-39.7	7.1	0
Ethylbenzene	0.043-2.2	0.06	94.9	1.2-35.6	14.9	0

1,2-dibromo-3-chloropropane

1,2-dibromo-3-chloropropane was previously used as a pesticide (registered by the US Environmental Protection Agency, EPA, as a soil fumigant to control nematodes during growth of crops). The US EPA banned all uses of 1,2-dibromo-3-chloropropane in 1985, and it is now used only as an intermediate in organic synthesis and for research purposes (ATSDR, 1992). Most of the 1,2-dibromo-3-

chloropropane released to the air disappears within several months. Most that enters surface water evaporates into the air within several days or a week.

In Flanders, eels from 20 sites were analysed (Figure 12.4). In 80% of the samples, 1,2-dibromo-3-chloropropane was below the detection limit (0.05 ng g^{-1} wet weight), but very high concentrations were found in eels from two canals, the Leuvense vaart and the Albertkanaal (265 and 706 ng g^{-1}, respectively). Both are important canals situated in the centre of Belgium. These data clearly indicate point sources, but the origin of these sources is unclear.

From the information presented by ATSDR (1992), 1,2-dibromo-3-chloropropane does not accumulate in sediments at the bottom of rivers, lakes, or ponds, and fish were not expected to accumulate large amounts of this chemical in their bodies. Our results nevertheless suggest that in some cases, fish may bioaccumulate this chemical.

There have been no ecotoxicological studies of the effect of this chemical on eel. Studies of workers in chemical factories that produced 1,2-dibromo-3-chloropropane showed that its main harmful effect was to the male reproductive system, resulting in a lower production of sperm and a reduced ability to reproduce.

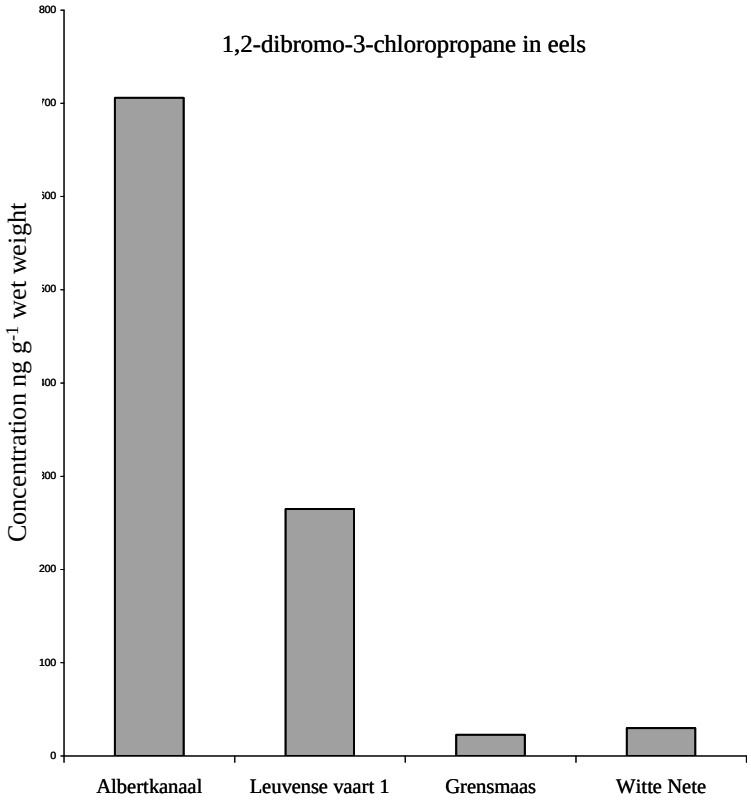


Figure 12.4. Concentrations of 1,2-dibromo-3-chloropropane in eels collected at 20 sites in Flanders (1996–1998). The values presented are the result of the analysis of one eel per location. Measurements on Leie, Groot Zuunbekken, Leuvense vaart 2, Oude Leie Ooigem, Hofstade, Maas, Weerde, Albertkanaal, A, Kanaal Bocholt-Herentals 1, Kanaal Bocholt-Herentals 2, Oude Leie Wevelgem, Putten van Niel, Warmbeek, Darse, and Kanaal Beverlo were below the detection limit and are not shown. Data from Roose *et al*. (2003).

BTEX compounds

Benzene, toluene, ethylbenzene, and the xylenes (BTEX) are important industrial compounds among the VOCs. Moreover, BTEX compounds are important constituents of unleaded gasoline and are present in crude oil. Benzene is on the list of priority substances defined by the WFD (CEC, 2007). Industrial processes are the main sources of benzene in the environment. Benzene concentrations in the air can be elevated by emissions from burning coal and oil, benzene waste and storage operations, motor vehicle exhaust, and evaporation from gasoline service stations. Industrial discharge, disposal of products containing benzene, and gasoline leaks from underground storage tanks release benzene into water and soil (ATSDR, 2005).

The concentrations of benzene, toluene, ethylbenzene, and *m*-xylene are presented in Figure 12.5. It is striking that all compounds were detectable at all sites (n = 20). The distribution of BTEX in Flanders is more widespread than most of the other chemicals studied. The variability of the data is also somewhat less than seen for other chemicals. Moreover, the BTEX compounds correlated very well with each other, with correlation coefficients between 0.77 and 0.98 (Roose *et al*., 2003). This indicates that contamination by BTEX is of a rather diffuse nature, supporting the conclusion that the use of fossil fuels in, e.g., motor vehicles is the major source of BTEX.

The high concentrations observed at the Groot-Zuunbekken station can possibly be explained by the fact that this is a pond in a densely populated and industrialized area just southwest of Brussels. Another source might be a large chemical industry located at Drogenbos (9 km from the sampling site), producing plastics in primary forms and reporting an emission of 0.46 t BTEX $year^{-1}$ to water in 2001 (EPER, 2006). In distinct contrast, eels from rural locations, such as river A (at Poppel) or the Warmbeek (at Achel), have significantly lower concentrations.

Once again, comparison of BTEX data in eels with the concentrations water (see Table 12.1) evidence that any monitoring strategy for these compounds should be based on analysis of biota rather than water.

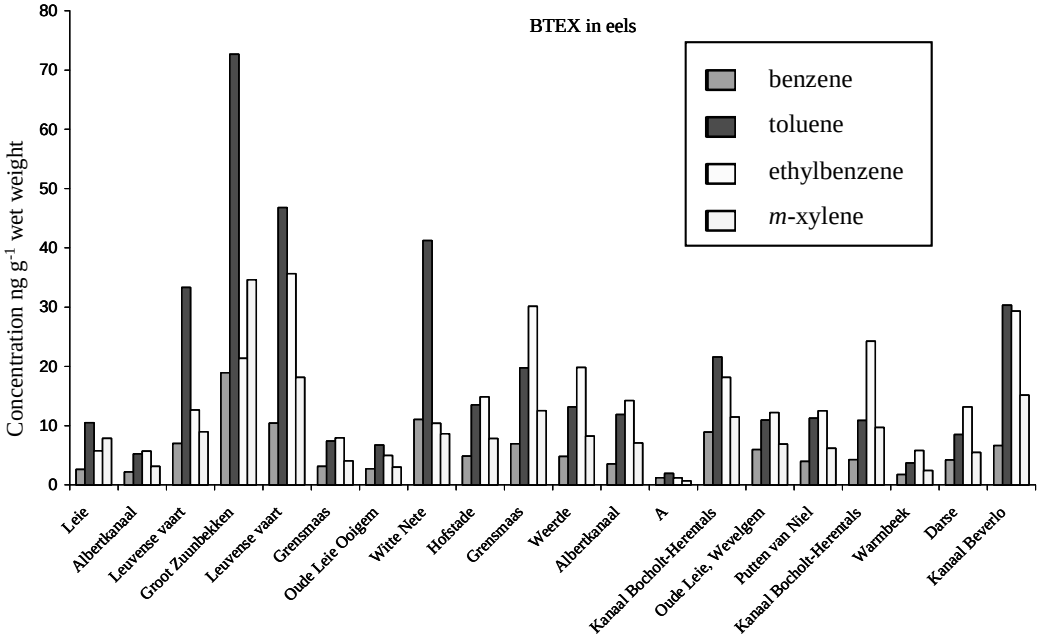


Figure 12.5. Concentrations of BTEX compounds in eels collected at 20 sites in Flanders (1996–1998). The values presented are the result of the analysis of one eel per location. Data from Roose *et al*. (2003).

Brominated flame retardants

Brominated flame retardants are chemicals used to inhibit or impede flammability in combustible products. Several groups of BFRs exist, e.g. hexabromocyclododecane (HBCD) and polybrominated diphenylethers (PBDEs), which have different applications. HBCD is used mainly to flame-retard extruded and expanded polystyrene used for thermal insulation, but also in upholstery textiles. PBDEs are produced as three commercial formulations: penta-BDE, octa-BDE, and deca-BDE. Penta-BDE is used primarily in foam products such as seat cushions and other household upholstered furniture, as well as in rigid insulation. Octa-BDE is used in high-impact plastic products, e.g. computers. Deca-BDE is used in plastics, such as wire and cable insulation, adhesives, textile, and other coatings. Typical end-products include housing for television sets, computers, stereos, and other electronics. Deca-BDE is also used as a fabric treatment and coating on carpets and draperies. Deca-BDE is not used on clothing.

BFRs are of major concern because their occurrence in all compartments of our environment have been increasing. Penta-BDE and octa-BDE products have been removed from production and use within the EU following risk assessments, and decreasing trends in BDE have been described in some studies (e.g. in human milk samples from Sweden). These compounds have a carcinogenic, neurotoxic, and endocrine-disrupting action. PBDEs are on the list of priority substances defined by the WFD (CEC, 2007).

Figure 12.6 illustrates the presence of PBDEs and HBCD in yellow eels from 18 sites in Flanders. At each site, the muscle tissue of ten eels was pooled for analysis. Both groups of chemicals were detected in all samples, indicating the widespread distribution of these chemicals (even in remote areas). The analysis of eel tissue has also highlighted significant local pollution by HBCD and PBDEs at some locations along the rivers Leie and Scheldt. Eels from the site at Oudenaarde, along the River Scheldt, showed extremely high concentrations of PBDEs and HBCD, respectively 31 639 and 33 000 ng g^{-1} lipid weight. These are among the highest concentrations reported worldwide in fish. Although measurements in water are not a good indicator of the concentration of these chemicals because of their lipophilic character, data are available and have been published for the sediment (Belpaire *et al*., 2003), and are more or less in line with the eel data.

The primary industry in Oudenaarde is textile production, with several companies involved in coatings, dyes, auxiliaries, and services for the textile industry.

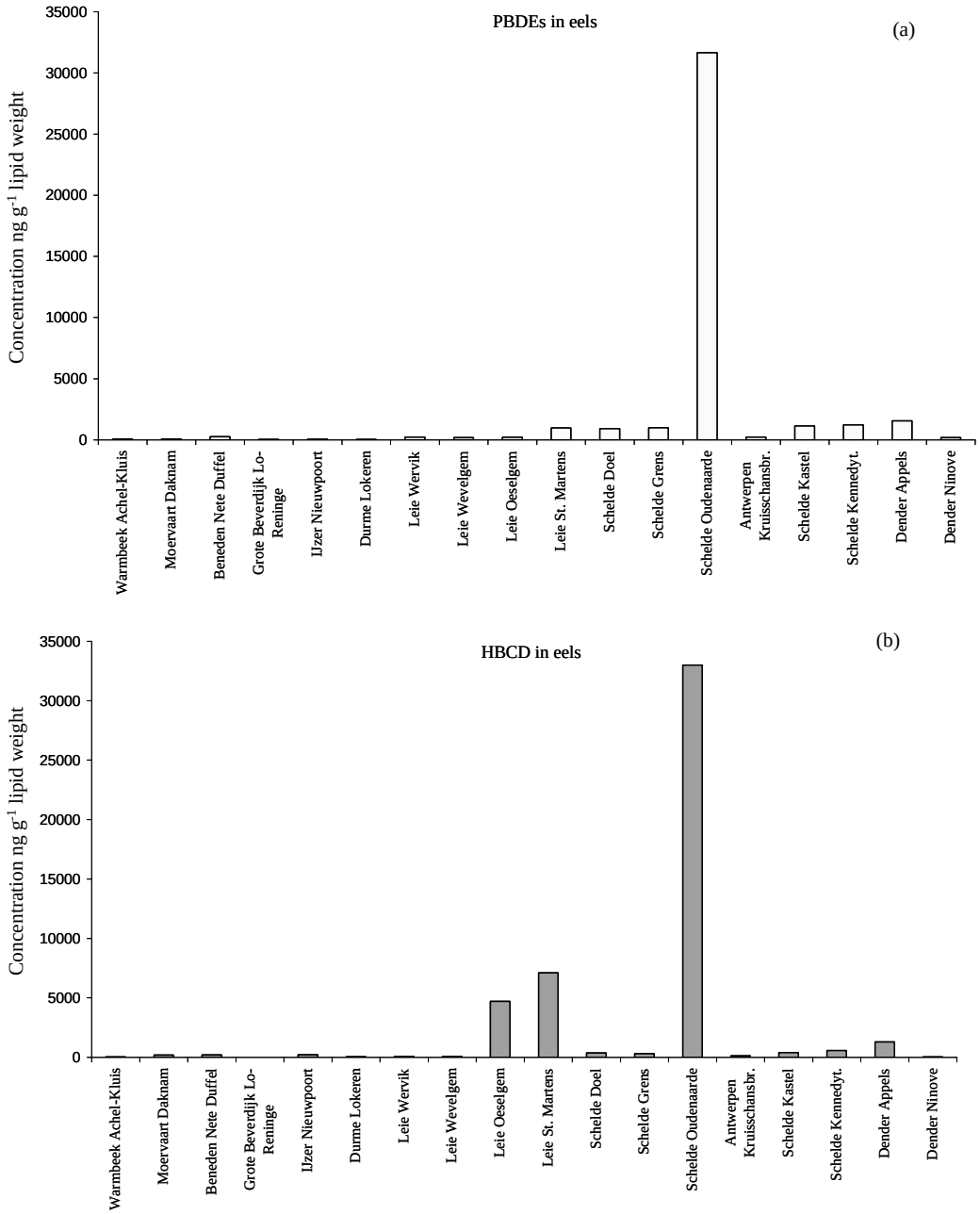


Figure 12.6. Concentrations of (a) PBDEs and (b) HBCD in eels collected at 18 sites in Flanders (2001). The values presented are the results of the analysis of pooled samples of ca. 10 eels per location (one survey in 2000). Data from de Boer *et al*. (2002) and Belpaire *et al*. (2003).

PCBs, organochlorine pesticides, and heavy metals

Ten PCBs, nine organochlorine pesticides, and nine heavy metals were analysed in each of the eels sampled. At each station, 5–10 eels were sampled and analysed individually. Results for each contaminant were averaged per station, so the data presented here represent means of 5–10 eels per station. We selected lindane and cadmium to illustrate the distribution pattern of the contaminants, because both are on the list of priority substances proposed by the WFD (CEC, 2007). Figure 12.7 shows that concentrations of lindane in eels can be very high, up to 9255 ng g^{-1} lipid weight, to our knowledge the highest recorded concentration in Europe. Lindane is an organochlorine insecticide, used on many crops, including sugar beet and oil seed rape. As it is a persistent organic pollutant known to be both carcinogenic and an endocrine disruptor, it has been banned in a number of countries for many years. However, in Belgium, it was banned only in June 2002. The pattern of distribution of this chemical in eels is related to agricultural activities. The highest values shown in Figure 12.7 are confined to areas situated in the subcatchments of the rivers IJzer, Demer, and Dijle, where there is intensive culture of sugar beet. Lindane is measured also in water and sediment. However, because of its lipophilic nature, concentrations in biota are some orders of magnitude higher than the concentrations in water or sediment. At all of our 357 sites, lindane was detectable in eels. In most cases, lindane is not detectable in sediment (15.5% above DL at 2445 sites), and in water lindane can only be detected during the season of application.

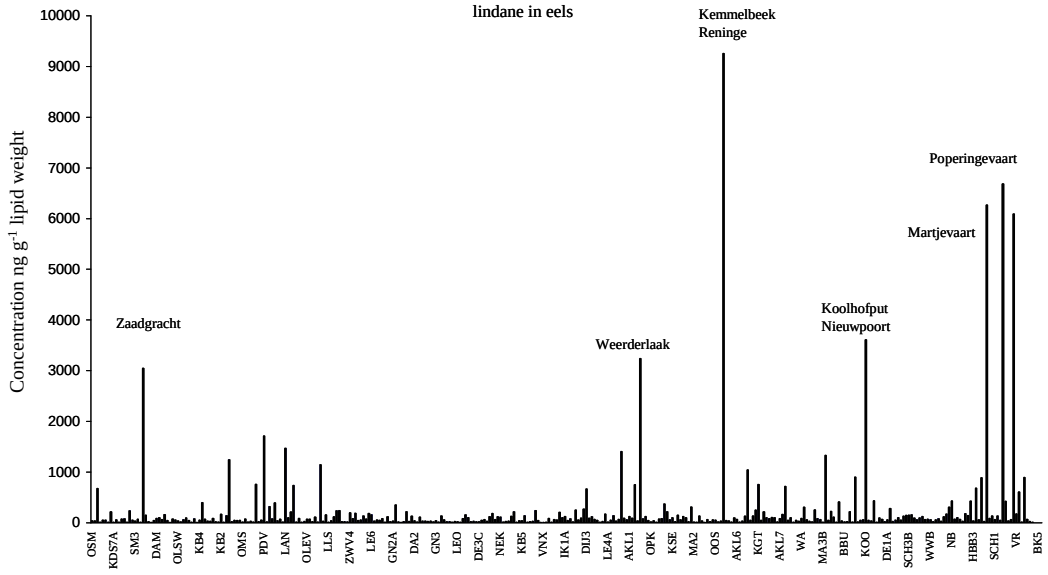


Figure 12.7. Concentrations of lindane in eels collected at 357 sites in Flanders (1994–2005). Data from the INBO Eel Pollutant Monitoring Network Database. Values represent the means of the individual analysis of 5–10 eels per location, for the most recent survey. Codes on the x-axis are location codes defined by Goemans *et al*. (2003).

Figure 12.8 shows cadmium concentrations in eels from 333 sites. The data clearly indicate local cadmium pollution. The sources may be variable, from historically polluted sediments to active industrial discharges. Some of these concentrations are above international health consumption limits. Heavy metals are well-known chemicals that are frequently determined in water and sediments. Generally spoken “black point” sites indicated by eel analyses confirmed what was known from measurements in water or sediment.

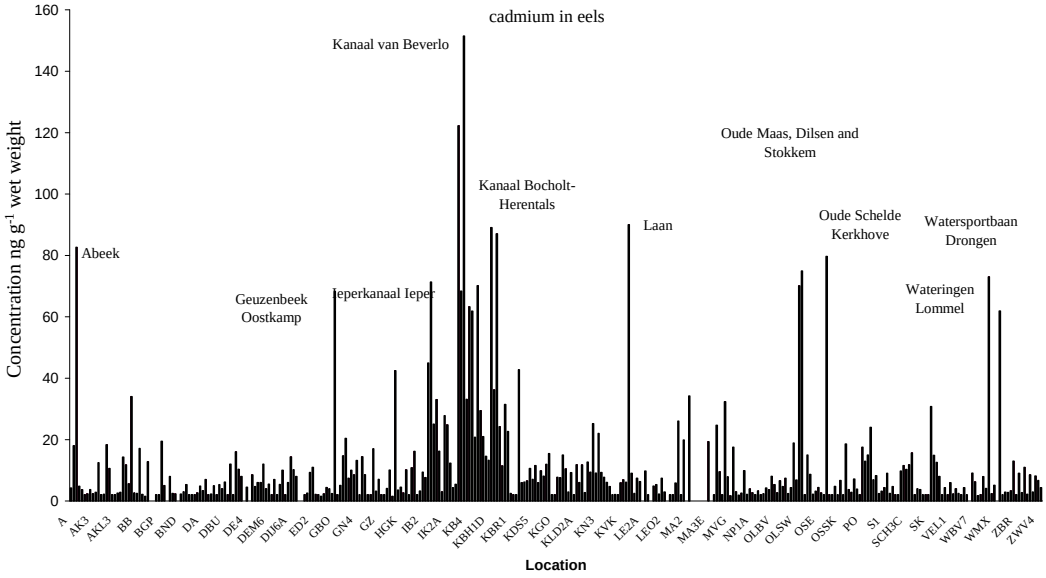


Figure 12.8. Concentrations of cadmium in eels collected at 333 sites in Flanders (1994–2005). Data from the INBO Eel Pollutant Monitoring Network Database. Values represent the means of the individual analysis of 5–10 eels per location, for the most recent survey. Codes on the x-axis are location codes defined by Goemans *et al*. (2003).

Pollutant monitoring of eels and the Water Framework Directive

The time schedule for the implementation of the EU WFD requires environmental and ecological monitoring to be in place by 2006, the development of a programme of measures by 2009, and the achievement of good ecological status by 2015. Within the Directive, emphasis is given to monitoring the ecological quality and chemical status of surface water. It is implicit in the spirit of the directive that implementation of the WFD should have a positive impact on the quantity and quality (e.g. with respect to the presence of contaminants) of silver eels migrating to the sea. It can be therefore argued that specific extensions should be implemented for eels as an indicator for river connectivity and ecological and chemical status. It was recommended by both the EIFAC/ICES Working Group on Eels (2006), and the CEC Scientific, Technical and Economic Committee for Fisheries (in its plenary meeting of April 2006) that the WFD should use the eel as a sentinel species for monitoring the

chemical status of surface waters with respect to hazardous substances, because of several ecological and physiological traits. Using the eel as a biomonitor will not only give us a powerful tool for measuring harmful substances, but using the species as a "target" organism for reaching good chemical status will also guarantee in a direct way achievement of a better status for the target species itself.

However, there is no specific reference made within the WFD to the use of eels for monitoring the chemical status of our waters. The monitoring guidance document states only that, besides monitoring in water, some fish species (as well as mussels) can be used in monitoring harmful organic substances and heavy metals, because they have a high bioaccumulation capacity (WFD–CIS, 2003). In the latest proposal (CEC, 2007) for a Directive on environmental quality standards in the field of water policy, amending the WFD (2000/60/EC), emphasis is still placed on measuring concentrations of hazardous substances in the water column. According to that proposal, there seems to be enough extensive and reliable information on concentrations of priority substances available from measuring in water to provide a sufficient basis to ensure comprehensive protection and effective pollution control of the aquatic environment. Member States have to ensure, on the basis of monitoring the chemical status of water, that concentrations of listed substances do not increase significantly in sediment and relevant biota (CEC, 2007).

Moreover, the Commission of European Communities (CEC, 2007) establishes 'Environmental quality standards' (EQSs) for priority substances and selected other pollutants. The EQSs are differentiated for inland surface waters (rivers and lakes) and other surface waters (transitional, coastal, and territorial waters). Two types of EQS are set: (i) annual average concentrations for protection against long-term and chronic effects, and (ii) maximum allowable concentrations for short-term, direct, and acute ecotoxic effects. However, for specific substances (hexachlorobenzene, hexachlorobutadiene, and methyl-mercury), it is not possible to ensure protection against indirect effects and secondary poisoning simply by setting EQSs for surface water at a Community level. Therefore, in those cases, EQSs for biota should also be set. The directive proposes limit concentrations for hexachlorobenzene, hexachlorobutadiene, and methyl-mercury, which may not be exceeded in prey tissue of fish, molluscs, crustaceans, and other biota (see below).

The directive allows Member States flexibility regarding their monitoring strategy. Member States should be able either to monitor and check compliance against EQSs in biota, or convert the Biota EQSs to equivalents for surface water. Where necessary and appropriate, more EQSs for sediment or biota can be set (CEC, 2007). In any case, the Member States should ensure that existing levels of contamination in relevant biota and sediments do not increase significantly.

Although CEC (2007) continues to focus on the analysis of those substances in the water column, there is growing awareness that sediment and biota should also be monitored (for instance, because many substances are lipophilic and are difficult to measure in water, but can be detected in high concentrations in biota). The need for a harmonized approach to monitoring the presence of hazardous substances through aquatic biota is becoming more and more acute. A good biomonitor needs to show a high capacity for bioaccumulation (see above). However, it is clear that to be adequate, potential biomonitoring organisms need more conditions to be fulfilled. These requirements are listed and discussed with respect to the eel in Table 12.2.

The WFD proposes (CEC, 2007) 33 substances or groups of substances in the list of priority substances, including selected existing chemicals, plant protection products, biocides, and metals. Other groups include polyaromatic hydrocarbons (PAHs), and polybrominated diphenylethers (PBDEs) used as flame retardants. Another eight pollutants are not on the priority list, but fall under the scope of older directives. From various published and unpublished data of concentrations in eels from Flanders collected between 1994 and 2005, we compiled the available

knowledge with respect to these WFD chemicals. Table 12.3 lists, where available, minimum and maximum concentrations, as well as the means for each. All data are expressed in ng g^{-1} wet weight. The percentage of the sites where values were below the detection limits is indicated. Data are available for more than half the substances. Table 12.3 indicates the proportion of sites under the detection limit for each substance. Of 21 (groups of) substances, just three show measurements under the DL for more than half the sampled sites. Considering the range of the measurements of these substances in eels (Table 12.3), it may be concluded that at some sites at least, some substances show extremely high levels in eels (see, e.g., maximum values for lindane, total DDT, lead, cadmium, mercury, and brominated diphenylethers). This dataset for eels in Flanders illustrates the potential of using the eel as a biomonitor over a broader geographical range, meeting the requirements of the WFD, at least for some priority substances.

CEC (2007) states that Member States have to ensure that the following concentrations of hexachlorobenzene, hexachlorobutadiene, and methyl-mercury are not to be exceeded in tissue (wet weight) of fish, molluscs, crustaceans, and other biota: 10 µg kg^{-1} for hexachlorobenzene, 55 µg kg^{-1} for hexachlorobutadiene, and 20 µg kg^{-1} for methyl-mercury. As can be seen from Table 12.3, hexachlorobutadiene is present in eels from half the sites but concentrations are always less than the limit value of 10 ng g^{-1} wet weight. However, for hexachlorobenzene the standard was exceeded at 14% of sites (total 357 sites). The situation is even more serious for mercury: the 20 ng g^{-1} wet weight was exceeded at 99% of sites (total 355 sites).

Finally, we are aware that the use of a now-endangered species, such as the eel, as biomonitor might raise some concerns. As several aspects such as fat levels, contaminants, condition, parasites, and disease are believed to play a major role in the decline of the species, we will have to monitor these to understand better the reasons for the decline. It has been calculated that our Flemish eel monitoring network, which is a very dense network, necessitates a quantity of ca. 25 kg eels annually, a negligible quantity compared with the total Belgian eel consumption (<0.005%). Still, in order to minimize culling eels for monitoring purposes, we recommend synergy in monitoring actions, e.g. by combining environmental monitoring through eel analyses with human health sanitary control of fisheries products. Also, maximum use of the eels sampled is urged (combining pollution monitoring with measuring other aspects such as condition, fat stores, and the prevalence of disease factors).

Table 12.2. Potential characteristics of a biomonitor appropriate for the monitoring of hazardous substances in the aquatic environment.

Prerequisites	Requirements	Eels : Advantage (+)/disadvantage (-)
Bioaccumulation capacity	In some species, particular ecological traits, habitat, or trophic status will enhance the bioaccumulation capacity.	+ Eels are benthic fish, carnivorous in their feeding behaviour and preying on insect larvae, worms, crustaceans, snails, mussels, and fish, in particular small bottom-dwelling species, resulting in high bioaccumulation of toxic residues. – Individual variations might occur through trophic specialization of some fish (Belpaire *et al*., 1992; Dörner *et al*., 2006). Dependent on local biotic conditions (e.g. chironomid biomass), eels might have different mean trophic positions (Dörner *et al*., 2006).
Bioavailability	The biomonitor should be at the top of the food chain, to obtain information on the degree of bioavailability of chemicals.	+ Eels are carnivorous predators (see above).
Range of chemicals measurable	The range of chemicals possible to quantify should be as broad as possible.	+ Eels have been demonstrated to be good indicators for a variety of chemical compounds, including PCBs, organochlorine pesticides, heavy metals (Goemans *et al*., 2003), brominated flame retardants (Belpaire *et al*., 2003), volatile organic pollutants (Roose *et al*., 2003), dioxins, perfluorinated chemicals (Hoff *et al*, 2005; Santillo *et al*., 2006), metallothionines (Langston *et al*., 2002), and polycyclic aromatic compounds (Ruddock *et al*., 2003). – Yellow eels are apparently not suited to indicating the extent of endocrine disruption by vitellogenin measurements (Versonnen *et al*., 2004).
Internationally accepted monitor species	Member States use diverse organisms as biomonitors: microbial assemblages, molluscs, algae, other fish species (trout, gudgeon, etc.), fish parasites, invertebrates, aquatic macrophytes, water birds, etc. There is definitely a need for harmonization and for a common approach and strategy for tracking chemicals in aquatic biota.	+ Eels have been used all over the world as (chemical) biomonitors, and studies on a local or a national scale are known for the European eel in Europe. In The Netherlands (Hendriks and Pieters, 1993; de Boer and Hagel, 1994; Pieters *et al*., 2004), France (Batty *et al*., 1996; Roche *et al*., 2002; Goursolle, 2002), Finland (Tulonen and Vuorinen, 1996), Sweden (van Leeuwen *et al*., 2002; Ankarberg *et al*., 2004), the UK (Mason and Barak, 1990; Mason, 1993; Weatherley *et al*., 1997), Spain (Usero *et al*, 2003), Italy (Bressa *et al*, 1995, 1997; Agradi *et al*., 2000; Corsi *et al*., 2005), Germany (Fromme *et al*., 1999; Wiesmüller and Schlatterer, 1999), and Belgium (Walloon region (Thomé *et al*., 2004)) and Flanders (Goemans *et al*., 2003; Goemans and Belpaire, 2004; Roose *et al*., 2003; Morris *et al*., 2004; Hoff *et al*., 2005; Maes *et al*., 2005). A regular monitoring network has been in place in The Netherlands (since 1977) and in Belgium (since 1994). More widespread studies over Europe have been presented by Greenpeace, using the eel as a bioindicator for the presence of brominated flame retardants and PCBs from rivers and lakes in 10 European countries (Santillo *et al*., 2005) and for perfluorinated chemicals (11 countries: Santillo *et al*., 2006).

Table 12.2. (continued)

Prerequisites	Requirements	Eels : Advantage (+)/disadvantage (-)
Seasonality	No or minimal seasonal changes through metabolic activities within annual cycles, linked with reproduction or seasonal environmental variation.	+ Because of the absence of annual reproductive cycles, there are no reproduction-linked seasonal metabolic variations (but see also Gorby *et al*., 2005 for seasonal variation in metallothioneins, cytochrome P450, bile metabolites and oxyradical metabolism) – Lipid content might fluctuate to some extent throughout the year (van Leeuwen *et al*., 2002).
Migratory behaviour	Sentinel species should be fairly resident to allow fingerprinting the local pollution load.	+ Yellow eels show explicit homing behaviour, and foraging movements are mostly restricted to a few hundred metres. Apparently, many eel species share this ecological trait (*A. anguilla* – Baras *et al*., 1998; Lafaille *et al*., 2005; *A. rostrata* – Oliveira, 1997; Goodwin, 1999; *A. australis* – Jellyman *et al*., 1996; *A. dieffenbachi* – Beentjes and Jellyman, 2003*; A. japonica* – Aoyama *et al*., 2002). The fingerprint value of eels has been demonstrated (Castonguay *et al*., 1989; Belpaire *et al*., 1999). – Although within tidal estuaries, home-site fidelity is obvious, home range may be larger than in fresh-water habitats (Parker, 1995), and seasonal movements might occur (Hammond, 2003). Also, seasonal migration activities have been reported, as well as the occurrence of erratic eels (nomads) (Feunteun *et al*., 2003). Because of migrations at the silver eel stage, the bioindicator value of the eel is restricted to the yellow eel phase.
Occurrence	The species should be widespread and should occur in a wide range of aquatic habitats. In the context of the WFD, an overall European distribution is recommended.	+ Eels are widespread and can be found in almost all aquatic habitats. They occur in fresh, brackish, and coastal waters in almost all Europe (even northern Scandinavia and from the Azores to the eastern Mediterranean), as well as in northern Africa. In Flanders, the species is the third most widespread fish species. – The presence of eels in upstream reaches might be limited by the presence of migration barriers. Mitigating management procedures such as restocking programmes can counter this.
Size	The size of the organism must be large enough to permit adequate analysis.	+ The targeted length of 40 cm means a weight of ca. 100 g, large enough to distribute eel tissue for the various analytical procedures and to laboratories linked to the various contaminants.
Standardization	Standardization on length and/or age is recommended.	+ Standardizing through the choice of an eel length class for monitoring is ~40 cm. – Bias attributable to growth heterogeneity.
Physiological properties	Besides size, physiological traits such as high lipid content will facilitate analysis of (mostly lipophylic) substances.	+ Eels show extremely high lipid values (mean for Flemish eels: 14.7%, n = 1164; Goemans *et al*., 2003). – There can be heterogeneity in lipid content between eels and sites.

Table 12.2. (continued)

Prerequisites	**Requirements**	**Eels : Advantage (+)/disadvantage (-)**
Reference values	Evaluation procedures, risk analysis, management decision trees, are dependent on the availability of normative values such as reference values, target values, action threshold values, and consumption standards.	+ On the basis of data distribution analysis, reference values for eels have been presented for Flanders for PCBs, OCPs, and heavy metals (Goemans *et al*., 2003, Belpaire and Goemans, 2004) and exist in The Netherlands (Hendriks and Pieters, 1993). Action threshold values are in place in The Netherlands. Many countries have national consumption standards, and EU consumption standards are in place or under development. – For many substances, threshold values are still missing
Life history	There should be a sufficiently long life cycle to be capable to accumulate hazardous substances.	+ The eel spends between 3 and 20 years in inland and coastal waters (Vøllestad, 1992)
Robustness of the biomonitor	It is essential that also in (highly) polluted waters, contaminants can be monitored through the sentinel species; therefore, the species should be (fairly) resistant to environmental degradation.	+ Eels are highly resistant to degradation of water quality and endure low levels of oxygen and high eutrophication levels. – Eels are sensitive to failure in river connectivity, but their presence is enhanced by restocking.
Multiple use biomonitor	Simultaneous use of one sentinel species for multiple goals is economical beneficial (cost-effficient).	+ Choosing eel as a chemical biomonitor allows triple usage: (i) Environmental health and chemical status (national level and WFD level); (ii) Human food safety and sanitary control of fisheries products; (iii). Monitoring of eel (spawner) quality within the requirements of the international eel restoration plan and the national Eel Management Plans (STECF, 2006).

Table 12.3. WFD substances mentioned under CEC (2006), and available data from measurements of Flemish eels. All data are expressed in ng g^{-1} wet weight. DL, detection limit.

Substance	Note	Range Min – Max (Mean)	% <DL	Number of sites	Years	Reference
Benzene	[a]	1.2-18.9 (5.7)	0	20	1996-1998	[j]
Brominated diphenylethers	[a]	6.9-5 284.4 (369.1)[c]	0	18	2001	[l]
Cadmium and its compounds	[a]	DL-151.4 (11.7)[d]	19	357	1994-2005	[k]
1,2-Dichloroethane	[a]	DL-4.9 (1,2)	55	20	1996-1998	[j]
Hexachlorobenzene	[a]	DL-61.6 (5.7)	<1	357	1994-2005	[k]
Hexachlorobutadiene	[a]	DL-12.2 (1.8)	50	20	1996-1998	[j]
Alfa-Hexachlorocyclohexane	[a]	DL-13.7 (0.8)[e]	13	357	1994-2005	[k]
(gamma-isomer, Lindane)	[a]	0.1-2 076.4 (46.9)	0	357	1994-2005	[k]
Lead and its compounds	[a]	DL-1 744.2 (56.6)[f]	3	357	1994-2005	[k]
Mercury and its compounds	[a]	10-535.4 (113.5)[g]	0	355	1994-2005	[k]
Naphthalene	[a]	1.5-63 (5.8)	20	20	1996-1998	[j]
Nickel and its compounds	[a]	DL-2 944.7 (186.2)[h]	16	297	1994-2005	[k]
(1,2,4-Trichlorobenzene)	[a]	DL-30.9 (6.0)	15	20	1996-1998	[j]
Trichloromethane (chloroform)	[a]	DL-96.0 (13.4)	25	20	1996-1998	[j]
DDT total	[b]	6.6-1 102.7 (90.2)[i]	0	357	1994-2005	[k]
p,p'-DDT	[b]	DL-62.6 (2.9)	38	357	1994-2005	[k]
Aldrin	[b]	DL-11.4 (1.3)	33	96	1994-2005	[k]
Dieldrin	[b]	DL-237.6 (19.1)	15	357	1994-2005	[k]
Endrin	[b]	DL-29.1 (1.1)	80	346	1994-2005	[k]
Tetrachloroethylene	[b]	DL-88.9 (13.4)	50	20	1996-1998	[j]
Trichloroethylene	[b]	DL-30.3 (2.0)	95	20	1996-1998	[j]

[a] Priority substances.
[b] Other pollutants, which fall under the scope of Directive 86/280/EEC and which are included in List I of the Annex to Directive 76/464/EEC, are not in the priority substances list. Environmental quality standards for these substances are included in the Commission's proposal to maintain the regulation of the substances at Community level.
[c] The data present the Sum of 10 BDEs.
[d] Cd.
[e] alpha-hexachlorocyclohexane.
[f] Pb.
[g] Hg.
[h] Ni.
[i] Sum of *p,p'*-DDD, p,p'-DDT, and *p,p'*-DDE.
[j] Data from Roose *et al*. (2003).
[k] INBO Eel Pollutant Monitoring Database.
[l] Data from de Boer *et al*. (2002) and Belpaire *et al*. (2003).

Conclusions

From the examples given, it is clear that the use of eels as sentinel species can pinpoint sources of pollutants. Owing to the ecological and physiological traits of the species, the European eel in its yellow eel phase is a suitable sentinel species for a variety of chemical substances. Its value as a biomonitoring tool for chemical environmental contamination, for both local and international purposes, is clear. The eel may be the best of all available aquatic species when monitoring lipophilic chemicals in aquatic biota for the purposes of the Water Framework Directive, whereas results show that, at least for some substances, monitoring in water is insufficient and does not guarantee sufficient protection of the aquatic environment. More effort is required to elaborate and optimize techniques for the analysis of additional chemicals in eel tissue. There is inadequate knowledge on the effects of these chemicals on eels but, considering the concentrations of some chemicals measured at some sites, these toxic substances are very likely to have detrimental

effects on the reproductive success of the species. Considering the variation in contaminant profile and concentrations, the degree and reproductive potential of eels leaving our system will vary considerably, depending on the level of pollution in the habitat where the eels grow and mature.

Acknowledgements

We acknowledge with thanks the support of the Flemish Environment Agency for their data, and Robin Law, Robert Rosell, and an anonymous referee for very helpful comments on the submitted draft.

References

Agradi, E., Baga, R., Cillo, F., Ceradini, S., Heltai, D., 2000. Environmental contaminants and biochemical response in eel exposed to Po river water. *Chemosphere* 41, 1555-1562.

Ahmad, N., Benoit, D., Brooke, L., Call, D., Carlson, A., DeFoe, D., Huot, J., Moriarity, A., Richter, J., Shubat, P., Veith, G., Wallbridge, C., 1984. Aquatic toxicity tests to characterize the hazard of volatile organic chemicals in water: a toxicity data summary,Parts I and II. Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, Minnesota.

Ankarberg, E., Bjerselius, R., Aune, M., Darnerud, P.O., Larsson, L., Andersson, A., Tysklind, M., Bergek, S., Lundstedt-Enkel, K., Karlsson, L., Törnkvist, A., Glynn, A., 2004. Study of dioxin and dioxin-like PCB levels in fatty fish from Sweden 2000-2002. *Organohalogen Compounds* 66, 2061-2065.

Anon., 2003. Waterkwaliteit – Lozingen in het water 2003 (uitgebreid rapport). Vlaamse Milieumaatschappij, Aalst.

Aoyama, J., Sasai, S., Miller, M.J., Shinoda, A., Nakamura, A., Kawazu, K., Tsukamoto, K., 2002. A preliminary study of the movements of yellow and silver eels, *Anguilla japonica*, in the estuary of the Fukui River, Japan, as revealed by acoustic tracking. *Hydrobiologia* 470, 31-36.

ATSDR, 1992. Data from the US Agency for Toxic Substances and Disease Registry, at http://www.atsdr.cdc.gov/toxprofiles/phs36.html

ATSDR, 2005. Toxicological profile for benzene. Draft for public comment, September 2005. US Agency for Toxic Substances and Disease Registry, at http://www.atsdr.cdc.gov/toxprofiles/tp3.html

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.

Batty, J., Pain, D., Caurant, F., 1996. Metal concentrations in eels *Anguilla anguilla* from the Camargue region of France. *Biological Conservation* 76, 17-23.

Beentjes, M.P., Jellyman, D.J., 2003. Enhanced growth of longfin eels, *Anguilla dieffenbachii*, transplanted into Lake Hawea, a high country lake in South Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 37, 1-11.

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 September 2001, p.170-174.

Belpaire, C., Goemans, G., 2004. Monitoring en normering van milieugevaarlijke stoffen in paling: bruikbaarheid en relevantie voor het milieubeleid. *Water* December 2004, 1-16.

Belpaire, C., Goemans, G., de Boer, J., Van Hooste, H., 2003. Verspreiding van gebromeerde vlamvertragers. In: van Steertegem , M. (Ed.), Mira-T 2003; Milieu- en Natuurrapport Vlaanderen. LannooCampus, Leuven. p.387-395.

Belpaire, C., van Driessche, H., Gao, F.Y., Ollevier, F., 1992. Food and feeding activity of glass eel *Anguilla anguilla* (L.) stocked in earthen ponds. *Irish Fisheries Investigations, Series A (Freshwater)* 36, 43-54.

Belpaire, C., van Thuyne, G., Callaars., S., Roose, P., Cooreman, K., Bossier, P., 1999. Spatial and temporal variation in organochlorine pesticide and polychlorinated biphenyl pollution in fresh water aquatic ecosystems in Flanders using the European eel (*Anguilla anguilla* L.) as an indicator. EIFAC/ICES, Working Group on Eel, Silkeborg (DK), 20–25 September 1999.

Black, J.A., Birge, W.J., McDonnell, W.E., Westerman, A.G., Ramey, B.A., Bruser, D.M., 1982. The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. Research Report 133, Water Resources Research Institute, University of Kentucky, Lexington, Kentucky. 61p.

Bosma, T. N. P., van der Meer, J.R., Schraa, G., Tros, M. E., Zehnder, A. J. B., 1988. Reductive dechlorination of all trichloro- and dichlorobenzene isomers. *FEMS Microbiology Ecology* 53, 223-229.

Bressa, G., Bronzi, P., Romano, P., Sisti, E., 1995. Chlorinated pesticides and PCBs in wild and farmed eels (*Anguilla anguilla* L): influence of water temperature and diet. *AgroFood Industry Hi-Tech* November/December, 46-48.

Bressa, G., Sisti, E., Cima, F., 1997. PCBs and organochlorinated pesticides in eel (*Anguilla anguilla* L.) from the Po Delta. *Marine Chemistry* 58, 261-266.

Castonguay, M., Dutil, J.D., Desjardins, C., 1989. Distinction between American eels (*Anguilla rostrata*) of different geographic origins on the basis of their organochlorine contaminant levels. *Canadian Journal of Fisheries and Aquatic Sciences* 46, 836-843.

Corsi, I., Mariottini, M., Badesso, A., Caruso,T., Borghesi, N., Bonacci, S., Iacocca, A., Focardi, S., 2005. Contaminants and sub-lethal toxicological effects of persistent organic pollutants in the European eel *(Anguilla anguilla)* in the Orbetello lagoon (Tuscany, Italy). *Hydrobiologia* 550, 237-249.

CEC., 2000. Directive of the European parliament and of the council 2000/60/EC establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* 22.12.2000 L 327/1-72.

CEC., 2007. 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, COM(2006) 397 final 2006/0129 (COD) 21.02.2007 11816/06 ENV 415 CODEC 782.

de Boer, J., Allchin, C., Zegers, B., Boon, J.P., Brandsma, S. H., Morris, S., Kruijt, A. W., *et al*., 2002. HBCD and TBBP-A in sewage sludge, sediments and biota, including interlaboratory study, RIVO report number C033/02, September 2002, 40p. + annexes.

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.

Dörner, H., Skov, C., Berg, S., Schulze, T., Beare, D.J., van der Velde, G., 2006. The diet of large eels (*Anguilla anguilla*) in relation to food availability. ICES Document CM 2006/J: 28.

Environment Canada, 1989. Environmental assessment of the Canadian textile industry. Chemical Industries Division, Industrial Programs Branch, Environment Canada. EPS 5/TX/1. 104p.

Environment Canada, 1993. Priority Substances List Assessment Report 1,2-Dichlorobenzene. Government of Canada, Health Canada, Canadian Environmental Protection, Act. Catalogue No. En40-215/33E. 27p.

EPER, 2006. The European Pollutant Emission Register, at http://eper.ec.europa.eu/eper/

Feunteun, E., Lafaille, 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, Tokyo, p.191-213.

Fromme, H., Otto, T., Pilz, K., Neugebauer, F., 1999. Levels of synthetic musks: bromocyclene and PCBs in eel (*Anguilla anguilla*) and PCBs in sediment samples from some waters of Berlin/Germany. *Chemosphere* 39, 1723-1735.

Goemans, G., Belpaire, C., 2004. The eel pollutant monitoring network in Flanders, Belgium. Results of 10 years monitoring. *Organohalogen Compounds* 66, 1834-1840.

Goemans, G., Belpaire, C., 2005. Congener profiles in European eel (*Anguilla anguilla* L.) as a method of tracing the origin of PCB contamination. *Organohalogen Compounds* 67, 1304-1307.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994–2001: gehalten aan polychloorbifenylen, 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.

Goodwin, K.R., 1999. American eel subpopulation characteristics in the Potomac river drainage, Virginia. Thesis, Faculty of Virginia Polytechnic Institute and State University. Blacksburg, Virginia. 116p.

Gorbi, S., Baldini, C., Regoli, F., 2005. Seasonal variability of metallothioneins, cytochrome P450, bile metabolites and oxyradical metabolism in European eel *Anguilla anguilla* L. (Anguillidae) and striped mullet *Mugil cephalus* L. (Mugilidae). *Archives of Environmental Contamination and Toxicology* 49, 62–70.

Goursolle, R., 2002. Etude de la contamination en polychlorobiphényles le long des réseaux trophiques estuariens. Application à l'estuaire de la Gironde. Université Bordeaux, Laboratoire de physico-toxicochimie des systèmes naturels. 82p. + annexes.

Hammond, S.D., 2003. Seasonal movements of yellow-phase American eels (*Anguilla rostrata*) in the Shenandoah River, West Virginia. Thesis. Davis College of Agriculture, Forestry, and Consumer Sciences at West Virginia University, Morgantown WV. 32p.

Hendriks, A.J., Pieters, H., 1993. Monitoring concentrations of microcontaminants in aquatic organisms in the Rhine delta: a comparison with reference values. *Chemosphere* 26, 817-836.

Hoff, P.T., van Campenhout, K., de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., *et al*., 2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Environmental Pollution* 137, 324-333.

Jellyman, D.J., Glova, G.J., Todd, P.R., 1996. Movements of shortfinned eels, *Anguilla australis,* in Lake Ellesmere, New Zealand: results from mark-

recapture studies and sonic tracking. *New Zealand Journal of Marine and Freshwater Research* 30, 371-381.

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.

Langston, W.J., Chesman, B.S., Burt, G.R., Pope, N.D., McEvoy, J., 2002. Metallothionein in liver of eels *Anguilla anguilla* from the Thames Estuary: an indicator of environmental quality? *Marine Environmental Research* 53, 263-293.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology* 73, 99-114.

Mason, C.F., 1993. Organochlorine pesticide residues and PCBs in eels *Anguilla anguilla* from some British reedbeds. *Chemosphere* 26, 2289-2292.

Mason, C.F., Barak, N.A.E., 1990. A catchment survey for heavy metals using the eel (*Anguilla anguilla*). *Chemosphere* 21, 695-699.

Morris, S., Allchin, C.R., Zegers, B.N., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P.E.G., *et al*., 2004. Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environmental Science and Technology* 38, 5497-5504.

Oliveira, K., 1997. Movements and growth rates of yellow phase American eels in the Annaquatucket River, Rhode Island. *Transactions of the American Fisheries Society* 126, 638-646.

Oliver, B.G., Nicol, K.D., 1982. Chlorobenzenes in sediments, water, and selected fish from Lakes Superior, Huron, Erie, and Ontario. *Environmental Science and Technology* 16, 532-533.

Oliver, B.G., Niimi, A.J., 1983. Bioconcentration of chlorobenzenes from water by rainbow trout: correlations with partition coefficients and environmental residues. *Environmental Science and Technology* 17, 287-291.

Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., and van den Thillart, G.E.E.J.M., 2006. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93, 145-148.

Parker, S.J., 1995. Homing ability and home range of yellowphase American eels in a tidally dominated estuary. *Journal of the Marine Biological Association of the UK* 75, 127-140.

Pieters, H., van Leeuwen, S.P.J., and de Boer, J., 2004. Verontreinigingen in aal en snoekbaars: monitorprogramma ten behoeve van de Nederlandse sportvisserij 2003. RIVO report, C063/04.

Robinet, T., Feunteun, E., 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265-277.

Roche, H., Buet, A., Ramade, F., 2002. Accumulation of lipophilic microcontaminants and biochemical responses in eels from the Camargue biosphere reserve. *Ecotoxicology* 11, 155-164.

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *Journal of Environmental Monitoring* 5, 876-884.

Ruddock, P.J., Bird, D.J., McEvoy, J., Peters, L.D., 2003. Bile metabolites of polycyclic aromatic hydrocarbons (PAHs) in European eels *Anguilla anguilla* from United Kingdom estuaries. *The Science of the Total Environment* 301, 105-117.

Santillo, D., Johnston, P., Labunska, I., Brigden, K., 2005. Widespread presence of brominated flame retardants and PCBs in eels *(Anguilla anguilla)* from rivers and lakes in 10 European countries. Greenpeace Research Laboratories, Technical Note 12/2005. Published by Greenpeace International, October 2005. 56p.

Santillo, D., Allsopp, M., Walters, A., Johnston, P., Perivier, H., 2006. Presence of perflurionated chemicals in eels from 11 European countries. Investigating the contamination of the European eel with PFCs, substances used to produce non-stick and water-repellant coatings for a multitude of products. Report of the Greenpeace Research Laboratories Technical Note 07/2006, publ. Greenpeace International, September 2006. 32p.

STECF, 2006. Scientific, Technical and Economic Committee for Fisheries of the Commission of the European Communities, 22nd Report of STECF, draft version 27 April 2006.

Thomé, J.P., Bertrand, A., Brose, F., Carabin, O., De Pauw, E., Dukmans, C., Eppe, G., *et al*., 2004. Evaluation du niveau de contamination des rivières par les PCBs et les dioxines. Report Université de Liège. Convention avec la Région Wallonne, Ministère de l'Aménagement du Territoire, de l'Urbanisme et de l'Environnement, Engagement no. 01/4143. 167p.

Tulonen, J., Vuorinen, P.J., 1996. Concentrations of PCBs and other organochlorine compounds in eels (*Anguilla anguilla* L.) of the Vanajavesi watercourse in southern Finland, 1990–1993. *Science of the Total Environment* 187, 11-18.

Usero, J., Izquierdo, C., Morillo, J., Gracia, I., 2003. Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain. *Environment International* 29, 949-956.

van Leeuwen, S.P.J., Traag, W.A., Hoogenboom, L.A.P., Booij, G., Lohman., M., Dao, Q.T., de Boer, J., 2002. Dioxines, furanen en PCBs in aal. Onderzoek naar wilde aal, gekweekte aal, geïmporteerde en gerookte aal. RIVO Nederlands Instituut voor Visserijonderzoek, IJmuiden, report CO34/02. 34p. + 7 annexes.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363-371.

Vøllestad, L. A., 1992. Geographic variation in age and length at metamorphosis of maturing European eel: environmental effects and phenotypic plasticity. *Journal of Animal Ecology 61,* 41–48.

Weatherley, N.S., Davies, G.L., Ellery, S., 1997. Polychlorinated biphenyls and organochlorine pesticides in eels (*Anguilla anguilla* L.) from Welsh rivers. *Environmental Pollution* 95, 127-134.

Wiesmüller, T., Schlatterer, B., 1999. PCDDs/PCDFs and coplanar PCBs in eel (*Anguilla anguilla*) from different areas of the rivers Havel and Oder in the state of Brandenburg (Germany). *Chemosphere* 38, 325-334.

WFD–CIS, 2003. Water Framework Directive, Common Implementation Strategy, Working Group 2.7, Monitoring, Guidance on Monitoring for the Water Framework Directive, Final Version, 23 January 2003.

WG Eel, 2006. FAO European Inland Fisheries Advisory Commission, and the International Council for the Exploration of the Sea. Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels, Rome, 23–27 January 2006. EIFAC Occasional Paper. 38. ICES Document CM 2006/ACFM: 16. 352p.

Young, C.M., Voorhees, K.J., 1989. Thermal decomposition of 1,2-dichlorobenzene. *American Chemical Society* 3, 280-287.

Eels as environmental indicators and as threat to mankind, seen by GAL (Gerard Alsteens).

Production of this artwork by GAL was ordered by the Flemish Environment Agency.

Chapter 13

Eels as chemical bioindicators for the Water Framework Directive

Claude Belpaire and Geert Goemans

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

This chapter is in published as:

Belpaire, C., Goemans, G., 2008.
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.

Summary

The Water Framework Directive recently (2006) proposed to monitor a selection of priority substances and to report on the chemical status of European water bodies. The final objective is the protection of aquatic life and human health. The majority of these substances are lipophilic, nevertheless it is proposed to monitor them in the water-phase. As there is serious concern about whether measurements of these lipophilic compounds in water will give results that will guarantee the protection of aquatic life, monitoring in biota seems to be more appropriate.

The advantages of using the European eel (*Anguilla anguilla*) as a model for evaluating the chemical status within the WFD is discussed. A wide range of studies over Europe exist and have pinpointed various types of environmental contamination. Eel contaminant profiles seem to be a fingerprint of the contamination pressure of a specific site. This is illustrated with results from 12 years of contaminant monitoring in eel in Flanders, where the database comprises at present analyses of 2946 eels from 365 sites. From this database, reference values and quality classes for PCBs, OCPs and heavy metals in eel were deduced and are presented.

The establishment of a harmonised, Europe-wide chemical monitoring programme of eels could enable three separate objectives to be addressed: (1) the evaluation of environmental health and chemical status, (2) the sanitary control of fisheries products within human food safety regulations, and (3) the monitoring of eel quality within the requirements of the international eel restoration plan. Because of the high concentrations of some contaminants in certain eel subpopulations and the ecotoxicological effects of these substances, achieving good chemical status of EU waters will directly be beneficial for restoration of eels stocks.

Introduction

During the last decade, many countries have reported the development of local monitoring programmes for specific contaminants within biotic matrices. Bioindicators belonging to very different classes are used for evaluating pollution in fresh water ecosystems, e.g. microbial assemblages, algae, Bryozoa, aquatic macrophytes, molluscs, fish parasites, invertebrates, fish, turtle eggs, aquatic birds and mammals.

As described by Belpaire and Goemans (2007), a good chemical status indicator should fulfil a number of requirements. It is essential that the species shows a high bioaccumulation capacity for a wide range of chemicals. Specific ecological traits of the indicator species should allow representative information of the chemical status of the sample site to be gained. Furthermore the species should present analytical advantages. Standard procedures for sampling and analysis should be available and a normative framework should be developed. It is an economic advantage when the data obtained through an indicator species can be used for multiple purposes (e.g. other (inter)national monitoring programmes), thus allowing better cost efficiency and effectiveness of the monitoring efforts. It is an additional benefit if monitoring networks are already in place in certain countries and expertise is already available. Self-evidently the indicator species should be widely distributed, to allow its use on a large geographical scale.

In recent years, an increasing number of studies have focused on the use of the eel to monitor harmful substances. It is known for many years that, due to specific physiological and ecological features, eels bioaccumulate many substances in their muscle tissue (e.g. Bruslé, 1991; de Boer and Hagel, 1994; Maes *et al*., 2008). Specific characteristics of the species (size, long life span, fat content, feeding and habitat ecology, distribution, euryhyalinity, one reproductive cycle) are considered as favourable for the choice of the eel as a chemical sentinel species. The European eel is distributed over a wide geographical area, extending from North Africa in the south to Northern Scandinavia in the north, and from the Azores in the west to the Eastern Mediterranean region in the south-east. The natural distribution of the eel covers most EC countries (Figure 13.1). Its distribution in remote places far from the sea where accessibility is hampered by migration barriers is quite often enforced by restocking with glass eel. Eels are thus widespread and can be found in a wide range of aquatic habitats of various typology. They occur in the fresh, brackish and coastal waters of a large part of the EC territory.

In this paper we will assess the indicator value of this species and, specifically, the possibility to use the eel as an indicator for the chemical status within the Water Framework Directive (WFD), using the results and experiences of 12 years of eel monitoring in Flanders. Since 1994 the Research Institute for Nature and Forest (INBO) has developed a pollutant monitoring network for public water bodies in Flanders (Belgium) using eel (*Anguilla anguilla*) as a sentinel species. During this monitoring within the river basins of Yser, Scheldt and Meuse (ca. 13 500 km²), 2946 eels have been sampled on 365 sites between 1994 and 2005. Muscle tissue of individual eels was routinely analysed for a series of ca.30 polychlorine biphenyls (PCBs), organochlorine pesticides (OCPs) and heavy metals (see Goemans *et al.*, 2003 and Maes *et al.*, 2008) for sampling and analytic procedures and quality assurance). In addition to this routine analysis, other contaminants were analysed on a restricted selection of sites. These contaminants included brominated flame retardants, volatile organic pollutants (VOCs), endocrine disruptors, dioxins, perfluorooctane sulfonic acids (PFOSs), metallothioneins and polycyclic aromatic compounds. These results are reported in various papers (Belpaire *et al.*, 2001, 2003;

Belpaire and Goemans, 2007; Goemans *et al*., 2003; Goemans and Belpaire, 2003, 2004, 2005; Hoff *et al*., 2005; Maes *et al*., 2005; Maes *et al*., 2008; Morris *et al*., 2004; Roose *et al.,* 2003; Versonnen *et al.,* 2004).

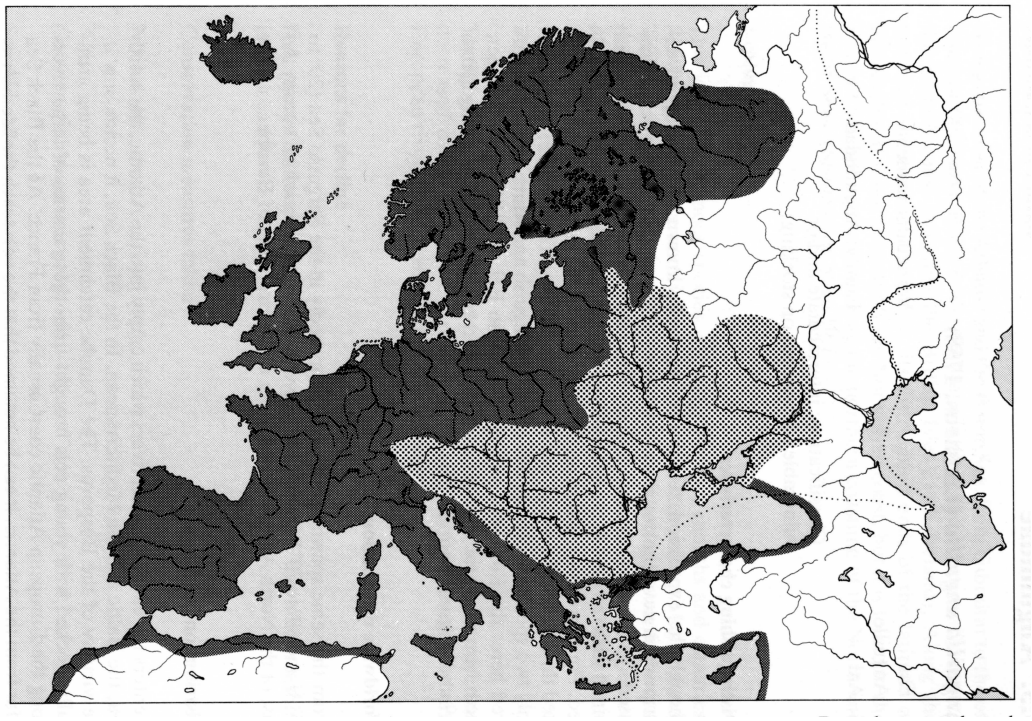


Figure 13.1. Distribution map of the European eel. Dark area: natural distribution area. Dotted area: enlarged distribution by stocking (Lelek, 1987).

The WFD (CEC, 2000) and, more specifically amendment CEC (2006a), enforces the monitoring of a selection of harmful substances in the aquatic environment. The monitoring strategy described sets out to measure most of these contaminants in the water-phase. However, the final aim of the Directive is to protect aquatic organisms and the aquatic ecosystem health. Belpaire and Goemans (2007) have discussed, and to some extent criticised, the monitoring strategy mainly on the basis of analytical features of those compounds. Basically, most of the substances selected under CEC (2006a) are highly lipophilic, and consequently are hardly (if ever) traceable in water. On the other hand, they may attain very high concentrations in organisms, as a result of bioconcentration and biomagnification. Belpaire and Goemans (2007) argued that within the WFD, at least for some substances, monitoring in water is inadequate and does not guarantee sufficient protection of the aquatic environment, and concluded that, as an alternative the eel, may be a suitable species for monitoring lipophilic chemicals in aquatic biota. From the INBO Eel Pollutant Monitoring Network (EPMN), specific examples of how eels can pinpoint environmental pollution by chemicals have been demonstrated. Belpaire and Goemans (2007) further illustrate the potential of using the eel as a biomonitor over a broader geographical range, meeting the requirements of the WFD for reporting on the chemical status of water bodies at least for some priority substances.

In this paper, we present evidence from results collected through the EPMN to further document and assess the potential advantage of using eel within the WFD chemical status monitoring. An overview of current eel monitoring work in Europe is given and possibilities for a standardised framework are described. Finally, other environmental constraints related to eel chemical monitoring will be discussed briefly.

Analytical issues

A number of specific traits of the eel, such as habitat preferences, trophic position, lipid content and size, give specific advantages when considering selecting eel as a chemical bioindicator species. Being benthic and carnivorous animals, eels are particularly vulnerable to high contamination levels through bioaccumulation and biomagnification. The lipid content of the eel is high compared to other species and especially the lipophilic contaminants can attain high levels. As a consequence these contaminants are easily traceable. Eel size is sufficient to provide the required quantity of tissue for the analyses of a series of different contaminants.

Table 13.1. Concentrations of PCB 153, lindane and cadmium measured simultaneously in water, sediment, suspended solids and eel at 5 stations in Flanders (2001). Concentrations are expressed as $\mu g.L^{-1}$ (water), in $\mu g.kg^{-1}$ dry matter (sediment and suspended solids) and in $\mu g.kg^{-1}$ wet weight of muscle tissue (eel). Stations are the canals Zuidwillemsvaart (ZWV) and Kanaal van Beverlo (KBL), a lake at Weerde (WEE), a polder water course Oude Avaart (OAV) and a river Leie (LEI) (after Belpaire and Goemans, 2004).

	Station	Water	Sediment	Suspended solids	Eel
PCB 153	ZWV	<DL	<DL	16	436
PCB 153	KBL	<DL	13	No data	142
PCB 153	WEE	<DL	12	54	429
PCB 153	OAV	<DL	<DL	<DL	13.5
PCB 153	LEI	<DL	5.2	16	128
Lindane	ZWV	0.006	<DL	<DL	9.3
Lindane	KBL	<DL	<DL	<DL	7.5
Lindane	WEE	<DL	<DL	<DL	1.1
Lindane	OAV	0.300	<DL	210	216
Lindane	LEI	0.057	0.7	7.9	40.4
Cadmium	ZWV	<DL	8	10	1.5
Cadmium	KBL	0.012	570	350	30
Cadmium	WEE	<DL	<DL	6	8.7
Cadmium	OAV	<DL	<DL	<DL	7.8
Cadmium	LEI	<DL	0.9	16	2.2

From an analytical perspective, biota have the advantage of containing much higher concentrations of contaminants compared with abiotic samples, as a result of processes like bioaccumulation and biomagnifications. Organisms at higher trophic levels are known to have higher contaminant levels than their prey. During an assessment of the occurrence and partitioning of an extended series of chemicals in the aquatic environment at 5 polluted sites in waters of different typology in Flanders, suspended solids, sediments and organisms of different trophic levels were analysed (Weltens *et al.*, 2002, 2003; Table 13.1). It is clear that even on sites with high levels

of various pollutants, a lot of measurements in the abiotic compartments fall under the detection limit (DL). In contrast, concentrations in eel are always measurable and attain higher values (and thus are better detectable) than in the sediment or suspended solids. For heavy metals, e.g. cadmium, differences in concentration levels between biotic and abiotic compartments are generally less pronounced. For monitoring heavy metals it could be recommended to measure eel liver concentrations instead of muscle tissue, as concentrations of most metals are higher in liver tissue (Durrieu *et al*., 2005). However metal measurements in muscle tissue are easily detectable (see below and Figure 13.2) and present an added value towards human health risk assessment (see below).

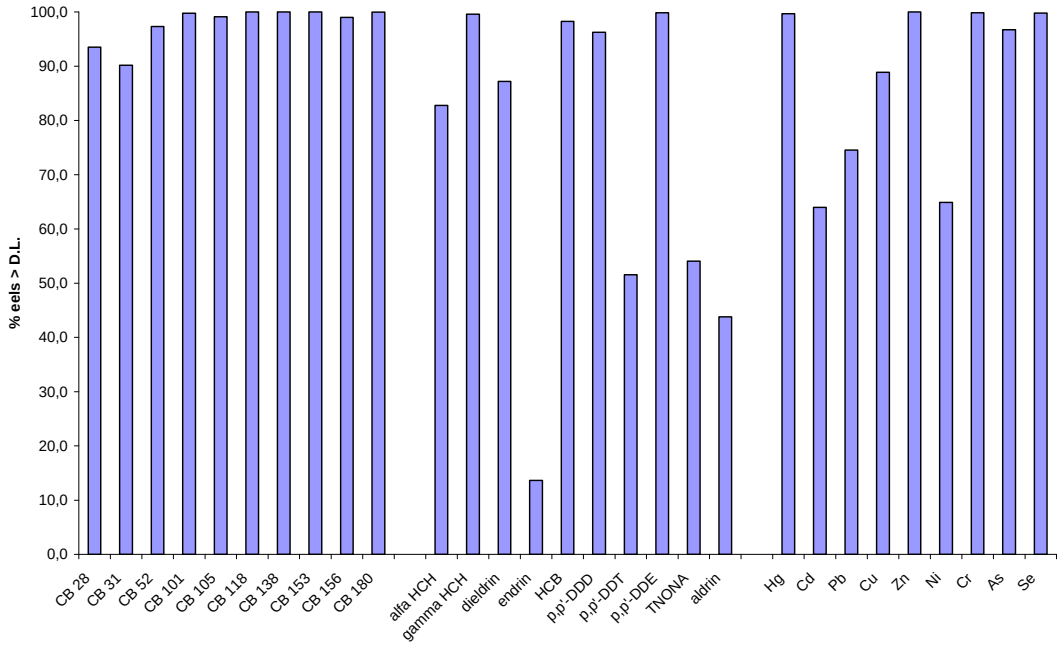


Figure 13.2. Percentage of individual yellow eel measurements above the detection limit for 10 PCB congeners and 10 organochlorine pesticides and 9 heavy metals in eels from Flanders collected in the period 1994-2005. N = 2528 for the PCBs, hexachlorocyclohexanes (HCHs), dieldrin, hexachlorobenzene (HCB), DDTs, trans-Nonachlor (T-nona) and endrin, N = 546 for aldrin, N = 2769 for Hg, Cd and Pb, N = 2117 for Cu, Zn, Ni and Cr and N = 1410 for As and Se (data from INBO Eel Pollution Monitoring Network). For an overview of the mean eel life history statistics (length, weight and lipid content) see Maes *et al*. (2008).

Trophic position is not the only factor determining the degree of contamination of a species. Top-predators like northern pike (*Esox lucius*) and pikeperch (*Sander lucioperca*) feeding exclusively on fish show 3 to 15 times lower levels of contamination by lipophilic substances than eel (on a muscle wet weight basis) dependent on the specific contaminant, due mainly to their significant lower muscle lipid contents (ca 0.5%) (Goemans, pers. comm.). Interspecific differences in contamination load within several field studies have been attributed to differences in lipid content (for an overview see Nowell *et al*., 1999). Amongst the various biota, eel has particular analytical advantages due to its very high fat content: Maes *et al*.

(2008) reported a mean muscle lipid content of 14.92% ± 10.18 (SD) in 2528 yellow eels collected over Flanders. High lipid content in eels is partly responsible for the high bioaccumulation of lipophilic contaminants in their tissues.

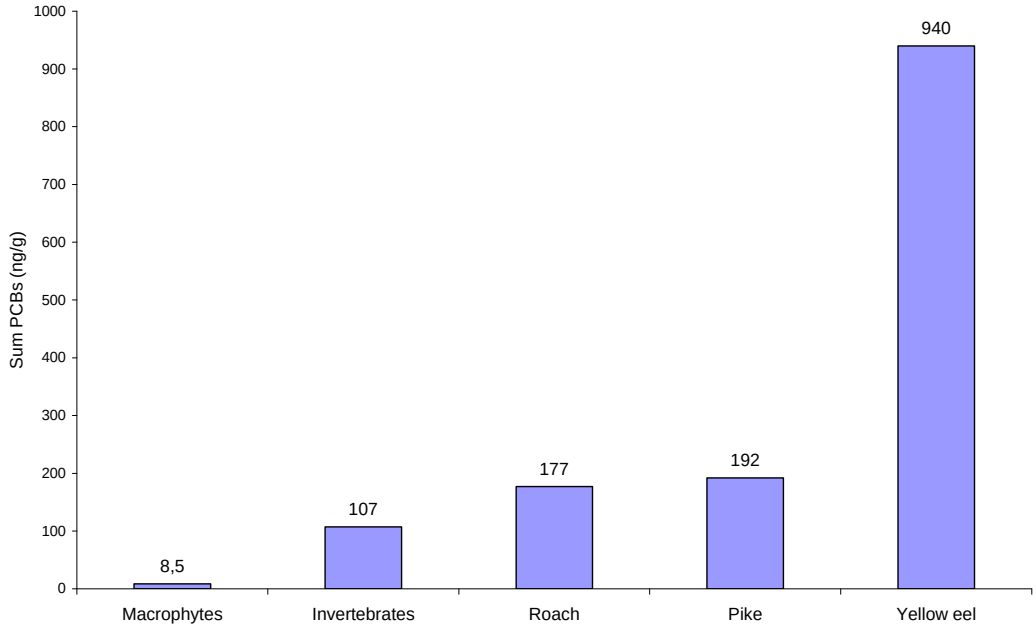


Figure 13.3. Concentration of Sum PCBs over various trophic levels in Lake Weerde (Flanders) in 2001 (spring). Data expressed as ng/g total wet weight for macrophytes and invertebrates and as ng/g wet weight of muscle tissue for fish (from Weltens *et al*., 2002). Fish analysis was performed on muscle tissue samples (N = 5 for roach and pike, N = 10 for yellow eel).

Figure 13.3 illustrates the concentrations of PCBs measured in various biota. Lipophilic contaminants like PCBs seem to be five times higher than in other fish species (on a muscle wet weight basis) and ten times higher in eel than in invertebrates (on a total wet weight basis), as can be deduced from measurements in Lake Weerde, a shallow contaminated lake in Flanders (Weltens *et al.*, 2002). Consequently tracing of these chemicals in eel, as an environmental indicator, is particularly meaningful, since only few fall under DL. From the results of the EPMN including quantitative data of 2946 eels collected from 365 sites between 1994-2005, it is clear that most of the PCBs, OCPs and heavy metals analysed are easily detectable. Figure 13.2 represents the proportion of eels above DL for the PCBs and OCPs. Of the higher chlorinated PCBs 99.0 - 100% are above DL, while for the lower chlorinated PCBs 28, 31 and 52, the proportion is slightly lower (90.2 - 97.3). For the OCPs the situation is more variable. Very high proportions (> 98%) are noticed for the γ isomer of hexachlorocyclohexane (gamma-HCH), hexachlorobenzene (HCB) and *p,p'*-DDE (1,1'-(2,2-dichlor-ethenylidene)- bis[4-chlorobenzene]). Also alfa-HCH, dieldrin and *p,p'*-DDD (1,1'-(2,2 dichloroethylidene)bis [4-chlorobenzene]) can be detected in at least 8 out of 10 samples. *P,p'*-DDT (dichlorodiphenyltrichloroethane) and trans-Nonachlor (T-nona) can be measured in more than 50% of the eels. The cyclodienes endrin and aldrin are obviously less common in Flanders and can be measured in 13 and 43% of the cases respectively.

Heavy metals were also measurable for the majority of sites. Mercury, zinc, chromium, arsenic and selenium were detectable in more than 96% of the samples. Cadmium, lead, cupper and nickel were measured in 60 to 90% of the samples. Similarly, brominated flame retardants and even a number of volatile organic compounds were described as omnipresent in eels (Belpaire and Goemans, 2007). Chemicals like HBCD (hexabromocyclododecane), PBDEs (polybrominated diphenylethers) and the volatile organic compounds BTEX (benzene, toluene, ethylbenzene and the xylenes) were found in all samples. This is in contrast with measurements in the water phase (as proposed by the WFD): as most of these compounds are lipophilic, measurements in water are frequently under the DL e.g. PCBs and VOCs are hardly traceable in water. For the VOCs this was documented by Belpaire and Goemans (2007). Even in sediments, the presence of PCBs and VOCs is quite often under the DL.

Another advantage of using eels as a chemical bioindicator is their size. Eels are long-lived and their size enables to obtain enough material for analysis of various contaminants in individual fish. An individual eel of 40 cm has a back-calculated weight of 110 g, allowing removal of enough muscle tissue for at least six samples (10 g wet weight each) to be labelled and frozen at - 20℃. In the EPMN two samples (from the mid part of the body) were analysed for heavy metals, OCPs and PCBs. Other samples can be sent to specialised laboratories and analysed for BFRs, VOCs, dioxins, … The remaining samples are routinely stored as back up in a tissue bank at -20℃.

From bioaccumulation studies in other fish species, it is known that the concentrations of lipophilic contaminants are related to length, weight or age, biological factors which are mostly covariant. Furthermore, length and age tend to correlate positively with lipid content. The relation between level of contamination and length or age is not always clearly positive : e.g. Reinert and Bergman (1974) described increasing DDT concentration with length in lake trout and in coho salmon from Lake Michigan, whereas in some other studies (e.g. Hubert and Ricci, 1981) effects related to size or age were smaller or nonexistent when contaminant concentrations were expressed on a lipid weight basis (Nowell *et al*., 1999). Size and age effects may vary depending of the contaminant. During a recent study assessing the contaminants in muscle of white perch (*Morone americana*) from Hackensack River (New Jersey, USA), Weis and Ashley (2007) found no significant correlations between PCB concentrations and length or weight. However for mercury a significant correlation for both length and weight was observed. For environmental monitoring purposes it should be recommended that the size of the eels sampled be standardised as much as possible. Sample selection within the EPMN focuses on eels between 35 and 45 cm, thereby precluding possible sex-related bias. We are well aware that for other monitoring purposes, like monitoring eel quality within the eel restoration plans or monitoring for human consumption quality (see below), it may be more appropriate to analyse eels from larger sizes, as these may attain higher contaminant concentrations.

Eels as chemical bioindicators of the contaminant pressure of their habitat

As was described earlier (Belpaire and Goemans, 2007) a sentinel species should be fairly sedentary to allow fingerprinting of the local pollution load. Yellow eels show explicit homing behaviour and foraging movements are mostly restricted to a few hundred meters. Apparently most eel species share this ecological trait (*A. anguilla*: Baras *et al.*, 1998; Laffaille *et al.*, 2005; *A. rostrata*: Oliveira, 1997; Goodwin, 1999; *A. australis*: Jellyman *et al.*, 1996; *A. dieffenbachi:* Beentjes and Jellyman, 2003*; A. japonica*: Aoyama *et al.*, 2002). Although home site fidelity is obvious also within tidal estuaries, the home range may be larger than in freshwater habitats

(Parker, 1995) and seasonal movements might occur (Hammond, 2003). The occurrence of erratic eels ('nomads') has also been reported (Feunteun *et al.*, 2003). Due to the migration activities in the silver eel stage, the bioindicator value of the eel is restricted to the yellow eel phase.

The potential of the eel to fingerprint the pollutant pressure at a specific site can be illustrated by several examples from within the EPMN. Belpaire and Goemans (2007) have illustrated with a number of examples (1,2-dichlorobenzene, 1,2-dibromo-3-chloropropane, BTEX, HBCD (hexabromocyclododecane), PBDEs (polybrominated diphenylethers), cadmium and lindane) the possibility of discovering environmental contamination through eel biomonitoring. They related high levels of specific contaminants in eel with local industrial or agricultural activities.

The EPMN covers a dense network of 365 sampling sites; each site is characterized by a series of ca. 30 chemicals for each individually analysed eel. This dataset allowed us to show how local land use at each site characterizes the pollution profile within eel muscle tissue. Belpaire *et al.* (1999) illustrated the usefulness of using eels as a sentinel species for measuring pollution by persistent pollutants. They presented (Figure 13.4) a cluster analysis of the PCB and OCP concentration in 129 yellow eels from 30 sites in Flanders and showed that intra-site variability between eels is generally lower than the inter-site variability. On the basis of their contaminant load, eels from the same location were mostly clustered together (Figure 13.4). The pollution profile of individual yellow eels from one site seems to be a fingerprint of the local contaminant pressure. Even within water bodies and on a small local scale eels may show variations depending on where they lived. A study on the canal Boudewijnkanaal demonstrated differences in pollution load in eels within the canal (Belpaire *et al.*, 1999). The Boudewijnkanaal is relatively short (14 km) and situated in the northwest of Flanders, mouthing in the North Sea at Zeebrugge harbour. The canal was divided into three zones each ca.4 km long : zone A which included the southernmost part nearby Brugge, zone B being the intermediate zone nearby Dudzele and zone C the northern part of the canal in front of the sea sluices (Zeebrugge) (Figure 13.5). Eels were analysed for PCBs and OCPs in 1991 (8 eels from zone B and 3 eels from zone C) and in 1995 (3 eels from zone A, 6 eels from zone B and 11 eels from zone C). Discriminant analysis (Figure 13.5) of the concentrations of 16 PCBs and OCPs (on a lipid weight basis) between these five groups showed differences between the 1991 and 1995 eels. With the exception of lindane, concentrations of most of the contaminants were higher in 1995 compared to the 1991 levels. Moreover, within a year, very distinct regional variations occurred, with eels from zone A being very distinct from the other zones. Also eels from B and C clearly belonged to separate groups, both in 1995 and in 1991. Differences between zones were explained by differences in local pollution pressure on the canal (with zone A being the most polluted zone). This gives strong evidence that eels do reflect differences in the pollution load of their habitat, even between locations which are relatively close to each other, as it was the case here with the 4 km zones. It also supports the hypothesis that eels are very sedentary.

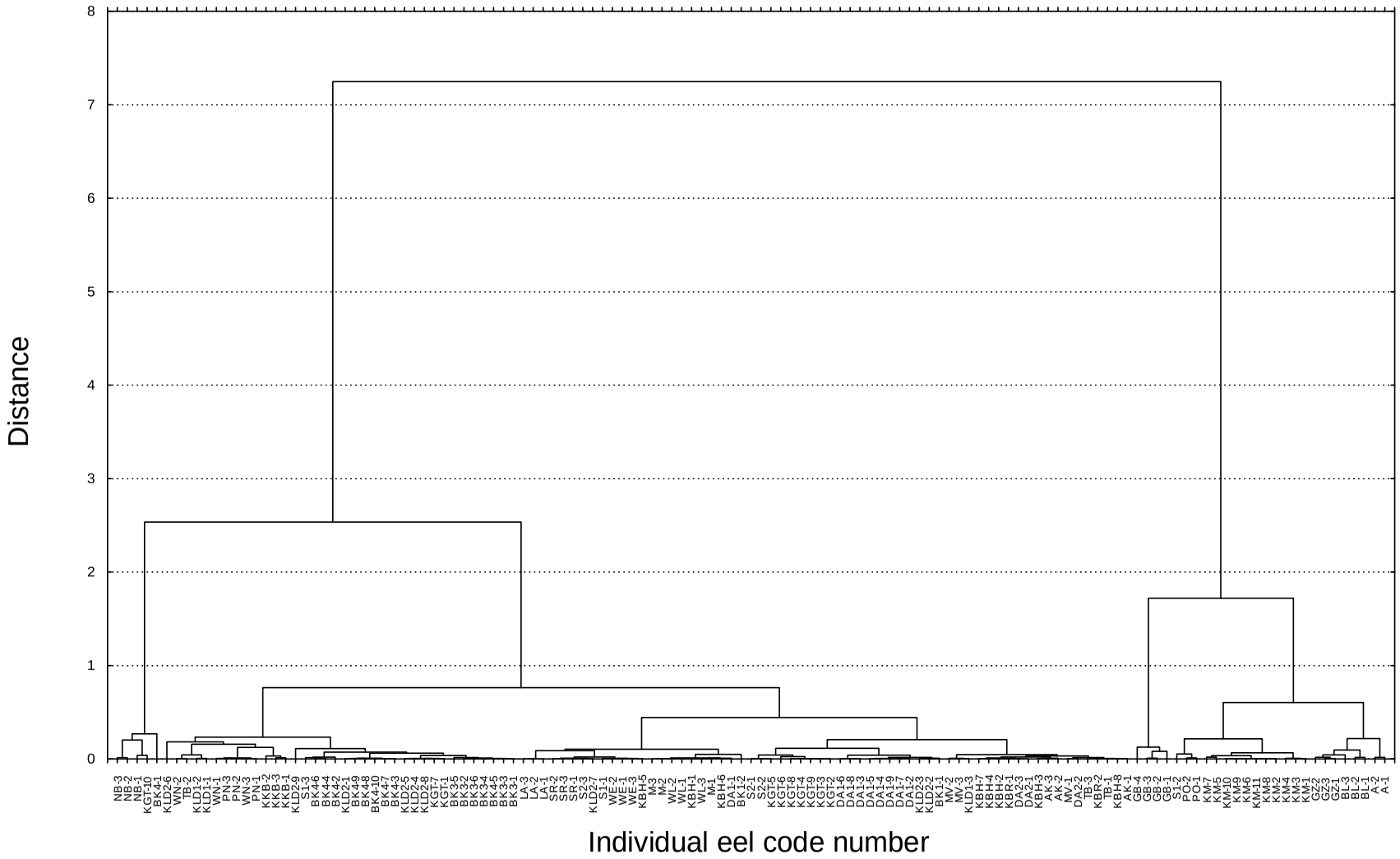


Figure 13.4. Cluster analysis based on the PCB and OCP (lipid weight basis) profiles of 129 yellow eels from 30 sites in Flanders sampled between 1994 and 1998 (Belpaire *et al*., 1999). Eels from the same site cluster mostly together.

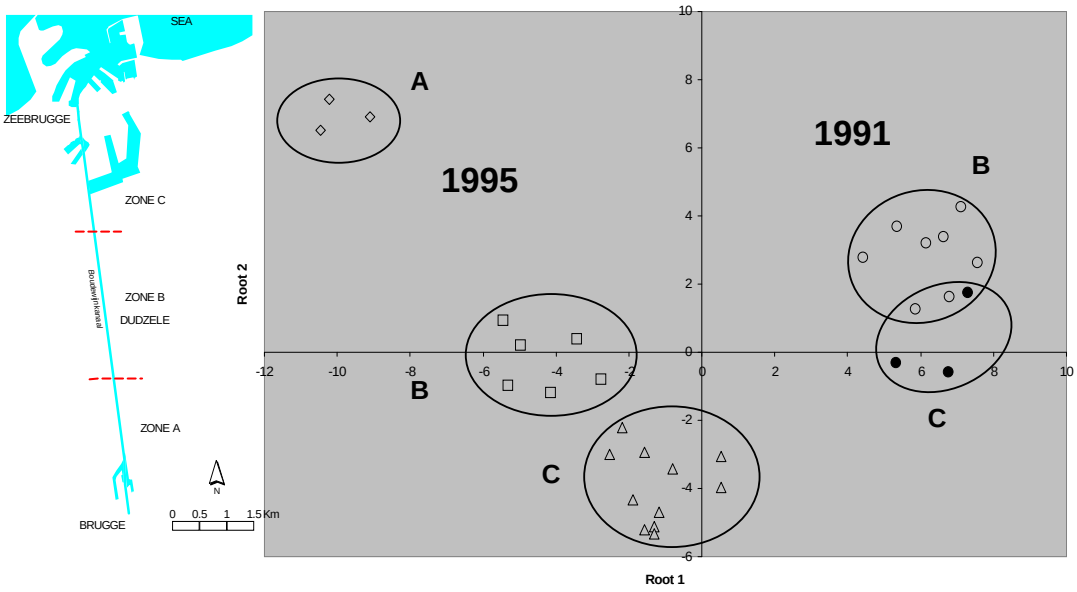


Figure 13.5. Discriminant analysis on the concentrations of 16 PCBs and OCPs (on lipid weight basis) in 31 eels from three zones in the Boudewijnkanaal from 1991 (11 eels) and 1995 (20 eels) (after Belpaire *et al*., 1999). Left: location of the three zones A, B and C on the Boudewijnkanaal.

Other evidence exists for stations within the Meuse river basin, as reported by Goemans and Belpaire (2003), combining data from Flanders and The Netherlands. Goemans and Belpaire (2005) also showed that within the group of the PCBs, congener profiles (e.g. ratio of PCB 118 to Sum PCBs) in eels from a specific location are almost constant, but can vary considerably between eels originating from different locations.

An example within a lacustrine environment has been presented by Belpaire *et al.* (2001). Lake Schulen is a 90 ha eutrophic, oblong lake (length not exceeding 3 km) in central Flanders. 17 eels captured from 4 areas of the lake were analysed individually for polychlorinated biphenyls, organochlorine pesticides and heavy metals. Although no significant differences were found between eels from the different areas for most of the individual pollutants, there seemed to be a variation in overall pollution pattern, as illustrated in the factor analysis in Figure 13.6. The study revealed significant differences in lindane (gamma HCH) concentrations in muscle tissue of eels from different areas. No evidence was found for potential causes of this pollution. This study illustrates the potential of using eel as a monitoring organism for pollution by some persistent substances within lacustrine environments, even within rather small lakes.

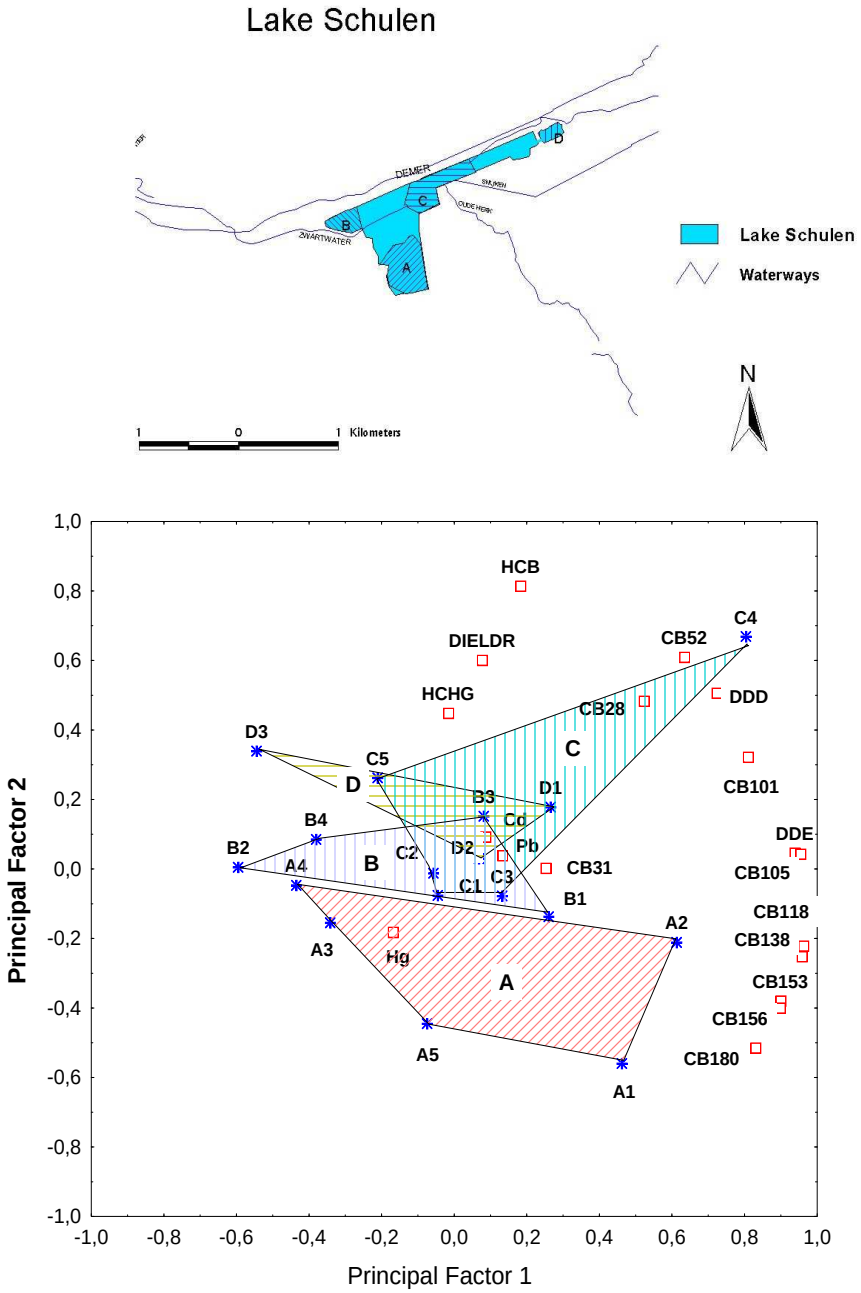


Figure 13.6. Factor analysis of contaminant concentrations (PCBs and OCPs) in 17 yellow eels from the zones A, B, C and D in Lake Schulen. Eels are numbered per zone (A1, A2, …). HCB: hexachlorobenzene, DIELDR: dieldrin, HCHG: γ-hexachlorocyclohexane (lindane), DDD: *p,p'*-DDD (1,1'-(2,2 dichloroethylidene)bis [4-chlorobenzene]), DDE: *p,p'*-DDE (1,1'-(2,2-dichlor-ethenylidene)-bis[4-chlorobenzene]) (Belpaire *et al*., 2001).

Weltens *et al*. (2002) described the results of a study investigating contamination through the various compartments of the aquatic ecosystem. PCBs, heavy metals and pesticides were analysed in water, suspended solids, sediment and biota of different trophic levels on 5 polluted sites in Flanders. Figure 13.7 presents the relationships between Sum PCBs in wet weight of muscle tissue of eel with Sum PCBs in wet weight of muscle tissues of predator fish species and roach and with Sum PCBs on total wet weight basis in invertebrates and macrophytes. Fairly good correlations were found.

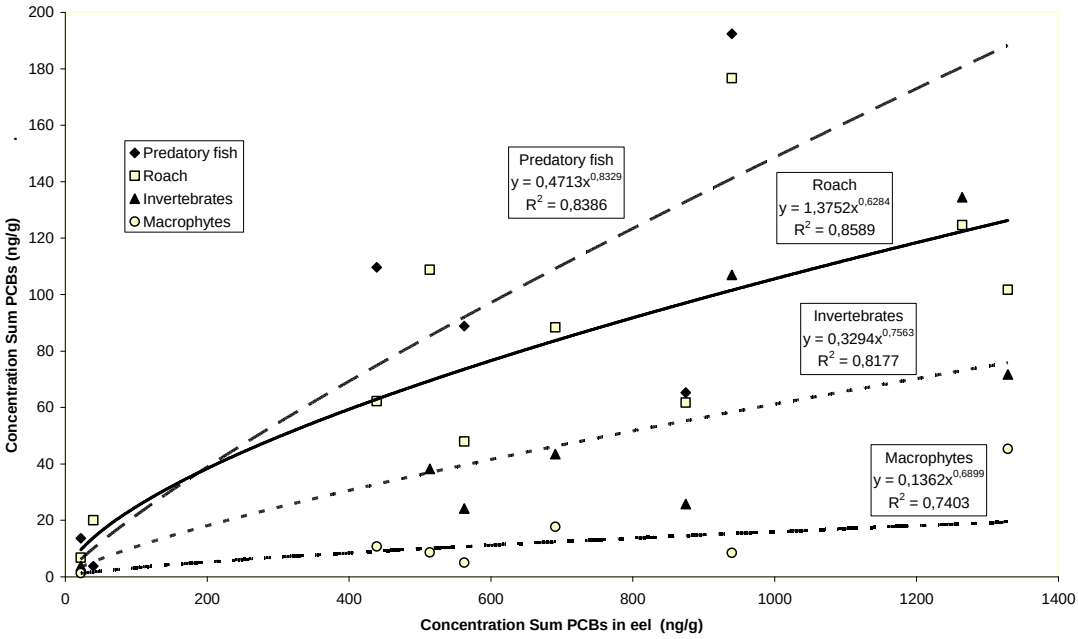


Figure 13.7. Correlation between the concentration of Sum PCBs measured simultaneously in predator fish species, roach, invertebrates and macrophytes, compared to the concentration of Sum PCBs in eel. Data acquired from sampling on five polluted water bodies in Flanders during spring and autumn 2001 (Weltens *et al*., 2002). Concentrations are expressed in ng/g wet weight of muscle tissue for fish and in ng/g total wet weight for invertebrates and macrophytes.

The contaminant fingerprint value of eels has already been illustrated, to some extent, in the 1980's for *A. rostrata* in the St. Lawrence River. Moreau and Barbeau (1982) distinguished eels of different origins on the basis of their heavy metal (Hg) content. Dutil *et al.* (1985) had similar results on the basis of the presence of mirex. They concluded that organic chemicals could be a better instrument for discriminating stocks than heavy metals. In the same region, Castonguay *et al.* (1989) found a relatively high discrimination among eels from various sampling sites based on their contamination level with organochlorines. More recently, many EC countries have reported the use of the European eel to monitor the presence of a variety of substances. Extensive reviews have been made by Bruslé (1990; 1991) for respectively, heavy metals, and OCPs and PCBs. He assembled reports on the bioaccumulation of contaminants within several eel species. Since then, for a whole variety of contaminants, reports on eels as bioindicators have been published all over the world. Knights (1997) made a review of available literature on persistent xenobiotic organochlorines in eel species and Robinet and Feunteun (2002) gave

examples of concentrations of some pollutants in yellow European and American eel. In Table 13.2 we summarize reports published recently for the EC countries. In some countries like The Netherlands and Belgium, a nationwide monitoring network is operational (respectively since 1977 and 1994). In other countries like Sweden, Finland, Denmark, Germany, United Kingdom and Northern Ireland, France, Spain and Italy, eel biomonitoring studies have been undertaken on a local scale. In Ireland investigations are in progress.

Table 13.2 shows that a whole variety of contaminants were analysed. The PCBs, OCPs and heavy metals are the most commonly analysed contaminants. Lately, groups of brominated flame retardants and dioxins are being analysed more frequently, illustrating the increasing concern for these compounds, and following the new EU dioxin regulation in foodstuffs (CEC, 2006e). Locally, other contaminants have been analysed within specific research programs (polycyclic aromatic hydrocarbons, volatile organic compounds, synthetic musks, perfluorinated compounds, metallothioneins …).

It is remarkable that, until now no pan-European comprehensive reports are available on the chemical status of the eel, considering the increasing number of recent papers that point towards chemicals as being responsible for the decline of the eel. Two studies have compared bioaccumulation data in eels from several countries with allowable values for human consumption: Karl and Lehmann (1993) reported on OCPs and PCBs in 54 eel samples, both wild and farmed, from 11 different countries, and van Leeuwen *et al.* (2002) compared PCBs, dioxins and furans in wild and farmed eels from The Netherlands, and in imported eels from 7 countries. More recently, two Europe-wide studies have been presented by Greenpeace using the eel as a bioindicator of brominated flame retardants and PCBs from rivers and lakes in 10 European countries (Santillo *et al.*, 2005) and of perfluorinated chemicals in 11 countries (Santillo *et al.*, 2006). These studies were however rather restricted with respect to the number of eels or sites analysed.

Table 13.2. Overview of recent reports describing bioaccumulation data of various chemicals in *Anguilla anguilla* within EC countries (PCBs: polychlorinated biphenyls, OCPs: organochlorine pesticides, HM: heavy metals, DIO: dioxines, BFRs: brominated flame retardants, PAHs: polyaromatic hydrocarbons, VOCs: volatile organic compounds, PFCs: perfluorinated compounds, MT: metallothioneins).

Reference	Country	PCBs	OCPs	HM	DIO	BFRs	PAHs	VOCs	PFCs	MT	Specification
van Leeuwen *et al*., 2002	EU	x			x						Wild, farmed, imported eels (8 countries)
Karl and Lehmann, 1993	EU	x	x								54 eels from 11 countries
Santillo *et al*., 2005	EU	x				x					10 countries, PBDEs, HBCD, TBBP-A
Santillo *et al*., 2006	EU								x		11 countries
Goemans *et al*., 2003	BE	x	x	x							Country wide (Flanders)
Maes *et al*., 2005	BE			x							Country wide (Flanders)
Hoff *et al*., 2005	BE								x		Country wide (Flanders)
Roose *et al*., 2003	BE							x			Country wide (Flanders)
Belpaire *et al*., 2003	BE					x					Country wide (Flanders)
Thomé *et al*., 2004	BE	x			x						Country wide (Walloonia)
Batty *et al*., 1996	FR			x							Camargue
Roche *et al*., 2002	FR		x				x				Camargue
Roche *et al*., 2003	FR	x	x				x				Camargue
Bragigand *et al*., 2006	FR					x					PBDEs in Seine and Loire
Oliveira Ribeiro *et al*., 2005	FR		x	x			x				Camargue Reserve

Table 13.2. Continued

Reference	Country	PCBs	OCPs	HM	DIO	BFRs	PAHs	VOCs	PFCs	MT	Specification
Jørgensen *et al.,* 2001	DK	x	x	x							Market eels
Food Standards Agency, 2004	UK					x					Skerne – Tees River System
Edwards *et al*., 1999	UK			x							River Yare and Ormesby Broad
Mason and Barak, 1990	UK			x							11 rivers
Mason, 1993	UK	x	x								11 reedbeds
Weatherley *et al*., 1997	UK	x	x								41 sites (Wales)
Ruddock *et al*., 2003	UK						x				Estuaries
Langston *et al*., 2002	UK									x	Thames estuary
Pieters *et al*., 2004	NL	x	x	x							Country wide
Hendriks and Pieters, 1993	NL	x	x	x							Rhine
de Boer and Hagel, 1994	NL	x									Country wide
van der Oost *et al*., 1996a&b	NL	x	x		x		x				PCBs, DDTs, HCB, PAHs, PCDFs and PCDDs in six Amsterdam freshwater sites
van den Heuvel-Greve *et al*., 2006	NL	x			x	x	x		x		Western Scheldt
Tulonen and Vuorinen, 1996	FI	x	x								Vanajavesi watercourse
Fromme *et al*., 1999	DE	x									synthetic musks; bromocyclene, Berlin
Wiesmüller and Schlatterer, 1999	DE	x			x						Rivers Havel and Oder in Brandenburg

Table 13.2. Continued

Reference	Country	PCBs	OCPs	HM	DIO	BFRs	PAHs	VOCs	PFCs	MT	Specification
Gaumert *et al.*, 2000	DE	x	x	x							River Elbe
Lehmann *et al*., 2005	DE				x						Nordrhein-Westfalen, 7 sites
Lehmann *et al*., 2006	DE	x	x	x	x						River Rhine, regular sampling
Linde *et al.,* 2004a	ES			x							Rivers
Linde *et al.,* 2004b	ES			x						x	
Usero *et al.,* 2004	ES			x							Salt marshes
Ankarberg *et al*., 2004	SE	x			x						PCDD/Fs and PCBs Baltic Sea
Poole and McCarthy, 2006	IR	x			x	x					Work under way
Corsi *et al*., 2005	IT	x	x								Orbetello lagoon
Bressa *et al.,* 1997	IT	x	x								Po delta
Mariottini *et al*., 2005	IT					x					PBDEs, Orbetello lagoon
Mariottini *et al*., 2006	IT	x									Orbetello and Santa Giusta lagoons
Storelli *et al*., 2007	IT	x	x	x							Lesina lagoon, Adriatic Sea

Eel contaminant quality classes and standards.

Analyses of a series of chemicals generates a database of quantitative data which have to be interpreted. There is a strong need for a normative framework with clear benchmarks to which the data should be compared. This framework can consist of various types of benchmarks. The WFD (CEC, 2006a) proposes 'Environmental quality standards' (EQS), limit concentrations (e.g. in hexachlorobenzene, hexachlorobutadiene and methyl-mercury) which can not be exceeded in 'prey'tissue of biota. No Observed Effect Concentrations (NOEC) have been described for specific chemicals for certain organisms, including eel (see PAN Pesticides Database, 2007). For some compounds (e.g. Hg, Pb, Cd, dioxins, furans and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ), ….) human health safety standards for fish have been set by the European Commission (CEC, 2001; 2006e) or by additional national legislation (e.g. consumption limit for indicator-PCBs for fisheries products in Belgium; Belgisch Staatsblad, 2002), some with special values for eel. In some countries (e.g. The Netherlands), concentrations of some substances in eel are used as environmental tolerance values and action thresholds (ecotoxicological values).

In Flanders, quality classes were developed based on quantitative distribution of the data (means per location) for PCBs, OCPs and heavy metals (Goemans *et al*., 2003). Reference values were fixed for each chemical. These reference values were defined as the 5 percentile value of the means of all sites. A common procedure was used to distinguish four quality classes as a measure of deviation from the reference value, and class boundary values were set. Class limits and reference values for each contaminant are listed in Table 13.3. Class boundary calculations were based on the distribution of the relationship between the recorded values and the reference value. Class 1 represents the '*not deviating'* class (blue colour) with 'unpolluted or low polluted' sites. Sites with a slight to moderate pollution level are classified as class 2 '*slightly deviating*' (green). The more polluted sites are assigned to class 3 '*deviating'* (yellow) or 4 '*strongly deviating'* (red).

In Figures 13.8 and 13.9, an example is given of a cartographic and graphic representation of the distribution of Sum PCBs in eel. Figure 13.9 indicates that, of a total of 351 sites, only 21% of the sites are relatively clean, while 57% of the sites are polluted and assigned to classes 3 or 4 (*deviating* or *strongly deviating* from the reference value). The map shows that most of the unpolluted or low polluted sites are located in the Yser basin, which is mainly characterized by agricultural land use.

In order to allow general status reports, more condensed reporting can be achieved by representing a combination of varieous chemicals e.g. within a region or as a function of time. This has been done in the annual state of the environment and the nature reports of Flanders. An example is given in Figure 13.10 (Peeters *et al.*, 2006). These representations are useful for showing temporal changes or spatial variation in environmental and biotic quality.

For Sum PCBs, possible management objectives and benchmarks have been proposed by Belpaire and Goemans (2004) and are illustrated in Figure 13.11. Action and target threshold values are proposed at 460 and 183 ng/g wet weight respectively. The action threshold can be seen as a limit which never may be exceeded; sites above this limit should be sanitized. The target threshold is the objective to attain within a planned timeframe.

Table 13.3. Reference values and boundary values of the quality classes for a series of heavy metals, PCB congeners and organochlorine pesticides as defined in the EPMN. Values are expressed in $ng.g^{-1}$ wet weight of muscle tissue, unless indicated as [1] in $ng.g^{-1}$ lipid weight or [2] in $\mu g.g^{-1}$ wet weight of muscle tissue. C: concentration.

Contaminant	Reference value (RV)	Not deviating log C/RV < 0.4	Slightly deviating 0.4 ≤ log C/RV < 0.8	Deviating 0.8 ≤ log C/RV < 1.2	Strongly deviating log C/RV ≥ 1.2
Mercury	40	< 100	100 - < 252	252 - < 634	≥ 634
Cadmium	2	< 5	5 - < 12.6	12.6 - < 31.7	≥ 31.7
Lead	10	< 25	25 - < 63	63 - < 158	≥ 158
Cupper[2]	0.25	< 0.6	0.6 - < 1.6	1.6 - < 4	≥ 4
Zinc[2]	14	< 35	35 - < 88	88 - < 222	≥ 222
Nickel	14	< 35	35 - < 88	88 - < 222	≥ 222
Chrome	96	< 241	241 - < 606	606 - < 1521	≥ 1521
Arsenic	41	< 103	103 - < 259	259 - < 650	≥ 650
Selenium	205	< 515	515 - < 1293	1293 - < 3249	≥ 3249
PCB 28	0.12	< 0.3	0.3 - < 0.8	0.8 - < 1.9	≥ 1.9
PCB 31	0.1	< 0.3	0.3 - < 0.6	0.6 - < 1.6	≥ 1.6
PCB 28+31	0.25	< 0.6	0.6 - < 1.6	1.6 - < 4	≥ 4
PCB 52	1	< 2.5	2.5 - < 6.3	6.3 - < 15.8	≥ 15.8
PCB 101	2.5	< 6	6 - < 16	16 - < 40	≥ 40
PCB 105	1.2	< 3	3 - < 7.6	7.6 - < 19	≥ 19
PCB 118	3.5	< 9	9 - < 22	22 - < 55	≥ 55
PCB 138	7.7	< 19	19 - < 49	49 - < 122	≥ 122
PCB 153	10	< 25	25 - < 63	63 - < 158	≥ 158
PCB 156	0.6	< 1.5	1.5 - < 3.8	3.8 - < 9.5	≥ 9.5
PCB 180	4.5	< 11	11 - < 28	28 - < 71	≥ 71
Sum PCBs	29	< 73	73 - < 183	183 - < 460	≥ 460
Sum PCBs[1]	240	< 603	603 - < 1514	1514 - < 3804	≥ 3804
α-HCH	0.05	< 0.1	0.1 - < 0.3	0.3 - < 0.8	≥ 0.8
γ-HCH	1.3	< 3.3	3.3 - < 8.2	8.2 - < 20.6	≥ 20.6
Dieldrin	1.1	< 2.8	2.8 - < 6.9	6.9 - < 17.4	≥ 17.4
HCB	0.5	< 1.3	1.3 - < 3.2	3.2 - < 7.9	≥ 7.9
p.p'-DDD	2.5	< 6	6 - < 16	16 - < 40	≥ 40
p.p'-DDT	0.005	< 0.01	0.01 - < 0.03	0.03 - < 0.08	≥ 0.08
p.p'-DDE	13	< 33	33 - < 82	82 - < 206	≥ 206
Sum DDTs	16	< 40	40 - < 101	101 - < 254	≥ 254

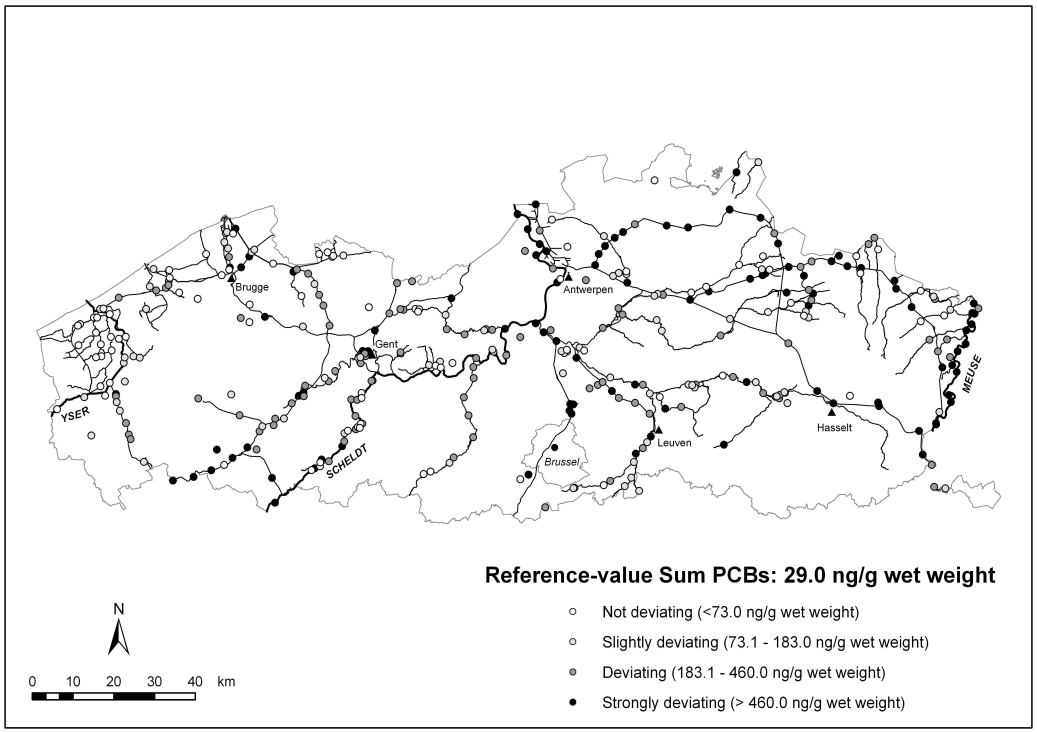


Figure 13.8. Sampling sites of the Eel Pollutants Monitoring Network in Flanders and geographical distribution of quality classes in Flemish eels for Sum PCBs (N = 351 sites, 1994-2005). Reference value and quality class boundaries are given. Sum PCBs equals the sum of the 7 indicator congeners (CB 28, 52, 101, 118, 138, 153 and 180).

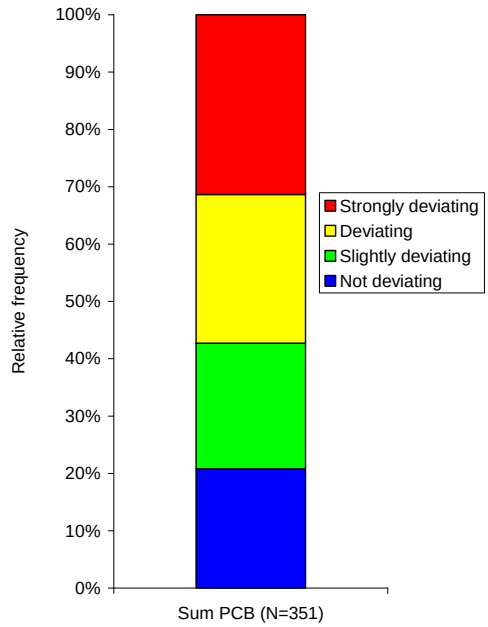


Figure 13.9. Distribution of Sum PCB quality classes in Flemish eels (N = 351 sites, 1994-2005). See Table 13.3 or Figure 13.8 for reference values and boundary values of the quality classes. At 57.2% of the sites, PCB levels in eels are *deviating* or *strongly deviating* from the reference value.

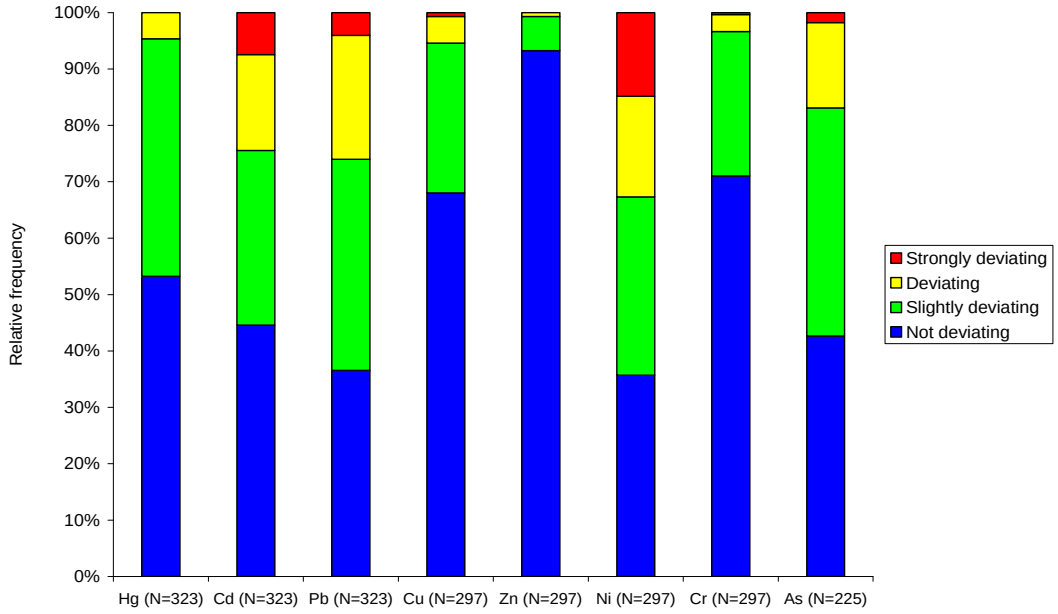


Figure 13.10. Status of heavy metals in eel in Flanders (after Peeters *et al*., 2006 in Flanders environmental report 2006). Data distribution is based on the means per site sampled between 1994 and 2005; the number of sites is indicated. See Table 13.3 for reference values and boundary values of the quality classes of the heavy metals.

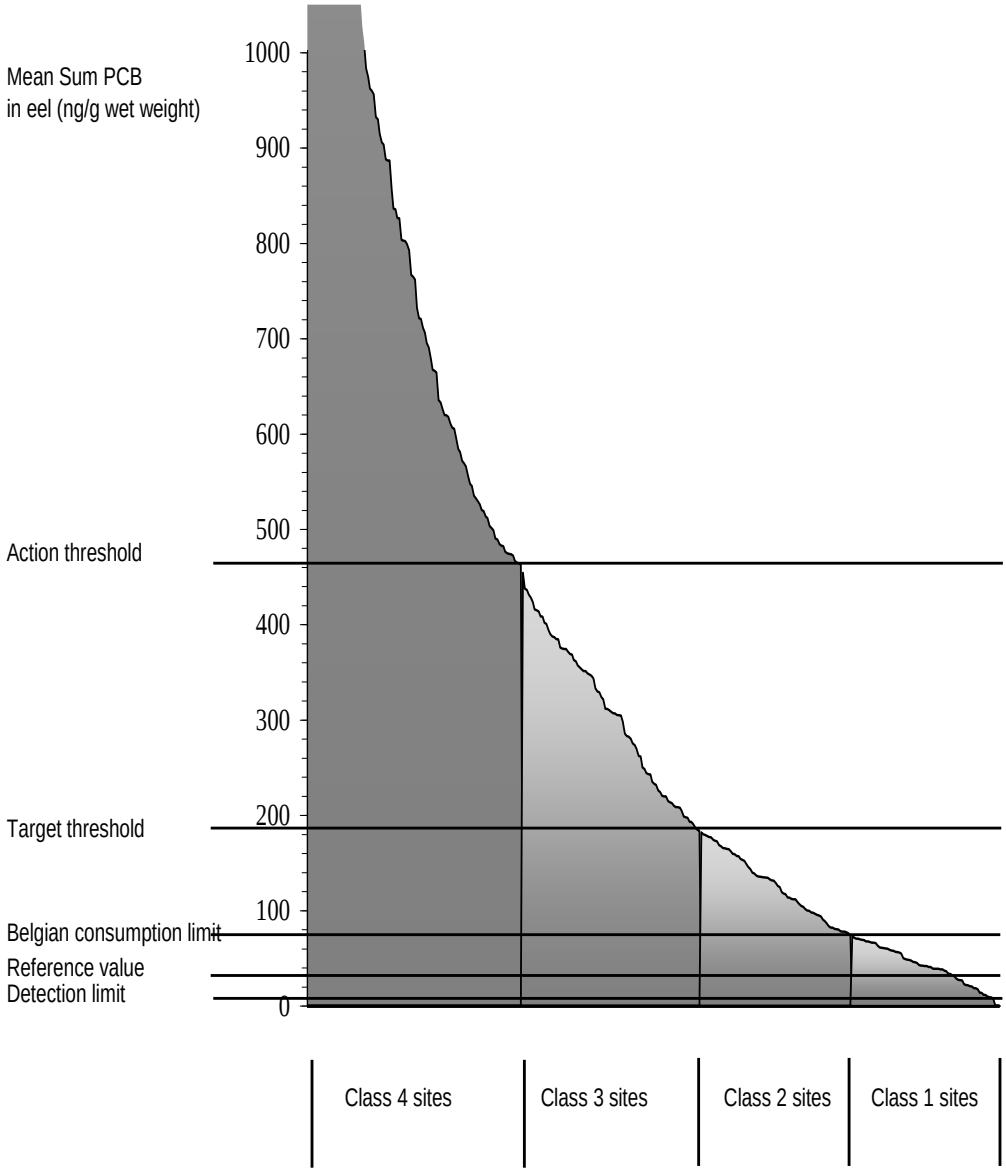


Figure 13.11. Mean Sum PCB values in eel from 351 sites in Flanders (1994-2005): distribution between quality classes and comparison with threshold values for action or target values as proposed by Belpaire and Goemans (2004). Detection limit (2 ng.g^{-1} wet weight), reference value (29 ng.g^{-1} wet weight) and the Belgian consumption limit (75 ng.g^{-1} wet weight) are included in the figure.

Eel biomonitoring for evaluating chemical status within the Water Framework Directive

The eel has a wide geographical, pan-European distribution range. It is exceptional that one bioindicator species occurs over such a vast diversity of habitats: the whole river trajectory from source to estuary and even in seawater, but also in canals, lakes, ponds and salt water lagoons. Consequently, eels can be used in reporting the chemical status of all categories of water bodies within the river basin approach of the WFD (rivers, lakes, transitional water bodies, coastal water bodies, artificial or heavily modified water bodies).

We are aware that some methodological problems still exist. Problems related to sampling procedures, laboratory procedures and quality assurance can hamper comparison and harmonisation. Some analytic procedures for the analysis of certain new chemicals will need further development. Nevertheless, from our own work presented in this paper and elsewhere, we are confident that the European eel is a suitable bioindicator species to use throughout its distribution area for monitoring a variety of priority substances in order to evaluate the chemical status of our waters.

In CEC (2006a), the latest amendment to the WFD (CEC, 2000), 33 substances or groups of substances were selected as priority substances, some of them of very high concern and identified as 'priority hazardous substances'. These include some existing chemicals, plant protection products, biocides, metals and other groups like polyaromatic hydrocarbons (PAHs) and some polybrominated biphenylethers (PBDEs). Another 8 pollutants are not on the priority list but fall under the scope of older directives. The environmental objectives of the WFD are to ensure the ecological integrity of aquatic ecosystems and the protection of humans (CEC, 2006b). In this approach, there is definitely a need to have a harmonised basis for assessment, in particular for international river basins (CEC, 2006b). Emphasis is placed on the measurement of these hazardous substances in the water column. It is important to define clear and harmonised standards for priority substances within the most cost-effective and appropriate approach. According to CEC (2006a), there seems to be enough extensive and reliable information on concentrations of priority substances from measurements made in water to provide a sufficient basis to ensure comprehensive protection and effective pollution control. Based on information concerning the toxicity, persistency and bioaccumulation potential of a substance, together with information on what happens to this chemical in the environment, it is possible to determine threshold concentrations to protect people, flora and fauna. This assessment will be based on 'environmental quality standards' (EQS) which are defined as *"the concentration of a particular pollutant or group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment"* (CEC, 2006a). It is recognised that sediment and biota remain important matrices for the monitoring of certain substances by member states in order to assess long term impacts of anthropogenic activity and trends. Furthermore, the member states have to ensure, on the basis of monitoring of the water status carried out in accordance with the WFD, that concentrations of substances listed do not increase in sediment and biota. It has been decided, however, that no EQS would be proposed for sediments and only three for biota (see above).

We found evidence that current legal chemical quality standards for the water column are wholly insufficient to guarantee the health of our aquatic ecosystems. After comparing the levels of contamination in all compartments of several polluted environments, Weltens *et al*. (2003) concluded that legal chemical criteria for the water column are not suitable to protect the health of the aquatic organisms. Simple partition models did not adequately predict the field concentrations in the different compartments nor in biota. We demonstrated that in particular lipohilic substances

are hard to trace in water and the majority of measurements fall under the DL, even on sites where these contaminants attain (very) high levels in fish. Therefore we strongly support the idea that monitoring programmes for lipophilic substances should be focused on biota.

It was admitted by CEC (2006d) that some of the substances are difficult to determine due to the low concentrations and that EQSs based on waterborne exposures are not protective of aquatic invertebrates and fish in all cases. It is stated by CEC (2006d) that monitoring programmes for lipophilic substances should be focused on biota (and possibly sediment). Following CEC (2006c), the biggest obstacle to develop EQS for sediment and biota was the considerable lack of data. Apparently, on the basis of the actual information, it was not possible to derive systematically such EQS for all those priority substances. It was however strongly recommended to produce the required ecotoxicological information for supporting sound EQS at least for these substances. In general CEC (2006d) believes that specific quality standards can and should be developed for sediment and biota. These should be based on direct assessment and monitoring of sediments and biota. Given the biological relevance of sediment and biota standards and the fact that many persistent substances accumulate in these media, CEC (2006c) underlined the priority need to develop the methodologies and gather further data in order to ensure that such EQS can be set in the near future.

We documented the availability of bioaccumulation data for various hazardous substances in one common aquatic organism within EC countries. Countrywide monitoring networks for eel are already in place in some member states and there is additionally a large amount of data available from short term local studies. The data within member states however are widely scattered over research institutes and universities, and not always available to national agencies committed in the WFD reporting.

We may conclude that, at the time being, the WFD urges the monitoring of toxic substances in the aquatic environment to protect aquatic organisms, but fails to present an appropriate model efficient enough to guarantee this protection.

However, monitoring of contaminants in biota and the development of biota based EQS is essential to preserve or restore the ecological integrity of the aquatic environment and the aquatic organisms themselves. Belpaire and Goemans (2007) recommended using eel for monitoring the chemical status of waters within the requirements of the Water Framework Directive. They give details about monitoring WFD substances in eels and the percentage of measurements above DL. In this paper we further discussed the analytical advantages of using eel among other aquatic biota and documented the suitability of this species for tracing local and specific chemical pressures. We provided a normative framework on the basis of the EPMN bioaccumulation data for a number of PCBs, OCPs and heavy metals. We compiled an overview of current monitoring work over the EC. As the eel seems to be a suitable model when monitoring chemical status in aquatic biota, we propose to further compile existing data on a European scale, as a basis to set up eel-based EQS and for further work. We recommend that a comprehensive research and monitoring project should be started and coordinated on a European level. A first initiative has been taken recently by the Working Group on eel (WG Eel, 2007) starting to compile data on contaminants and diseases in eel within an European Eel Quality Database. Twelve countries submitted data on contaminants in eel for inclusion into this database. Monitoring of the quality of eel received increased attention. Countries like The Netherlands and Belgium continue their monitoring programmes on contaminants, whilst other countries have initiated eel quality studies. Preliminary interrogation of the database illustrates the wide variability of contaminants and the presence of 'black spots' over the distribution area of the eel. Such examples highlight the benefits of an eel quality database, and the need for a

harmonised eel quality monitoring network across Europe to feed such a database (WG Eel, 2007).

Using eel as an indicator for the chemical status within the WFD forms the basis for other required monitoring programmes, i.e. the required monitoring of the quality of human foodstuffs (fisheries products) (e.g. CEC, 2001; 2006e) and the sampling for eel quality within the European efforts for the restoration of the species (Data Collection Regulation) as proposed by the Working Group on Eel (WG Eel, 2006) and the Scientific, Technical and Economic Committee for Fisheries of the EC (STECF, 2006) (see Figure 13.12). Of course, by combining sampling procedures and analytic efforts, these monitoring programmes become more cost-efficient and -effective. The set up of a harmonised, Europe wide chemical monitoring programme of eels could stand for triple usage: the evaluation of environmental health and chemical status (national level and WFD level), the sanitary control of fisheries products within human food safety regulations, and the monitoring of eel (spawner) quality within the requirements of the international eel restoration plan and the national Eel Management Plans (STECF, 2006). To this end, it might be envisaged to extend the contaminant monitoring in yellow eel with analysis in silver eel populations from specific locations, to trace the quality of the spawners (e.g. in European basins with high production of spawners), and to measure against food safety standards (e.g. within exploited silver eel stocks).

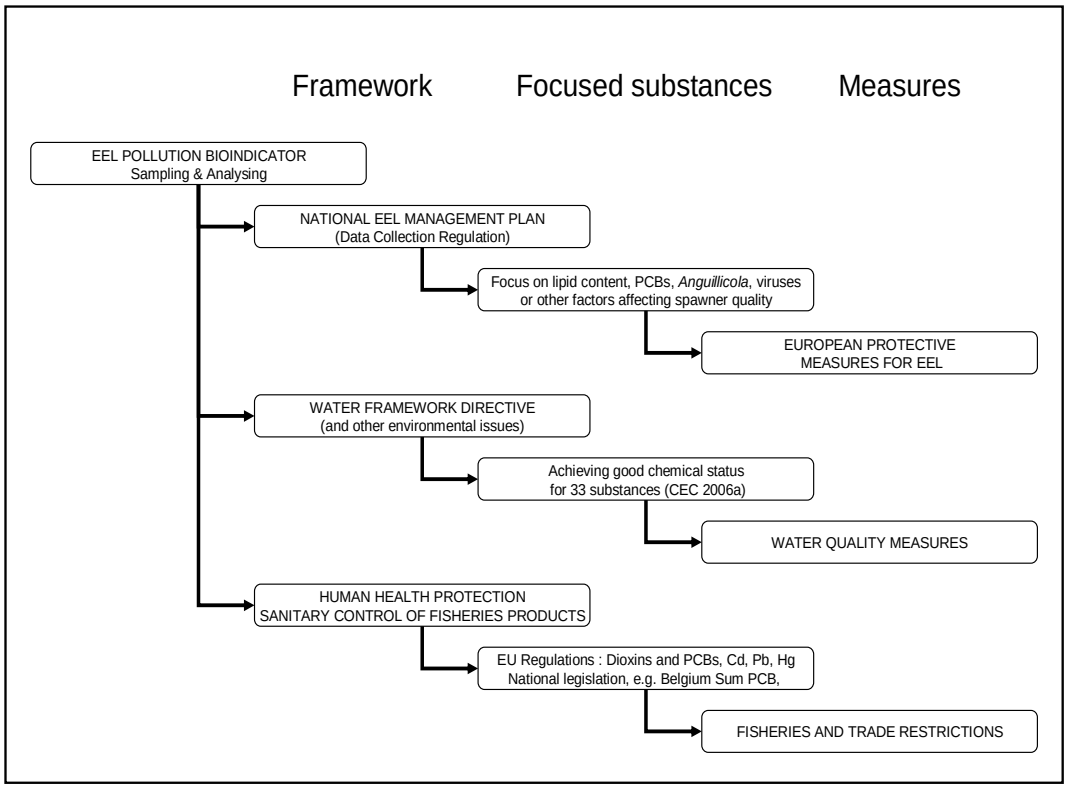


Figure 13.12. Possibilities of combined use of monitoring contaminants in the eel.

Up-scaling the European monitoring strategy of chemicals in the European eel to a worldwide scale seems to be possible. Other eel species occur in other parts of the world, and at least some of them share similar ecological and physiological traits (migration and homing behaviour, trophic position, fat content, …). In the U.S. and Canada (Hodson *et al.*, 1994, Castonguay *et al.*, 1994) and in New Zealand (Buckland *et al.*, 1998) there is already a long history in using anguillids as sentinel species for selected chemicals.

Taking into account the high concentration of some contaminants in certain eel subpopulations (Maes *et al*., 2008; WG Eel, 2007), and the ecotoxicological and reprotoxic effects of these substances (e.g. Maes *et al*., 2005; Palstra *et al*., 2006), the authors believe that achieving good chemical status of EU waters will directly benefit eel restoration efforts. How better to assess the status of its environment, than using the eel itself?

Acknowledgements

We would like to thank all Groenendaal collaborators for technical support in sampling and treatment of eels and Yves Maes and Tom De Boeck for preparing maps and datasets. We very much acknowledge Kieran McCarthy, Tara Higgins, Hugo Verreycken and two anonymous referees for their critical comments on the manuscript.

References

Ankarberg, E., Bjerselius, R., Aune, M., Darnerud, P.O., Larsson, L., Andersson, A., Tysklind, M., Bergek, S., Lundstedt-Enkel, K., Karlsson, L., Törnkvist, A., Glynn, A., 2004. Study of dioxin and dioxin-like PCB levels in fatty fish from Sweden 2000-2002. *Organohalogen Compounds* 66, 2061-2065.

Aoyama, J., Sasai, S., Miller, M.J., Shinoda, A., Nakamura, A., Kawazu, K., Tsukamoto, K., 2002. A preliminary study of the movements of yellow and silver eels, *Anguilla japonica*, in the estuary of the Fukui River, Japan, as revealed by acoustic tracking. *Hydrobiologia* 470, 31-36.

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.

Batty, J., Pain, D., Caurant, F., 1996. Metal concentrations in eels *Anguilla anguilla* from the Camargue region of France. *Biological Conservation* 76, 17-23.

Beentjes, M.P., Jellyman, D.J., 2003. Enhanced growth of longfin eels, *Anguilla dieffenbachii*, transplanted into Lake Hawea, a high country lake in South Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 37, 1-11.

Belgisch Staatsblad, 2002. Koninklijk besluit tot wijziging van het koninklijk besluit van 19 mei 2000 tot vaststelling van maximale gehaltes aan dioxines en polygechloreerde bifenylen in sommige voedingsmiddelen. Brussel, 6 march 2002. Publication 2002-04-16.

Belpaire, C., Van Thuyne, G., Callaars, S., Roose, P., Cooreman, K., Bossier, P., 1999. Spatial and temporal variation in organochlorine pesticide and polychlorinated biphenyl pollution in fresh water aquatic ecosystems in Flanders using the European eel (*Anguilla anguilla* L.) as an indicator. EIFAC/ICES, Working Group on Eel, Silkeborg (DK), 20-25 Septembre 1999.

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., *et al.*, (eds.), Proceedings of the International Symposium 'Advances in Eel Biology', University of Tokyo, 28-30 September 2001, p.170-174.

Belpaire, C., Goemans, G., de Boer, J., Van Hooste, H., 2003.Verspreiding van gebromeerde vlamvertragers. In: van Steertegem, M., (ed.), Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, LannooCampus, Leuven, p.387-395.

Belpaire, C., Goemans, G., 2004. Monitoring en normering van milieugevaarlijke stoffen in paling: bruikbaarheid en relevantie voor het milieubeleid. *Water* 2004, 1-16.

Belpaire, C., Goemans, G., 2007. Eels: contaminant cocktails pinpointing environmental pollution. *ICES Journal of Marine Science* doi:10.1093/icesjms/fsm121

Bragigand, V., Amiard-Triquet, C., Parlier, E., Boury, P., Marchand, P., El Hourch, M., 2006. Influence of biological and ecological factors on the bioaccumulation of polybrominated diphenyl ethers in aquatic food webs from French estuaries. *The Science of the Total Environment* 368 (2006), 615-626.

Bressa, G., Sisti, E., Cima, F., 1997. PCBs and organochlorinated pesticides in eel (*Anguilla anguilla* L.) from the Po Delta. *Marine Chemistry* 58, 261-266.

Bruslé, J., 1990. The effect of heavy metals on eels (*Anguilla* sp). *Aquatic Living Resources* 3, 131-141.

Bruslé, J., 1991. The eel (*Anguilla* sp) and organic chemical pollutants. *Science of the Total Environment* 102, 1-19.

Buckland, S.J., Jones, P.D., Ellis, H.K., Salter, R.T., 1998. Organochlorines in New Zealand: Ambient concentrations of selected organochlorines in rivers. Ministry for the Environment, Wellington (New Zealand), 72p.

Castonguay, M., Dutil, J.D., Desjardins, C., 1989. Distinction between American eels (*Anguilla rostrata*) of different geographic origins on the basis of their organochlorine contaminant levels. *Canadian Journal of Fisheries and Aquatic Sciences* 46, 836-843.

Castonguay, M., Hodson, P.V., Moriarty, C., Drinkwater, K.F., Jessop, B.M., 1994. Is there a role of ocean environment in American and European eel decline? *Fisheries Oceanogr*aphy 3, 197-203.

Corsi, I., Mariottini, M., Badesso, A., Caruso, T., Borghesi, N., Bonacci, S., Iacocca, A., Focardi, S., 2005. Contaminants and sub-lethal toxicological effects of persistent organic pollutants in the European eel *(Anguilla anguilla)* in the Orbetello lagoon (Tuscany, Italy). *Hydrobiologia* 550, 237-249.

CEC, 2000. Directive of the European parliament and of the council 2000/60/EC establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* 22.12.2000 L 327/1.

CEC, 2001. Commission Regulation (EC) 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, 16 March 2001, L77/1.

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. Communication from the Commission to the Council and the European Parliament. Integrated prevention and control of chemical pollution of surface waters in the European Union {SEC(2006) 947} {COM(2006) 397 final} Commission of the European Communities Brussels, 17.7.2006 COM(2006) 398 final.

CEC, 2006c. Commission Staff Working Document *Impact Assessment* 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 {COM(2006) 397 final} {COM(2006) 398 final} Commission of the European Communities, Brussels, 17.7.2006 SEC(2006) 947.

CEC, 2006d. 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 Commisssion, Health & Consumer Protection Directorate-General, Directorate C – Public Health and Risk Assessment C7 – Risk Assessment Brussels, C7/GF/csteeop/WFD/280504 D(04).

CEC, 2006e. Commission Regulation (EC) 199/2006 of 3 February 2006 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. *Official Journal of the European Communities* 04.02.2006 L 32/34.

de Boer, J., Hagel, P., 1994. Spatial differences and temporal trends of chlorobiphenyls in yellow eel (*Anguilla anguilla*) from inland waters of the Netherlands. *Science of the Total Environment* 141, 155-174.

Durrieu, G., Maury-Brachet, R., Girardin, M., Rochard, E., Boudou, A., 2005. Contamination by heavy metals (Cd, Zn, Cu, and Hg) of eight fish species in the Gironde estuary (France). *Estuaries* 28 (4), 581-591.

Dutil, J.D., Legare, B., Desjardins, C., 1985. Discrimination d'un stock de poisson, l'anguille (*Anguilla rostrata*), basée sur la présence d'un produit chimique de synthèse, le mirex (Discrimination of a fish stock, *Anguilla rostrata*, on the basis of the presence of synthetic chemical product, the mirex). *Canadian Journal of Fisheries and Aquatic Sciences* 42 (3), 455-458.

Edwards, S.C., MacLeod, C.L., Lester, J.N., 1999. Mercury contamination of the eel (*Anguilla anguilla*) and roach (*Rutilus rutilus*) in East Anglia, UK. *Environmental Monitoring and Assessment* 55, 371-387

Feunteun, E., Lafaille, 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.

Food Standards Agency, 2004. Brominated flame retardants in trout and eels from the Skerne-Tees river system and total diet study samples. *Food Surveillance Information Sheet* No. 52/04, April 2004, FSA, London, available at http://www.food.gov.uk/science/surveillance/fsis2004branch/fsis5204

Fromme, H., Otto, T., Pilz, K., Neugebauer, F., 1999. Levels of synthetic musks: bromocyclene and PCBs in eel (*Anguilla anguilla*) and PCBs in sediment samples from some waters of Berlin/Germany. *Chemosphere* 39, 1723-1735.

Gaumert, T., Löffler, J., Bergemann, M., Reincke, H., 2000. Schadstoffe in Elbefischen. Belastung und Vermarktungsfähigkeit. Arbeitsgemeinschaft für die Reinhaltung de Elbe, 103p.

Goemans, G., Belpaire, C., 2003. The Flemish eel monitoring network: PCB-concentrations in eel from the Meuse catchment area. Proceedings of the First International Scientific Symposium on the River Meuse, November 27-28, 2002, Maastricht, Netherlands, p.58-62.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollution 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.

Goemans, G., Belpaire, C., 2005. Congener profiles in European eel (*Anguilla anguilla* L.) as a method of tracing the origin of PCB contamination. *Organohalogen Compounds* 67, 1304-1307.

Goodwin, K.R., 1999. American Eel subpopulation characteristics in the Potomac river drainage, Virginia. Thesis Faculty of Virginia Polytechnic Institute and State University. Blacksburg, Virginia, 116p.

Hammond, S.D., 2003. Seasonal movements of yellow-phase American eels (*Anguilla rostrata*) in the Shenandoah River, West Virginia. Thesis. Davis College of Agriculture, Forestry, and Consumer Sciences at West Virginia University, Morgantown WV, 32p.

Hendriks, A.J., Pieters, H., 1993. Monitoring concentrations of microcontaminants in aquatic organisms in the Rhine delta: a comparison with reference values. *Chemosphere* 26, 817-836.

Hodson, P.V., Castonguay, M., Couillard, C.M., Desjardins, C., Pelletier, E., McLeod, R., 1994. Spatial and temporal variations in chemical contamination of American eels, *Anguilla rostrata,* captured in the estuary of the St. Lawrence River. *Canadian Journal of Fisheries and Aquatic Sciences* 51 (2), 464-478.

Hoff, P.T., Van Campenhout, K., de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., Goemans, G., Belpaire, C., Blust, R., De Coen, W., 2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Enviromental Pollution* 137, 324-333.

Hubert, W., Ricci, E.D., 1981. Factors influencing dieldrin and DDT residues from Des Moines Rivers, Iowa, 1977–1980. *Pesticides Monitoring Journal* 15 (3), 111-116.

Jellyman, D.J., Glova, G.J., Todd, P.R., 1996. Movements of shortfinned eels, *Anguilla australis,* in Lake Ellesmere, New Zealand: results from mark-recapture studies and sonic tracking. *New Zealand Journal of Marine and Freshwater Research* 30, 371-381.

Jørgensen, K., Larsen, E.H., Petersen, A., Lund, K.H., Hilbert, G., Andersen, N.L., Hallas-Møller, T., Larsen, J.C., 2001. Chemical contaminants. Food monitoring, 1993-1997. Part 2. Ministry of Food, Agriculture and Fisheries, Danish Veterinary and Food Administration.

Karl, H., Lehmann, I., 1993. Organochlorine residues in the edible part of eels of different origins. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 197, 385-388.

Knights, B., 1997. Risk assessment and management of contamination of eels (*Anguilla* spp.) by persistent xenobiotic organochlorine compounds. *Chemistry and 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.

Langston, W.J., Chesman, B.S., Burt, G.R., Pope, N.D., McEvoy, J., 2002. Metallothionein in liver of eels *Anguilla anguilla* from the Thames Estuary: an indicator of environmental quality? *Marine Environmental Research* 53, 263-293.

Lehmann, J., Stürenberg, F.J., Kullmann, Y., Kilwinski, J., 2005. Umwelt- und Krankheitsbelastungen der Aale in Nordrhein-Westfalen. *LÖBF-Mitteilungen* Nr. 2/2005, 35–40.

Lehmann, J., Stürenberg, F., Ingendahl, D., 2006. Analysis of health status of yellow eel and silver eel of the River Rhine. In: WG EEL 2006. FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea. Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels. Rome, 23–27 January 2006. EIFAC Occasional Paper. No. 38, ICES CM 2006/ACFM:16. Rome, FAO/Copenhagen, ICES. 2006. 352p.

Lelek, A., 1987. The freshwater fishes of Europe. Vol. 9 Threatened fishes of Europe. Aula Verlag Wiesbaden, 343p.

Linde, A.R., Sanchez-Galan, S., Garcia-Vazquez, E., 2004a. Heavy metal contamination of European eel (*Anguilla anguilla*) and brown trout (*Salmo trutta*) caught in wild ecosystems in Spain. *Journal of Food Protection* 67(10), 2332-2336.

Linde, A.R., Sanchez-Galan, S., Klein, D., Garcia-Vazquez, E., Summer, K.H., 2004b. Metallothionein and heavy metals in brown trout (*Salmo trutta*) and European eel (*Anguilla anguilla*): a comparative study. *Ecotoxicology and Environmental Safety* 44 (2), 168-173.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology* 73, 99-114.

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.

Mariottini, M., Bianchini, A., Focardi, S., 2005. Distribution and profile of polybrominated diphenyl ethers in European eel (*Anguilla anguilla*) from a Mediterranean coastal lagoon (Orbetello, Italy). *Organohalogen Compounds* 67, 467-470.

Mariottini, M., Corsi, I., Focardi, S., 2006. PCB levels in European eel (*Anguilla anguilla*) from two coastal lagoons of the Mediterranean. *Environmental Monitoring and Assessment* 117, 519-528.

Mason, C.F., Barak, N.A.E., 1990. A catchment survey for heavy metals using the eel (*Anguilla anguilla*). *Chemosphere* 21, 695-699.

Mason, C.F., 1993. Organochlorine pesiticide residues and PCBs in eels *Anguilla anguilla* from some British reedbeds. *Chemosphere* 26, 2289-2292.

Moreau, G., Barbeau, C., 1982. Les métaux lourds comme indicateurs d'origine géographique de l'anguille d'Amérique (*Anguilla rostrata). Canadian Journal of Fisheries and Aquatic Sciences* 39, 1004-1011.

Morris, S., Allchin, C.R., Zegers, B.N., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P.E.G., Van Leeuwen, S.P.J., de Boer, J., 2004. Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environmental Science and Technology* 38, 5497-5504.

Nowell, L.H., Capel, P.D., Dileanis, P.D., 1999. Pesticides in stream sediment and aquatic biota. Distribution, trends, and governing factors. Boca Raton, Fla., CRC Press, Pesticides in the Hydrologic System series, v. 4, 1040p.

Oliveira, K., 1997. Movements and growth rates of yellowphase American eels in the Annaquatucket River, Rhode Island. *Transactions of the American Fisheries Society* 126, 638-646.

Oliveira Ribeiro C.A., Vollaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and

heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. *Aquatic Toxicology* 74, 53-69.

Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., van den Thillart, G.E.E.J.M., 2006. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93, 145-148.

PAN Pesticides Database, 2007. Chemical Toxicity Studies on Aquatic Organisms. Available at http://www.pesticideinfo.org/List_AquireAll.jsp?Species=574&Effect=&offset=0

Parker, S.J., 1995. Homing ability and home range of yellowphase American eels in a tidally dominated estuary. *Journal of the Marine Biological Association of the United Kingdom* 75, 127-140.

Peeters, B., De Cooman, W., Vos, G., Gabriels, W., Martens, K., Timmermans, G., Van Hoof, K., Breine, J., Van Thuyne, G., Goemans, G., Belpaire, C., Geeraerts, C., 2006. Oppervlaktewater: Biologische kwaliteit nog steeds ondermaats. In: Van Steertegem, M., (ed.), Mira-T 2006 Milieurapport Vlaanderen: Focusrapport. Leuven Lannoo Campus, p.89-104.

Pieters, H., van Leeuwen, S.P.J., de Boer, J., 2004.Verontreinigingen in aal en snoekbaars: monitorprogramma ten behoeve van de Nederlandse sportvisserij 2003. RIVO report C063/04, 20p.

Poole, R., McCarthy, K., 2006. Report on the eel stock and fishery in the Republic of Ireland 2005. In: WG EEL 2006. FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea. Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels. Rome, 23–27 January 2006. EIFAC Occasional Paper. No. 38, ICES CM 2006/ACFM:16. Rome, FAO/Copenhagen, ICES. 2006. 352p.

Reinert, R.E., Bergman, H.L., 1974. Residues of DDT in lake trout (*Salvelinus namaycush*) and Coho salmon (*Oncorhynchus kisutch*) from the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 31(2), 191-199.

Robinet, T., Feunteun, E., 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels*? Ecotoxicology* 11, 265-277.

Roche, H., Buet, A., Ramade, F., 2002. Accumulation of lipophilic microcontaminants and biochemical responses in Eels from the Camargue biosphere reserve. *Ecotoxicology* 11, 155-164.

Roche, N., Buet, A., Ramade, F., 2003. Characterization and validation of ecotoxicological biomarkers in an eel population exposed to persistent organic pollutants in the Vaccares lake, French national reserve of Camargue. *Revue d' Ecologie – La Terre et La Vie* 58, 127-141.

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *Journal of Environmental Monitoring* 5, 876-884.

Ruddock, P.J., Bird, D.J., McEvoy, J., Peters, L.D., 2003. Bile metabolites of polycyclic aromatic hydrocarbons (PAHs) in European eels *Anguilla anguilla* from United Kingdom estuaries. *The Science of the Total Environment* 301, 105-117.

Santillo, D., Johnston, P., Labunska, I., Brigden, K., 2005. Widespread presence of brominated flame retardants and PCBs in eels *(Anguilla anguilla)* from rivers and lakes in 10 European countries. Greenpeace Research Laboratories, Technical Note 12/2005, Greenpeace International, October 2005, 56p.

Santillo, D., Allsopp, M., Walters, A., Johnston, P., Perivier, H., 2006. Presence of perflurionated chemicals in eels from 11 European countries. Investigating the contamination of the European eel with PFCs, substances used to produce non-stick and water-repellant coatings for a multitude of products.

Report Greenpeace Research laboratories Technical Note 07/2006, publ. Greenpeace International, September 2006, 32p.

STECF, 2006. Scientific, Technical and Economic Committee for Fisheries of the Commission of the European Communities, 22nd Report of STECF, draft version of 27 April 2006.

Storelli, M.M., Barone, G., Garofalo, R., Marcotrigiano, G.O., 2007. Metals and organochlorine compounds in eel (*Anguilla anguilla*) from the Lesina lagoon, Adriatic Sea (Italy). *Food Chemistry* 100, 1337-1341.

Thomé, J.P., Bertrand, A., Brose, F., Carabin, O., De Pauw, E., Dukmans, C., Eppe, G., Gaspar, P., Leroy, A., Louvet, M., Maghuin-Rogister, G., Marneffe, Y., Massart, A.C., Philippart, J.C., Rimbaut, G., Scippo, M.L., 2004. Evaluation du niveau de contamination des rivières par les PCBs et les dioxines.Report Université de Liège. Convention avec la Région Wallonne, Ministère de l'Aménagement du Territoire, de l'Urbanisme et de l'Environnement, Engagement n°01/4143, 167p.

Tulonen, J., Vuorinen, P.J., 1996. Concentrations of PCBs and other organochlorine compounds in eels (*Anguilla anguilla* L.) of the Vanajavesi watercourse in southern Finland, 1990–1993. *The Science of the Total Environment* 187, 11-18.

Usero, J., Izquierdo, C., Morillo, J., Gracia, I., 2004. Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain. *Environment International* 29, 949-956.

van den Heuvel-Greve, M.J., Leonards, P.E.G., Vethaak, D., 2006. Food web transfer of dioxins and other organic contaminants in the Western Scheldt: implications for human and environmental health. ICES Annual Science Conference, Maastricht, CM 2006/G:08.

van der Oost, R., Opperhuizen, A., Satumalay, K., Heida, H., Vermeulen, N.P.E., 1996a. Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*): I. Bioaccumulation: biota sediment ratios of PCBs, OCPs, PCDDs and PCDFs. *Aquatic Toxicology* 35, 21-46.

van der Oost, R., Goksøyr, A., Celander, M., Heida, H., Vermeulen, N.P.E., 1996b. Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*) II. Biomarkers: pollution-induced biochemical responses. *Aquatic Toxicology* 36, 189-222.

van Leeuwen, S.P.J., Traag, W.A., Hoogenboom, L.A.P., Booij, G., Lohman, M., Dao Q.T., de Boer, J., 2002. Dioxines, furanen en PCBs in aal. Onderzoek naar wilde aal, gekweekte aal, geïmporteerde en gerookte aal. RIVO Nederlands Instituut voor Visserijonderzoek, IJmuiden, report CO34/02, 34p., 7 annexes.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363-371.

Weatherley, N.S., Davies, G.L., Ellery, S., 1997. Polychlorinated biphenyls and organochlorine pesticides in eels (*Anguilla anguilla* L.) from Welsh rivers. *Environmental Pollution* 95, 127-134.

Weis, P., Ashley, J.T.F., 2007. Contaminants in Fish of the Hackensack Meadowlands, New Jersey: Size, Sex, and Seasonal Relationships as Related to Health Risks. *Archives of Environmental Contamination and Toxicology* 52, 80-89

Weltens, R., Goemans, G., Huyskens, G., Witters, H., Belpaire, C., 2002. Wetenschappelijke onderbouwing voor de normering van polluenten in Vlaamse oppervlaktewateren en selectie van ecologische en exotoxicologische indicatoren voor de waterkwaliteit. Report for VMM, VITO and IBW, IBW.Wb.VR.2002.88.

Weltens, R., Goemans, G., Huyskens, G., Belpaire, C., Witters, H., 2003. Evaluatie van de kwaliteitsnormering voor oppervlaktewater in Vlaanderen: een praktijkstudie. *Water* 2003, 1-9.

Wiesmüller, T., Schlatterer, B., 1999. PCDDs/PCDFs and coplanar PCBs in eel (*Anguilla anguilla*) from different areas of the rivers Havel and Oder in the state of Brandenburg (Germany). *Chemosphere* 38 (2), 325-334.

WG Eel, 2006. FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea. Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels. Rome, 23–27 January 2006. EIFAC Occasional Paper. No. 38, ICES CM 2006/ACFM:16. Rome, FAO/Copenhagen, ICES. 2006. 352p.

WG Eel, 2007. Report of the 2007 Session of the Joint EIFAC/ICES, Working Group on Eels, FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Bordeaux, 3–7 September 2007, EIFAC Occasional Paper No. 38, ICES CM 2007/ACFM:23, Draft, 524p. Available from www.ices.dk/reports/ACFM/2007/WGEEL/WGEEL_07_draft.pdf

May 25th 2002: Flemish authorities set a catch interdiction for eels in Flanders.
January 1st 2006: Flemish authorities released the catch interdiction for eels in Flanders.
June 15th 2006: Walloon authorities set a catch interdiction for eels in Wallonia.

Illustration: Cathy Wilcox

Chapter 14

Towards a European Eel Quality Database

Claude Belpaire[1], Caroline Geeraerts[1], Derek Evans[2], Eleonora Ciccotti[3] and Russell Poole[4]

1 - Research Institute for Nature and Forest, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium
2 - Agri-Food & Biosciences Institute for Northern Ireland, Newforge Lane, Belfast, Northern Ireland
3 - Universita di Roma Tor Vergata, Dipartimento di Biologia, Via della Recerca Scientifica, 00133 Rome, Italy
4 - Marine Institute, Aquaculture & Catchment Management Services, Newport, Co. Mayo, Ireland

This chapter is presented as an unpublished manuscript.

Summary

The stocks of the European eel *Anguilla anguilla* are in decline and there is an increasing awareness that contaminants and/or diseases might be key elements for this decline. Many countries have started compiling data on the quality of eels in their water bodies. Objectives for these monitoring actions are diverse and there is a large amount of information collected by member countries. However, this information is widely scattered over Europe in agencies, institutes and universities. As there is a growing need to collect and report on quality data of the eel, the EIFAC/ICES Working Group on Eel initiated in September 2007 the set up of a European Eel Quality Database to collect recent data of contaminants and diseases over the distribution area of the eel. It represents now the first comprehensive pan-European compilation of eel quality data, including data from over 3500 eels from approximately 550 sites over twelve countries. Preliminary work has indicated a number of shortcomings and future developments will be needed. Guaranteeing further development of the database, harmonisation of methods, quality assurance, and setting up harmonised eel monitoring strategies over Europe will be a great challenge and will need pan-European cooperative work.

Introduction

The stocks of the European eel (*Anguilla anguilla*) are in decline and there is an increasing awareness that contaminants and/or diseases might be key elements for this decline (Robinet and Feunteun, 2002; van den Thillart *et al.*, 2005; Palstra *et al.*, 2006, 2007). The ICES/EIFAC Working Group on Eel recommended the monitoring and collection of information on the pollution and disease status of eels (WG Eel, 2006). During the last decade, many EU member states have collected data on the quality of eels in their water bodies. Such actions are normally focused on identifying the presence of contaminants and diseases in the eel. But the objectives of these national initiatives are quite diverse, ranging from academic epidemiological studies into an individual disease agent, studies to quantify eel spawner quality, monitoring programs for the presence of chemicals in the environment or even analytical work into hazardous substances in eels to determine if safe human consumption limits have been exceeded.

The spatial coverage of sampling is also highly variable and is dependent upon the focus of the study given that some studies are focused on a specific water body, (e.g. to study the biology of a specific eel parasite or to follow a certain pollution problem) whilst others may cover the whole national territory generating nation-wide information.

If the degree of spatial coverage does not guarantee at all a European wide coverage and if in many cases a regular periodical planning of the monitoring actions is not guaranteed, still, the amount of information collected by member countries is huge, but widely scattered over Europe in agencies, institutes and universities. The collection and reporting of eel quality data is recommended within the international framework for the restoration of the species (Data Collection Regulation) as proposed by the Working Group on Eel (WG Eel, 2006) and the Scientific, Technical and Economic Committee for Fisheries of the EC (STECF, 2006). The collection of such data is now included in the guidelines for the preparation of Eel Management Plans. As previous studies have reported that contamination (e.g. by polychlorine biphenyls (PCBs) (Palstra *et al*., 2006)) impairs fertility and diseases cause major problems for migrating eels (Palstra *et al*., 2007), the WG Eel (2006) recommended the collection of data on the pollution and disease status of eels in order to identify areas producing high quality spawners (i.e. with low contaminant and parasite burdens) and thus maximise protection for these areas. It was advised that member states should set up a national program to evaluate the quality of their migrating spawners. At the very least this should include body burden of PCBs, brominated flame retardants (BFRs), and infection parameters of *Anguillicola*, and EVEX. It should be included in the national eel management plans whilst special emphasis should be placed on the standardisation and harmonisation of methodologies (units and methods). Under the monitoring of the chemical status of European water bodies as required by the Water Framework Directive (European Commission, 2006b) it has been recently proposed (Belpaire and Goemans, 2007b) to use the eel as a bio-monitor model to record the presence of a selection of priority substances in the biota. As eels are fished for the human consumption market, monitoring of their quality is required within both, European and national legislative frameworks[1] to protect human health.

Considering the above there is a need for a comprehensive compilation of these national data at an international level. The ICES/EIFAC Working Group on Eel

[1] For pesticides in products of animal origin established by Council Directive 86/363/EEC and Regulation (EC) 396/2005 (European Commission, 1986 and 2005), for metals Commission Regulation (EC) 466/2001 (European Commission, 2001) and, recently, for dioxins, furans and dioxin-like PCBs in muscle meat of eel by Commission Regulation (EC) 199/2006 (European Commission, 2006a).

underlined the need for international coordination (WG Eel, 1999). To this end, an initiative has been taken by the Working Group during its 2007 session (WG Eel, 2007). The objectives were (1) to collect recent data of contaminants and diseases of eels available over the distribution area of the eel, (2) to initiate the set up of a European Eel Quality Database (EEQD), (3) to get a view on constraints and problems, (4) to achieve preliminary analysis of the data and (5) to assess the possibilities of elaborating a strategy for Europe wide monitoring of eel quality.

The European Eel Quality Database

The database is coordinated by the Research Institute for Nature and Forest (Belgium) and includes data on eel quality elements, such as condition, contaminant concentrations and epidemiological parameters, in addition to the relevant descriptors of date and place of sampling and sample characteristics (eel life stage, number and morphometrics). Table 14.1 gives an overview of recorded quality elements.

The availability of eel quality data in the various member countries has been assessed and described by WG Eel (2007). Eleven countries submitted data on fat content, twelve on contaminants and ten on eel pathogens for inclusion into the European Eel Quality Database (EEQD) (Table 14.2). Eel quality data were provided for approximately 550 different sites over Europe, however, it should be mentioned that a considerable number of sites are situated in Belgium. Most information is available for PCBs (672 records), heavy metals (625) and organochlorine pesticides (OCPs) (514) whilst 538 observations on lipid content were also included. The EEQD includes information on more than 3500 individual eels. Apart from some observations on bacterial diseases at three sites in Spain, the number of disease agents included in the database is restricted to one, the swimbladder nematode *Anguillicola crassus* (Kuwahara, Niimi & Itagaki, 1974*)*, as this is believed to be one of the most invasive and debilitating parasites to eel (Palstra *et al*., 2007) and many studies have been carried out throughout Europe since its initial introduction in the early 1980s (Kirk, 2003). The database includes epidemiological data on *A. crassus* from 280 sites across Europe, although at present half of these are situated in Belgium. We are aware that some information is missing and the database has to be completed and activated in the future. For instance some countries (e.g. France and The Netherlands) have published reports that show considerable information is available but were not presented for inclusion in the EEQD. It is also presumed that many unpublished results are available in some countries and should be utilized by inclusion in the database.

Table 14.1. Overview of quality elements included in the European Eel Quality Database. For condition and contaminants data are presented as minimum, maximum and mean values.

Condition	
Fat content	Percent fat in muscle tissue
Contaminants	
Polychlorine biphenyls [1]	PCB28, PCB31, PCB52, PCB77, PCB95, PCB101, PCB105, PCB114, PCB118, PCB123, PCB126, PCB138, PCB153, PCB156, PCB157, PCB167, PCB169, PCB170, PCB180, PCB183, PCB189, PCB194, PCB209
Pesticides [1]	α-HCH, β-HCH, γ-HCH (Lindane), Dieldrin, Aldrin, Endrin, Hexachlorobenzene (HCB), *p,p*'-DDD (TDE), *p,p'*-DDT, *p,p'*-DDE, trans-nonachlor
Heavy metals [2]	Cd, Hg, Pb, Cr, Ni, Cu, Zn, As, Se, Mn, Co, V, Ba, Sr
Diseases	
Parasites	*Anguillicola crassus* (Abundance [3], prevalence [4] and mean intensity of infection [5])
Bacteria and other lesions	*Edwardsiella*, *Vibrio* or *Aeromonas* septicaemia, Skin injuries caused by bacteria or fungi (Prevalences [4])

[1] Expressed as $ng.g^{-1}$ body weight or $ng.g^{-1}$ lipid weight
[2] Expressed as $ng.g^{-1}$ body weight
[3] Total number of nematodes per eel including uninfected specimens
[4] Number of infected eels divided by the total number of eels investigated at each site
[5] Mean of the number of adult nematodes per infected eel

Table 14.2. Number of records of eel quality data reported by European countries and compiled by WG Eel (2007) in the European Eel Quality Database.

Country	Fat content	PCBs	OCPs	Heavy metals	*Anguillicola crassus*	Other diseases
Belgium	409	408	373	373	140	
Denmark	7	6	6		3	
France		19		3		
Germany	12	10	9	9	23	
Ireland	2	2			6	
Italy	18	18	14	7	10	
Norway	8	8	8			
Poland					7	
Portugal	1	1		4	3	
Spain	18	52	65	24	26	3
Sweden	25	10	1	179	51	
The Netherlands	37	99	23	14		
United Kingdom	1	39	15	12	11	
Total	**538**	**672**	**514**	**625**	**280**	**3**

Shortcomings and future development of the eel quality database

The database was initially restricted to a limited number of quality elements (lipid content, ca 30 chemicals and *A. crassus* infection parameters). During WG eel (2007) some countries reported on some more elements, and the list of ICES7 (CB28, CB52, CB101, CB118, CB138, CB153 and CB180) congeners was extended with non-*ortho* and mono-*ortho* congeners, as they exhibit the highest dioxin-like toxicity and contribute most to the TEQ (toxic equivalency). Also one pesticide, several metals and some bacterial disease agents were added (Table 14.1). There is still a need to broaden the list of quality elements in the database. It may be necessary to prioritise the inclusion of chemical quality elements (1) which have been reported as harmful for eel and which may impair normal migration and/or reproduction, or (2) which have been identified as priority hazardous substances to monitor in our water bodies under the Water Framework Directive (WFD, European Commission, 2006b) or which are recognized as harmful by other international conventions or agreements (e.g. OSPAR guidelines for marine environmental assessments, ICES (2006)) or (3) which are regulated for the protection of human health and where consumption limits are available. In the mean time substances like dioxins, brominated flame retardants, fluorinated compounds will be included, as well as more disease agents (e.g. EVEX).

Initial interrogation of the database revealed some variation in methodologies. Most monitoring focuses on analysis of yellow eels, but in some countries data were submitted for the silver eel stage. Most contaminant analyses are carried out on eel muscle tissue, but some data e.g. heavy metal contents refer to analysis on whole eel, or specific organs, such as the liver or gill, or it is simply not indicated. Analytical methodology is likely to vary between labs, but it is not integrated into the database and can be found in the referred report. Data reported from one site may be the result from the analysis of a single eel, the mean of several individual eels, or the result from a pooled sample of several eels. Data are indicated as minimum, maximum and mean values, but in some cases data are submitted as median values. These considerations urge for caution when analysing and interpreting these data, yet emphasise the need for standardisation and harmonisation. Besides, quality assurance issues need to get special attention. Both, harmonisation of the methodology and quality assurance are essential elements when developing an international monitoring network on eel quality. It is important to link our efforts for harmonisation and quality assurance to initiatives taken nowadays to improve other biota based contaminant databases over the world (Weisbrod *et al*., 2007).

One major objective of the database is to collect and compile available information on eel quality, which at the moment is widely scattered over environmental agencies, research institutes, administrations, fisheries managers, eel workers, parasitologists, toxicologists, food safety monitoring agencies. Efforts will have to be made to reach all of these potential data providers and as part of the present study information has been acquired through national representatives and members of the Working Group on Eel. However, for various reasons, not all existing information could be provided, and not every country over the European distribution area of the eel is an active participant in the Working Group. Therefore, it should be envisaged to use other information channels, beyond the WG (such as this paper), to reach a maximal numbers of data providers.

Another challenge will be to further develop the current database version into a powerful user-friendly and web-based database, available for all interested parties,

allowing easy access and data submission, and enabling analytic queries and cartographic applications.

Applications

The availability of an international up-to-date database compiling a whole range of eel quality parameters over the distribution area of the European eel is an essential instrument within the national and international eel recovery programs. Use of the database and application of the results are multiple. The database enables the identification and designation of good quality sites where special measures for maximum protection of stocks and emigrating spawners of good quality can be proposed (e.g. restriction of fisheries, priority places for restocking, priority for habitat restoration measures, etc). The WG Eel (2007) constructed some preliminary graphs on the lipid content and concentrations of cadmium and PCBs across Europe. One general conclusion was the wide variability in the levels of these contaminants and the presence of 'black spots' over the distribution area of the eel (WG Eel, 2007). As an example (on a larger scale) it could be deduced from the database that overall, PCB load in U.K. and in Denmark seemed to be lower than in many other countries. From data on *A. crassus* in the EEQD (280 sites) it is clear that the parasite is widespread over Europe, and only a few countries (Ireland, Italy, Spain, Sweden and Belgium) have reported sites free of the parasite. On a local scale Fazio (2007) monitored *A. crassus* in a series of lagunes in the Golfe du Lion (southern France) and suggested differential stock protection measures as a function of the sporadic distribution of the parasite.

From an environmental point of view it is clear that the database will give information about specific environmental chemical pressures and will indicate pollution areas for specific contaminants (Belpaire and Goemans, 2007a). The database will allow quick overview and follow-up of emerging problems of a chemical or epidemiological nature and can be used as an early warning system for the spread of new eel diseases or contaminants. It will also permit the in-depth analysis of eel quality on a Europe wide scale.

Yellow eels have been proposed as a sentinel organism (Belpaire and Goemans, 2007b, Belpaire *et al*., 2008) for evaluating the chemical quality of priority hazardous substances in biota in accordance with the WFD (European Commission, 2006b). EEQD can integrate these data and make them available for eel stock management.

In some well known heavily polluted areas it has been advised that fishermen are prevented from consuming their catch of eels, as human intake of PCBs via the consumption of eels is of concern to human health (Bilau et al, 2007). The database will pinpoint sites where the quality of eels is below that deemed suitable for human consumption (i.e. maximum PCB human consumption limits exceeded), so adequate fisheries management measures, like closing fisheries or preventing consumption of eels, can be taken in these areas.

Conclusion

The European Eel Quality Database was a major and innovative outcome of the ICES/EIFAC Working Group on Eel 2007. It represents the first comprehensive pan-European overview of eel quality data, including data from over 3500 eels from approximately 550 sites over twelve countries. The database will contain the eel quality data collected in the context of the monitoring within the eel management

units, but will also contain data collected for a variety of reasons such as chemical monitoring in the biota for the WFD, monitoring of consumption quality of fisheries products, academic research on toxicology or disease epidemiology. The EEQD provides a useful instrument for the compilation and scrutiny of these data, enabling the use of these results for the production of future eel management plans aimed at the restoration of the stocks.

The WG Eel (2007) has recommended that the European Eel Quality Database should be further developed and maintained. Initiated in 2007, the database has indicated a number of shortcomings and future developments will be needed, especially regarding expansion of the quality elements recorded, harmonisation of the methodology, quality assurance, communication, and database design. WG Eel (2007) endorses the need to develop an international monitoring network on eel quality, and member states should initiate harmonised monitoring strategies for eel. Guaranteeing further development of the database, harmonisation of methods, quality assurance, and setting up eel monitoring strategies over Europe will be a great challenge and will need pan-European cooperative work.

Acknowledgements

We thank all members of the Working Group on Eel at the Bordeaux 2007 meeting for valuable comments and contributions, in particular those people providing eel quality data (published and non-published) for inclusion in the European Eel Quality Database. Full reference of the origin of the data included in the database can be found in the Annex 6 of WG Eel (2007).

References

Belpaire, C., Goemans, G., 2007a. Eels: contaminant cocktails pinpointing environmental pollution. *ICES Journal of Marine Science* 64 (7), 1423-1436.

Belpaire, C., Goemans, G., 2007b. The European eel *Anguilla anguilla*, a rapporteur of the chemical status for the Water Framework Directive? *Vie et Milieu- Life and Environment* 57 (4), 235-252.

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,* in press.

Bilau, M., Sioen, I., Matthys, C., De Vocht, A., Goemans, G., Belpaire, C., Willems, J.L., De Henauw, S., 2007. Polychlorinated biphenyl (PCB) exposure through eel consumption in recreational fishermen as compared to the general population, using a probabilistic approach, *Food Additives and Contaminants* 24 (12), 1386-1393.

European Commission, 1986. Council Directive 86/363/EEC of 24 July 1986 on the fixing of maximum levels for pesticide residues in and on foodstuffs of animal origin. *Official Journal of the European Union* L221, 43-47.

European Commission, 2001. Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union* L70, 1-13.

European Commission, 2005. Commission Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. *Official Journal of the European Union* L70, 1-16.

European Commission, 2006a. Commission Regulation (EC) 199/2006 of 3 February 2006 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. *Official Journal of the European Union* 04.02.2006 L 32/34.

European Commission, 2006b. 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).

Fazio, G., 2007. Epidémiologie et biologie du parasite invasif *Anguillicola crassus* (Nematoda) et son impact sur la physiologie de son hôte, l'anguille européenne, *Anguilla anguilla.* Doctoral thesis. Académie de Montpellier, 281p.

ICES, 2006. Report of the Working Group on Marine Chemistry (MCWG), 27–31 March 2006, ICES Headquarters, Copenhagen. Computer Management. 36p. Available from http://www.ices.dk/reports/MHC/2006/MCWG06.pdf

Kirk, R.S., 2003 The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology* 10, 385-94.

Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., van den Thillart, G.E.E.J.M., 2006. Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93, 145-148.

Palstra, A.P., Heppener, D.F.M., van Ginneken, V.J.T., Székely, C., van den Thillart, G.E.E.J.M., 2007. Swimming performance of silver eels is severely impaired by the swim-bladder parasite *Anguillicola crassus*. *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2007

Robinet, T., Feunteun, E., 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? *Ecotoxicology* 11, 265-277.

STECF, 2006. Scientific, Technical and Economic Committee for Fisheries of the Commission of the European Communities, 22nd Report of STECF, draft version 27 April 2006.

van den Thillart, G.E.E.J.M., *et al*., 2005. Estimation of the reproduction capacity of European eel. EELREP Final Report. 272p. Available from http://www.fishbiology.net/EELREP_final_report.pdf

Weisbrod, A.V., Burkhard, L.P., Arnot, J., Mekenyan, O., Howard, P.H., Russom, C., Boethling, R., Sakuratani, Y., Traas, T., Bridges, T., Lutz, C., Bonnell, M., Woodburn, K., Parkerton, T., 2007. Review of fish bioaccumulation databases used to identify persistent, bioaccumulative, toxic substance. Environ Health Perspectives 115:255–261 (2007). doi:10.1289/ehp.9424. Available from http://dx.doi.org/ [Online 30 October 2006].

WG Eel, 1999. Report of the eleventh session of the Joint EIFAC/ICES Working Group on Eels. Silkeborg, Denmark, 20-24 September 1999. EIFAC Occasional Paper. No. 34. Rome, FAO. 2001. 31p.

WG Eel, 2006. Report of the 2006 session of the Joint EIFAC/ICES Working Group on Eels. FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Rome, 23–27 January 2006. EIFAC Occasional Paper. No. 38, ICES CM 2006/ACFM:16. Rome, FAO/Copenhagen, ICES. 352p. Available from http://www.ices.dk/reports/ACFM/2006/WGEEL/WGEEL06.pdf

WG Eel, 2007. Report of the 2007 Session of the Joint EIFAC/ICES, Working Group on Eels, FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Bordeaux, 3–7 September 2007, EIFAC Occasional Paper No. 38, ICES M 2007/ACFM:23, Draft, 524p.

Available from
www.ices.dk/reports/ACFM/2007/WGEEL/WGEEL_07_draft.pdf

Part V

Contaminants in eel and human health

Paling in 't Groen (or Anguille au Vert)
Eel in a green sauce

"This famous dish is easily made. It is agreeable to join the Belgians in passionate discussions about which greens should be used, and I give below what Louis Paul Boon regards as the ideal list; but the important thing is to have several and to be sure to include sorrel, since its flavour should be the most noticeable.
Finely chop and stew gently for half an hour in a little butter, with salt and pepper, the following greens : sorrel, celery tops, parsley, mint, sage, chervil, lemon balm and summer savory. A little minced onion may be added with advantage to this brew.
Meanwhile, select a fat eel, skin it, clean it and cut it into sections. Bring to the boil some water to which a little vinegar has been added. Boil the pieces of eel in this for 5 to 10 minutes, according to their thickness. Then drain them and put them in with the stewed greenery to finish cooking. When the pieces of eel are quite tender, add to the sauce a lump of butter into which you have worked some flour, and bind it thus. Serve hot, with thickly buttered brown bread."
(Alan Davidson, 1979).

Eels are much appreciated by Flemish people and some traditional recipes are world famous. However feral eels must be regarded as risky food, considering PCB body burden is far above legal consumption limit.

Davidson, A., 1979. North Atlantic Seafood. Penguin Books, London, 512 pages.

Chapter 15

Polychlorinated biphenyl exposure through eel consumption in recreational fishermen

Maaike Bilau[1], Isabelle Sioen[1,2], Christoffe Matthys[1], Alain De Vocht[3], Geert Goemans[4], Claude Belpaire[4], Jan Willems[1] and Stefaan De Henauw[1]

1 - Department of Public Health, Ghent University, UZ 2 Blok A, De Pintelaan 185, B-9000 Ghent, Belgium

2 - Department of Food Safety and Food Quality, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

3 - Centre for Environmental Sciences, Hasselt University, Campus Diepenbeek, Agoralaan, B-3590 Diepenbeek, Belgium

4 - Institute for Forestry and Game Management, Duboislaan 14, B-1560 Groenendaal-Hoeilaart, Belgium

This chapter was published as:

Bilau, M., Sioen, I., Matthys, C., De Vocht, A., Goemans, G., Belpaire, C., Willems, J.L., De Henauw, S., 2007.
Probabilistic approach to polychlorinated biphenyl (PCB) exposure through eel consumption in recreational fishermen vs. the general population.
Food Additives & Contaminants 24 (12), 1386-1393

Summary

Concentrations of the sum of the 7 indicator PCBs (Σ_7 iPCBs) measured in non-commercial European eel (*Anguilla anguilla* L.*)* in Flanders are high: in 80% of all sampled localities, the Belgian PCB standard for fish is exceeded. The objective of this study was to assess the intake of the Σ_7 iPCBs through consumption of eel by recreational fishermen and to compare it with the intake of a background population.

The median estimated intake for recreational fishermen varies between 18.4 and 237.6 ng iPCBs/kg BW/day, depending on the consumption scenario, while the estimated intake of the background population (consumers only) is 4.3 ng Σ_7 iPCBs/kg BW/day. Since the levels of intake via eel for 2 intake scenarios are respectively 50 and 25 times higher than the intake of the background population, body burden (BB) might be quite higher and reach levels of toxicological relevance**.** The intake of the 7 iPCBs via consumption of self-caught eel in Flanders seems to be at a level of high concern. The Flemish catch-and-release obligation for eel, established in 2002, should be maintained and supervised (more) carefully.

Introduction

Polychlorinated biphenyls (PCBs) exist in many different technical mixtures and were mainly used in electronic appliances, heat transfer systems and hydraulic fluids, but also in other applications such as paints, coatings and flame retardants. The use of PCBs was considerably restricted in the seventies. However, most PCB congeners are very lipophilic and persistent and tend to accumulate in the environment and the human food chain. Mixtures of PCBs are generally assessed on the basis of a chemical analysis of the (sum of the) so-called indicator PCBs (Σ_7 iPCBs, i.e. congeners 28, 52, 101, 118, 138, 153, 180). None of these PCB congeners exhibits dioxin-like activity, except for PCB 118, that has a toxic equivalence factor (TEF) value of 0.00003 (van den Berg *et al.*, 2006). They are known to bioaccumulate in the human diet and are assumed to be representative for all PCBs, as they are the predominant congeners in biotic and abiotic matrices (Bakker *et al.*, 2003). The sum of 6 indicator PCBs (congeners 28, 52, 101, 138, 153 and 180) represents about 50% of the total non-dioxin like PCBs in food (EFSA, 2005).

European eel (*Anguilla anguilla* L.*)* is known to bioaccumulate lipophilic contaminants such as PCBs and organochlorine pesticides through carnivorous feeding behaviour. Moreover, eel is a so-called benthic fish, living near and in the contaminated sediment. Consequently, eel is expected to have a large exposure to contaminants and is therefore commonly used as an environmental bio-indicator for a variety of contaminants (Wiesmuller and Schlatterer, 1999; Versonnen *et al.*, 2004). Human dietary exposure to iPCBs might be driven by the consumption of highly-contaminated fishes, at least for a subpopulation of eel consumers (Harrad and Smith, 1999).

Since 1994, the Flemish Eel Pollutant Monitoring Network monitors about 300 different sites in Flanders (the northern part of Belgium, a region of 13,500 km²) by measuring contaminants in European eel. The monitoring sites are situated in rivers, canals, polder waters and closed water bodies. The monitoring program includes PCBs, organochlorine pesticides (e.g. hexachlorobenzene, lindane, dieldrin, …), polybrominated flame retardants (polybrominated diphenyl ethers, …) and heavy metals (such as mercury, cadmium, lead, arsenicum, …) (Goemans *et al.*, 2003; Goemans and Belpaire, 2004).

The concentrations of the Σ_7 iPCBs measured by this monitoring network are very high: in 80% of all sampling sites, the mean concentration in eel exceeds the Belgian PCB standard for fish (75 ng/g fresh weight) (Goemans and Belpaire, 2004). For this reason, in 2002, the Flemish authorities have issued a catch-and-release obligation for all fish in the 5 most polluted waters in Flanders and an overall catch-and-release obligation for eel in the whole region of Flanders. It has been demonstrated that, in spite of this restriction, some recreational fishermen still take their eel home, most likely for consumption (Vandecruys, 2004).

The objective of this study was to assess the intake of Σ_7 iPCBs via eel consumption in this subgroup of recreational fishermen and to compare it to the intake of a Flemish background population.

Materials and Methods

In order to estimate the exposure to Σ_7 iPCBs through eel consumption, two approaches were used. For the subpopulation of fishermen (and their family), a simple distribution approach was used in which a point estimate for eel consumption was combined with a contaminant distribution, based on the available data for iPCB contamination of eel (Lambe, 2002). On the other hand, for the background

population (eel consumers only), two distributions were combined in a full probabilistic model (Cullen and Frey, 1999): a distribution for eel consumption and a distribution for PCB contamination (using @Risk® 4.5 for Excel®, Palisade Corporation, Ivybridge, Devon).

Recreational fishermen

In 2003, 61,245 individuals in Flanders had a fishing license for public waters. A survey on specific aspects of recreational fisheries, including the issue of taking home a catch, was carried out (Vandecruys, 2004). The survey included questions on the fish species caught and taken home as well as the number and the weight of the fish caught and taken home. A systematic random sampling of the dataset of anglers on public waters was carried out and 10,000 entries were selected. After omitting foreign anglers and undelivered mail, the real sample size was 9,492. A total number of 3,001 of the licensed anglers completed this questionnaire about recreational fishing. Respectively 1.9% and 5.3% of these anglers indicated that they "always" (group A) or "sometimes" (group B) take home the eel they have caught. No information was obtained about what these fishes were used for. Therefore, some assumptions had to be made concerning the consumption of these fishes. However, personal or familial consumption can be expected based on the small number of eels caught per fishing trip. Based on extrapolation to all licensed fishermen, the number of people taking home the eel, caught in Flemish public waters, is estimated to be more than 4,000.

For group A (the group of fishermen always taking home the eel caught), it is calculated that an average of 25.88 kg/year of edible eel (or a mean of 498 g/week) is taken home, based on the number of fishing occasions (average of 41.67 trips/year), the number of eels caught per occasion (average of 4.14) and a mean weight of edible portion per eel (150 g). For group B, the fishermen stating that they only "sometimes" take home their catch, it was assumed that on average one eel out of five caught, is taken home. The same calculation has been done (average number of fishing occasions = 42.03/year, the number of eels caught per occasion and taken home = 3.12/5, the mean weight of edible portion per eel = 150 g), resulting in 3.93 kg edible eel per year (76 g/week).

We further considered two different consumption scenarios for both groups:

- In scenario A1, the fisherman takes home 498 g/week (cf. supra) or 71.14 g/day. In this worst case scenario, it was assumed that this was consumed by the angler himself;
- In scenario A2, the fisherman takes home the same amount of eel (498 g/week). Here it was assumed that he eats only half of this amount (i.e. 35.57 g/day). The other half could be consumed by friends and/or family;
- In scenario B1, the fisherman takes home 76 g/week (cf. supra) or 10.86 g/day. This is consumed by the fisherman himself;
- In scenario B2, the fisherman takes home the same amount (76 g/week) and eats half of it (i.e. 5.43 g/day).

Fishermen were assumed to have a mean body weight (BW) of 70 kg.

Data on the iPCB contamination of eel in the Flemish water bodies were based on the Eel Pollutant Monitoring Network in Flanders, 1994-2001 *(*Goemans *et al.*, 2003; Goemans and Belpaire, 2004). The concentration of iPCB was analysed in 261 samples. Length of sampled eels varied between 30 and 50 cm. The sampling sites are spread over Flanders.

A distribution of iPCB concentrations in eel was fit, using BestFit®-software (BestFit Probability Distribution Fitting for Windows; Palisade Corporation, Ivybridge, Devon). BestFit® determines the optimal distribution and the optimal parameters for each data set, performing three standard tests to determine the goodness of fit: Chi-squared, Anderson-Darling and Kolmogorov-Smirnov. The probability distributions evaluated by BestFit include 28 possible distributions (e.g. binomial, exponential, gamma, logistic, log-logistic, lognormal, the normal distribution, …). All these distributions were tested. In this study, the Anderson-Darling test was used in order to determine the optimal distribution: this test focuses on the differences between the tails of the fitted distribution and input data, rather than on the center of the distribution. In order to preclude too high contamination data, the distribution was truncated at the upper level, at twice the maximum value measured during monitoring (13,466 ng/g). Also at the lower end the distribution was truncated (half of the minimum value: 5.5 ng/g).

The background population

For the background population, the most recent data on eel consumption available in Belgium were used. Within the context of a large Flemish biomonitoring study, in the field of environmental health, a food frequency questionnaire (FFQ) was used to estimate the daily consumption of fat-containing food items. This FFQ contained a question on the frequency ("how often do you consume eel?" with 7 response categories, ranging from "never or less than 1 day a month" to "6 to 7 days a week") and the portion ("how much do you consume on that day?") of eel consumption. This FFQ was completed by 1,179 women of childbearing age (18-44 years). The data were collected between September 2002 and December 2003.

In this study population, a total of 132 women (11.2%) consumed eel at least once during the last year. The mean intake among consumers was 2.87 (± 1.28) g/day.

Again, BestFit®-software was used to determine a distribution describing these consumption data. In order to preclude unrealistic consumption data, the distribution was truncated at 0.16 g/day (half of the minimal estimated consumption) and at 15 g/day (double of the maximal estimated consumption).

For this population, contamination data on the Σ_7 iPCBs measured in commercially available eel in Flanders were used (Belpaire *et al.*, 2000). A total of 80 samples of commercially available eel was analysed for iPCBs. Again, a distribution was fit on these data using BestFit®-software. In order to preclude unrealistic contamination values, the distribution was truncated at both ends: 0.7 ng/g (half of the minimal contaminant concentration) and 11,472 ng/g (double of the maximum contaminant concentration).

The consumption and the contamination distributions were combined using a probabilistic approach (@Risk®, Risk Analysis Add-in for Microsoft Excel; Palisade Corporation, Ivybridge, Devon). The mean body weight (self-reported) of the women was 64.6 (± 11.4) kg.

Results

Distributions

For the contamination data of eel (commercially available eel and eel caught by Flemish recreational fishermen), two lognormal distributions were chosen. In Figure 15.1, the original contamination data are compared via a Box and Whisker plot. In Figure 15.2, the fitted distributions, based on these contamination data, are shown.

Also for the consumption of the background population, a lognormal distribution was used.

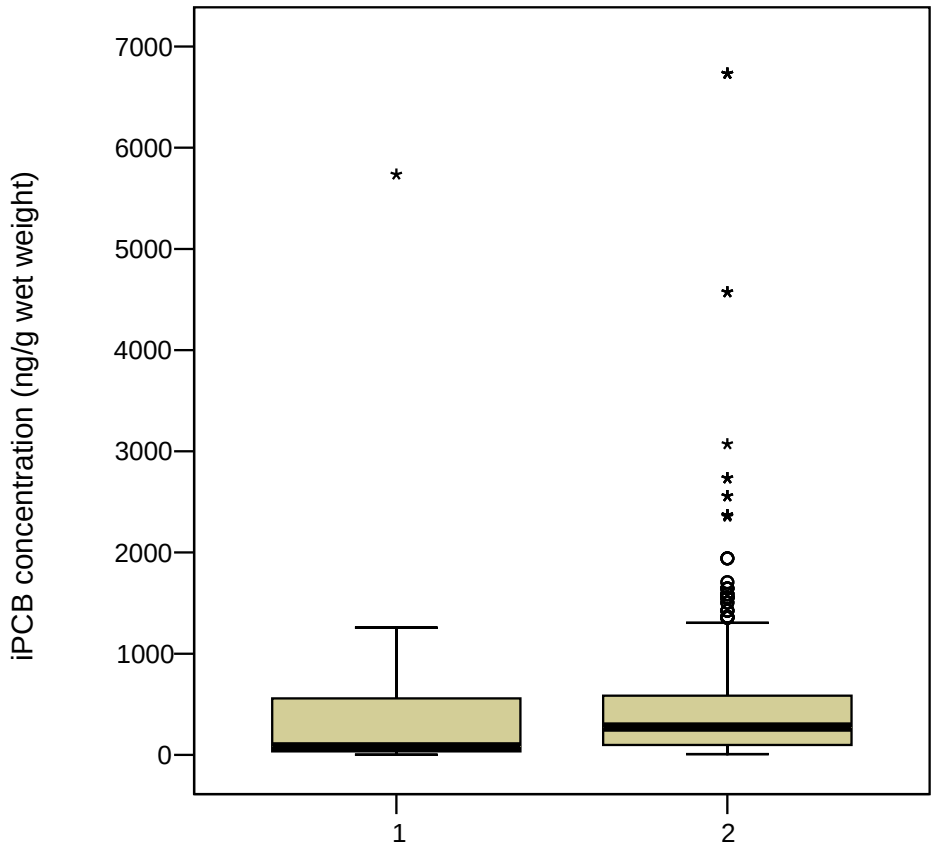


Figure 15.1. Box and Whisker plots[1] for concentrations of the Σ_7 iPCBs (ng/g wet weight), analysed in (1) commercially available eel (n = 80) and (2) eel in Flemish waterbodies (n = 261).

[1] Each box represents the interquartile range (P25 – P75). The bold line expresses the median value. The whiskers extend from the boxes and indicate the upper and lower values not classified as statistical outliers or extremes. Stars are statistical outliers (i.e. cases with values between 1.5 and 3 times the interquartile range). Open circles are statistical extreme values (i.e. cases with values more than 3 times the interquartile range).

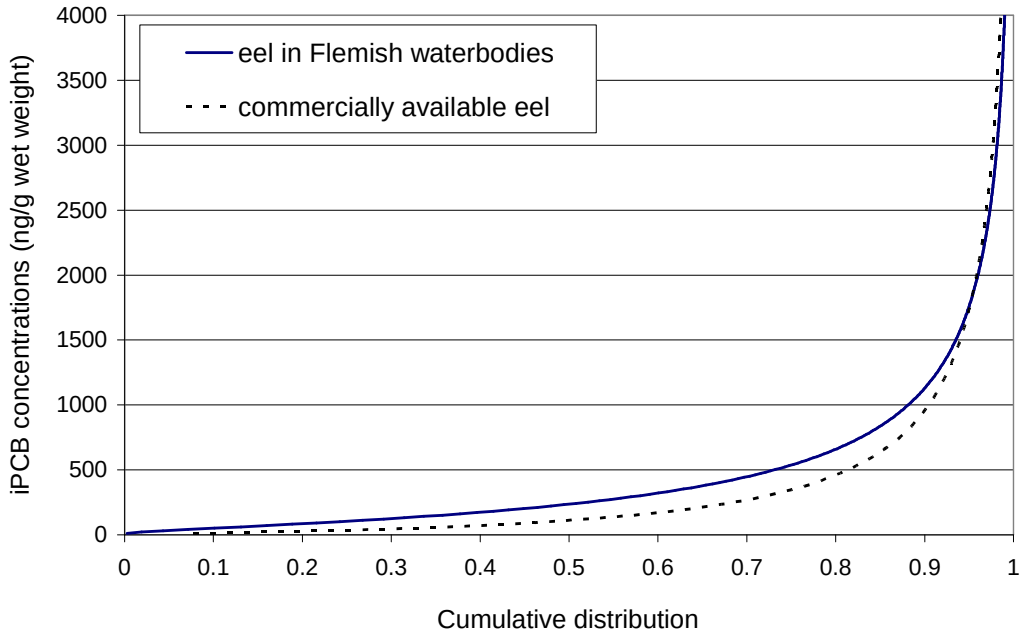


Figure 15.2. Fitted cumulative distribution functions for the concentrations of the Σ_7 iPCBs (ng/g wet weight) for eel in Flemish waterbodies and commercially available eel.

Based on the distribution of the data (see Figure 15.1), the truncation of the distribution at the double of the maximum seems reasonable, since the probability of measuring concentrations higher than twice the maximum is very low.

iPCB-exposure

The median intake for recreational fishermen varies between 18.4 ng iPCBs/kg BW/day (scenario B2: consumption of 5.4 g eel/day) and 237.6 ng iPCBs/kg BW/day (worst case scenario A1: consumption of 71.1 g eel/day). At median level, the estimated intake of the background population (consumers only) is 4.3 ng iPCBs/kg BW/day. At the 90th percentile, the estimated intake for the fishermen varies between 86 (consumption scenario B2) and 1118 ng iPCBs/kg BW/day (scenario A1), while the intake for the background population (consumers only) is 42.9 ng iPCBs/kg BW/day. The estimated intakes for the Σ_7 iPCBs are presented in Table 15.1 for both the background population and the fishermen. Cumulative distribution functions for the estimated intake of Σ_7 iPCBs are shown for the background population and for the different consumption scenarios of the fishermen in Figure 15.3.

Table 15.1. Estimated intake of the Σ_7 iPCBs (ng/kg BW/d) for the background population and the recreational fishermen. The estimates for the fishermen are presented for the different consumption scenarios (A1, A2, B1, B2).

		Estimated iPCB intake (ng/kg BW/d)			
Percentile	Background	Recreational fishermen			
		A1	A2	B1	B2
5	0.2	31.9	16.8	5.2	2.5
25	1.3	105.2	52.8	16.1	8.1
50	4.3	237.6	118.6	36.7	18.4
95	80.4	1727.8	861.6	285.2	140.3
97.5	135.2	2513.1	1282.7	425.0	203.9
99	238.2	4032.2	1946.0	647.9	296.7
99.9	707.9	8582.8	4181.3	1362.3	656.0

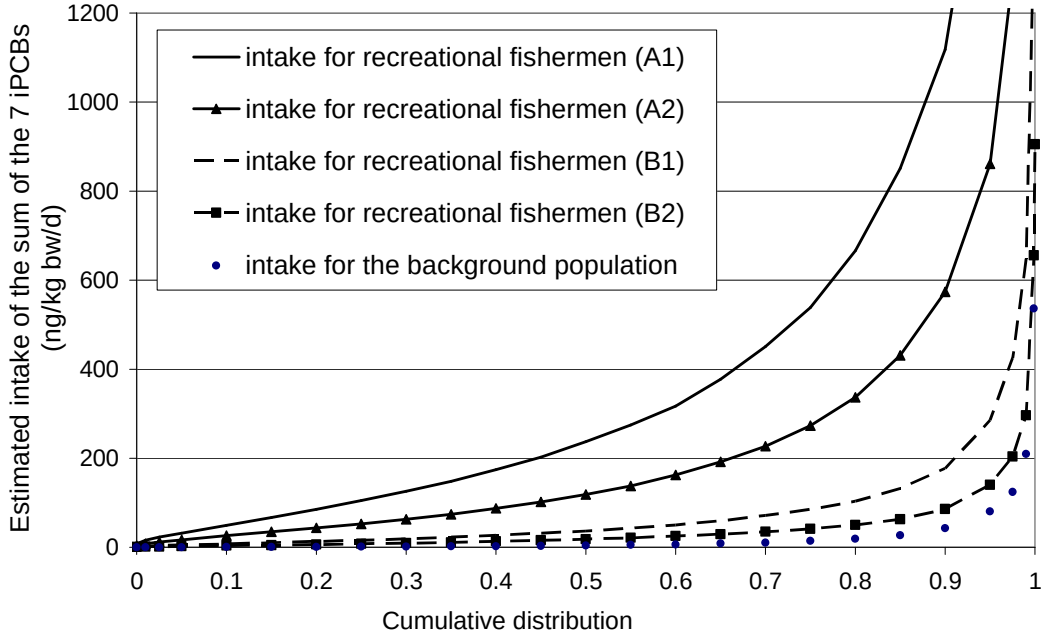


Figure 15.3. Cumulative distribution functions of the estimated intake of the Σ_7 iPCBs (ng/kg BW/d) for the background population and the recreational fishermen. The results for the fishermen are presented for the different consumption scenarios (A1, A2, B1, B2).

It should be noted that the results, presented in this study (Table 15.1 and Figure 15.1), are based on eel consumers only: 7.2% of the recreational fishermen consume their self caught eel, while 11.2% of the background population are eel consumers. When extrapolating these results to an intake assessment for the population at large (consumers and non consumers together), the assessed intakes of this study would be situated at the higher end of the overall distribution.

On the other hand, only the intake via eel is taken into account. Also other food items, such as other fish and food items containing animal fat, will contribute to the overall PCB intake. In a previous dietary intake assessment of polychlorinated dibenzodioxins/furans (PCDD/Fs) and dioxin-like PCBs in Belgium, Vrijens and co-authors reported that fish remains an important source of dioxin-like contaminants for the higher percentiles of the population. At the 90th percentile, fish becomes the greatest contributor to dietary PCB exposure (Vrijens *et al.*, 2002).

Discussion

The intake of iPCBs via eel consumption was estimated using a probabilistic model, based on Monte Carlo techniques, for a population that could be at risk, i.e. eel fishermen, and compared with a background population. Large differences of estimated intake have been found between the different scenarios.

Methodological considerations

Probabilistic techniques such as Monte Carlo analysis have been used since about 1990 to characterize the health risks of populations exposed to various chemicals (Carrington *et al.*, 1996; McKone, 1994). Many papers have been published showing that probabilistic methods represent a significant improvement over deterministic approaches (Finley and Paustenbach, 1994; Finley *et al.*, 1993; Thompson, 2002). As in deterministic techniques, however, the quality of the output depends largely on the quality of the input data.

The available information on consumption for the population of recreational fishermen is rather elusive and several assumptions had to be made: fishermen stated that they take home the fish they have caught, still it is not known who is consuming this eel. We have chosen to consider four different scenarios, as a reflection of a range of true variation. In the worst case scenario the mean intake is 498 g eel/week. Other available consumption data from Flanders (a seven day food record, 341 adolescents, 12-18 years old, 1997) (Matthys *et al.*, 2003), showed that a consumption of 500 g fish/week corresponds to the 97th percentile of the distribution for total fish consumption for adolescents. Our worst case scenario, therefore, seems not to be exceptional, as compared to the general population. It is perhaps not unrealistic to assume that at least some anglers are among the highest consumers of fish in the population.

Considering the background population, it could be stated that women of childbearing age (18-44 years) are not a representative group for the general population in order to assess the consumption of eel. It is clear that there are differences in consumption between men and women and between different age groups. Nevertheless, these data were used because no other, recent consumption data on eel were available for Belgium or Flanders. The FFQ used, focused on consumption during the last year.

Concerning the contamination data, two different data sets were used since the contamination of eel commercially available on the Belgian market (exposure for the background population) is known to be different from the contamination of eel caught in public waters in Flanders (exposure for the recreational fisherman).

Contamination levels can be influenced by several factors. It is possible and even probable that some individuals of the background population, consuming eel in a restaurant, are served eel from an unofficial circuit. This eel might be caught in private waters. PCB levels of those eels are unknown, but suspected to be in the range of the eels living in public waters in Flanders. This can be a reason for an underestimation of exposure of the background population. Secondly, it is known that consumers can reduce the contaminant level by removing the skin and fat from fish before cooking them (Sidhu, 2003). Also, other processing or cooking procedures will influence the contaminant level. Furthermore, the dataset of contaminants in feral eel from Goemans *et al.* (2003) are originating from eels of a specific length class (30-50 cm). Many eels caught and consumed by fishermen are larger, and therefore containing higher contaminant levels. In this way, our calculation of PCB exposure might be biased and data presented here might be an underestimation. From the dataset it is obvious that regional variations in PCB contamination throughout Flanders are important (Goemans *et al.*, 2003). Refined analysis of intake levels from heavily contaminated eels in specific areas might point towards more severe risks.

Available data on intake of iPCBs in other countries

Comparable data in literature are scarce, due to several reasons (Baars *et al.*, 2004; Bakker *et al.*, 2003; Fattore *et al.*, 2005; Wilhelm *et al.*, 2002). The most important reason is the use of different methodologies, such as (1) a different number of congeners (e.g. Σ_3 PCBs, Σ_6 PCBs, Σ_7 PCBs, Σ_{10} PCBs) that are taken in account, (2) intake via total diet versus via specific food groups or food items, (3) total population versus consumers only, (4) different age groups, etc. In spite of this, a limited number of intake estimates from other countries are presented here.

In Italy, the intake of Σ_6 iPCBs (PCB 28, 52, 101, 138, 153 and 180) was estimated based on a food diary of 3 to 7 consecutive days, completed by 1940 subjects (age 0-94 years) (Fattore *et al.*, 2005). The estimated intake for adolescents and adults (13-94 years) varied from 5.9 over 10.9 to 23.8 ng/kg BW/day for the 5th percentile, mean and 95th percentile respectively. On average, 42% could be attributed to fish and fish products. This means that on average 4.6 ng/kg BW/day (Σ_6 iPCBs) is due to the consumption of fish and fish products.

A Dutch intake assessment of Σ_7 iPCBs via the whole diet resulted in following estimated median intake: 4.8 ng iPCBs/kg BW/day (Baars *et al.*, 2004; Bakker *et al.*, 2003). At the 90th percentile, an intake of 8.6 ng iPCBs/kg BW/day was estimated.

In France, the average intake of Σ_7 iPCBs among French high seafood consumers (Calipso Study) was estimated to be 57 ng/kg BW/day through seafood consumption only (Sirot *et al.*, 2006).

Recent European studies estimated the average daily intake of total non dioxin-like PCBs for adults to be in the range of 10-45 ng/kg BW/day (EFSA, 2005).

Risk evaluation

Non dioxin-like PCBs are less toxic than PCDD/Fs and dioxin-like PCBs. Nevertheless, it is recommended that the intake is as low as possible. Unlike for dioxin-like substances (Tolerated Daily Intake (TDI) = 1 - 4 pg TEQ/kg BW/day) (Scientific Committee on Food, 2001) or total PCBs (TDI = 20 ng/kg BW/day, in Aroclor Equivalent) (WHO, 2003), no specific health based guidance value (e.g. a tolerated daily or weekly intake, TDI or TWI), has been proposed for the non-dioxin like PCBs only (EFSA, 2005). The major problem encountered was that it is very difficult to distinguish between effects of non dioxin-like PCBs and effects of dioxin-like PCBs and PCDD/Fs that may be part of PCB mixtures. No definite relationship,

however, has been found between levels of non dioxin-like PCBs and levels of dioxin-like PCBs and PCDD/Fs in these mixtures. Only occasionally a certain relationship could be found, e.g. in the PCB animal feed contamination case in Belgium in 1999 or in geographically defined sampling areas (EFSA, 2005; Vrijens *et al*., 2002).

The WHO (2003) proposed a TDI for total PCBs, expressed in Aroclor equivalent, of 20 ng/kg BW/day, while Sirot *et al.* (2006) stated that the concentration of Σ_7 iPCBs must be multiplied by two to be expressed in Aroclor equivalent. If our calculated exposure (the exposure of Σ_7 iPCBs multiplied by two) is compared with the TDI, it can be seen that more than 30% of the eel consumers of the background population exceeds this TDI, without taking in account other PCB sources. In comparison: between 70% and 99% of the recreational fishermen exceed this TDI, depending on the consumption scenario used.

In a recent publication, a statistically significant relationship has been observed between individual dioxin-like PCBs and total PCBs, measured in a number of fishes, caught mainly in Canada and Northern America (Bhavsar *et al.*, 2007). This correlation can be an interesting application for risk assessment estimations executed in that region. However, it has not been demonstrated that this relationship is also valuable in other geographical regions. In contrast, clear spatial and temporal variations have been observed in the ratio of PCB118 to the sum of the remaining 6 iPCBs in eel in Flemish water bodies (Goemans and Belpaire, 2005). Therefore, this extrapolation has not been used in the current estimation, since this paper handles the intake of eel, locally caught in Belgium.

EFSA concluded that the margin of body burden (MoBB) – which was calculated by comparing the body burden (BB) in the rat at the no observed adverse effect level (NOAEL) of 500 µg/kg BW (liver and thyroid toxicity) with the estimated median human BB for total non dioxin-like PCBs (48 µg/kg BW) in the general population – was about 10. We do not know how much PCBs the fishermen ingest via the total diet, but since the levels of intake via eel in scenario A1 and A2 are respectively 50 and 25 times higher than the intake of the background population, BB might be quite higher and reach levels that become toxicologically relevant.

Since other animal based food items are very likely to contain some concentration of iPCBs, it, therefore, remains advisable to maintain the catch-and-release obligation for eel and to sensitize the recreational fishermen about the contamination problem of eel in the Flemish waters.

Attention has to be paid to the background population too, since high eel consumers might also be at risk. In other countries, e.g. the USA, advisories on fish consumption were formulated, especially focusing on pregnant women, young children (under 15) and women of childbearing age (MDCH Environmental and occupational epidemiology division, 2004; Scientific Advisory Committee on Nutrition and Food Standard Agency, 2004; US EPA, 2005; US EPA and US FDA, 2004). Also, the Swedish National Food Administration has recommended pregnant and lactating women to refrain from eating some predatory species, including eel (Bjornberg *et al.*, 2005).

Conclusion

In conclusion, the intake of the Σ_7 iPCBs via the consumption of self-caught eel seems to be at a level of high concern. Further monitoring seems appropriate. Although risk assessment would be easier if, in analogy with PCDD/Fs and dioxin-like PCBs, a reference TDI or TWI could be established for the Σ_7 iPCBs only, it is very unlikely that this will be possible in the near future (EFSA, 2005). In the meantime, it should be advised to maintain the public health measure of preventing fishermen from consuming their self-caught eel. The catch-and-release obligation should be maintained and supervised (more) carefully.

Acknowledgements

The authors would like to thank Kristof Vlietinck for his contribution concerning the eel consumption data of the fishermen. Food safety research at the Department of Public Health, Ghent University, is funded by the Centre for Health and Environment of the Flemish Ministry of Public Health, the Belgian Science Policy through the SPSD II project CP/02/56 and the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen).

References

Baars, A.J., Bakker, M.I., Baumann, R.A., Boon, P.E., Freijer, J.I., Hoogenboom, L.A., Hoogerbrugge, R., van Klaveren, J.D., Liem, A.K., Traag, W.A., de Vries, J., 2004. Dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs: occurrence and dietary intake in The Netherlands. *Toxicology Letters* 151, 51-61.

Bakker, M., Baars, A.J., Baumann, B., Boon, P., Hoogerbrugge, R., 2003. Indicator PCBs in foodstuffs: occurrence and dietary intake in The Netherlands at the end of the 20th century. RIVM and RIKILT, Bilthoven, The Netherlands.

Belpaire, C., Van Thuyne, G., Cooreman, K., 2000. PCB measurements in eel for consumption, on the market in Flanders. Note for the Belgian Health Council, 22 november 2000. (in Dutch: 'PCB metingen in consumptiepaling uit de handel in Vlaanderen. Nota voor de Hoge Gezondheidsraad, 22 november 2000').

Bhavsar, S.P., Fletcher, R., Hayton, A., Reiner, E.J., Jackson, D.A., 2007. Composition of Dioxin-like PCBs in Fish: An Application for Risk Assessment. *Environmental Science & Technology* 41, 3096-3102.

Bjornberg, K.A., Vahter, M., Grawe, K.P., Berglund, M., 2005. Methyl mercury exposure in Swedish women with high fish consumption. *The Science of The Total Environment* 341, 45-52.

Carrington, C.D., Bolger, P.M., Scheuplein, R.J., 1996. Risk analysis of dietary lead exposure. *Food Additives and Contaminants* 13, 61-76.

Cullen, A.C., Frey, H.C., 1999. Probabilistic techniques in exposure assessment. Plenum Press, New York and London.

EFSA, 2005. Opinion of the scientific panel on contaminants in the food chain on a request from the Commission related to the presence of non dioxin-like polychlorinated biphenyls (PCB) in feed and food. *The EFSA Journal* 284, 1-137.

Fattore, E., Fanelli, R., Turrini, A., Dellatte, E., Fürst, P., di Domenico, A., 2005. Assessment of dietary intake of non dioxin-like polychlorinated biphenyls in the Italian general population. *Organohalogen Compounds* 67, 1749-1752.

Finley, B.L., Scott, P., Paustenbach, D.J., 1993. Evaluating the adequacy of maximum contaminant levels as health-protective cleanup goals: an analysis based on Monte Carlo techniques. *Regulatory Toxicology and Pharmacology* 18, 438-455.

Finley, B., Paustenbach, D.J., 1994. The benefits of probabilistic exposure assessment: three case studies involving contaminated air, water, and soil. *Risk Analysis* 14, 53-73.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. The Flemish eel pollutant monitoring network 1994-2001: polychlorine biphenyls, organochlorine pesticides and heavy metals in eel. (in Dutch). Institute for Forestry and Game Management, Belgium.

Goemans, G., Belpaire, C., 2004. The eel pollutant monitoring network in Flanders, Belgium. Results of 10 years monitoring. *Organohalogen Compounds* 66, 1834-1840.

Goemans, G., Belpaire, C., 2005. Congener profiles in European eel (*Anguilla anguilla* L.) as a method of tracing the origin of PCB contamination. *Organohalogen Compounds* 67, 1304-1307.

Harrad, S., Smith, D., 1999. Eel consumption as a pathway of human exposure to PCBs. *International Journal of Environmental Health Research* 9, 31-37.

Lambe, J., 2002. The use of food consumption data in assessments of exposure to food chemicals including the application of probabilistic modelling. *The Proceedings of the Nutrition Society* 61, 11-18.

Matthys, C., De Henauw, S., Devos, C., De Backer, G., 2003. Estimated energy intake, macronutrient intake and meal pattern of Flemish adolescents. *European Journal of Clinical Nutrition* 57, 366-375.

McKone, T.E., 1994. Uncertainty and variability in human exposures to soil contaminants through home-grown food: a Monte Carlo assessment. *Risk Analysis* 14, 449-463.

MDCH Environmental and occupational epidemiology division, 2004. Michigan family fish consumption guide. Important facts to know if you eat Michigan fish. http://www.michigan.gov/documents/FishAdvisory03_67354_7.pdf

Scientific Advisory Committee on Nutrition, Food Standard Agency, 2004. Advice on fish consumption: benefits and risks. Crown copyright, Norwich, UK.

Scientific Committee on Food, 2001. Opinion of the SCF on the risk assessment of dioxins and dioxin-like PCBs in food. Update based on new scientific information available since adoption of the SCF opinion of 22nd November 2000. Brussels, Belgium. http://ec.europa.eu/food/fs/sc/scf/out90_en.pdf

Sidhu, K.S., 2003. Health benefits and potential risks related to consumption of fish or fish oil. *Regulatory Toxicology and Pharmacology* 38, 336-344.

Sirot, V., Tard, A., Marchand, P., Le Bizec, B., Venisseau, A., Brosseau, A.., Volatier, J.L., Leblanc, J.C., 2006. Food exposure to persistent organic pollutants among French high seafood consumers (Calipso study). *Organohalogen Compounds* 68, 383-386.

Thompson, K.M., 2002. Variability and uncertainty meet risk management and risk communication. Risk Analysis 22, 647-654.

US EPA, 2005. Fish advisories. http://www.epa.gov/waterscience/fish/

US EPA, US FDA, 2004. What you need to know about mercury in fish and shelfish. http://www.epa.gov/waterscience/fishadvice/advice.html EPA-823-F-04-009:

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker,

N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences* 93, 223-241.

Vandecruys, W., 2004. Economical and social aspects of angling in public waters in Flanders (Dissertation, In Dutch). Limburgs Universitair Centrum, Diepenbeek, Belgium.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363-371.

Vrijens, B., De Henauw, S., Dewettinck, K., Talloen, W., Goeyens, L., De Backer, G., Willems, J.L., 2002. Probabilistic intake assessment and body burden estimation of dioxin-like substances in background conditions and during a short food contamination episode. *Food Additives and Contaminants* 19, 687-700.

WHO, 2003. Polychlorinated biphenyls: Human health aspects. WHO, Geneva, Switzerland.

Wiesmuller, T., Schlatterer, B., 1999. PCDDs/PCDFs and coplanar PCBs in eels (*Anguilla anguilla*) from different areas of the rivers Havel and Oder in the state of Brandenburg (Germany). *Chemosphere* 38, 325-334.

Wilhelm, M., Schrey, P., Wittsiepe, J., Heinzow, B., 2002. Dietary intake of persistent organic pollutants (POPs) by German children using duplicate portion sampling. *International Journal of Hygiene and Environmental Health* 204, 359-362.

Part VI

Conclusions and perspectives

19 APRIL 2002

Ministerieel besluit houdende een tijdelijk meeneemverbod van paling in alle openbare wateren en een tijdelijk meeneemverbod van alle vissen op bepaalde openbare wateren

De Vlaamse Minister van Leefmilieu en Landbouw,
Gelet op de wet van 1 juli 1954 op de riviervisserij, inzonderheid op de artikelen 14 en 15;
Gelet op het besluit van de Vlaamse regering van 20 mei 1992 tot uitvoering van de wet van 1 juli 1954 op de riviervisserij;
Gelet op het besluit van de Vlaamse regering van 13 juli 2001 tot bepaling van de bevoegdheden van de leden van de Vlaamse regering;
Overwegende dat de consumptie van paling, afkomstig uit de openbare wateren, dient voorkomen te worden in het belang van de volksgezondheid en dat op bepaalde sites waar zeer hoge verontreiniging werd vastgesteld de consumptie van alle vis dient voorkomen te worden in het belang van de volksgezondheid;
Overwegende dat op basis van wetenschappelijk onderzoek naar de aanwezigheid van verontreinigende stoffen in paling in de openbare wateren, in het overgrote deel van de openbare wateren concentraties aan polychloorbifenylen in paling werden aangetroffen die de norm overschrijden, vastgelegd in het koninklijk besluit van 6 maart 2002 tot wijziging van het koninklijk besluit van 19 mei 2000 tot vaststelling van maximale gehaltes aan dioxines en polygechloreerde bifenylen in sommige voedingsmiddelen;
Overwegende dat de norm van maximale gehaltes aan dioxines en polygechloreerde bifenylen in sommige voedingsmiddelen is gebaseerd op cijfers van jarenlange metingen door het Instituut voor Veterinaire Keuring, in samenwerking met het Wetenschappelijk Instituut Volksgezondheid en gelet op het advies dat de Hoge Gezondheidsraad uitbracht over deze norm;
Overwegende dat eenieder recht heeft op de bescherming van een gezond leefmilieu en gelet op het voorzorgsbeginsel, zoals bepaald in artikel 1.2.1, § 2, van het Decreet van de Vlaamse Raad van 5 april 1995 houdende algemene bepalingen inzake milieubeleid,
Besluit :
Artikel 1. In alle wateren waarop de wetgeving op de riviervisserij van toepassing is, is het voor iedere visser verboden om paling (levend of dood) in zijn bezit te houden. Iedere gevangen paling dient onmiddellijk te worden vrijgelaten in het water van herkomst.
Art. 2. In de waterlopen of gedeelten ervan, beschreven in artikel 3, is het voor iedere visser verboden om vis (levend of dood) in zijn bezit te houden. Iedere gevangen vis dient onmiddellijk te worden vrijgelaten in het water van herkomst. Het gebruik van aasvisjes is verboden. Het gebruik van een leefnet of enig ander tuig om vis in te bewaren is verboden.
Art. 3. 1° Kanaal van Dessel over Turnhout naar Sch oten : vanaf de baan Beerse-Merksplas tot het sas voor de monding in het Albertkanaal;
2° Laan : volledige lengte;
3° Maas : vanaf de weg naar het toeristisch voetvee r Rotem-Grevenbricht tot de autosnelwegbrug E-314;
4° Kanaal van Bocholt naar Herentals (inclusief Con govaart) : vanaf de baan Eksel-Eindhoven (Overpelt) tot de baan Geel-Kasterlee (Ten Aard);
5° Kanaal naar Beverlo : volledige lengte.
Art. 4. In afwijking van artikel 2 is het houden van vis in een leefnet toegelaten tijdens hengelwedstrijden toegestaan door of namens de Vlaamse minister bevoegd voor Riviervisserij, in de waterlopen of gedeelten ervan genoemd in artikel 3.
Art. 5. Dit besluit treedt in werking op de dag van bekendmaking ervan in het Belgisch Staatsblad en houdt op van kracht te zijn op 1 januari 2006.
Brussel, 19 april 2002.
V. DUA

A temporary catch and release obligation for eel fishermen (2002-2006) in Flanders.

Chapter 16

Summary, conclusions and recommendations

Summary

In this chapter we review and summarize the main results and conclusions of this thesis. We present the Flemish Eel Pollution Monitoring Network and give an overview of related studies. Current status and recent trends of contamination in eels are described. Our results induced several management measures, which are reviewed here. The implication of our research for human health management is discussed. We further summarize our findings concerning potential effects of contaminants on the eel population. Finally, recommendations for future work are suggested.

Introduction

This work constitutes a summary of results of a 14-year research programme (1994-2007) carried out at the Research Institute for Nature and Forest (Flanders, Belgium). The main objectives of this programme related to the status, effects and dynamics of pollution in the European eel:

1. to provide a comprehensive overview of the status and trends of a selection of contaminants in eel in Flanders;
2. to study the effects of pollution in eel;
3. to assess the potential of eel as chemical bioindicator in a regional and international context;
4. to estimate human health risks for eel consumers.

To this end, a monitoring network for contamination in yellow eel over Flanders was set up: the Flemish Eel Pollution Monitoring Network (EPMN). This network has been further developed and constitutes a major outcome of this work. In addition to methodological and analytical work presented in several chapters (Chapters 2, 3, 12 and 13), we describe the current state of the EPMN and review additional results, especially regarding communication and support to policy makers.

We summarize the main conclusions of this work, focusing on the use of the eel to report on status and trends of contaminants in the environment. We discuss human health hazards through the consumption of feral eels. An important issue is the adverse effect of these contaminants on the eel's breeding potential. To conclude, we present recommendations for future work.

Flanders' Eel Pollution Monitoring Network

Fish Contaminant Networks and Databases over the world

There is a long tradition to survey freshwater fish for the presence of contaminants on the North-American continent. Long-running and large-scale programmes are operational e.g. in Michigan (Bohr, 2007), Ontario (Health Canada, 2004) and New Jersey (State of New Jersey, 2006). These programmes primarily focused on monitoring consumption quality for safeguarding human and wildlife health, but extended their objectives to environmental issues.

As an example, the Michigan Fish Contaminant Monitoring Programme (FCMP) started in 1980 and includes regular monitoring over 45 watersheds in the Michigan Great Lakes area. The specific objectives of the FCMP are to:

1. determine whether fish from the sampled waters are safe for human consumption.
2. measure whole fish contamination concentrations.
3. assess whether contaminant levels in fish are changing with time.
4. assist in the identification of waters that may exceed standards and target additional monitoring activities.
5. evaluate the overall effectiveness of programmes aiming to reduce contaminant levels in fish.
6. identify high quality, non-contaminated water bodies.
7. determine if new chemicals are bioaccumulating in fish from Michigan waters.

This fish contaminant programme consists of several components that, in combination, provide data necessary to achieve these objectives. These include (1) edible fish portion monitoring; (2) native whole fish trend monitoring; (3) young-of-the-year perch monitoring; and (4) caged fish monitoring. Fish tissues are analyzed for bioaccumulative contaminants of concern, including mercury, polychlorinated biphenyls (PCBs), chlorinated pesticides (e.g. DDT/DDE/DDD), dioxins, furans and polybrominated biphenyl ethers (PBDEs). Data are under review each year to determine whether there are additional parameters of concern for which the fish should be analyzed. All fish contaminant data are maintained in a database, and communicated via annual (fish contaminant) reports. Monitoring activities in each watershed include not only fish contaminants, but also macro-invertebrate and fish community evaluations, water chemistry, wildlife contaminant studies, and sediment chemistry. This integration of the FCMP with other monitoring results is a sound basis to allow recommendations for resource management decisions. All American programmes have a solid data communication and dissemination component.

In some other networks, objectives are much broader defined, and the focus is also directed towards effect-monitoring and facilitation of related research work by providing comprehensive contaminant data. The objectives of the U.S. Large River Monitoring Network (USGS, 2007) for monitoring contaminants and their effects in large rivers include assessing the status and distribution of contaminants and effects in large rivers and monitoring changes over long time scales, but also providing information for scientists conducting site-specific investigations (information that is currently lacking in most of the biological effect measures). Furthermore, the network aims to identify topics to be addressed through applied research and to guide follow-up investigations. A wide variety of scientific results has been generated through this network (CERC, 2007).

American environmental monitoring networks are using large number of fish species to follow-up the presence of contaminants, but eel is not included. Distribution of the eel over the American continent is restricted mostly to the lower parts of the large rivers. Eel stocks of *Anguilla rostrata* are hampered by fisheries, dams and natural barriers (e.g. Niagara falls), and stocks are characterized by a similar decrease as the European eel.

Only few long term monitoring programmes of contaminants in freshwater fish are running in Europe. Apart from Flanders, a long term programme exists in The Netherlands. This network started in 1992 and is monitoring a selection of sites on large Dutch rivers and canals. The European eel is the main indicator species used to follow the presence of PCBs, organochlorine pesticides (OCPs) and mercury through this Dutch network. As this monitoring network was an initiative of the fisheries sector, also pikeperch (*Sander lucioperca*) tissue was analysed because of its value to both recreational and professional fisheries.

The eel as environmental sentinel

A considerable part of this thesis consists of debating the potential use of the European eel as chemical bioindicator. A chemical bioindicator is a species used to monitor the status or the effects of (specific) chemicals in an ecosystem. Eels are fat, long-lived, benthic carnivores, and spawn only once during their lives. They are prone to bioaccumulation of especially lipophilic compounds. Several physiological and ecological traits are beneficial for its use as chemical indicator and were reviewed in Chapter 12 (see Table 12.2). Yellow eels are highly sedentary. Their pollution load is thus expected to be indicative of the contaminant pressure of the site where they live. In this stage, within their on-growing habitat, movements (foraging behaviour or other) seem to be very limited, and they reflect the contamination present in this particular site.

Compared to other species, the yellow eel is by far more sensitive as chemical bioindicator. Eels have extremely high fat levels (Chapter 6), whereas other species usually do not exceed a muscle lipid content of 5%. Thus, especially lipophilic compounds are accumulated to a much higher extent in eels compared to other fish. Body burdens of contaminants in eels are in general a tenfold of the levels measured in other species (Figure 13.3, Chapter 13 and Weltens *et al*., 2002). Compared to other fish species in freshwater eel's home range is quite restricted: during recapture experiments in a fish assessment survey in a 100 ha lake (Lake Schulen, Simoens *et al.*, 2002), 92% of the eels (n = 48) were recaptured in the same zone of capture. During an experiment in another lake (Lake Weerde) 77% of the tagged eels (n = 1381) were recaptured within 50m of the initial capture place (44% within 10m) (Maes, 2003). Most other species show more or less marked moving behaviour with some having pronounced seasonal migration activities, and thus are less appropriate as indicators of local pollution pressure. Unlike the eel, freshwater fish species are iteroparous; seasonal reproduction cycles and associated changes in lipid metabolization, and loss of contaminants through passing to their offspring, complicate interpretations of contaminant analyses in these species. Another aspect that makes the yellow eel a better chemical bioindicator compared to other fish species is its wide distribution, eels are eurytopic and can be found in almost all aquatic habitats, whereas many other freshwater species are limited to certain water typologies. In Flanders, the eel is the third most widespread fish species.

Eels captured in the same sampling site show similar pollution profiles. Eels originating from other sites even within the same water body show distinct contamination profiles. Spatial resolution for distinguishing pollution variation between eels from one water body is quite high. In lacustrine environment, eels from several areas within a lake (maximum width 3 km) have distinct body burdens (Figure 9.3, Chapter 9). Eels from 3 different zones along a 14 km long canal were characterized by different levels of PCBs and OCPs (Figure 13.5, Chapter 13). After analyzing the contaminant data in eels from the River Nete catchment (Chapter 10) similar conclusions were drawn. Intra-site variation in eel body burden of lipophilic compounds is smaller than the inter-site variation, even between sites as close as 5 km. Future work will have to demonstrate if longitudinal gradients in pollution profiles in riverine eels still can be detected on even smaller spatial scale (≈1 km or less) which would allow for directed geographical identification of pollution sources and would further illustrate the restricted home range of eel. In addition, temporal variation in contaminant fingerprinting should be the object of a future study.

We have demonstrated that in Flanders current measuring strategy fails to protect aquatic life. Status and trend monitoring of contaminants in Flanders is based on measuring chemicals in water and sediments, but many analytical results of lipophilic compounds like PCBs and OCPs like DDT, drins or HCB, fall under the detection limit, whereas in fish, those compounds are detectable in nearly all cases, sometimes peaking to very high levels. From analyses of eel tissues, new centres of pollution for specific substances became apparent, and some substances seem to be omnipresent (like PCBs and DDTs) in Flemish rivers (see below). Results of fish stock assessment programmes and status reports on the ecological integrity of our water bodies (Peeters *et al*., 2006 and Chapter 1) are clearly indicative of the poor state of our aquatic environment. Recent studies have shown that these contaminants adversely impact individual, population and community levels of aquatic life in Flanders (Bervoets *et al*. (2005), Weltens *et al*., 2002, Berckmans *et al*., 2007, Geeraerts *et al*., 2007 and Belpaire *et al*., submitted (Chapter 6)).

We therefore strongly recommend critically assessing the monitoring strategy of chemical substances in our aquatic environment. Possibly, an efficient strategy needs to be based on measuring the presence of chemicals in several compartments, depending on the type of substance. In addition to monitoring specific contaminants

in biotic tissue, we suggest that chemical monitoring networks should include monitoring of the effects of environmental contaminants on the health of the biota. These biological effects can be measured by evaluating biochemical, physiological, morphological, and histopathological responses of organisms.

We provided the basis for a reference framework for contamination in eel, presenting reference values and quality classes for a selection of contaminants (See Chapter 13 and especially Table 13.3). This allows easy representation of results, both on spatial scale (see maps under Annex I) and for general reporting on the status and trends of contaminants for policy makers (Peeters *et al*., 2006; Mira 2007a,b,c). We recommend critical assessing and further fine-tuning of these reference values. Also for new compounds like brominated flame retardants (BFRs) and dioxins, reference values and quality classes should be defined. It should be envisaged to develop such framework considering total pollution load per contaminant group (e.g. total metal load) taking into account variation in toxicity of different compounds (using TEFs).

Flanders' Eel Pollutant Monitoring Network

A major outcome of this work has been the development of the Flemish Eel Pollutant Monitoring Network: a set of sites (Annex II) located on water bodies of diverse typology covering Flanders, where on a regular basis yellow eels were sampled and analysed for a selection of contaminants.

The basis of the EPMN dates back to 1994, when a study was initiated to assess the degree of pollution in fishes of the canal Boudewijnkanaal (Van Thuyne *et al*., 1995a). During this research it became apparent that there was not only a risk for human health by consuming eels from this canal, but – interestingly – that spatial variations in the contaminant load within eels caught at different locations were evident (Belpaire and Goemans, 2007b, Chapter 13). Apparently, eels were good indicators for the presence of a variety of contaminants in the environment, as was described earlier on in the literature (de Boer and Hagel, 1994). As a result we sampled eels from different sites collected during fish stock assessments by INBO in the late 1990s (Van Thuyne *et al*., 1995b, Van Thuyne *et al*., 1999, Belpaire *et al*. 1999). These samples were analysed for a series of PCBs, OCPs and heavy metals by the analytic laboratories of ILVO (the Sea Fisheries Department, Institute for Agricultural and Fisheries Research) at Ostend and CODA (the Veterinary and Agrochemical Research Centre) at Tervuren. The results were alarming. Overall, many of these substances attained very high concentrations in the muscle of the eel, which in Flanders is a much appreciated species in the local cuisine. The authorities were warned and, within the aftermath of the Belgian dioxin crisis (Dujardin *et al*., 2001), reacted quick and vigorously. They advised fishermen to stop eating self-caught freshwater fishes and initiated the monitoring of eel quality over Flanders. This, in 1999, was the real start of the EPMN.

At the moment the network consists of 376 sites (Annex II, Table II.1). Each of these sites is sampled for eels by electrofishing along both river banks or by fyke net fishing a river stretch of 100 or 250 m (dependent of typology). The objective is to catch 10 eels per site in the length class 35-45 cm, five of them are used for analysis of a variety of contaminants, the remaining are kept as archived samples for future analysis if required. In many cases it was not possible to achieve this objective, and smaller or larger individuals had to be taken. In some cases during sampling for the EPMN, also other species of fish were collected and analysed in the framework of specific research projects (Table 16.1 and 16.2).

Table 16.1. Overview of EPMN related research, cooperating laboratories or institutes and results valorising maximally eel samples. CODA - the Veterinary and Agrochemical Research Centre at Tervuren; ILVO - the Sea Fisheries Department, Institute for Agricultural and Fisheries Research at Ostend; IMARES - Netherlands Institute for Fisheries Research at IJmuiden (NL); KUL - University of Leuven, Laboratory for Animal Diversity and Systematics; UA - University of Antwerp, Laboratory for Ecophysiology, Biochemistry and Toxicology; UG[1] - Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology; UG[2] - Ghent University, Evolutionary Morphology of Vertebrates; ULg - University of Liège, Physical Chemistry, Mass Spectrometry Laboratory; UV - University of Vienna, Institute of Zoology, Austria; VITO - Vlaamse Instelling voor Technologisch Onderzoek (Flemish Institute for Technological Research); VMM - Vlaamse Milieumaatschappij (Flanders Environmental Agency); WG Eel - Joint EIFAC/ICES Working Group on Eels, FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea.

Tissue/organ	Category	Description	Laboratory	Reference
Muscle tissue	Heavy metals	cadmium, mercury, lead, chromium, nickel, copper, zinc, arsenic, selenium	CODA	Van Thuyne *et al*., 1995a, b; ; Van Thuyne and Belpaire, 1999; Nouwen *et al*., 2001, 2002, 2003; Belpaire, 2003; Goemans *et al.*, 2003; Belpaire and Goemans, 2004, Goemans and Belpaire, 2004; Maes *et al.*, 2005a,b ; Peeters *et al*., 2006 Maes *et al*, 2008; Belpaire and Goemans, 2007a,
Muscle tissue	PCBs	PCB 28/PCB 31, PCB 52, PCB 101, PCB 105, PCB 118, PCB138, PCB153, PCB 156, PCB 180	ILVO	Vandecasteele *et al*., 2001; Van Gerven *et al.*, 2002, 2003; Belpaire *et al.*, 1999; Belpaire, 2003; Goemans *et al*, 2003; Belpaire and Goemans, 2004, Goemans and Belpaire, 2004, 2005; Maes *et al*, 2005b, 2007; Peeters *et al.*, 2006; Belpaire and Goemans, 2007a, b; MIRA, 2007b; Belpaire *et al*., 2008.
Muscle tissue	OCPs	Hexachloro-cyclohexanen (α-HCH, γ-HCH), cyclodienes (Dieldrin, Aldrin, Endrin), Polychlorobenzenes (Hexachlorobenzene), chloroethanes (p,p'-DDD (TDE), p,p'-DDT, p,p'-DDE, trans-nonachlore)	ILVO	Belpaire *et al*., 1999; Steurbaut *et al*., 2001; Overloop *et al*., 2003; Belpaire, 2003; Goemans *et al.*, 2003; Belpaire and Goemans, 2004, Goemans and Belpaire, 2004; Maes *et al.*, 2005b, 2008; Peeters *et al*., 2006; Belpaire and Goemans, 2007a,b; MIRA, 2007c; Belpaire *et al*., 2008.
Muscle tissue	Lipid content		ILVO	Geeraerts *et al.*, 2007; Belpaire *et al.*, submitted
Muscle tissue	Brominated flame retardants	HBCD, TBBP-A, PBDE's	IMARES, UA	Belpaire *et al.*, 2003a; Morris *et al.*, 2004; Covaci *et al.*, 2005; Roosens *et al*., 2008.
Muscle tissue	Volatile organic compounds	50 substances (see Roose *et al*, 2003)	ILVO	Roose *et al.*, 2003, Block *et al*., 2003; Belpaire and Goemans, 2007a.
Bile	Polyaromatic hydrocarbons		ILVO	To be reported.
Muscle tissue	Dioxins		UL	To be reported.
Liver	Metallothionein		UA	Van Campenhout *et al.*, 2008.

Blood	Endocrine disruption	Plasma vitellogenin	UG[1]	Versonnen *et al*., 2004
Liver and blood	Fluorinated compounds	PFOS (perfluorooctane sulfonic acid)	UA	Hoff *et al.*, 2005
Morfometrics	Condition	Length – weight relation	INBO	Maes *et al.*, 2005a; Geeraerts *et al.*, 2007, Belpaire *et al.*, submitted.
Muscle and liver tissue	Genotype		KUL	Maes *et al.*, 2005a
Swim bladder	Parasites	*Anguillicola crassus*	UV, KUL	Schabuss *et al*., 1997; Audenaert *et al*., 2003
Intestine	Parasites	*Proteocephalus macrocephalus, Bothriocephalus claviceps, Camallanus lacustri, Acanthocephalus lucii, Acanthocephalus anguillae*	UV	Schabuss *et al*., 1997
Head	Head morfology	Broad headed – narrow headed	UG[2]	De Schepper *et al.*, submitted; Ide *et al.*, 2007

Data use	**Interdiciplinary**	**Description**	**Laboratory**	**Reference**
		Fish metal load and IBI	UA	Bervoets *et al.*, 2005, 2007 a
		Evaluation of the sanitation of River Dommel	UA	Bervoets *et al.*, 2007 b
		Statistical quality assurance of strategy and methodology of the EPMN	INBO	Onkelinx *et al*., 2007
		Comparison EPMN-SMN	INBO/VMM	Belpaire *et al*., 2007
		European database	INBO/WG Eel	Chapter 14
		Contaminant distribution over the abiotic and biotic aquatic compartments. Effect measures.	VITO/INBO/ VMM	Weltens *et al*., 2002, 2003.
		Endocrine disruption in roach and the EPMN.	VITO/INBO/ VMM	Berckmans *et al*., 2007

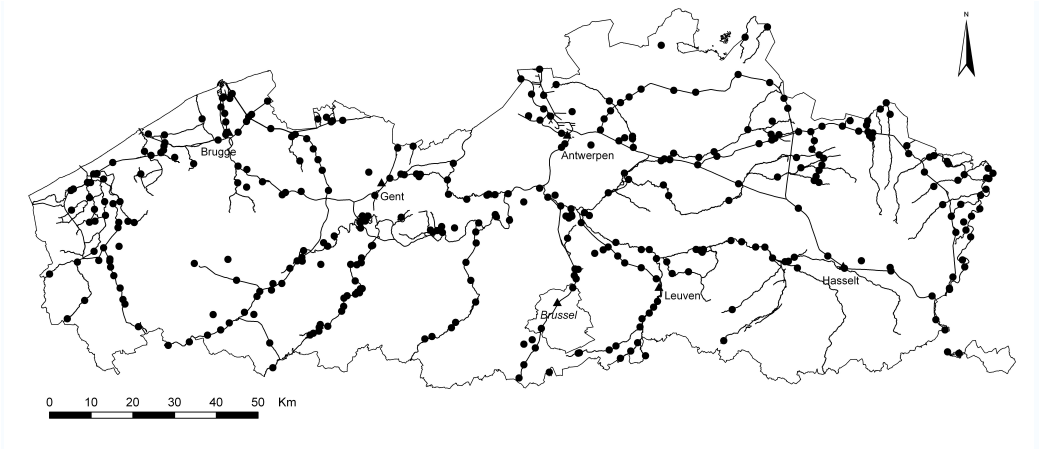


Figure 16.1. Position of sampling sites of the Eel Pollution Monitoring Network (n=376).

Table 16.2. Description of data from the EPMN available in VIS (February 2008). * Other fish species comprised mostly perch (*Perca fluviatilis*) - 34%, pikeperch (*Sander lucioperca*) - 23%, pike (*Esox lucius*) - 21% and roach (*Rutilus rutilus*) - 18%.

Period	01/05/1994 – 04/12/2006
Number of different sites visited	376
Number of sampling occasions	593
Total number of eels analysed	3093
Total number of other fish* analysed	433
Mean number of eels per sampling occasion	8.3
Total number of contaminant observations in eel (records)	82262

Detailed information on the EPMN and its analytic methodology and quality assurance is provided elsewhere (Goemans *et al*., 2003, Belpaire and Goemans, 2004, Belpaire *et al*., 2007; and Chapters 2, 3, 12 and 13). After validation, analytic results are entered in a database (VIS ('Vis Informatie Systeem') available at http://vis.milieuinfo.be/). An overview of the current results, as available via the VIS database is presented in Table 16.1. Currently, 82262 analytic results, from 3093 individual eels are available. Analytic data for PCBs, OCPs, and heavy metals in eel collected by EPMN during 1994-2001 have been published by Goemans *et al.* (2003). These tables have now been updated (Annex II) with new data from samples taken in the period 2002-2005. This information includes details about the sampling and location characteristics, and data presenting means per location and per sampling date for PCBs, OCPs and heavy metals, expressed on a wet weight basis. For individual data per eel, and for data on a lipid weight basis for PCBs and OCPs we refer to the authors or to the VIS website.

Our pollution network uses the eel as bioindicator, but undoubtedly, there is a need to assess the contamination in other species appreciated by the recreational fisheries for their consumption value. Especially predatory fish like pikeperch, perch and pike, are highly appreciated and are frequently taken home for consumption. Some cases are known where fishermen, or even poachers, sell their catch to locals. However, due to their predatory feeding habits, these species are prone to bioaccumulation and allowable consumption limits are exceeded on many sites

(Goemans and Belpaire, 2006) as shown for PCBs in Figure 16.2. Weltens *et al*. (2002, 2003) studied the behaviour of contaminants within the various compartments (including several fish species) of 5 Flemish aquatic ecosystems with different contamination pressure, and presented the differences in contaminant load between fishes of various trophic level (Chapter 13, Figure 13.3). A better understanding of the relationships between body burden in eels and in other fish species should be a focus for future work. Another focus should be to analyse the fate and strengths of eel as chemical bioindicator in the freshwater environment compared to other fish species (e.g. flounder, *Platichthys flesus*) used as indicators of the presence (or effects) of contaminants in marine (Roose *et al*., 1996) or estuarine systems (Richardson *et al*., 2001).

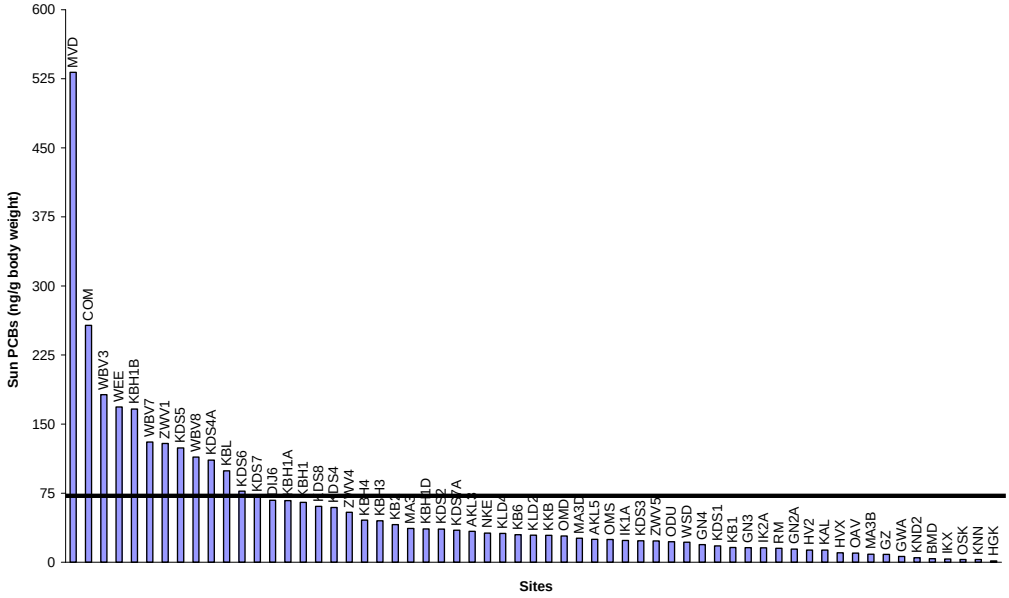


Figure 16.2. Sum PCBs (means) in predatory fish in 62 locations over Flanders (2001-2005) (Goemans and Belpaire, 2006). Black line represents Belgian legal consumption limit for PCBs. See Annex II (Table II.1) for abbreviations of sites.

We are aware that the use of the European eel, a now-endangered species, as biological monitor might raise some concerns. However as several aspects such as fat levels, contaminants, condition, parasites and diseases are believed to play a major role in the decline of the species, it is necessary to continue monitoring these aspects to better understand the reasons for the decline. Still, in order to minimize the number of eels killed for monitoring purposes, we recommend synergy in monitoring actions, e.g. by combining environmental monitoring through eel analyses with human health sanitary control of fisheries products. In Chapter 12 we recommended maximum use of the eels sampled to combine pollution monitoring with measuring other aspects such as condition, fat stores, pathology, genetics and morphometrics. This certainly has been our ambition during this work. To valorise the samples maximally, special effort has been made to use the samples for many purposes. From the sampled eels several tissues and organs have been taken for specific studies. This resulted in a variety of studies and results based on the samples collected during the EPMN. An overview of these studies is given in Table 16.1.

During this work a comprehensive database was generated with analytic data of a broad set of contaminants indicative of environmental pollution pressure over Flanders. This database has important potential for biological, environmental or interdisciplinary studies on spatial scale, providing data indicative of environmental pressure for diverse contamination. It may be used e.g. in the framework of effect studies, to analyse effects of contamination on individuals, populations or communities. Berckmans *et al*. (2007) recently studied relations between eel pollution load and endocrine disruption in roach. At the community level, the impact of heavy metals in fish was found to be correlated with a fish community index (IBI) (Bervoets *et al*., 2007a). Another future application which should be worked out is the comparison of aquatic pollution pressure with other pollution monitoring (e.g. terrestrial) or with emission monitoring networks. Finally an important challenge is to analyse causal interactions between the environmental EPMN database and human epidemiology. A human biomonitoring programme is currently carried out by the Flemish Centre for Environment and Health (Schroijen *et al*., 2008). The objectives are to measure and compare internal exposure to pollutants in various areas differing in pollution pressure and to assess whether observed differences in internal concentrations of pollutants are associated with biological and health effects. Incidences of some human diseases are characterized by spatial variation and may be influenced by environmental pollution pressure. To this end a special application of the EPMN has been cross-tabulated within the human health database ('Kruisdatabank'). This interdisciplinary work certainly needs further elaboration.

As stated earlier, due to the lipophilic character of many chemicals, monitoring strategies for contaminants in aquatic ecosystems solely based on water are insufficient to safeguard biotic quality. Analysis of sediments in the Flemish river Sediment Monitoring Network (SMN) however may to some extent reflect contamination with lipophilic compounds. This network monitors 600 sites over Flanders. Belpaire *et al*. (2007) discussed dissimilarities and complementarities between EPMN and SMN. To some extent a harmonisation has been carried out by localizing common sampling sites (for ca. 100 sites, Figure 16.3). On the other hand there is a complementarity in site distribution. The EPMN sites are restricted to localities where eels are present. Currently, the distribution of eel – and thus monitoring possibilities - in Flanders is hampered by low stock recruitment, numerous migration barriers and poor water quality conditions, resulting in eels being absent or only present at very low densities in a number of tributaries of larger rivers (Belpaire *et al*., 2003b). In the SMN, evaluation of sediment quality is restricted to river sediment consisting of sand, silt or mud. Rivers with stony river beds (as is the case in the Maas basin) can not be sampled. Future work will include further harmonization of both networks (also in time by simultaneous sampling), and concurrent analysis of data and studies of the relationship of contaminants in sediments and biota (e.g. through Biota Sediment Accumulation Factors).

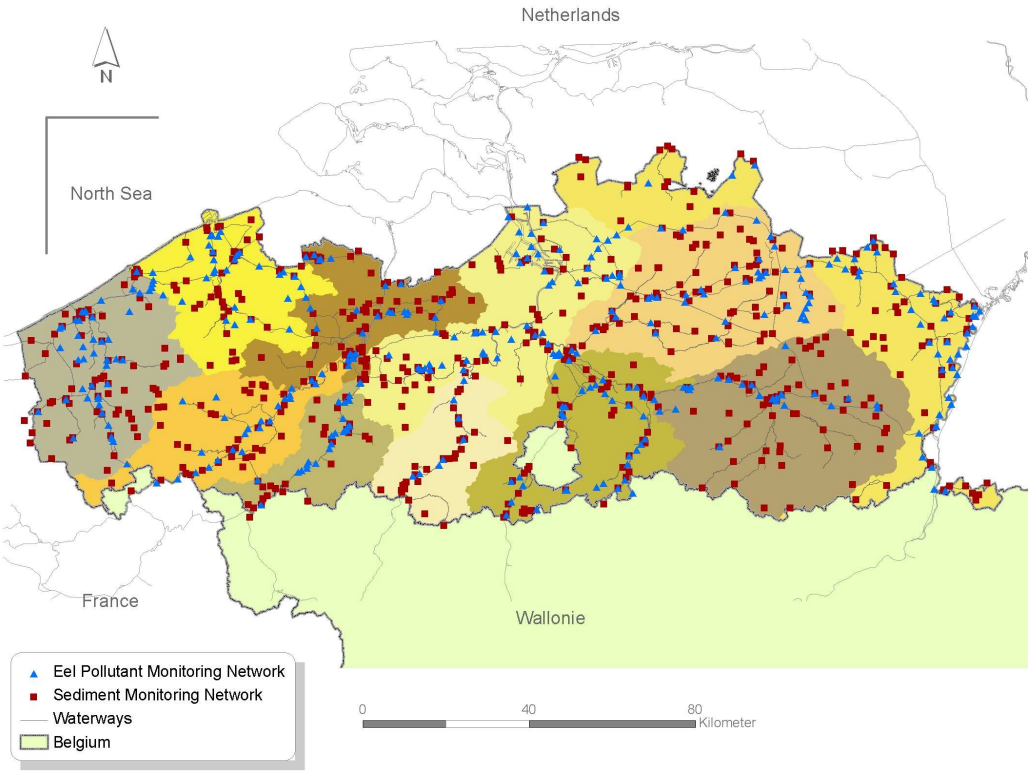


Figure 16.3. Sampling sites of the Eel Pollution Monitoring Network (blue triangles) and the river Sediment Monitoring Network (red squares) (adapted from Belpaire *et al.*, 2007).

Obviously, the EPMN has generated a variety of results and have shown to be a useful tool for the follow up and risk management of chemical compounds in our environment, enabling management measures to be taken (see below 'Management issues'). Especially for monitoring lipophilic compounds in our aquatic ecosystems measuring in eel is far more sensitive than in other compartments. However, apart from strengths and opportunities, the EPMN also has its weaknesses and shortcomings. A SWOT analysis is a valuable tool to critically assess strengths and weaknesses of the EPMN, to be able to achieve selected objectives in further planning. SWOT analysis incorporates analysis of **s**trengths, **w**eaknesses, **o**pportunities and **t**hreats, combining internal and external factors. Table 16.3 presents major components in this analysis. Strengths and opportunities have been discussed in several chapters of Part IV (The use of the eel as an indicator of pollution).

Weaknesses include insufficient understanding of the processes involved in bioaccumulation in eel. Contaminants may enter the fish through various ways (respiration, food intake, skin) but processes are strongly depending of the contaminant. Many factors might influence the uptake of contaminants by fish. Environmental factors such as temperature and oxygen might influence the rate of uptake through the gills; biotic assemblages and trophic interactions influence the uptake through food ingestion; the structure of the sediment has an effect on the bioavailability of sediment-bound contaminants. Typology of the habitat is another important factor: in lacustrine environments eel contamination is the result of the eel's

immediate surrounding environment (local pollution sources), whereas under riverine conditions eels will be polluted by chemicals both from local (sediment) or upstream (water, suspended solids) origin. Other factors affecting body burden of a certain chemical measured in fish are the rate of elimination and detoxification, chemical transformation through metabolic processes and lipid content of the fish. Another weakness is related to sampling procedure. Although length-standardised samples were purchased, the restricted presence and low densities of eel in certain basins, forced us to broaden the length range of sampled eels. Body burden is to some extent influenced by size, larger eels being more contaminated than small sized eels. Within the EPMN neither the gender nor the age of the eels is identified, but it may not be excluded that also age and sex of the eels might have influenced the results. As growth may be variable between individuals, gender and sites, analytic results may reflect contamination pressure during a variable period (e.g. 5-10 years for 40cm eels).

Another restriction of the EPMN is the rather limited set of contaminants (ca 30) being routinely analysed, compared to the large quantity of chemicals used in our environment (> 30 000). Criteria for selection of the PCBs, OCPs, heavy metals, BFRs, dioxins to be measured were primarily influenced by the expertise and the analytic possibilities of the laboratories and by available budget. Analytic limitations but also insufficient financial means are actually bottlenecks for further expanding the list of measured compounds. As discussed under Chapter 14, it may be necessary to prioritise to compounds which have been reported as harmful for eel, or which have been identified as priority hazardous substances to monitor under the Water Framework Directive or which are recognized as harmful by other international conventions or agreements, or which are regulated for the protection of human health and where consumption limits are available.

Onkelinx *et al*. (2007) critically assessed the structural basis and set-up of the EPMN. It was concluded that clear objectives are essential to lead the development process of this kind of networks. An adapted sampling strategy (number and distribution of samples and a fixed sampling periodicity depending on the objectives) is essential to be able to guarantee sufficient discriminating power, and allow sound conclusions. The choice of the variables to be measured and reporting should be organised in function of the objectives. Inadequate structural framework and insufficient financial means were the weak points of the EPMN. It will be a challenge to further optimize the EPMN, taking into account these recommendations, and guaranteeing a solid structural basis within the current reorganization of INBO (under the research section ‘Species and ecosystem diversity’).

In Chapters 12 and 13 we discussed extensively the rationales of choosing for the European eel as chemical indicator despite the fact the stocks have declined drastically. This might be perceived as contradictory to the principles of the Convention of Biodiversity. But studying and monitoring contaminants in eel might lead to understanding the reasons for its decline and setting up benchmarks of chemicals in the eel itself may guarantee its survival. But if, in contrast, the stock of the eel is further declining and the species might become close to extinction, this certainly represents a threat to the EPMN. In this case an alternative chemical indicator species for freshwaters must be searched for, but it will be difficult to found one which can compete with the sedentarity, the wide distribution and the fat concentration of the eel.

Table 16.3. SWOT analysis of the Eel Pollution Monitoring Network.

	STRENGTHS	WEAKNESSES
Internal factors	- sensitive chemical bioindicator, nearly always above detection limits - international dimension, extension to Europe-wide - global indicator of environmental contaminant pressure, hence essential tool for environmental management of contaminants (spatial and trend analysis) - allows for risk assessment for human health - possibilities for measuring effect respons (gene expression, biomarkers) - indicator for some substances in biota as demanded by the WFD - methodology, reference values, quality classes defined	- possible bias through impact of length, age, sex and growth heterogeneity of the eel - insufficient knowledge of processes involved in bioaccumulation (accumulation through, water, sediment, food, depuration time, detoxification) - restricted presence and low densities of the eel in certain basins - restricted set of contaminants - objectives not clearly defined - sampling strategy (number and distribution of samples and sampling periodicity) is not guaranteeing sufficient discriminating power - inadequate structural framework - insufficient financial means
	OPPORTUNITIES	**THREATS**
External factors	- international initiatives for monitoring eel quality in the framework of the eel stock protection measures - international harmonisation required - a key element directing further research into the causes of stock decline - complementarity with Sediment Monitoring Network - the use of EPMN data as indicator of global environmental pressure in relation to ecological health indicators - the use of EPMN data as indicator of global environmental pressure in human disease epidemiology - measuring and focusing on new compounds	- status of the eel as endangered species and further stock decline to minimal densities?

VIS: a web-based Eel Contaminant Database

There was a strong need to compile all contaminant data and make them available for internal and external use. 'VIS' is a database compiling data on freshwater fish in Flanders, currently comprising raw data of fish stock assessments, but also derived data like fish-based ecological integrity evaluations and fish contamination data. This database is available on the net since 2007, and is accessible to all external users, like policy makers, river basin managers, fish stock managers, water quality managers, inspection services, recreational fishermen.

The database allows queries to report on contamination status for a certain species in a catchment, river or site. Data can be downloaded and trends can be visualised by figures, an example is presented in Figure 16.4. Since February 2008, a site-based consumption advice based on the most recent results, has been added in the reports.

The contaminant database has been the initial model for the European Eel Quality Database (Chapter 14).

Further work on VIS will include implementation of more flexible tools for data storage, input and treatment as well as customer oriented communication (including an English version).

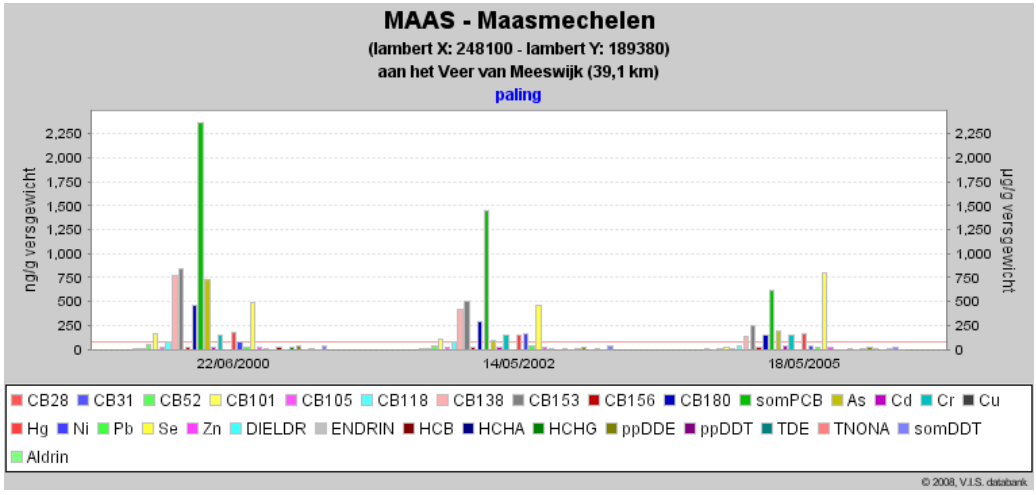


Figure 16.4. Example of a query for contamination data in eel from a site on River Maas (VIS, 2008). For more information about units and abbreviations we refer to the VIS website.

Status and trends

Analysing a number of contaminants in eel in a standardized way over a relatively dense monitoring network in Flanders gives a comprehensive overview of the contamination in Flemish waters fully covering the area of Flanders (Goemans *et al*, 2003, and Annex I). Especially for the lipophilic compounds like the PCBs and

OCPs measured, this is innovative research. As debated before, lipophilic compounds are very hard to trace in water or sediments.

Since the network is running now for 14 years, and many sites have been sampled twice or more, it becomes possible to draw trends. The maps and the database VIS allow now to analyse in detail the status and the trends for a specific contaminant, or a group of contaminants. They also allow detailed analysis of status and trends of contamination on a certain spatial scale (site, river, catchment, town, province, region). In VIS these trends can be viewed in reports via predefined queries on the database.

High peaks of some substances in eel tissue confirmed the previously known high pollution load of some specific areas e.g. the high lead and cadmium pollution in the canal Kanaal van Beverlo, historically related to the metallurgy activities. In many cases however, eel analyses revealed unknown environmental problems, like for instance the presence of 1,2-dibromo-3-chloropropane in eels from two canals (Albertkanaal and Leuvense Vaart) and 1,2-dichlorobenzene in eels of some sites along the River Leie, indicating some point sources. In a few cases analysis of eels from a specific location has demonstrated unsuspected high pollution levels of several contaminants, this was the case for Lake Weerde, possibly indicating local spilling or dumping of contaminated material. Other compounds measured in eels had distribution patterns which can be explained by specific agricultural or industrial pressures (e.g. lindane in the basins of IJzer, Demer and Dijle or HCB in the subbasin of the Grote Nete). But several contaminants were omnipresent in Flemish eels. BTEX (benzene, toluene, ethylbenzene and the xylenes) compounds were found at all places. This was also the case for PCBs and some very persistent OCPs like DDTs which were banned a long time ago. From the profiles of DDT and derivatives it was concluded that in some river basins, DDT must still be in use (see below). But maybe the most striking and threatening observations are the very high levels of some BFRs measured in eels at several sites along the rivers Leie and Schelde, peaking at Oudenaarde (River Schelde). This eel contamination is most likely related to the intensive textile industry from this area.

Eels from different river basins differ in contamination. In Chapter 10, PCB and OCP contamination profiles have been presented for some basins. Eels from the river IJzer are characterized by high OCPs, especially dieldrin and lindane (γ-HCH), and low PCB levels. River Leie shows a distinctive profile of PCBs, with a high proportion of lower chlorinated congeners. Rivers Dender and Schelde fingerprints are generally intermediate compared to the other rivers, but show considerably high PCB levels. River Demer eels usually have high lindane and DDT levels, whereas eels from River Grote Nete are characterized by peaking HCB and high DDT concentrations. In the River Maas PCB concentrations are peaking, and the PCB profile is totally different from that in the River Leie. It is dominated by the higher chlorinated PCBs. OCP levels in the River Maas eels are low.

Data on other contaminants (e.g. polyaromatic hydrocarbons (PAHs), dioxins) will be reported but are currently too limited to allow sound conclusions. Preliminary results of measurements of dioxins on a restricted set of locations indicate some reason for concern: dioxins seem omnipresent in eels and in ca. half of the sites data are above European consumption levels (unpublished data).

Trend analysis (Maes *et al*., 2008) over the period 1994-2005 indicated that there were significant decreases in the average wet weight concentration of all PCB congeners, nearly all pesticides and four metals.

The observed decline of PCBs in eel tissue was in agreement with other studies reporting on time series of contaminants in fish. PCBs were banned from the EU in 1985 and since then, several time series have indicated decreasing levels of contamination.

Also concentrations of most pesticides decreased significantly over time. This was especially evident for α-HCH and lindane, demonstrating that the ban of lindane in 2002 has positive effects on the accumulation in biota. Similar reductions were modelled for HCB, dieldrin and endrin; however these compounds were banned many years ago. Unexpectedly, concentrations of *p,p'*-DDT increased while at the same time, *p,p'*-DDD and *p,p'*-DDE showed significant decreases. At first sight, the ratio of DDE over DDT was in all eels analysed > 1, suggesting that remaining DDT had not been recently reapplied. However, at some locations in Flanders (Kanaal Dessel Schoten, Handzamevaart and Ieperkanaal) the ratio of DDE over DDT rapidly decreased over a few years by an order of magnitude of three. Such a steep decrease, even if the ratio was higher than one, probably indicates recent application of DDT and shows that not all stock was depleted. These results, as well as the recent observation that human blood samples, particularly of the juvenile population living outside urban areas, still contain DDT (Schroijen *et al*., 2008) urged regional policy makers to make a serious attempt in order to collect the remaining stock of banned pesticides.

Also for some heavy metals, concentrations decreased in the eel. Especially lead, arsenic, nickel and chromium were notably reduced. The concentration of lead in eel muscle tissue was consistently decreasing between 1994 and 2005, which possibly is related to the gradual changeover from leaded to unleaded fuels and a reduction of industrial emissions. For arsenic, nickel and chromium, the trend may be biased as data were available only since 2000. Cadmium and mercury, however, did not show decreasing trends and remain common environmental pollutants in the industrialized region of Flanders.

Following the very high levels of BFRs encountered in eels from Oudenaarde, new measurements were carried out in 2006. A descending trend in the contamination with BFRs was observed from 2000 to 2006 on this site. For PBDEs, levels have decreased by a factor 35 (26 500 to 780 ng/g LW), whereas for hexabromocyclododecane (HBCD), the decrease was less conspicuous, (35 000 to 10 000 ng/g LW). Based on these results we can conclude that in 2006 fish seem to be less exposed to PBDEs than 6 years earlier. This is probably due to the restriction regarding the use of the Penta-BDE technical mixture (since 2004), a better environmental management and a raising awareness concerning PBDEs. However, since there are no restrictions regarding its usage, HBCD can still be detected in large quantities, especially in aquatic environmental samples taken next to industrialized areas, where it is used in specific applications. The slight decrease in the concentrations of HBCDs in eels observed between 2000 and 2006 might indicate that HBCD is slowly being replaced by other BFRs for which no risk assessment is available. BFR-levels have decreased in the Oudenaarde area, but still remained higher than in other locations in Flanders. Also compared to several European studies the reported PBDE levels are still one order of magnitude higher in Oudenaarde eels. The textile industry is likely the cause of elevated BFR levels in fish on this part of the river Schelde, but further studies should be set up to determine the exact origin and how far this contaminated area extends over the whole river.

For other contaminants like volatile organic compounds and dioxins data series are too restricted to allow trend analysis.

We may conclude that the results from the Flemish Eel Pollution Monitoring Network are unique as they allow getting a comprehensive overview of a set of contaminants indicating environmental pressure over Flanders, and in an innovative way they are able to document the temporal evolution of some of these pressures.

Management issues

Belpaire and Goemans (2004) have presented and discussed the significance and relevance of this work for Flanders' environmental policy. During our study we communicated the results of our work to managers and made several recommendations to policy makers on local and international scale.

On a local scale, actions of policy makers were inspired by human health and environmental considerations.

Human health issues

Following the first results (Van Thuyne *et al.*, 1995a,b, Van Thuyne *et al.*, 1999, Belpaire *et al.*, 1999), and indications of – overall – quite severe pollution in Flemish eels, the Flemish Ministry of the Environment warned recreational fishermen not to consume eels of the 11 most polluted sites, and announced future measures (Dua, 1999):

- a temporary stop on restocking inland waters with eel;
- the start of the EPMN to get a more complete overview of fish contamination in Flanders;
- the continuation of the river Sediment Monitoring Network Flanders;
- initiatives for sanitation of river sediments.

In 2002, new data were made available for 250 sampling sites indicating in many cases very high levels of PCBs (Goemans *et al.*, 2003).

Subsequently, a Royal Decree was issued fixing new maximum allowed concentrations of dioxins and PCBs in fish and derived products. The maximum limit for Sum PCBs was set at 75 $ng.g^{-1}$ body weight (Belgisch Staatsblad, 2002a).

As our results showed that in 81% of the sites, legal PCB consumption limits in eels were exceeded, the Flemish Ministry of the Environment took more stringent measures to protect the health of recreational fishermen and their families (Dua, 2002a,b):

- a temporary catch and release obligation for eels over all public waters in Flanders (Belgisch Staatsblad, 2002b);
- a temporary catch and release obligation for all fish species in five water bodies (Kanaal Dessel-Schoten (partly), River Laan, River Maas (partly), Kanaal Bocholt-Herentals (partly, including Congovaart), Kanaal naar Beverlo) (Belgisch Staatsblad, 2002b) (Figure 16.5);
- the temporary prohibition of fishing with eel fyke nets and square nets in some parts of Flanders (Belgisch Staatsblad, 2002c).

In 2006, the catch and release obligation for eels over Flanders and for all fish species on specific sites, was not prolonged, despite the fact that human health risks had not significantly diminished. Moreover, several universities, agencies and advisory boards produced advices to warn for human health risks after consumption and to support strong measures to protect human health (Belpaire *et al.*, 2005, Hoge Gezondheidsraad, 2005, Section Human Health Care, 2005, Bilau *et al.*, 2007). The Flemish government issued a new leaflet discouraging fishermen to consume self caught freshwater fish. It is worth mentioning that in 2006, the same year the catch and release obligation in Flanders ended, the Walloon government, after being informed of a similar degree of contamination in eels in Wallonia, issued a catch and release obligation for eels in the Walloon region.

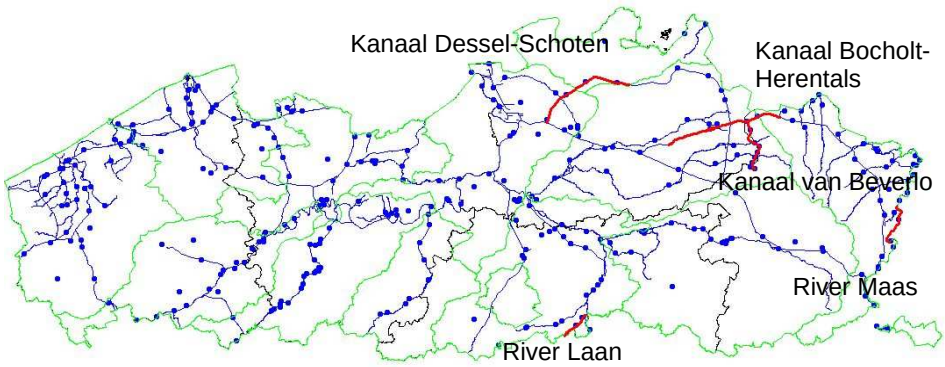


Figure 16.5. Localisation of stretches of rivers or canals (indicated in red) where a total catch and release obligation for all fish species was established in 2002. Selection of sites was based mainly on the very high concentrations of PCBs in eel (>2000 $ng.g^{-1}$ body weight) measured in the EPMN (Goemans *et al.*, 2003).

Environmental issues

The contaminant data in eel obtained through the EPMN, as an indication of the contaminant quality of our aquatic environment, have been generally accepted in the environmental management of PCBs, OCPs and heavy metals in Flanders, and data are published in the annual environmental reports (Peeters *et al.*, 2006, MIRA, 2007a,b,c,). In these reports, the concentration in eel represents three MIRA-indicators for respectively PCBs, OCPs and heavy metals.

Subsequent to the high levels of lindane recorded in eels from rural areas (especially in areas with intensive beet culture like in the IJzer, Demer and Dijle basins), the use of lindane was banned in Belgium in 2002, which initiated a decreasing trend in the lindane levels in eels in following years (Chapter 2).

After detecting and reporting very high concentrations of BFRs in eels from some sites in the Schelde basin (Belpaire and Goemans, 2002, Belpaire *et al.*, 2003a), specific measures were taken by inspection services and Flanders' Environmental Agency. A descending trend in the contamination with BFRs (especially for PBDEs) was observed from 2000 to 2006 at Oudenaarde. It is not clear whether this decrease is due to these specific measures and a better environmental management or to the restriction regarding the use of the Penta-BDE technical mixture (since 2004) (Chapter 3).

Other specific pollution problems initiated targeted measures for certain contaminants in local areas. The high levels of Cd (and Pb) in the northeast of Flanders (Kempen area) induced special measures (the 'Cadmium-plan') including intensified fish contaminant monitoring and communication actions in this area. Detection of DDT in fish (Goemans *et al.*, 2003, Belpaire *et al.*, 2008) and humans (Koppen *et al.*, 2002) in Flanders gave rise to a special DDT action plan, with intensive communication and sensitization in order to collect old stocks of these pesticides ('Pilot Project DDE').

In some cases measures in eels gave evidence to suspect specific and local points as sources for the contamination (Chapter 12). Further studies, including analysis of contamination profiles of derived products, congeners or isomers, may be

carried out in order to localise the exact origin. We reported that apparently DDT is still in use in Flanders. The proportion DDT/break-down products may lead to the discovery of these sites (Chapter 11). Further study of HCB distribution in eels from River Grote Nete will be able to pinpoint its origin. Also for BFRs and PCBs we have evidence that further study of the isomer (e.g. a, ß, γ-HBCD; Belpaire *et al.*, 2002, Morris *et al.,* 2004) and congener profiles will be able to localise exact pollution source. We recommend further research on this in consultation with environmental agencies and inspection services.

Management plans to clear out polluted river sediments have been announced by policy makers in 1999 (Dua, 1999). Priorities within river sanitation programmes for ecotoxicological reasons are currently based on the results of the river sediment quality evaluation (Triad method: De Deckere *et al.*, 2000). However there is a need to include the EPMN data, as they are an indication of bioavailability of those toxicants in the sediment (Belpaire *et al.*, 2007). Ecotoxicological sanitation of River Dommel, where sediments and biota were heavily polluted by heavy metals, started end 2006, and in this pilot case a follow-up study is currently evaluating to what extent aquatic life is restoring after the clearing out of polluted sediments (Bervoets *et al.*, 2007b).

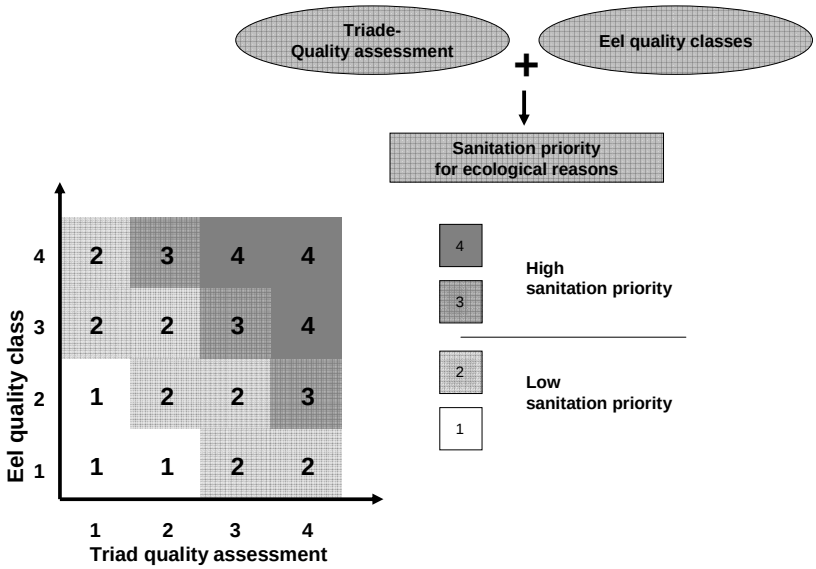


Figure 16.6. Schematic representation of the proposed integration of the bioaccumulation data in biota with sediment quality evaluation for prioritizing water bodies for clearing out sediment for ecological or ecotoxical reasons (Belpaire *et al.*, 2007).

On an international scale, management issues are mainly related to the international eel restoration plan and the monitoring of the chemical status of our waters for the Water Framework Directive.

International eel management

Within the international framework of the management of the eel we have reported our results to advising commissions and working groups (Joint ICES – EIFAC Working Group on Eel, the Scientific, Technical and Economic Committee for Fisheries of the EC) on many occasions. The aspect 'Quality of eel' and more specifically 'Quality of spawners' got increasing attention as an important management issue (WG Eel, 2007). This work, and related papers to this issue (Chapter 6) will certainly contribute to the acceptance of the idea that pollution by lipophilic contaminants might be a key element in the stock decline.

European Commission (2007) set up the framework and took measures for the recovery and sustainable use of the stock of the European eel. This directive requires the preparation of national Eel Management Plans (EMPs). In the draft guidance document for the preparation of these EMPs, member states have to indicate for each Eel Management Unit the proportion of eels of each life stage affected by contaminants, pathogens and parasites.

Our EPMN and its database modeled for the recent initiative taken by ICES/EIFAC Working Group (WG Eel, 2007) to set up the European Eel Quality Database to compile all information on quality elements of the eel, including lipid content and contamination data in the European eel over its distribution area.

Water Framework Directive

The Water Framework Directive recently (European Commission, 2006a) proposed to monitor a selection of priority substances and to report on the chemical status of European water bodies. The final objective is the protection of aquatic life and human health. The majority of these substances are lipophilic, nevertheless it is proposed to monitor them in the water-phase. As there is serious concern about whether measurements of these lipophilic compounds in water will give results that will guarantee the protection of aquatic life, monitoring in biota seems to be more appropriate. We found evidence that current legal chemical quality standards for the water column are insufficient to guarantee the health of our aquatic ecosystems. In Chapters 12 and 13 we discussed these issues and brought under attention of the international policy that eels can be used to report on the chemical status of all categories of water bodies within the river basin approach of the WFD (rivers, lakes, transitional water bodies, coastal water bodies, artificial or heavily modified water bodies). How to monitor the chemical status within the WFD is still under international discussion.

Human health hazards through consumption of feral eels

The potential human health risk of consuming contaminated fish has been the initial motive to start this work. After a local study on the Boudewijnkanaal in 1994, high levels of heavy metals were reported (Van Thuyne *et al.*, 1995a). Later on Van Thuyne *et al.* (1999) and Belpaire *et al.* (1999) measured and reported concentrations of metals and lipophilic compounds like PCBs and pesticides in eels from a small set of places. As the level of contamination was quite alarming, the Ministry of the Environment decided to start a comprehensive study to assess consumption quality in eels in fishing waters over Flanders. Primary objective was the

protection of human health of the freshwater fishermen and their families. The results indicating high levels of contamination over several sites were reasons for serious concern, since self caught fish might be an important part of the diet of fishermen. In this work, on several occasions (e.g. Chapters 2, 3 and 15), we discussed potential human health hazards by consuming wild eels.

Maes *et al.* (2008) summarized both the European and national legislative framework on maximum residue and contaminant levels in food (Chapter 2, Table 2.6) and calculated the proportion of Flemish eels in this study that exceeded these limits. Relative to maximum quantities as adopted by legislation, it appears that eel tissue was, in general terms, compliant with European regulations for pesticides and heavy metals. However, for several pesticides in some parts of the country under intensive agricultural pressure, concentrations measured in eel were not compliant to this legislation. For PCBs, the maximum limit, based on the sum of seven indicator PCBs, was fixed at 75 $ng.g^{-1}$ wet weight basis for fish (Belgisch Staatsblad, 2002a). PCB concentrations in eel muscle tissue remain problematic. About 76 % of the analysed individuals and 78 % of the sampling stations exceeded the maximum level for human consumption. For dioxins and furans a maximum level was recently (2006) established by the European Commission (European Commission, 2006b). On a small set of locations (N = 8) recent, unpublished data indicate that in 50 % of the sites concentrations in eels exceed this maximum level.

Maes *et al.* (2008) calculated that a meal consisting of 100 grams wild eel would result in a dietary uptake of 24 pg TEQ g^{-1} body weight for an adult person of 70 kg. This indicates that dietary exposure to PCBs by eating wild eel exceeds the tolerable weekly intake that was advanced by the Scientific Committee on Food of the EU, which is 14 pg TEQ kg^{-1} body weight.

Roosens *et al.* (2008, in press) reported a wide concentration range of BFRs in eel samples collected from River Schelde around Oudenaarde and wondered wether the consumption of contaminated eel had an important impact on human exposure. Therefore, exposure profiles to PBDEs and HBCDs through eel consumption originating from less and most contaminated locations were calculated, and a variable contribution to the total human exposure through local eel consumption was estimated. The calculated daily intake ranged from 3 ng to 330 ng PBDEs/day for normal eel consumers, but was as high as 9 800 ng PBDEs/day for anglers, whom may be considered at risk. These values indicate a high contribution of contaminated fish to the total dietary intake of PBDEs by the local anglers. Contributions to the dietary intake were in the same order of magnitude as for the highly contaminated lake Mjøsa in Norway (Thomsen *et al*., 2008 in press). For obvious reasons, stakeholders (fish stock managers and human health protectors) should avoid fish consumption of this part of River Schelde with all legal and practical means.

Bilau *et al.* (2007) assessed the intake of the Sum PCBs through consumption of eel by recreational fishermen and compared this with the intake of a background population. The median estimated intake of Sum PCBs for recreational fishermen varies between 18.4 and 237.6 ng/kg BW/day, depending on the consumption scenario, while the estimated intake of the background population (consumers only) is 4.3 ng/kg BW/day. Since the levels of intake via eel for two intake scenarios are respectively 50 and 25 times higher than the intake of the background population, fishermen's body burden might be quite higher and reach levels of toxicological relevance. They concluded that the intake of PCBs via the consumption of self-caught eel is at a level of high concern.

All along our work the measures to protect human health have been object of much debate by politicians in the Flemish parliament and the federal senate (see e.g. Crevits, 2007). From the studies presented above, it may be deduced that the consumption of eels caught in the wild should be firmly discouraged. All practical

means should be taken to prevent fishermen from consuming their self-caught fish. During our work two leaflets have been published to warn fishermen. Very recently (February 2008), a site specific contaminant advice can be obtained via the VIS website. We are aware that communication and sensitization alone is not sufficient to protect human health. Many fishermen still take their eels (and some other species) home for own consumption and in some cases fish are even sold in local circuit. As current measures fail to protect the health of fishermen and their families, we urge for more stringent measures with all legal and practical means.

One of the objectives of this work was to quantify the risk of eating feral eels from Flanders for the human health. We are aware that through our theoretical approach, this objective was only partly met, as it warrants proper field surveys involving the study and follow-up of risk groups (eel anglers and consumers) by ecotoxicologists. We therefore recommend cooperation with the Flemish Centre for Environment and Health to study potential effects (body burden) after prolonged consumption of self-caught fish by (eel) fishermen.

Contamination in eel and its role in the collapse of the stock

Potential causes of the decline of the eel (Chapter 1) have been summarized by Dekker (2004). *Fisheries* have an impact on all stages. The glass eel exploitation is reducing the upstream migration of recruits, in some cases (e.g. the French River Vilaine) virtually all glass eel are caught. But also the exploitation of the yellow eel can reduce the local stock and limits silver eel production to almost zero in some areas (e.g. Lake IJsselmeer). Silver eel fisheries exploit migrating eels and hence have direct impact on the quantities of potential spawners. *Habitat loss and obstruction of migration* are important anthropogenic pressures. The surface of suitable areas where yellow eels can grow up in inland waters has decreased considerably due to river embankment, canalisation and river fragmentation. Available area of marshes and reed fringes decreases all over Europe. Many constructions on European waterways block the upriver migration of glass eel and elvers. Sluices hamper the tidal driven movement of the glass eel entering the estuaries, and the many dams and weirs hinder the passage to upstream parts of the river system. Many reports show the total absence of eels in upper parts of rivers obstructed by dams. The need for more 'green energy' has triggered the establishment of more *hydropower stations*, impeding the downward migration of the silvers eels, injured through their passage of the turbines. Turbines can cause immediate death, serious injuries or damages with delayed effects. In polder areas *draining pumps* have been shown to kill migrating silver eels. But also natural processes such as *predation*, especially by cormorants, have been reported as an important threat causing considerable loss of eels. The number of breeding pairs of the cormorant population has multiplied by a factor 60 compared to 1970. Information about predation in marine waters is missing. Other hypotheses put forward to explain the eel stock collapse deal with *changes in oceanic processes*. The migratory phase of adults and larvae as well as the egg and larvae production might have been influenced by climate variation. The migration of the silver eel to the Sargasso Sea might be hampered by an increased strength of the Gulf Stream. Altered climate might have changed the local conditions (strength or position of the thermal fronts) in the spawning area affecting mating success. Other potential climate linked changes relate to nutrient availability and productivity influencing larval development and growth. Changes in oceanic currents may affect migration, growth, survival and metamorphosis of the leptocephali. Another (anthropogenic) pressure is the

introduction and spread of non-native *parasites and diseases* diminishing the quality of the spawners. Some of these parasites (e.g. *Anguillicola crassus*, a parasite of the swimbladder) have been reported to impair swimming performance; infected eels spend more energy for migration and increase overall energy consumption. Finally, *pollution and contamination* by hazardous substances is affecting the eel in various ways.

We summarize the main findings of our work in this field in the following section and draw some conclusion related to the potential role of contamination in the collapse of the stock.

In the eel, the impacts of contaminants on metabolic functions and on behaviour of the eel are widely divergent and act through various mechanisms. Chapter 5 reviewed recent literature describing the effects of the different groups of contaminants on the European eel.

Endocrine disruption seems a widely distributed phenomenon among freshwater fishes. Also in Flanders this was recently documented in a comprehensive study (Berckmans *et al.*, 2007) assessing reproductive functions in Flemish roach (*Rutilus rutilus*). This study demonstrated that in 50% of male roach, testes were feminized. In eel, Versonnen *et al.* (2004) (Chapter 8) investigated potential effects of xenoestrogens, and measured plasma vitellogenin (VTG) content in 142 eels sampled at 20 different locations of variable pollution levels. The plasma VTG content of eels was very low, despite a very high internal load of endocrine disrupters. Therefore, no indications were found for estrogenic effects to occur in natural freshwater eel populations in Flanders. These results suggest that immature yellow European eel might not be the best sentinel species to study the effects of estrogenic compounds on VTG levels of wild fish populations. Most probably, endocrine disrupting effects of pollutants related with reproduction, will only become apparent during the maturing silver eel stage.

Maes *et al.* (2005a) studied the effects of pollutants on the genome of eels with variable metal load. They analysed the relationship between heavy metal bioaccumulation, fitness (condition) and genetic variability. A significant negative correlation between heavy metal pollution load and condition was observed, suggesting an impact of pollution on the health of sub-adult eels. In general, a reduced genetic variability was observed in strongly polluted eels, as well as a negative correlation between level of bioaccumulation and allozymatic multi-locus heterozygosity.

Van Campenhout *et al*. (2008) studied the effect of metal exposure on the accumulation and cytosolic speciation of metals in livers of European eel by measuring metallothioneins (MT) induction. This research was carried out in four sampling sites in Flanders showing different degrees of heavy metal contamination (Cd, Cu, Ni, Pb and Zn). It was concluded that the metals, rather than other stress factors, are the major factor determining MT induction. The effects of perfluorooctane sulfonic acids (PFOS) in Flemish eels were studied by Hoff *et al*. (2005), indicating that PFOS induces liver damage.

Geeraerts *et al.* (2007) analysed our extensive dataset of contaminants by statistical modelling and concluded that PCBs, especially the higher chlorinated ones, and DDTs, have a negative impact on lipid content of the eel. We further demonstrated that fat stores and condition decreased significantly during the last 15 years in eels in Flanders (Geeraerts *et al.*, 2007) and in The Netherlands (Belpaire *et al.*, submitted, Chapter 6)), jeopardizing a normal migration and successful reproduction of this endangered species.

As was discussed in Chapter 6 these findings are of utmost importance for eel management, and may represent a key element in the search for understanding the causes of the decline of the eel.

We postulate that contaminant pressure is a very plausible causative factor for the collapse of the eel stocks and summarize major arguments and hypotheses to underpin this.

1. Contamination has been demonstrated as the cause of population collapse of many other biota from the 1970s on (e.g. the collapse of several birds of prey in the 1960s due to DDT).
2. Many chemicals have been developed and put on the market, simultaneous with the intensification of agricultural and industrial activities during the 1970s. The timing of this increase in the production and release of chemicals may fit with the timing of the decrease in recruitment from 1980 on.
3. Eels bioaccumulate many chemicals to a very high extent.
4. The more or less simultaneous decreases in recruitment in the Northern-hemisphere *Anguilla* species, like *A. rostrata* and *A. japonica*, during the last 30 years, is an additional argument endorsing the idea that some new contaminants quickly spreading over the industrialized world, are key elements in the decline.
5. Many reports have been dealing with direct adverse effects of contamination on individual, population and community level in fish. In eel, many detrimental effects of contaminants on the individual level have been demonstrated, including impact on cellular, tissue and organ level. Also genetic diversity seems to be lowered by pollution pressure.
6. Considering the high levels of contamination in eels from many areas, endocrine disruption in mature silver eels might be expected, jeopardizing normal reproduction. Dioxin-like contaminants have been reported to hamper normal larval development.
7. Fat levels in eels have decreased considerably over the past 15 years, suggesting failure of successful migration and reproduction. This decrease is mainly induced by contamination.

As described above many pressures have been suggested or demonstrated to negatively impact the eel stock. Maybe these pressures acted in a synergetic way, resulting in the collapse of the stock. Dekker (2004) suggested that the most likely proximate cause of the collapse in recruitment observed in the European eel after a prolonged period of gradually declining abundance in continental waters is caused by an insufficient quantity of spawners. From the evidence presented here, *we may conclude that not only the quantity, but also the quality of the potential spawners leaving continental waters, is insufficient, and has contributed to the decline of the stock*. Contaminant pressure in continental waters seems to represent a major threat for the European eel stock and will limit the possibilities of restoration of the stock. Hence, we believe that within the (inter)national eel restoration plans, measures to decrease contaminant pressure are an essential issue.

Recommendations for future research and management

Several upcoming papers not included in this thesis deal with processes related to (the effect of) bioaccumulation. One paper describes results of an in depth analysis of the impact of contaminants and possible confounding factors (such as eel length and water typology) on the fitness (lipid content and condition) of the eel. Another paper will present the results of field work following tagged eels under closed lacustrine condition (Lake Weerde). During seven years the home range, the growth

and the spatial and temporal trends in contamination have been studied. This will certainly give more details about the sedentarity of the yellow eel.

During our work, on several occasions, we have put forward recommendations for future management measures and for further research focus. Many recommendations towards policy makers were published in the annual environmental and nature reports (MIRA/NARA). In several chapters of this work specific recommendations were put forward, some of them were repeated in this summary chapter.

Besides, some reports included an extensive recommendation part. Belpaire *et al.* (2007) issued recommendations for further co-operation and harmonization between the Eel Pollution Monitoring Network and the Sediment Monitoring Network, not only within the monitoring rationale but also in the application of the results. Onkelinx *et al.* (2007) made clear that the structural framework and financial means of the EPMN were insufficient and recommended an adaptation of the sampling strategy. Geeraerts *et al.* (2007) summarized perspectives for future work, focusing on the eel as a bioindicator on a national and international scale, and on future research studying the ecotoxicological impact on the eel.

Internationally, during the joint EIFAC/ICES Working Group on Eel (WG Eel, 2007) seven formal recommendations were put forward, two of them being directly related to our work:

- *The European Eel Quality Database (EEQD) should be further developed and maintained and Member States should initiate harmonised monitoring strategies for eel*.
- *Under the implementation of the WFD eel specific extensions should be included, using the eel as an indicator of river connectivity and ecological and chemical status, and making cost-effective use of collected data, also for the benefit of the EU Eel Regulation and recovery of the eel stock*.

Finally, we conclude by listing major recommendations for research and management:

Research

- To continue on local scale the long term monitoring of contaminants in eel with emphasis on the optimization of the methodological approach, the analysis of new compounds, an appropriate communication and a robust data management. To scale up Flanders' eel quality monitoring to a European scale.

- To carry out studies, on local and international scale, to comprehend relationships between contamination and eel stock decline. An important focus should be to study the effects of contaminants on lipid metabolism and condition.

- To stimulate studies on a more multidisciplinary scale by using and valorising the EPMN data as a global indicator of environmental contamination pressure. In particular by analysing relations between EPMN data and ecological quality of rivers (e.g. fish community or other biotic quality indices) or indicators of health effect in fishes or other organisms; by carrying out comparative studies of aquatic and terrestrial monitoring series (soils, free-range eggs, birds, mammals); and by using the EPMN contaminant data within human health studies (e.g. studies of spatial variation in internal concentrations of pollutants in humans and incidence of human diseases associated to environmental pollution pressures).

Management

- Harmonisation of monitoring strategies. Currently, local and international contaminant assessment methods and legal frameworks fail to protect aquatic life. Monitoring strategies for contaminants in the different aquatic compartments in Flanders should be harmonized taking into account the specific properties of these substances (in particular lipophilicity and capacity to bioaccumulate). Furthermore the normative framework for contaminants should be adjusted taking into account bioavailability and bioaccumulation. Also on an international scale, the chemical monitoring required by the Water Framework Directive should be directed – for lipophilic compounds – towards monitoring in biota. The eel could serve as a model.

- Environmental management of contaminants. To undertake by all means all necessary actions to decrease the level of contaminants in our aquatic habitats, and by extension in our environment, in order to decrease bioaccumulation in and effects on eel (and other fish). Contaminant management should include conducting studies to find pollution sources, to allow remediation by taking goal-oriented and successful measures. (e.g. stop discharging contaminated water, ban chemicals, collect old stocks, clear out polluted river sediments, …).

- Human health. As current measures fail to protect the health of fishermen and their families, we recommend to take more stringent measures to prevent fishermen from consuming their self-caught eel (and other freshwater fish), with all legal and practical means.

References

Audenaert, V., Huyse, T., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2003. Spatio-temporal dynamics of the nematode *Anguillicola crassus* in Flanders, Belgium. *Diseases of Aquatic Organisms* 56, 223-233.

Belgisch Staatsblad, 2002a. Koninklijk besluit tot wijziging van het koninklijk besluit van 19 mei 2000 tot vaststelling van maximale gehaltes aan dioxines en polygechloreerde bifenylen in sommige voedingsmiddelen, Belgisch Staatsblad 16 april 2002.

Belgisch Staatsblad, 2002b. Ministerieel besluit houdende een tijdelijk meeneemverbod van paling in alle openbare wateren en een tijdelijk meeneemverbod van alle vissen op bepaalde openbare wateren, Belgisch Staatsblad 25 mei 2002.

Belgisch Staatsblad, 2002c. Ministerieel besluit houdende een tijdelijk verbod op het gebruik van de palingfuik en het kruisnet in de grensscheidende Maas en de niet bevaarbare noch vlotbare waterlopen en kanalen in de provincies Oost-Vlaanderen en West-Vlaanderen. Belgisch Staatsblad 25 mei 2002.

Belpaire, C., Van Thuyne, G., Callaars., S., Roose, P., Cooreman, K., Bossier, P., 1999. Spatial and temporal variation in organochlorine pesticide and polychlorinated biphenyl pollution in fresh water aquatic ecosystems in Flanders using the European eel (*Anguilla anguilla* L.) as an indicator. EIFAC/ICES, Working Group on Eel Silkeborg (DK), 20-25 September 1999.

Belpaire, C., Goemans, G., 2002. Hoge meetwaarden van vlamvertragers in paling en sediment van waterlopen in het Scheldebekken. Nota voor Vera Dua, Vlaams minister van Leefmilieu. Instituut voor Bosbouw en Wildbeheer, 2002, IBW.Wb.V.Adv.2002.092.

Belpaire, C., 2003. Het Vlaamse palingpolluentenmeetnet : resultaten en toepassingen. In: Proceedings PDL Symposium : Effecten van polluenten op plant en dier, p. 41-47.

Belpaire, C., Goemans, G., de Boer, J., Van Hooste, H., 2003a. Verspreiding van gebromeerde vlamvertragers. In: Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, p. 387-395.

Belpaire C., Goemans G., Van Thuyne, G., Verreycken H., Maes, J., 2003b. Eel Fisheries and Management in Flanders, Belgium: Status and Trends. International Eel symposium, Quebec, 10-15 august, 2003. Available at http://www.giuliodeleo.it/AFS-Index.html

Belpaire, C., Goemans, G., 2004. Monitoring en normering van milieugevaarlijke stoffen in paling: bruikbaarheid en relevantie voor het milieubeleid. *Water* 2004, 1-14.

Belpaire, C., Goemans, G., Van Slycken, J., 2005. Advies van het Instituut voor Bosbouw en Wildbeheer over het meeneemverbod voor paling. Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.V.Adv.2005.116, 7p.

Belpaire, C., De Cooman, W., Goemans, G., Onkelinx, T., Quataert, P., 2007. Waterbodem- en palingpolluentenmeetnet: een tandem voor de waterbodemsanering, *Water* 2007, 1-8

Belpaire, C., Goemans, G., 2007a. Eels: contaminant cocktails pinpointing environmental contamination. *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? *Vie et Milieu – Life and Environment* 57 (4), 235-252.

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,* in press.

Belpaire, C., Goemans,' G., Geeraerts,' C., Quataert,' P., Parmentier, K., Hagel, P. and De Boer, J., submitted. Decreasing eel stocks: The Survival of the Fattest? *Ecology of Freshwater Fish.*

Berckmans, P., Witters, H., Goemans, G., Maes, J., Belpaire, C., 2007. Ondersteunend studiewerk en verdere karakterisatie van de Vlaamse toestand inzake hormoonverstoring: vraagstelling inzake ecologische relevantie. Studie uitgevoerd in opdracht van VMM door Vlaamse Instelling voor Technologisch Onderzoek, Mol, VITO rapport nr. 2007/TOX/R071 - Instituut voor Natuur- en Bosonderzoek, Brussel, INBO report nr. R.2007.37.

Bervoets, L., Goemans, G., Belpaire, C., Van den Boeck, H., De Boeck, G., De Jonge, M., Van Thuyne, G., Breine, J., Joosen, S., Van De Vijver, B., De Temmerman, L., De Cooman, W., 2007b. Ecologische en ecotoxicologische toestand van de Dommel vóór de sanering van de waterbodem. University Antwerp and INBO, Report for Flanders Environmental Agency.

Bervoets, L., Knaepkens, G., Eens, M., Blust, R., 2005. Fish community responses to metal pollution. *Environmental Pollution* 138, 338-349.

Bervoets, L., Reynders,H., Knapen, D., De Cooman, W., Goemans, G., Belpaire, C., Van Thuyne, G., Blust, R., 2007a. Is de visindex bruikbaar voor de evaluatie van effecten van metalen op vislevensgemeenschappen? *Water* 2007, 1-8.

Bilau, M., Sioen, I., Matthys, C., De Vocht, A., Goemans, G., Belpaire, C., Willems, J.L., De Henauw, S., 2007. Probabilistic approach to polychlorinated biphenyl (PCB) exposure through eel consumption in recreational fishermen vs. the general population. *Food Additives & Contaminants* 24 (12), 1386-1393.

Block, C., Vandecasteele, C., Andriessens, S., Goemans, G., Belpaire, C., Roose, P., Van Hooste, H., 2003. Verspreiding van vluchtige organische stoffen (VOS). In: Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, p.137-146

Bohr J., 2007. Fish contaminants. The Official State of Michigan Website. Department of Environmental quality. Michigan Fish Contaminant Monitoring Program (FCMP). Available at http://www.michigan.gov/deq/0,1607,7-135-3313_3686_3728-32393--,00.html. Assessed January 2008.

Boer, J. de, Hagel, P., 1994. Spatial differences and temporal trends of chlorobiphenyls in yellow eel (*Anguilla anguilla*) from inland water of The Netherlands*. The Science of the Total Environment* 141, 155-174.

CERC, 2007. Biomonitoring of Environmental Status and Trends (BEST) Large Rivers Monitoring Network. BEST-LRMN Related References. Columbia Environmental Research Center, 2007. Available at http://www.cerc.usgs.gov/data/best/search/lrmn-related_references.htm. assessed January 2008.

Covaci, A., Bervoets, L., Hoff, P., Voorspoels, S., Voets, J., Van Campenhout, K., Blust, R., Schepens, P., 2005. Polybrominated diphenyl ethers (PBDEs) in freshwater mussels and fish from Flanders, Belgium. *Journal of Environmental Monitoring* 7, 132-136.

Crevits, H., 2007. Vraag om uitleg van mevrouw Margriet Hermans tot mevrouw Hilde Crevits, Vlaams minister van Openbare Werken, Energie, Leefmilieu en Natuur, over de aanwezigheid van vervuilende stoffen in zoetwatervis, in het bijzonder paling. Vlaams Parlement, Commissie voor Leefmilieu en Natuur, Landbouw, Visserij en Plattelandsbeleid en Ruimtelijke Ordening en Onroerend Erfgoed Vergadering van 04/10/2007. Available at http://jsp.vlaamsparlement.be/website/htm-vrg/498318.html

De Cooman, W., Detemmerman, L., 2003. Waterbodemonderzoek in Vlaanderen: Hoe gaan we te werk en wat zijn de eerste meetresultaten? *Water* 2003, 1-7.

De Deckere, E., De Cooman, W., Florus, M., Devroede-Vander Linden, M.P., 2000. Karakterisatie van de bodems van de Vlaamse bevaarbare waterlopen. AMINAL / AWZ, 56p.

De Schepper, N., Christiaens, J., Van Liefferinge, C., Herrel, A., Goemans, G., Meire, P., Belpaire, C., Adriaens, D., submitted. Variation in cranial morphology of the European eel: broad- and narrow-headedness. *Biological Journal of the Linnean Society.*

Dekker, W. 2004. Synthesis and discussion: Population dynamics of the European eel. In : Slipping through our hands - Population dynamics of the European eel. Ph D thesis. University of Amsterdam. P. 127-145.

Dua, V., 1999. PCB's, pesticiden en zware metalen in rivierpaling. Persmededeling van het kabinet van Minister Vera Dua, Vlaams Minister van Leefmilieu en Landbouw, 30 november 1999.

Dua, V., 2002a. Minister ontraadt consumptie van rivierpaling. Persmededeling van het kabinet van Minister Vera Dua, Vlaams Minister van Leefmilieu en Landbouw, 5 maart 2002.

Dua, V., 2002b. Teruggooiplicht voor paling uit Vlaamse rivieren. Persmededeling van het kabinet van Minister Vera Dua, Vlaams Minister van Leefmilieu en Landbouw, 12 maart 2002.

Dujardin, M., Narbonne, J.F., Alexander, S., 2001. The Belgian "Dioxin" crisis. In: Robertson, L.H., Hansen, L.W., eds. PCBs: recent advances in environmental toxicology and health effects. University Press of Kentucky, Kentucky. pp. 155-159.

European Commission, 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).

European Commission, 2006b. Commission Regulation (EC) No 199/2006 of 3 February 2006 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. *Official Journal of the European Union* L32, 34-46.

European Commission, 2007. Council Regulation (EC) No 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. *Official Journal of the European Union* 22.9.2007 L 248/17-23.

Geeraerts, C., Goemans, G., Quataert, P., Belpaire, C., 2007. Ecologische en ecotoxicologische betekenis van verontreinigende stoffen in paling. Studie uitgevoerd in opdracht van de Vlaamse Milieumaatschappij, MIRA, MIRA/ 2007/05, INBO/R/2007/40. Instituut voor Natuur- en Bosonderzoek. p. 207.

Goemans, G., Belpaire, C., 2004. The eel pollutant monitoring network in Flanders, Belgium. Results of 10 years monitoring. *Organohalogen Compounds* 66, 1834-1840.

Goemans, G., Belpaire, C., 2005. Congener profiles in European eel (*Anguilla anguilla* L.) as a method of tracing the origin of PCB contamination. *Organohalogen Compounds* 67, 1304-1307.

Goemans, G., Belpaire, C., 2006. Polluentconcentraties in roofvis: de cijfers. Poster contribution. Roofvissymposium Brussels, November 23th, 2006.

Goemans, G., Belpaire, C., Raemaekers, M., Guns M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollution 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.2003.99.169 p.

Health Canada, 2004. Ontario Ministry of the Environment, Sport Fish Contaminant Monitoring Program. Environmental Monitoring and Reporting Branch. Available at http://www.hc-sc.gc.ca/ewh-semt/pubs/eval/inventory-repertoire/fishbase_e.html. Assessed January 2008.

Hoff, P.T., Van Campenhout, K., Van de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., Goemans, G., Belpaire, C., Blust, R., De Coen, W., 2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium) : relationships with biochemical and organismal effects. *Environmental pollution* 137, 324-333.

Hoge Gezondheidsraad, 2005. Schatting van de inname van PCB's door sportvissers en het hieraan gebonden gezondheidsrisico (Hoge Gezondheidsraad 7747 – 23 February 2005).

Ide, C., De Schepper, N., Christiaens, J., Van Liefferinge, C., Herrel, A., Goemans, G., Belpaire, C., Adriaens, D., 2007. A non-invasive analysis of head shape dimorphism in a European eel population (*Anguilla anguilla*). *Journal of Morphology* 268 (12), 1088.

Koppen, G., Covaci, A., Van Cleuvenbergen, R., Schepens, P., Winneke, G., Nelen, V., Van Larebeke, N., Vlietinck, R., Schoeters, G., 2002. Persistent organochlorine pollutants in human serum of 50–65 years old women in the Flanders. Environmental and Health Study (FLEHS). Part 1: Concentrations and regional differences. *Chemosphere* 48, 811–825.

Maes, Y., 2003. Onderzoek naar de grootte van het foerageergedrag van palingen uit de Weerdse visvijver in functie van het Vlaamse palingpolluentenmeetnet. Instituut voor Bosbouw en Wildbeheer and Hogeschool Brabant, 49 p + annexes.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005a. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology : Relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology* 73, 99-114.

Maes, J., Belpaire, C., Breine, J., Goemans, G., 2005b. Vissen als gezondheidsindicatoren voor de toestand van het Zeeschelde-ecosysteem. *Water*, mei 2005, 1-7.

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.

MIRA, 2007a. Milieurapport Vlaanderen, MIRA Achtergronddocument 2007, Kwaliteit oppervlaktewater, De Cooman W., Peeters B., Theuns I., Vos G., Lammens S., Debbaudt, W., Timmermans G., Meers, B., Van Erdeghem M., Van Wauwe P., Callebaut R., Barrez I., Van den Broeck S., Emery J., Van Volsem S., Bursens K., Van Hoof K., D'Heygere T., Soetaert H., Martens K., Baten I., Goris M., Haustraete K., Breine J., Van Thuyne G., Belpaire C., Smis A., Vlaamse Milieumaatschappij, http://www. milieurapport.be.

MIRA, 2007b Milieurapport Vlaanderen, MIRA Achtergronddocument 2007, Verspreiding van bestrijdingsmiddelen, Claeys, S., Steurbaut, W., Theuns, I., De Cooman, W., De Wulf, E., Eppinger, R., D'hont, D., Dierckxens, C., Goemans, G., Belpaire, C., Wustenberghs, H., den Hond, E., Peeters, B., Overloop, S., Vlaamse Milieumaatschappij, http://www. milieurapport.be.

MIRA, 2007c. Milieurapport Vlaanderen, Achtergronddocument 2007, Verspreiding van PCB's, M. Wevers, M., Voorspoels, S., Covaci, A., Schepens, P., D'Havé, H., De Coen, W., Van Gerven, T., Vandecasteele, C., Goemans, G., Belpaire, C., Van Hooste, H., MIRA H. Van Hooste, Vlaamse Milieumaatschappij, http://www. milieurapport.be.

Morris, S., Allchin, C., Zegers, B., Haftka, J., Boon, J., Belpaire, C., Leonards, P., Van Leeuwen, S., De Boer, J., 2004. Distribution and Fate of HBCD and TBBPA Brominated Flame Retardants in North Sea Estuaries and Aquatic Food Webs. *Environmental Science & Technology* 38, 5497-5504.

Nouwen, J., Geuzens, P., Den Hond, E., Nawrot, T., Staessen, J., Goemans, G., Belpaire, C., Van Hooste, H., 2003. Verspreiding van zware metalen. In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, p.157-170.

Nouwen, J., Geuzens, P., Goemans, G., Van Thuyne, G., Belpaire, C., Guns, M., Schneiders, A., Van Hooste, H., 2001. Verspreiding van zware metalen in Mira-T 2001; Milieu- en Natuurrapport Vlaanderen, p. 177-191.

Nouwen, J., Geuzens, P., Goemans, G., Van Thuyne, G., Belpaire, C., Schneiders, A., Van Hooste, H., 2002. Verspreiding van zware metalen. In : MIRA-T 2002. Milieu-en natuurrapport Vlaanderen : thema's. VMM, p. 139-148

Onkelinx, T., Quataert, P., Wouters, J., Bauwens, D., Genouw, G., Vandecasteele, B., 2007. Gevalstudie II: waterbodemmeetnet en palingpolluentenmeetnet. Kwaliteitsvolle monitoring voor het beleid. Afwegingskader en rekenmodel voor de bepaling van de steekproefgrootte bij beleidsgerichte monitoring. INBO.IR.2006.8. Instituut voor Natuur- en Bosonderzoek, Geraardsbergen, 149 p.

Overloop, S., Steurbaut, W., De Smet, B., Theuns, I., De Cooman, W., Goemans, G., Belpaire, C., 2003. Verspreiding van bestrijdingsmiddelen. In: Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, p. 171-181.

Peeters, B., De Cooman, W., Vos, G., Gabriels, W., Martens, K., Timmermans, G., Van Hoof, K., Breine, J., Van Thuyne, G., Goemans, G., Belpaire, C., Geeraerts, C., 2006. Oppervlaktewater: Biologische kwaliteit nog steeds ondermaats. In: Van Steertegem, M., (ed.), Mira-T 2006 Milieurapport Vlaanderen: Focusrapport. Leuven Lannoo Campus, p.89-104.

Richardson, D.M., Davies, I.M., Moffat, C.F., Pollard, P., Stagga, R.M., 2001. Biliary PAH metabolites and EROD activity in flounder (*Platichthys flesus*) from a contaminated estuarine environment. *Journal of Environmental Monitoring* 3, 610-615.

Roose, P., Cooreman, K. and Vyncke, W., 1996. PCBs in cod (*Gadus morhua*), flounder (*Platichthys flesus*), blue mussel (*Mytilus edulis*) and brown shrimp (*Crangon crangon*) from the Belgian Continental Shelf - Relation to biological parameters and trend analysis. *Chemosphere*, 37, 2199-2210.

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *Journal of Environmental Monitoring* 5, 876-884 .

Roosens, L., Dirtu, A., Goemans, G., Belpaire, C., Gheorghe, A., Neels, H., Blust, R., Covaci, A., 2008. Brominated flame retardants and polychlorinated biphenyls in fish from the River Scheldt, Belgium. *Environment International,* in press.

Schabuss, M., Konecny, R., Belpaire, C., Schiemer, F., 1997. Endoparasitic helminths of the European eel, *Anguilla anguilla*, from our disconnected meanders from the rivers Leie and Scheldt in western Flanders, Belgium. *Folia Parasitologica* 44 : 12-18.

Schroijen, C., Baeyens, W., Schoeters, G., Den Hond, E., Koppen, G. Bruckers, L., Nelen, V., Van De Mieroop, E., Bilau, M., Covaci, A., Keune, H., Loots, I. , Kleinjans, J., Dhooge, W., Van Larebeke, N., 2008 in press. Internal exposure to pollutants measured in blood and urine of Flemish adolescents in function of area of residence. *Chemosphere*, doi:10.1016/j.chemosphere.2007.11.053.

Section Human Health Care, 2005. Advies polluenten in paling: consumptie. Letter advise. Afdeling Preventieve en Sociale Gezondheidszorg to the Visserijfonds.

Simoens, I., Breine, J., Verreycken, H., Belpaire, C., 2002. Fish stock assessment of Lake Schulen, Flanders: a comparison between 1988 and 1999. In: Cowx, I., *et al.* (eds.), Management and ecology of lake and reservoir fisheries. Fishing News Books, Oxford, p.46-57.

State of New Jersey, 2006. Department of environmental Protection. Routine Monitoring Program for Toxics in Fish. Available at http://www.nj.gov/dep/dsr/2004data.htm. Assessed January 2008.

Steurbaut, W., De Smet, B., Vercruysse, F., Belpaire, C., Goemans, G., Overloop S., 2001.Verspreiding van bestrijdingsmiddelen. In Mira-T 2001; Milieu- en Natuurrapport Vlaanderen, p.193-206

Thomsen, C., Knutsen, H.K., Liane, V.H., Froshaug, M., Kvalem, H.E., Haugen, M., Meltzer, H.M., Alexander, J., Becher, G., 2008. Consumption of fish from a contaminated lake strongly affects the concentrations of polybrominated diphenyl ethers and hexabromocyclododecane in serum. *Molecular Nutrition and Food Research*, in press.

USGS, 2007. U.S. Department of the Interior, U.S. Geological Survey. A National Network to Monitor Contaminants and their Effects in Large Rivers. Available at http://biology.usgs.gov/status_trends/best_site/bestprojects.html and interactive database on line at: http://www.cerc.usgs.gov/data/best/search/index.htm

Van Campenhout, K., Goenaga Infante, H., Goemans, G., Belpaire, C., Adams, F., Blust, R., Bervoets, L., 2008. A field survey of metal binding to metallothionein and other cytosolic ligands in liver of eels using an on-line isotope dilution method in combination with size exclusion (SE) high pressure liquid chromatography (HPLC) coupled to Inductively Coupled Plasma time-of-flight Mass Spectrometry (ICP-TOFMS). *The Science of the Total Environment*, 2008, doi:10.1016/j.scitotenv.2008.01.026.

Van Gerven, T., Vandecasteele, C., Goemans, G., Belpaire, C., Van Hooste, H., 2002. Verspreiding van PCB's. In : MIRA-T 2002. Milieu- en natuurrapport Vlaanderen : thema's. VMM, p. 327-335.

Van Gerven, T., Vandecasteele, C., Goemans, G., Belpaire, C., Van Hooste, H., 2003. Verspreiding van PCB's. In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, p. 379-385

Van Thuyne G., Belpaire C., Guns M., Denayer B., 1995a. Monitoring van de vispopulaties en de visvleeskwaliteit op het Boudewijnkanaal. Rapport Instituut voor Bosbouw en Wildbeheer, Groenendaal, IBW.Wb.V.R.94.031.

Van Thuyne, G., Belpaire, C., Guns, M., 1995b Onderzoek van gehalten aan zware metalen in vis van enkele Vlaamse binnenwateren. Rapport Instituut voor Bosbouw en Wildbeheer, Groenendaal, IBW.Wb.V.R.95.033.

Van Thuyne, G., Belpaire, C., 1999. Zware metalen in paling als indicator voor de immissietoestand in het aquatisch milieu. MIRA-T 1999. Milieu-en natuurrapport Vlaanderen : Wetenschappelijke rapporten.

Vandecasteele, C., Van Gerven, T., Geysen, D., Belpaire, C., Goemans, G., Raemaekers, M., Van Hooste, H., 2001. Verspreiding van producten van onvolledige verbranding (POV's) en PCB's, deel PCB's. In Mira-T 2001; Milieu- en Natuurrapport Vlaanderen, 167-175.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363–371.

VIS, 2008. Fish Information System. Flanders' database with fish-related data. World Wide Web electronic publication. Research Institute for Nature and Forest (INBO). http://vis.milieuinfo.be/, version (02/2008). Accessed Februray 2008.

Weltens, R., Goemans, G., Huyskens, G., Witters, H., Belpaire, C., 2002. Wetenschappelijke onderbouwing voor de normering van polluenten in

Vlaamse oppervlaktewateren en selectie van ecologische en exotoxicologische indicatoren voor de waterkwaliteit. Rapport i.o.v. VMM, VITO en IBW, IBW.Wb.VR.2002.88.

Weltens, R, Goemans, G. Huyskens, G., Belpaire, C., Witters, H., 2003. Evaluatie van de kwaliteitsnormering voor oppervlaktewater in Vlaanderen: een praktijkstudie. *Water*, 2003, 1-9.

WG Eel 2007. Report of the 2007 Session of the Joint EIFAC/ICES, Working Group on Eels, FAO European Inland Fisheries Advisory Commission; International Council for the Exploration of the Sea, Bordeaux, 3–7 September 2007, EIFAC Occasional Paper No. 38, ICES CM 2007/ACFM:23, Draft, 524 p. Available from www.ices.dk/reports/ACFM/2007/WGEEL/WGEEL_07_draft.pdf

Eels are a favourite food of otters. Bioaccumulating contaminants were reported as a cause of the rapid decline of the European otter populations, and the otter has disappeared in Flanders in the early 1960s. Considering the high levels of contaminants in fish, Flemish aquatic ecosystems are currently not capable to sustain a viable otter population.

Photo: An otter eating an eel (Scotland) - David Carss

Annex I

The Eel Pollutant Monitoring Network: results for 2002-2005. Cartography.

Geert Goemans, Yves Maes and Claude Belpaire

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

These maps are available on the INBO website.

Goemans, G. Maes, Y., Belpaire, C., 2008.
The Eel Pollutant Monitoring Network: results for 2002-2005. Cartography.
Available on www.inbo.be

Cartography

In 2003 a report was published describing the results of the Flemish eel Pollutant Network for the period 1994-2001 (Goemans *et al*, 2003). This report included cartographic representation of the means of the PCBs, OCPs and heavy metals measured in eel for each sampling site. The level of pollution was represented in colour-scaled quality classes defined in function of deviation from the reference value. For description of the methodology for setting reference values and defining quality classes we refer to Goemans *et al*. (2003) and Belpaire and Goemans (2007). The report included also maps representing sites where legal consumption limits were exceeded (for Sum PBCs, Cd, Hg and Pb).

In this annex an update of these maps for the data obtained in the period 2002-2005 are reported. For three substances (endrin, *p,p'*-DDT and trans-Nonachlor) it was necessary to recalculate the reference value and quality classes presented in Goemans *et al*. (2003), see Figures XV, XVIII and XXI. As a consequence the quality class distribution for these substances may not be compared with the previous ones presented in Goemans *et al*. (2003).

The map with sites exceeding the Belgian consumption limit for mercury, cadmium and lead, is not included as this is only the case for one site (site KB6 on Kanaal van Beverlo).

For exact location of the sites, mean concentration values and additional information of samples we refer to the tables in Annex II. Additional and up-to-date information can be gained by visiting the database via the VIS-website at http://vis.milieuinfo.be/.

Following maps are included:

Figure I:	Polychlorine biphenyls in eel (Flanders, 2002-2005): Sum 7 indicator PCBs.
Figure II:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 28.
Figure III:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 31.
Figure IV:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 52.
Figure V:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 101.
Figure VI:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 105.
Figure VII:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 118.
Figure VIII:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 138.
Figure IX:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 153.
Figure X:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 156.
Figure XI:	Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 180.
Figure XII:	Organochlorine pesticides in eel (Flanders, 2002-2005): γ- hexachlorocyclohexane (lindane).
Figure XIII:	Organochlorine pesticides in eel (Flanders, 2002-2005): α- hexachlorocyclohexane.
Figure XIV:	Organochlorine pesticides in eel (Flanders, 2002-2005): Hexachlorobenzene.
Figure XV:	Organochlorine pesticides in eel (Flanders, 2002-2005): Endrin.
Figure XVI:	Organochlorine pesticides in eel (Flanders, 2002-2005): Dieldrin.

Figure XVII: Organochlorine pesticides in eel (Flanders, 2002-2005): Sum DDTs.
Figure XVIII: Organochlorine pesticides in eel (Flanders, 2002-2005): *p,p'*-DDT.
Figure XIX: Organochlorine pesticides in eel (Flanders, 2002-2005): *p,p'*-DDD (TDE).
Figure XX: Organochlorine pesticides in eel (Flanders, 2002-2005): *p,p'*-DDE.
Figure XXI: Organochlorine pesticides in eel (Flanders, 2002-2005): trans-Nonachlor.
Figure XXII: Heavy metals in eel (Flanders, 2002-2005): Mercury.
Figure XXIII: Heavy metals in eel (Flanders, 2002-2005): Cadmium.
Figure XXIV: Heavy metals in eel (Flanders, 2002-2005): Lead.
Figure XXV: Heavy metals in eel (Flanders, 2002-2005): Copper.
Figure XXVI: Heavy metals in eel (Flanders, 2002-2005): Zinc.
Figure XXVII: Heavy metals in eel (Flanders, 2002-2005): Nickel.
Figure XXVIII: Heavy metals in eel (Flanders, 2002-2005): Chromium.
Figure XXIX: Heavy metals in eel (Flanders, 2002-2005): Arsenic.
Figure XXX: Heavy metals in eel (Flanders, 2002-2005): Selenium.
Figure XXXI: Sites exceeding Belgian consumption limit for PCBs.

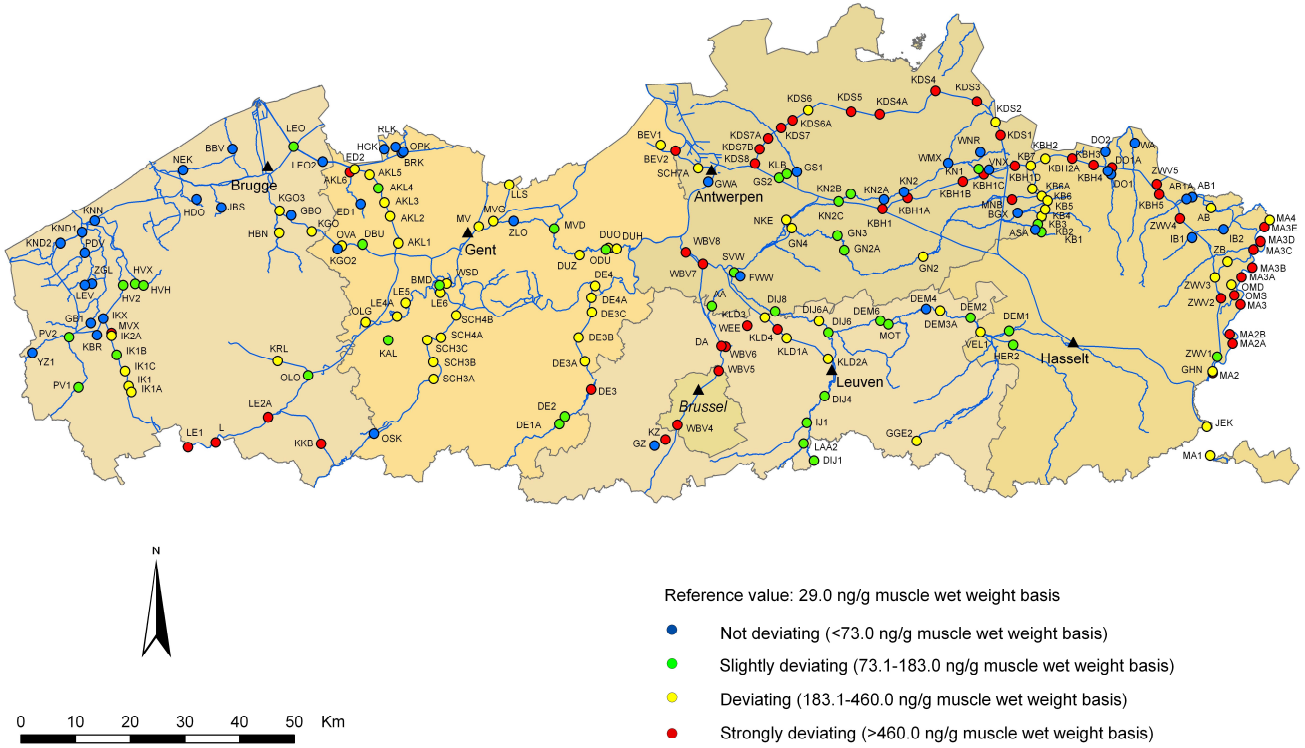
References

Belpaire, C., Goemans, G., 2007. The European eel (*Anguilla anguilla*) a rapporteur of the chemical status for the Water Framework Directive? *Vie et Milieu – Life and Environment* 57 (4), 235-252.

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollution monitoring network 1994-2001: polychlorinated biphenyls, organochlorine pesticides and heavy metals in eel]. Report of the Institute for Forestry and Game Management, IBW. Wb.V.R.2003.99., 169p.

Figure I: Polychlorine biphenyls in eel (Flanders, 2002-2005): Sum 7 indicator PCBs

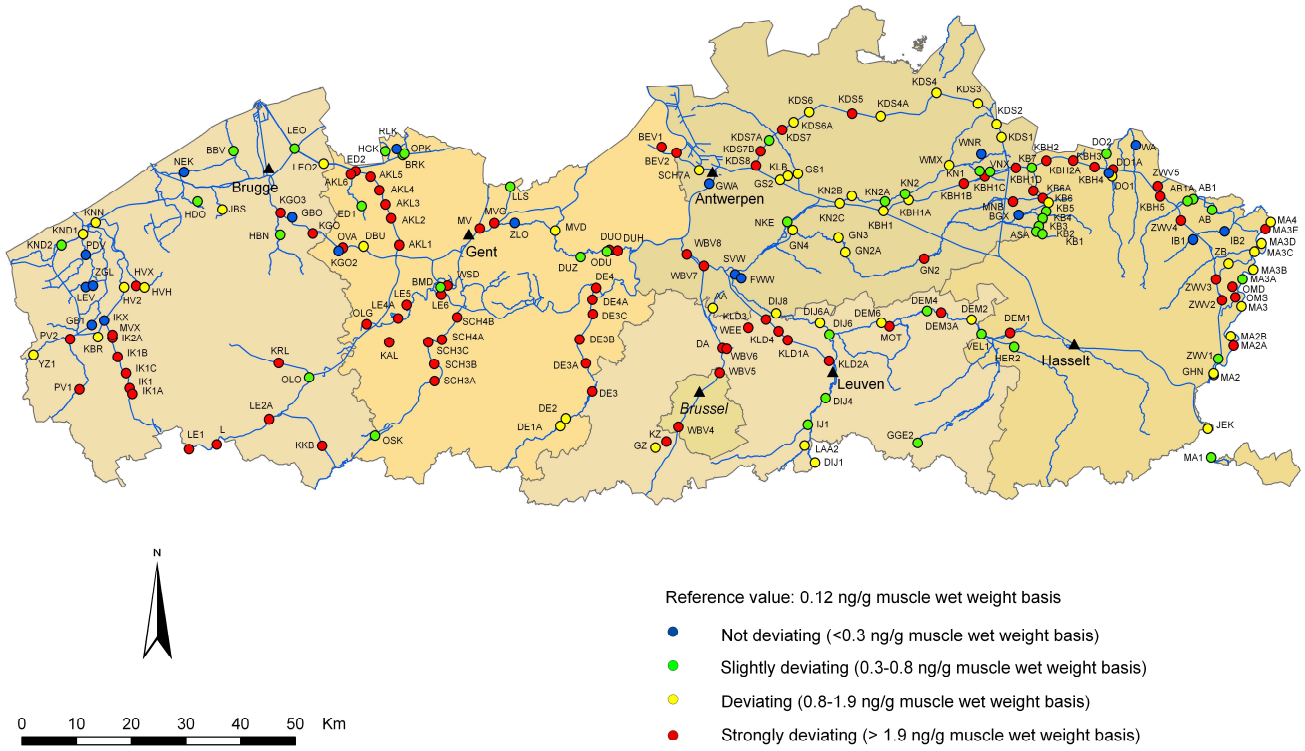
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure II: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 28

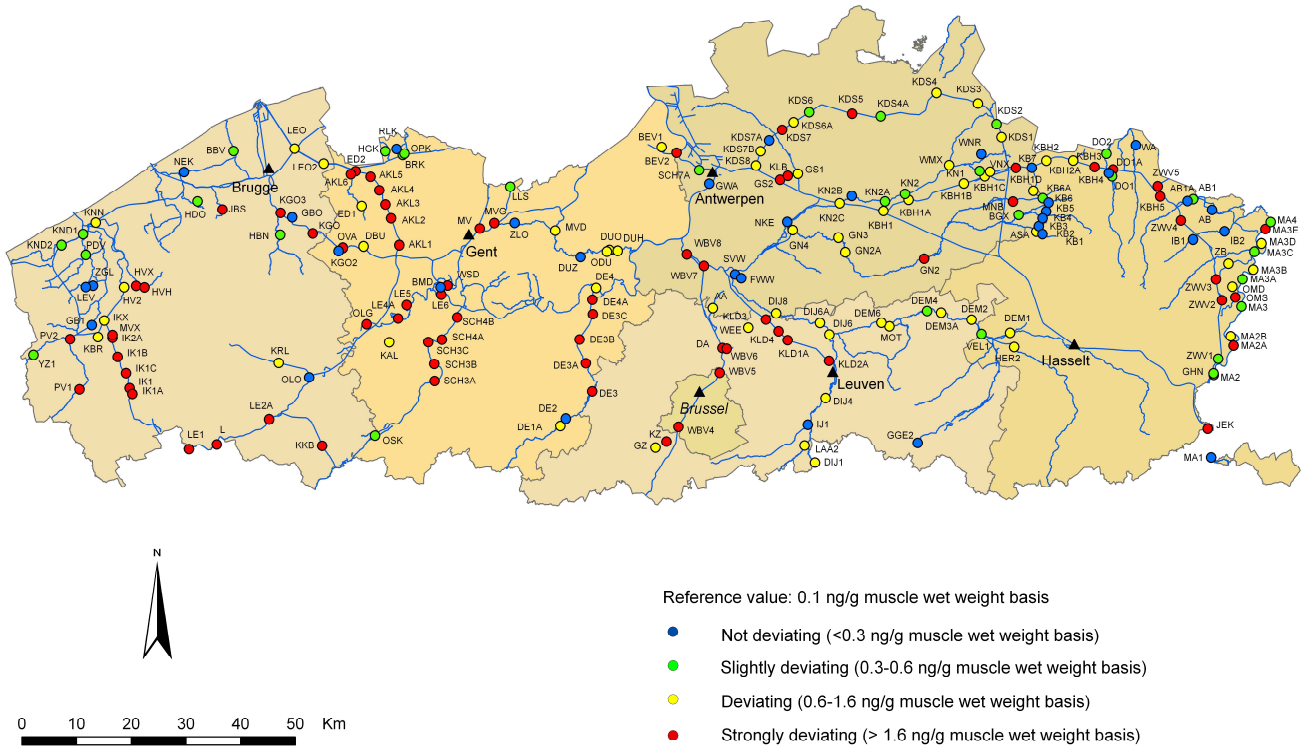
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure III: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 31

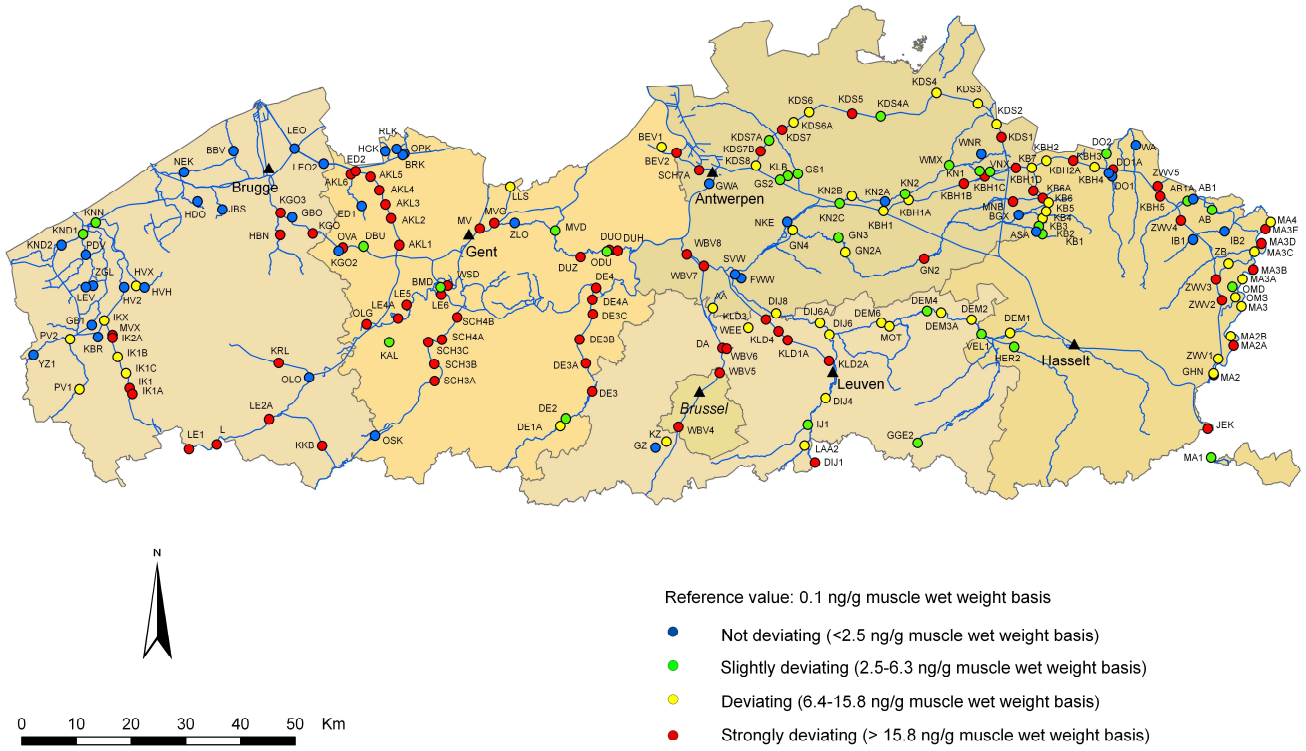
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure IV: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 52

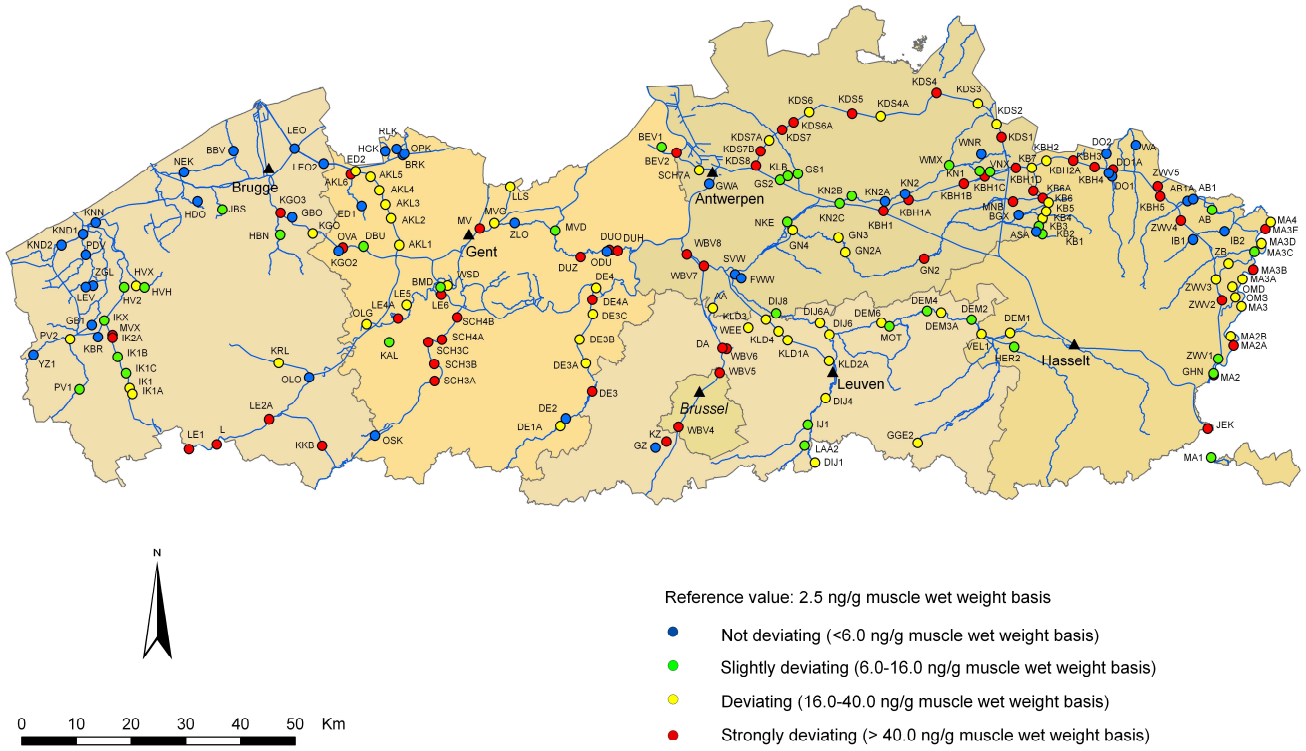
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure V: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 101

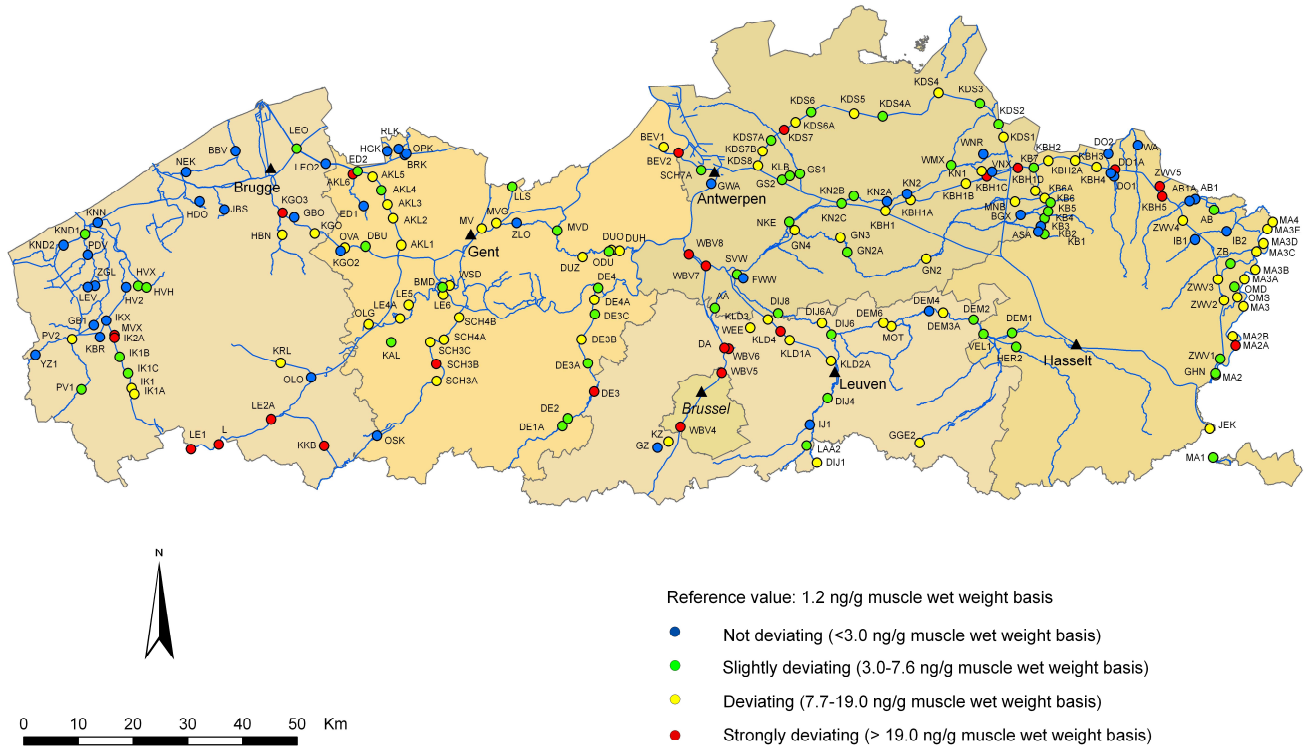
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure VI: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 105

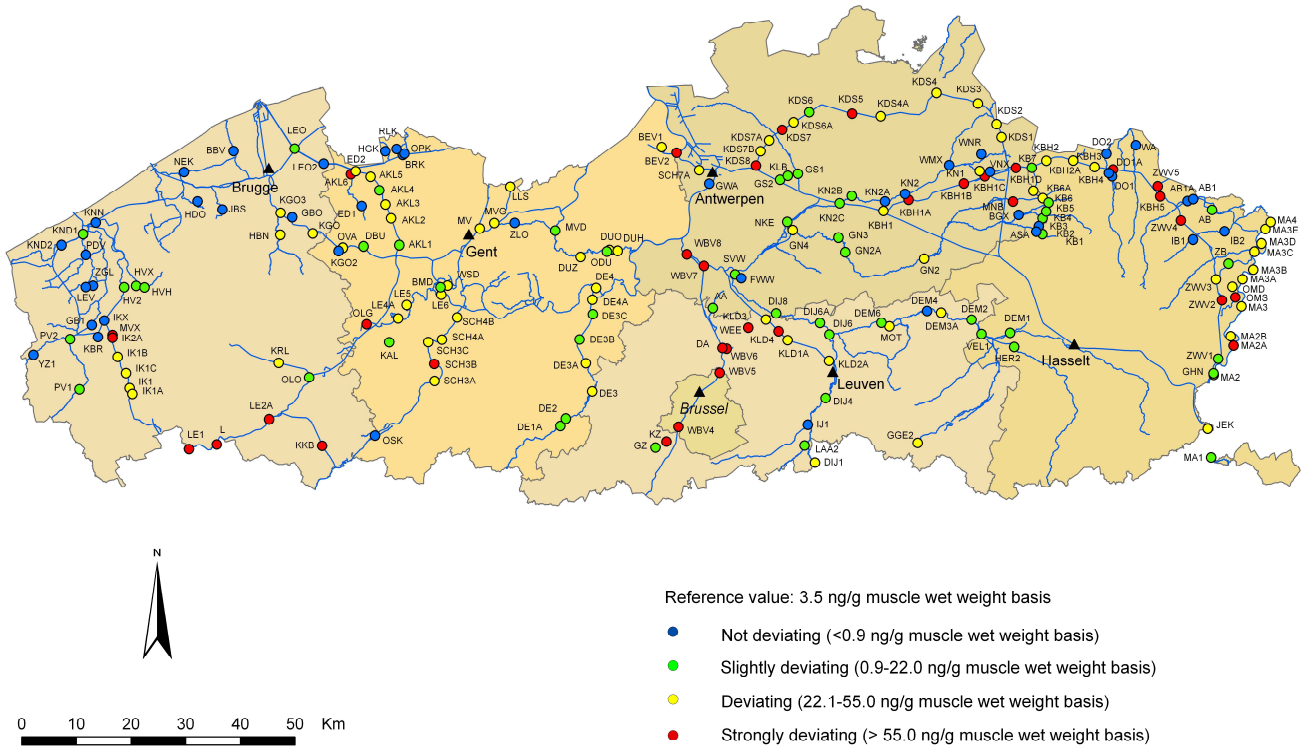
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure VII: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 118

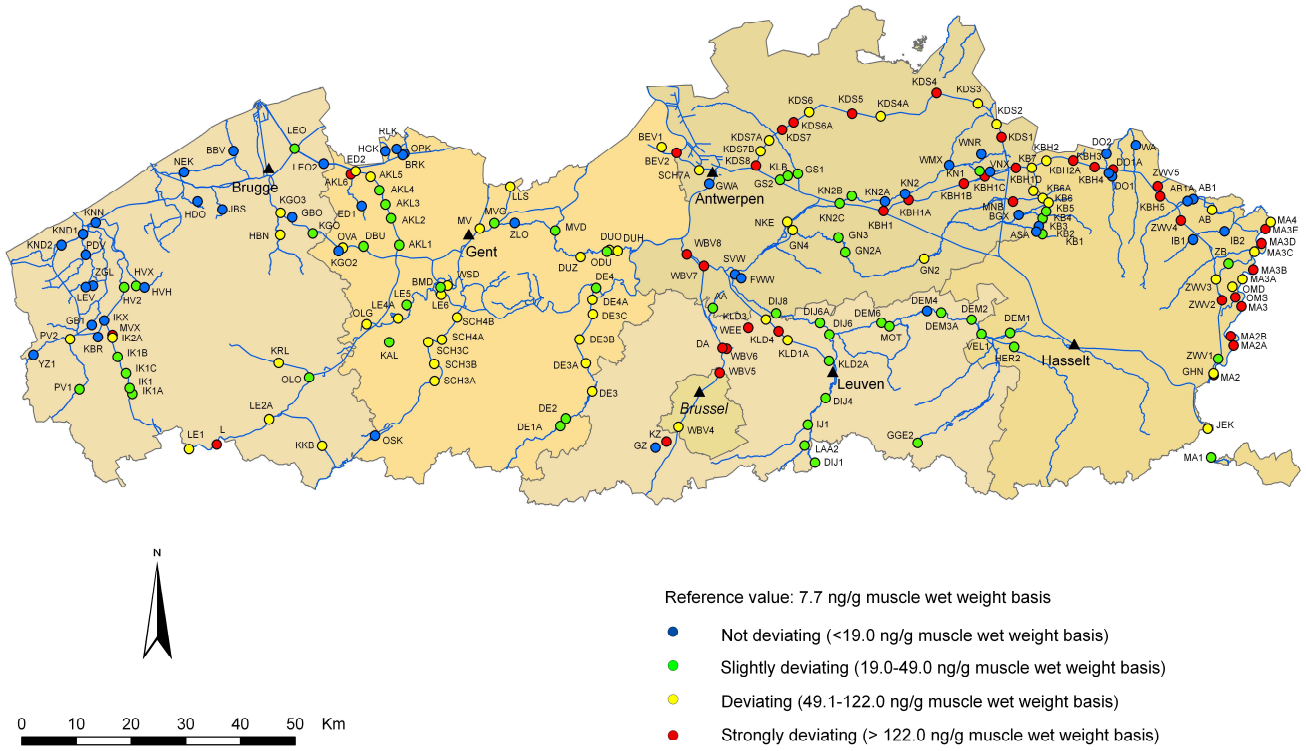
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure VIII: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 138

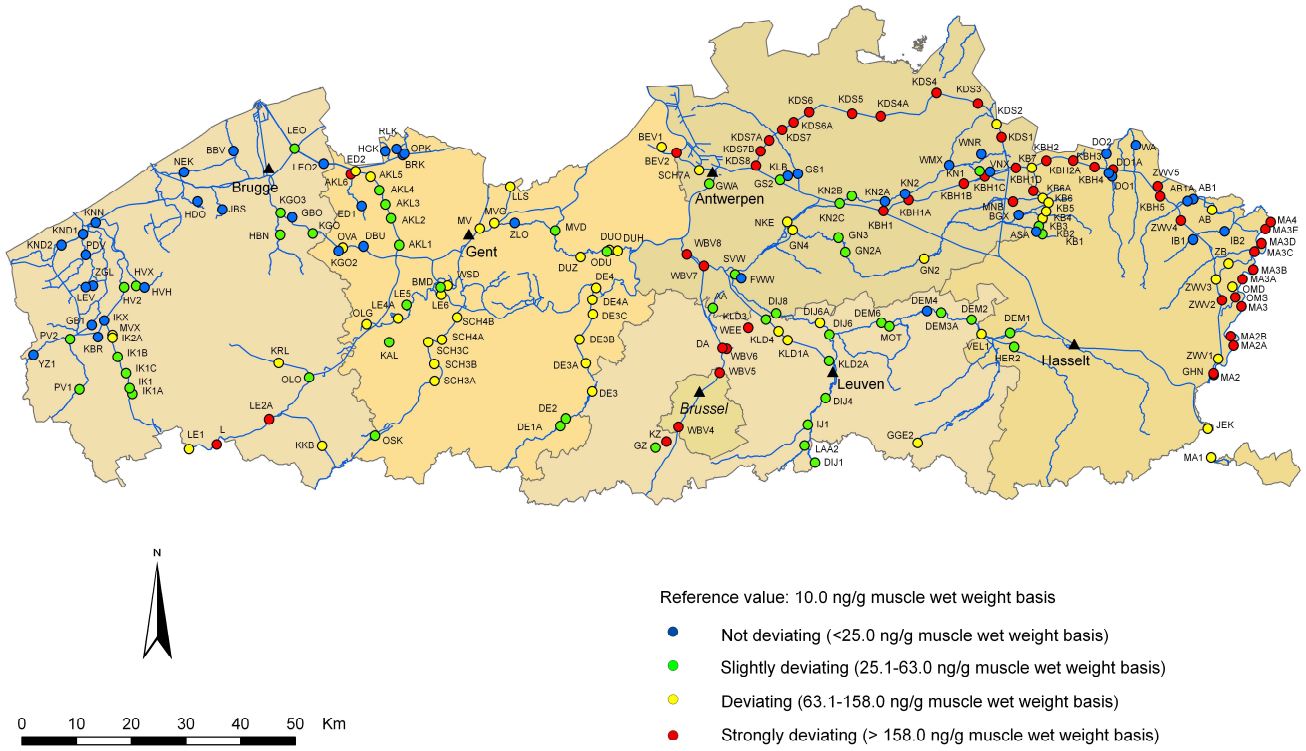
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure IX: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 153

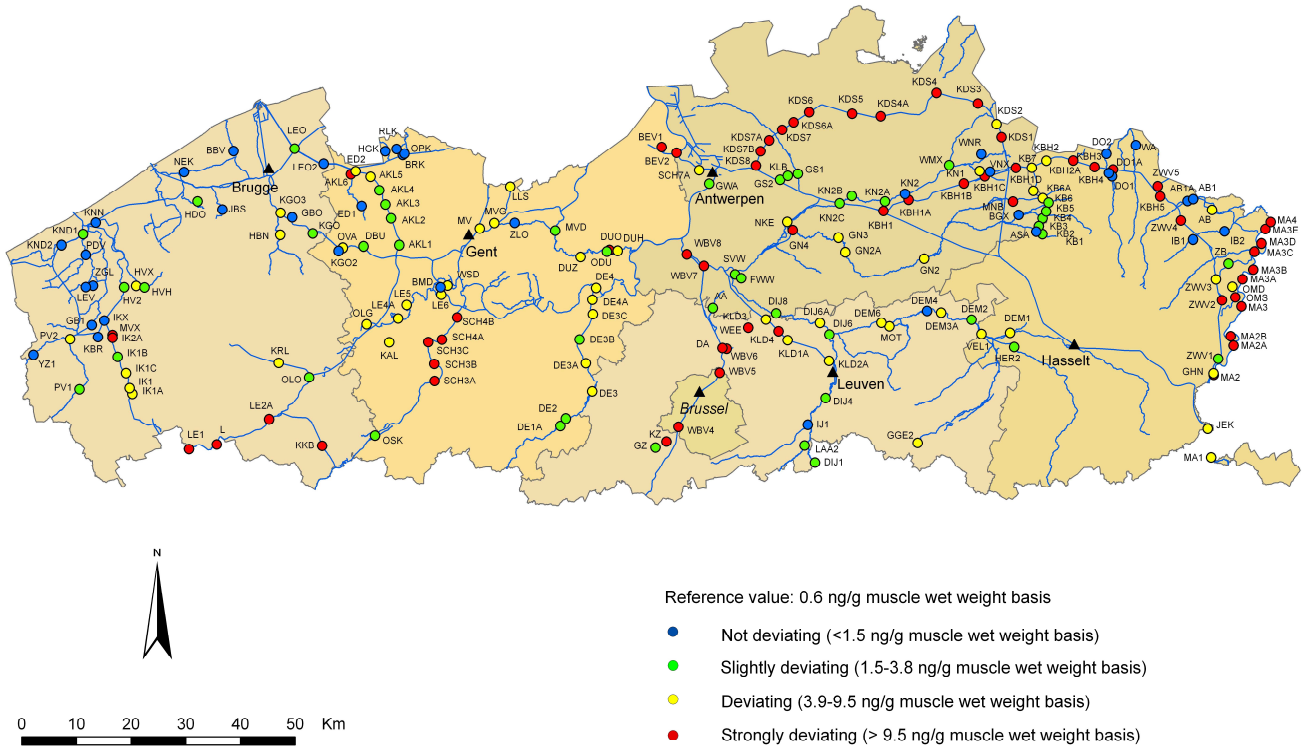
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure X: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 156

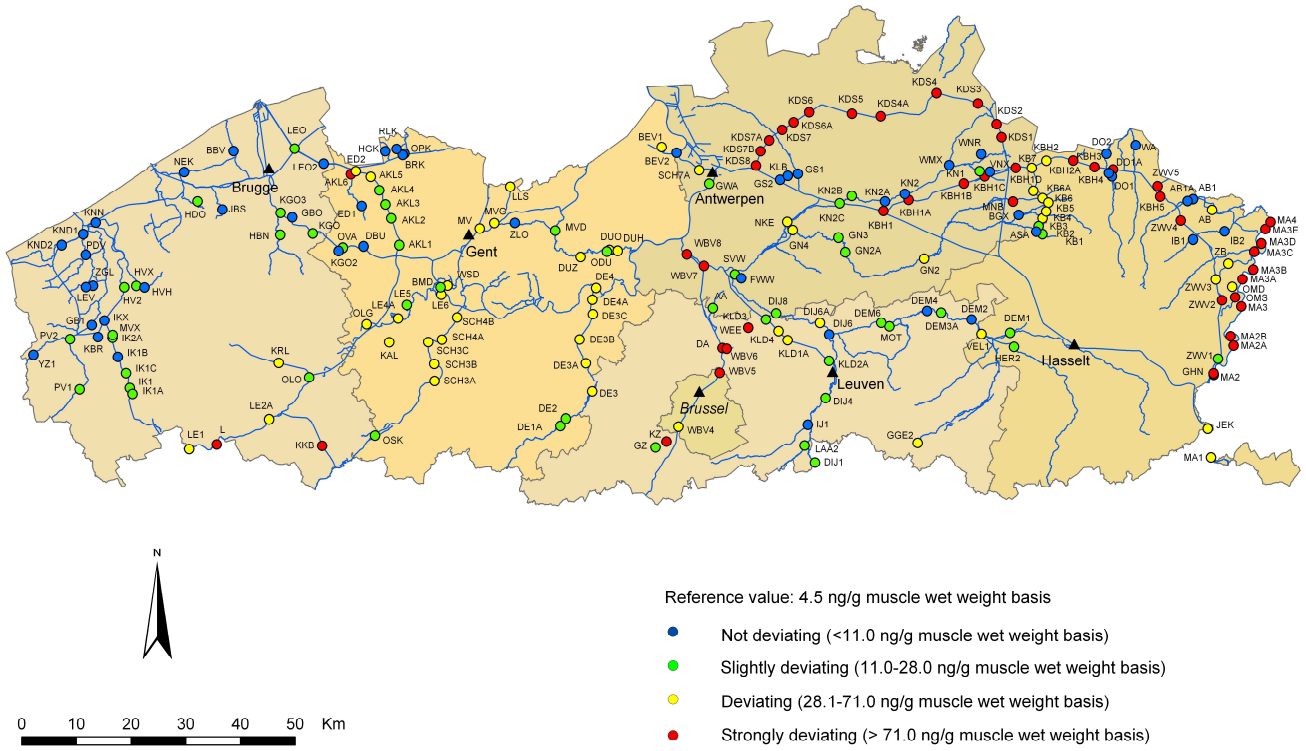
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XI: Polychlorine biphenyls in eel (Flanders, 2002-2005): PCB 180

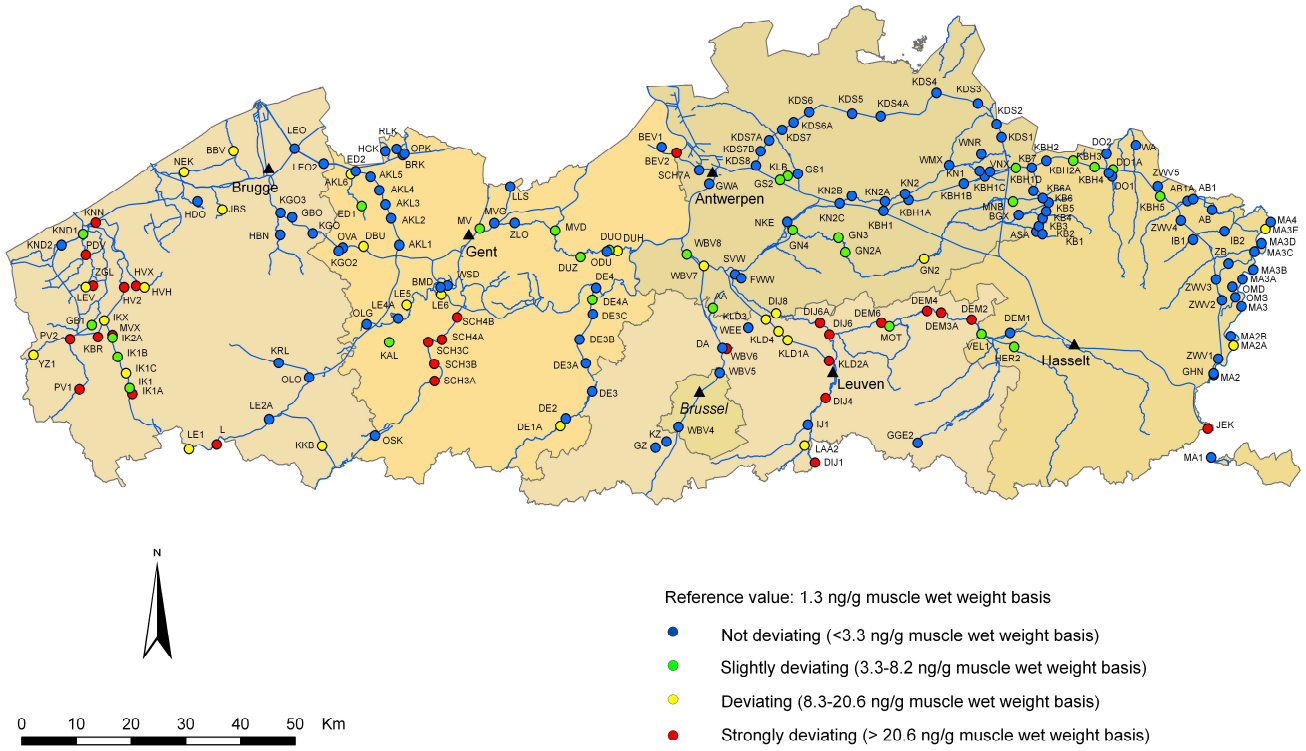
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XII: Organochlorine pesticides in eel (Flanders, 2002-2005): γ-hexachlorocyclohexane (lindane)

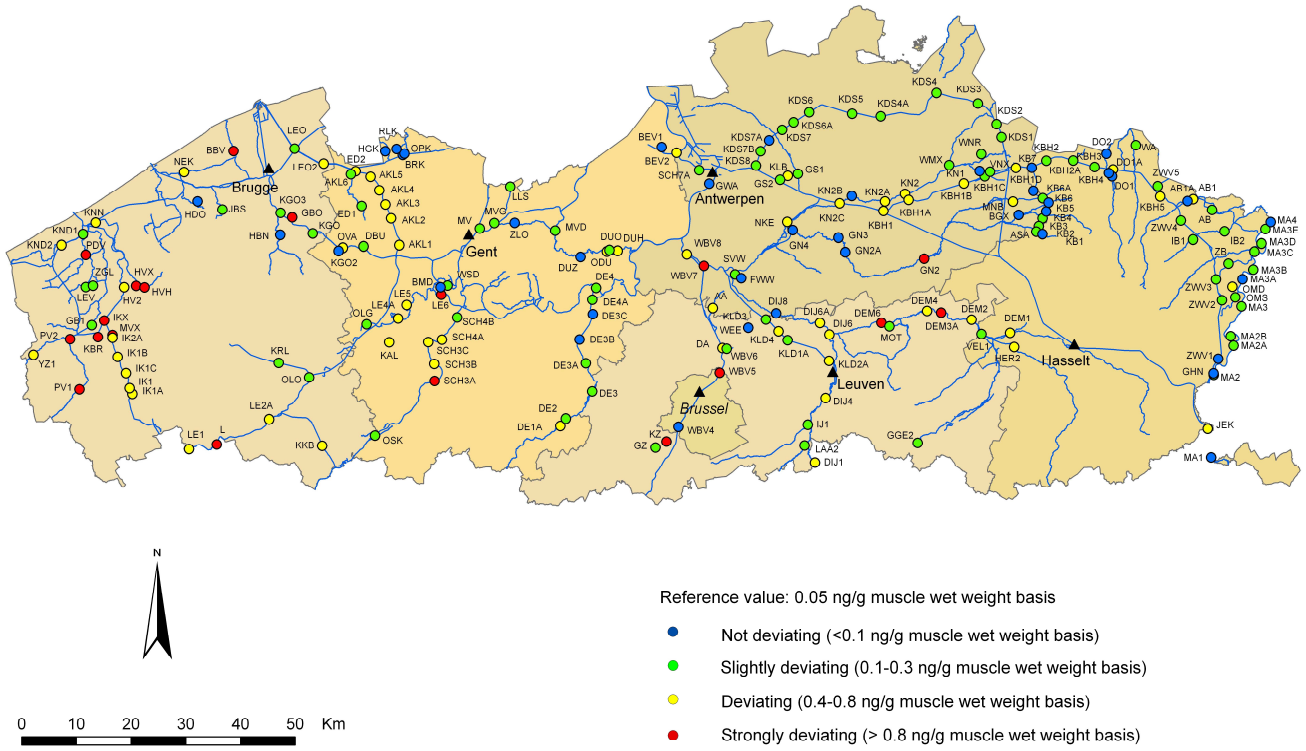
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XIII: Organochlorine pesticides in eel (Flanders, 2002-2005): α-hexachlorocyclohexane

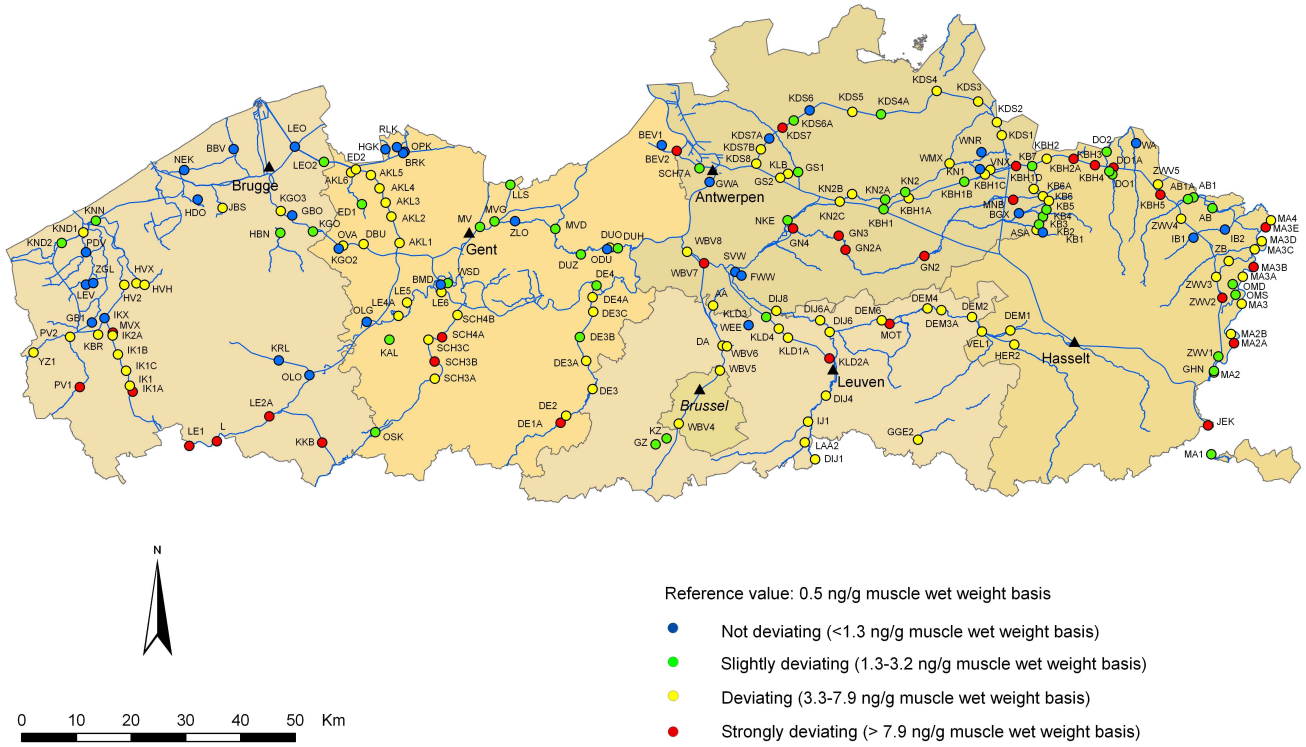
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XIV: Organochlorine pesticides in eel (Flanders, 2002-2005): Hexachlorobenzene

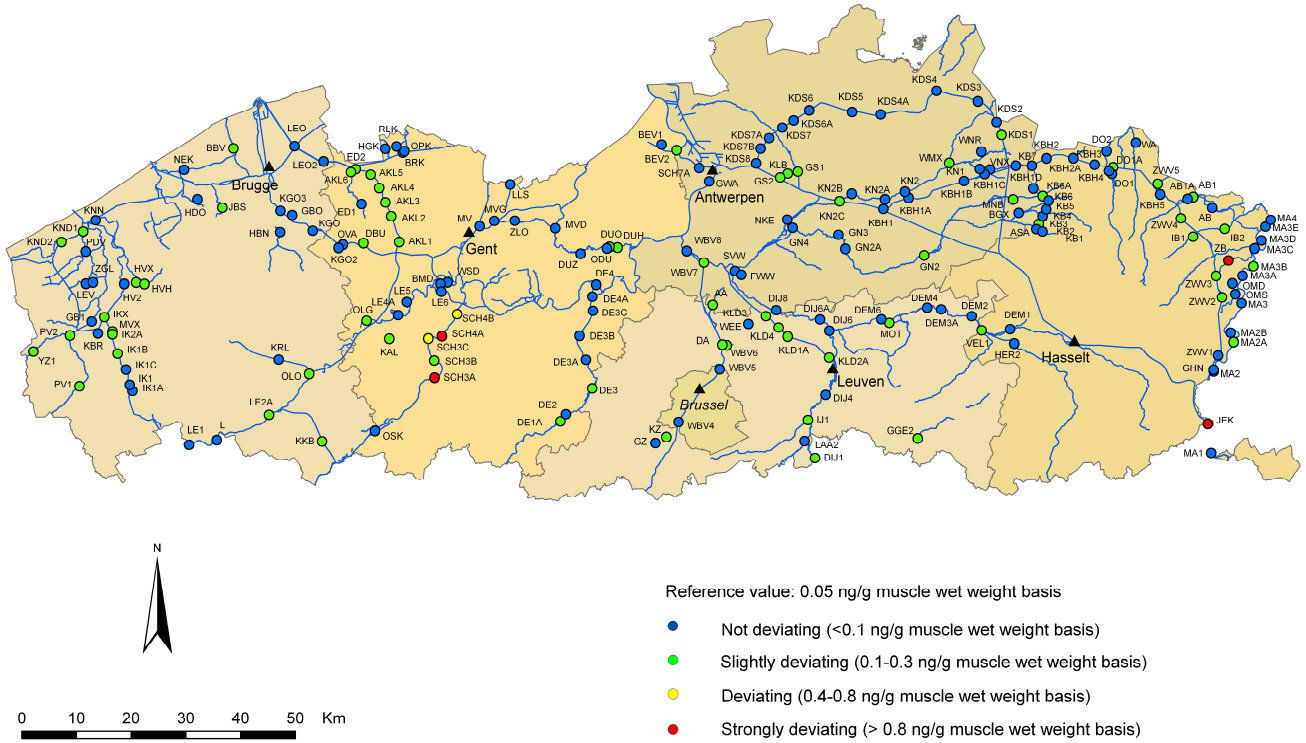
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthograph.

Figure XV: Organochlorine pesticides in eel (Flanders, 2002-2005): Endrin

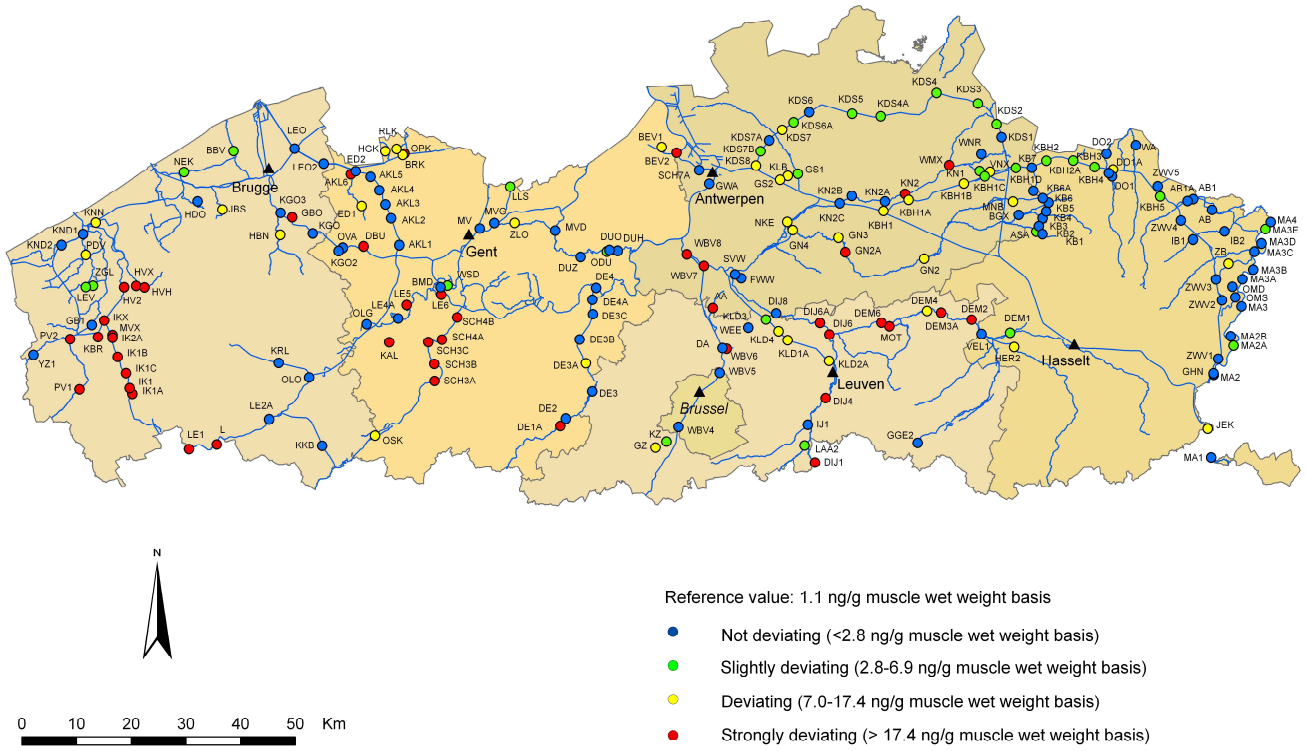
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XVI: Organochlorine pesticides in eel (Flanders, 2002-2005): Dieldrin

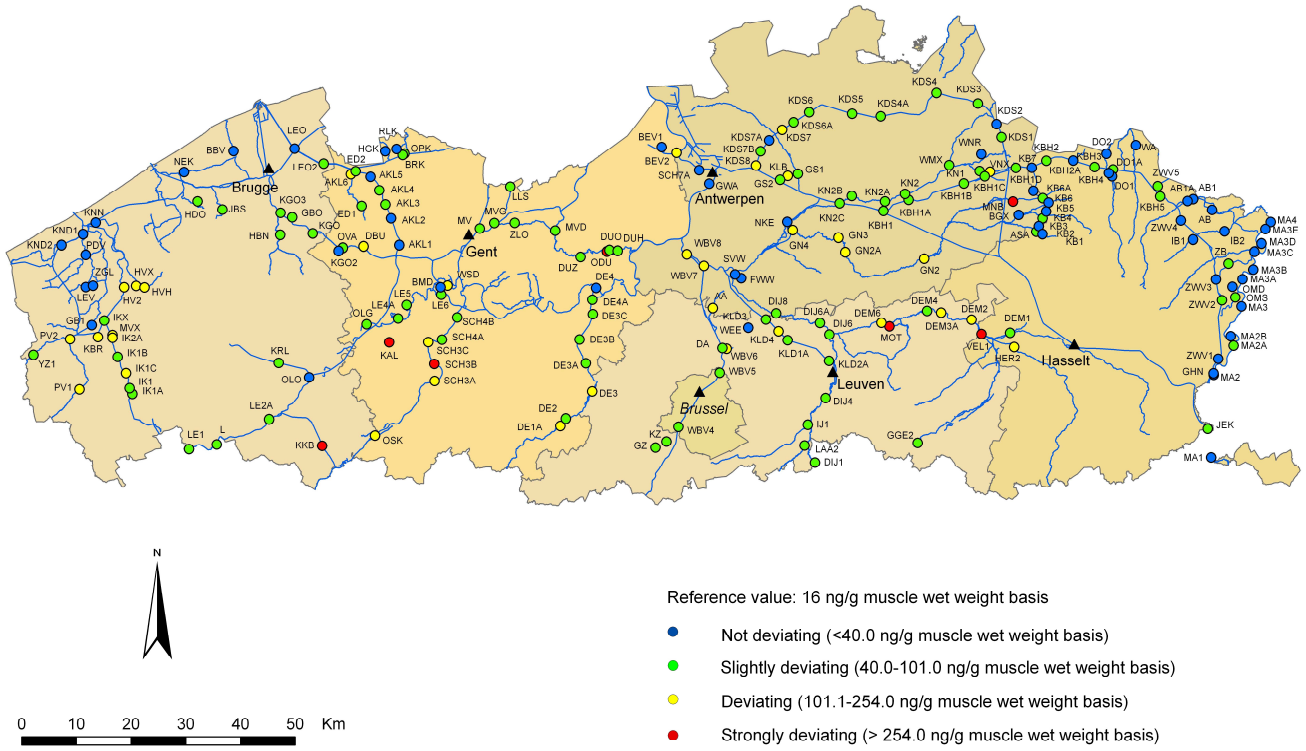
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XVII: Organochlorine pesticides in eel (Flanders, 2002-2005): Sum DDTs

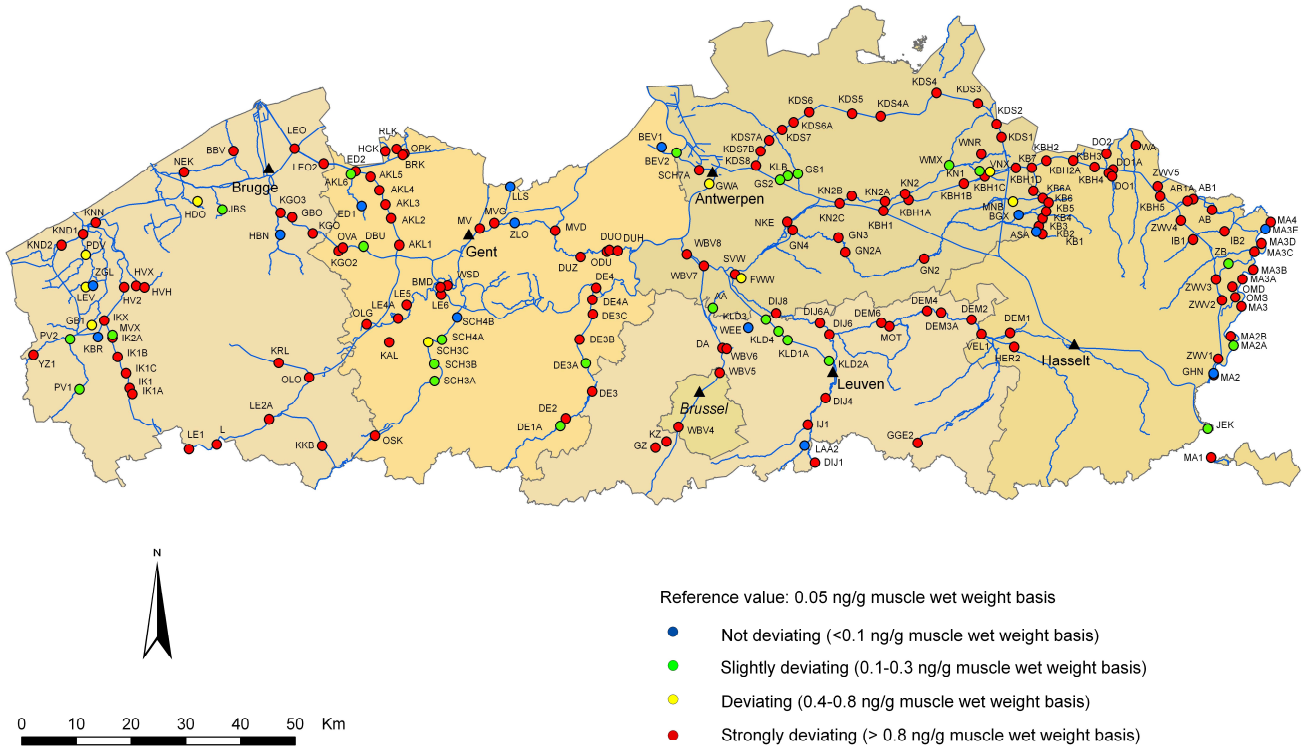
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XVIII: Organochlorine pesticides in eel (Flanders, 2002-2005): p,p'-DDT

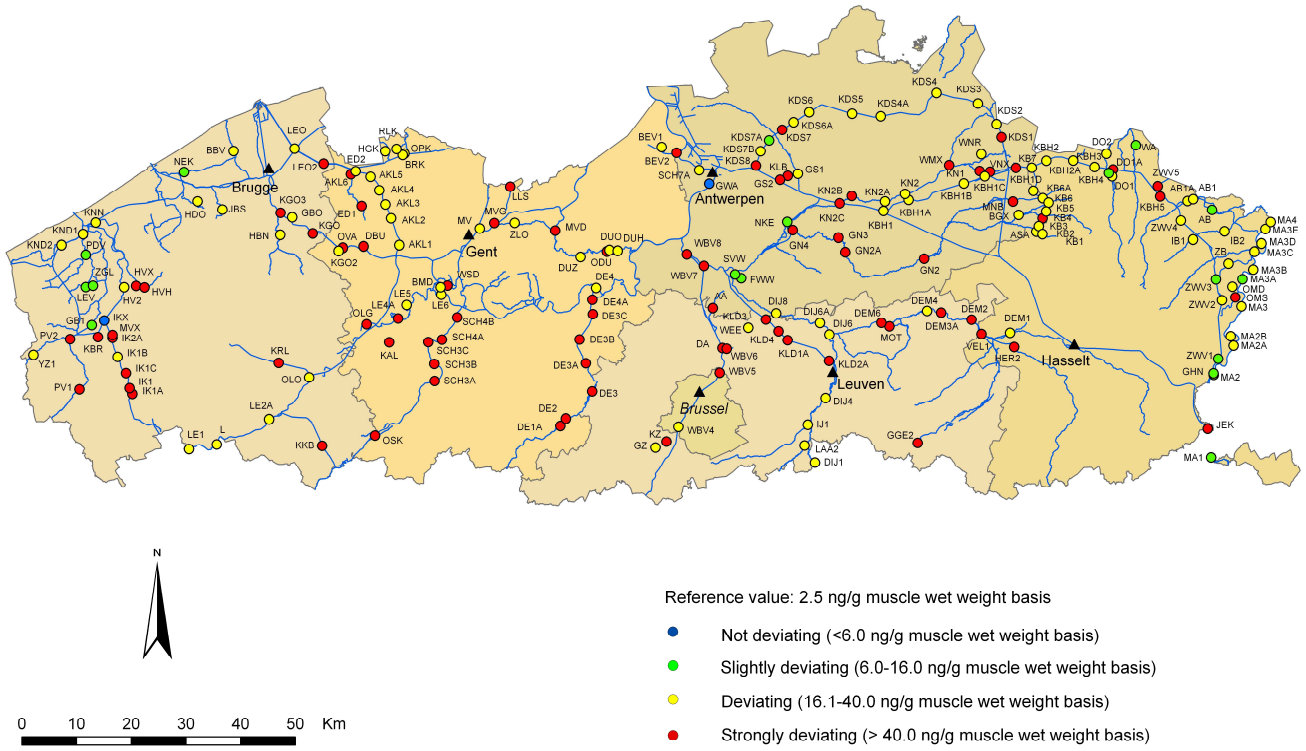
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XIX: Organochlorine pesticides in eel (Flanders, 2002-2005): p,p'-DDD (TDE)

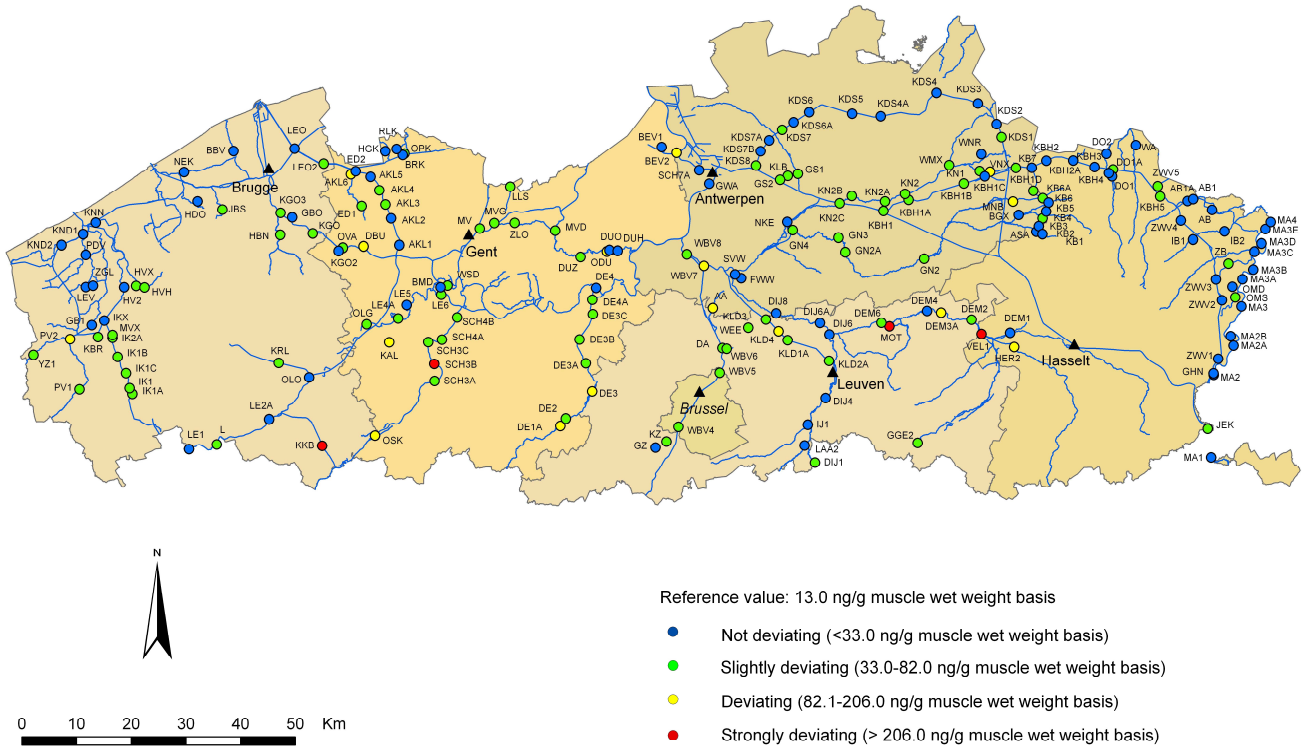
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XX: Organochlorine pesticides in eel (Flanders, 2002-2005): p,p'-DDE

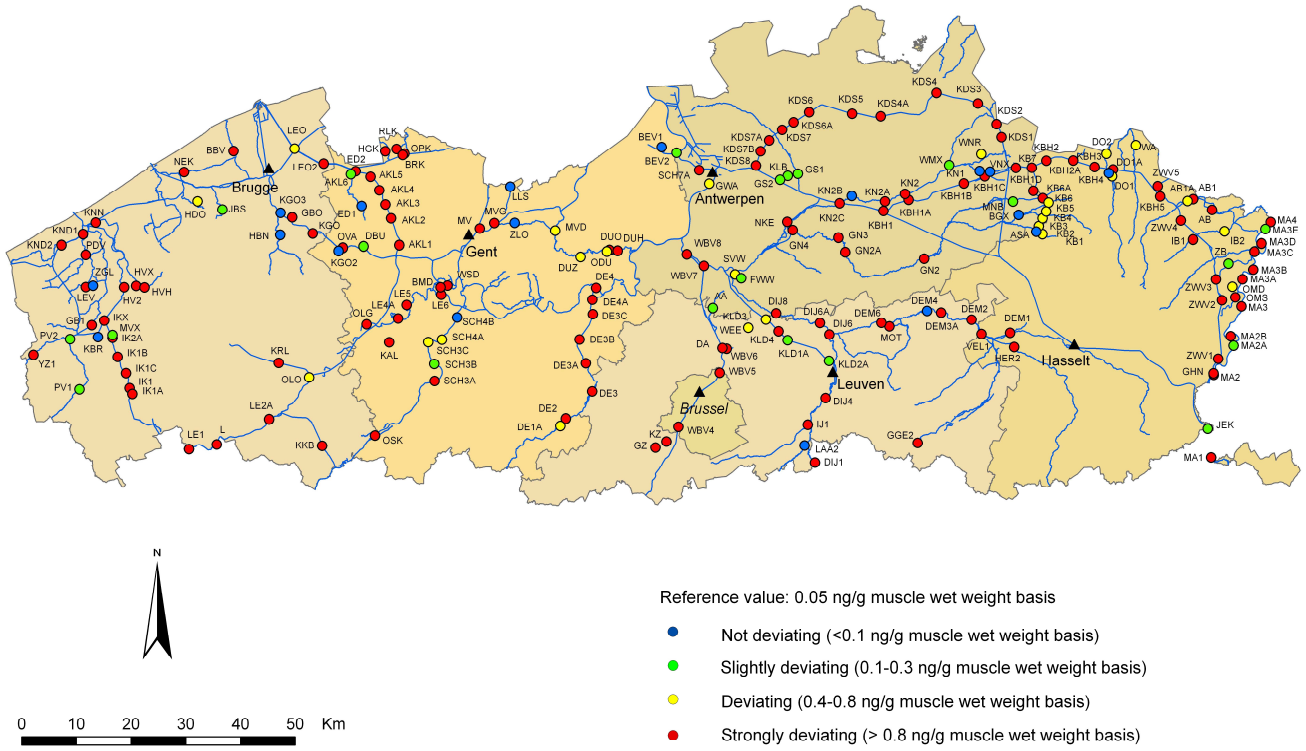
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXI: Organochlorine pesticides in eel (Flanders, 2002-2005): trans-Nonachlor

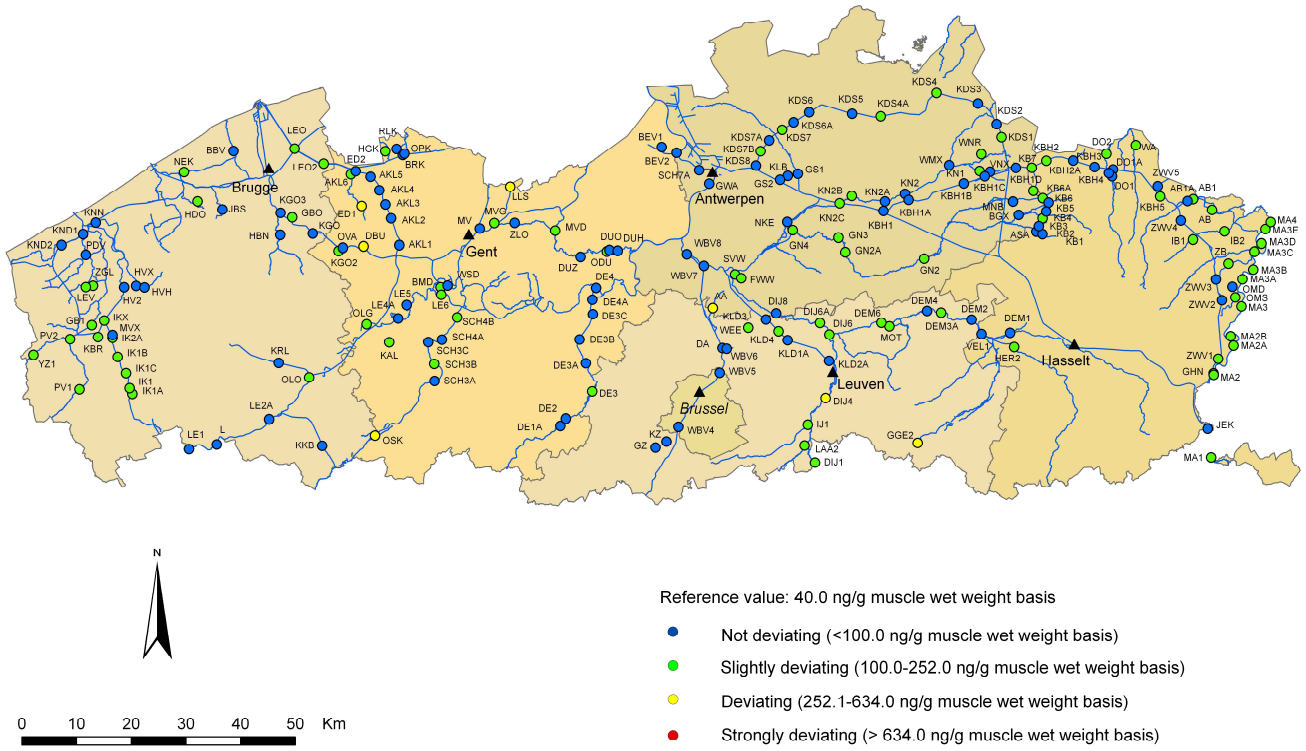
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXII: Heavy metals in eel (Flanders, 2002-2005): Mercury

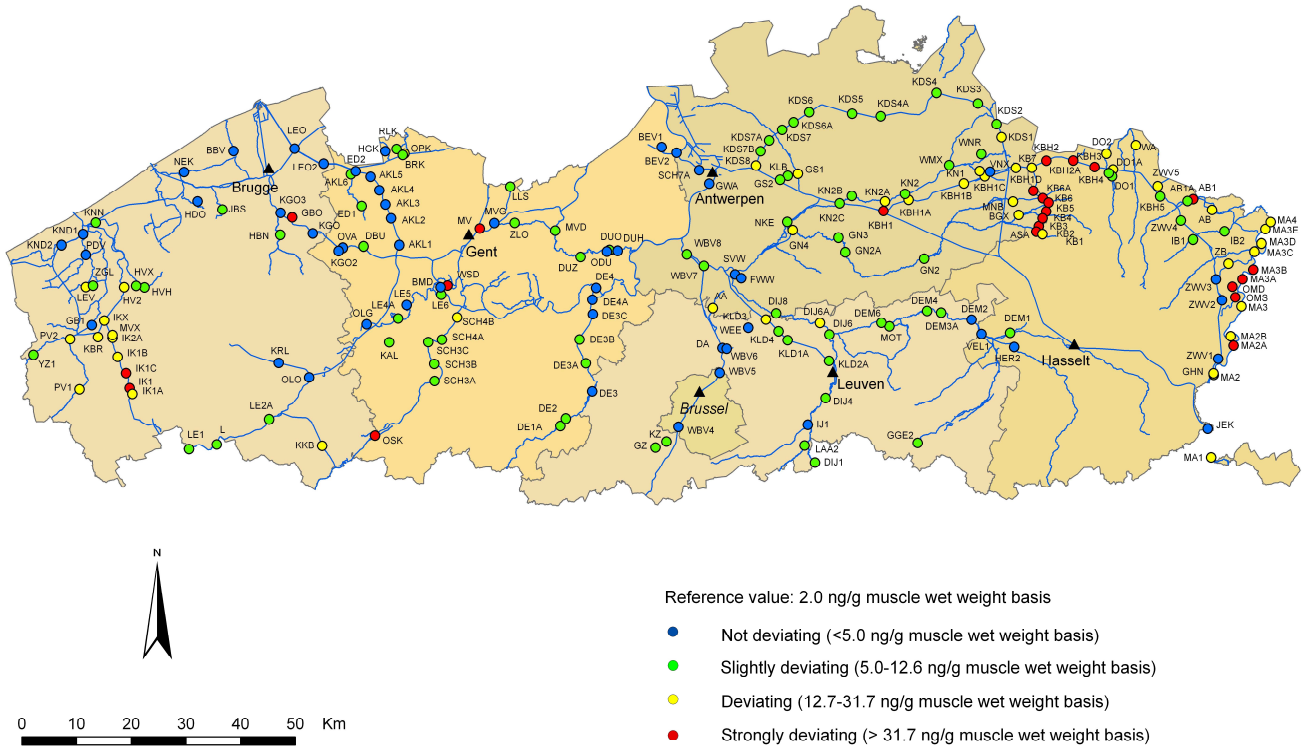
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXIII: Heavy metals in eel (Flanders, 2002-2005): Cadmium

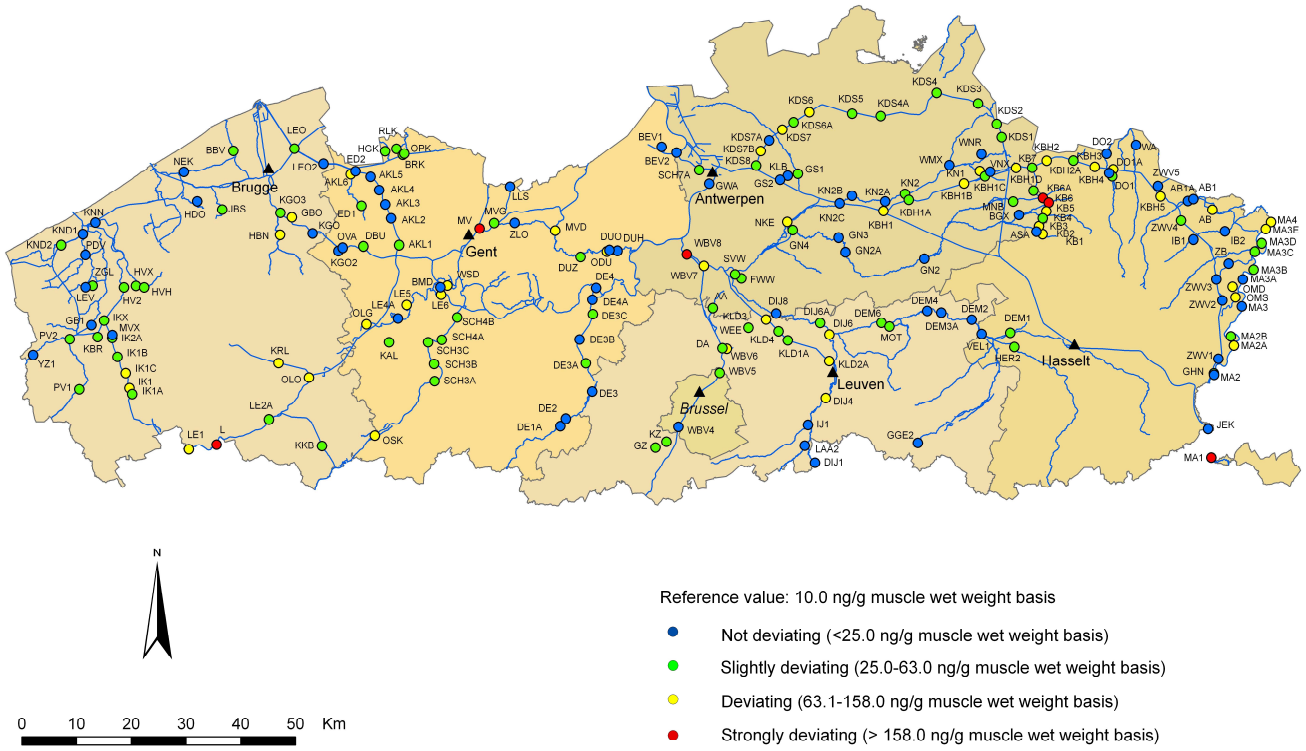
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXIV: Heavy metals in eel (Flanders, 2002-2005): Lead

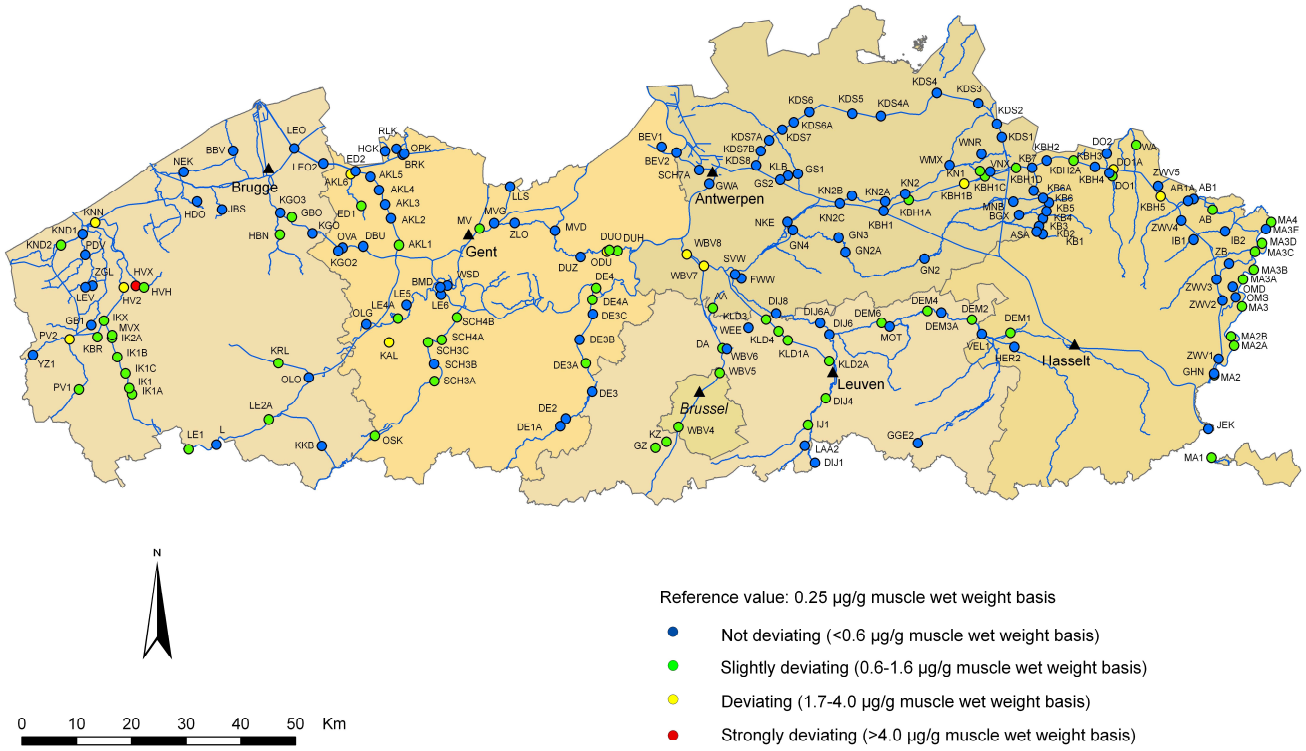
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXV: Heavy metals in eel (Flanders, 2002-2005): Copper

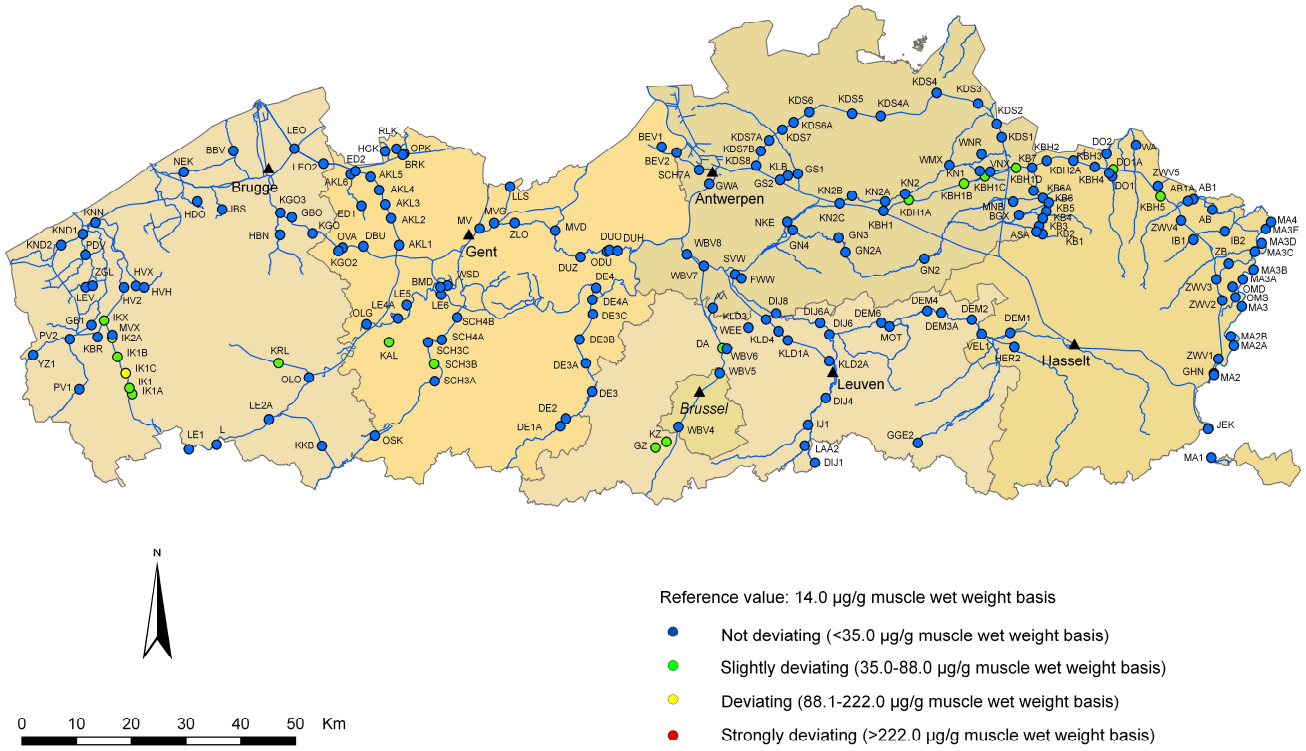
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXVI: Heavy metals in eel (Flanders, 2002-2005): Zinc

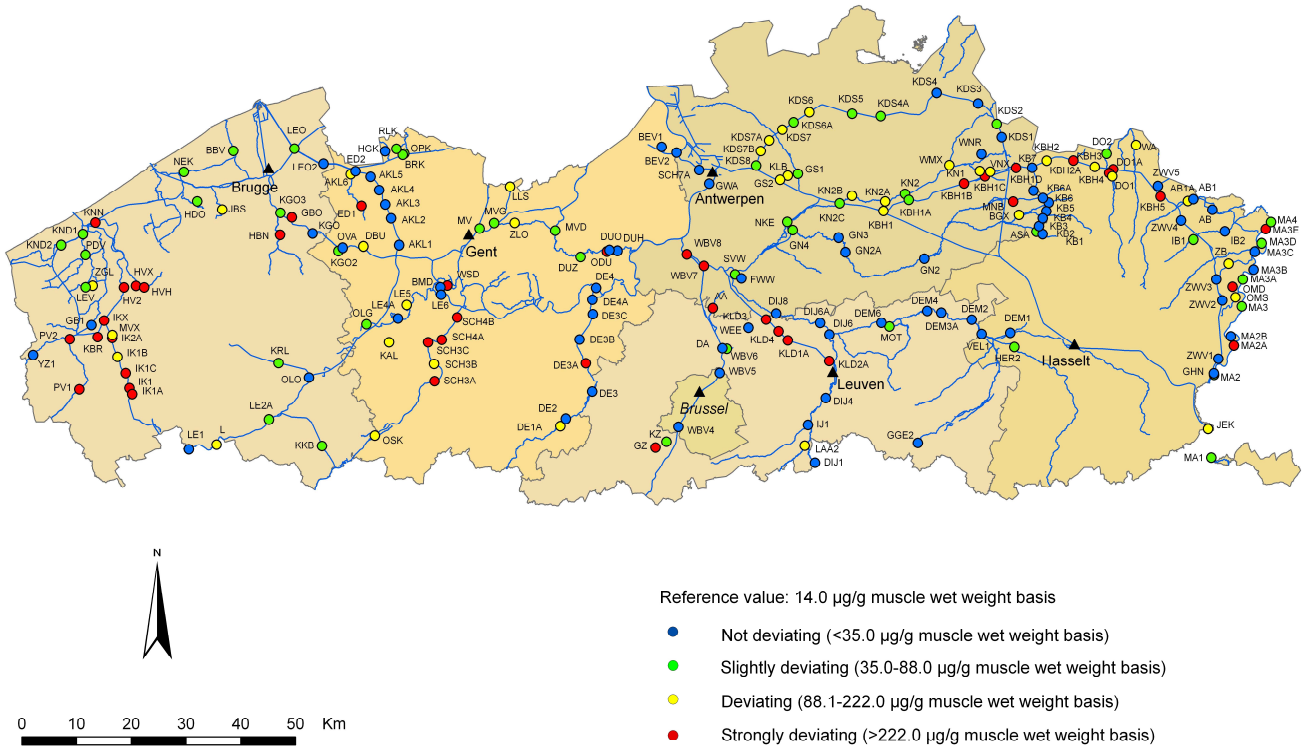
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXVII: Heavy metals in eel (Flanders, 2002-2005): Nickel

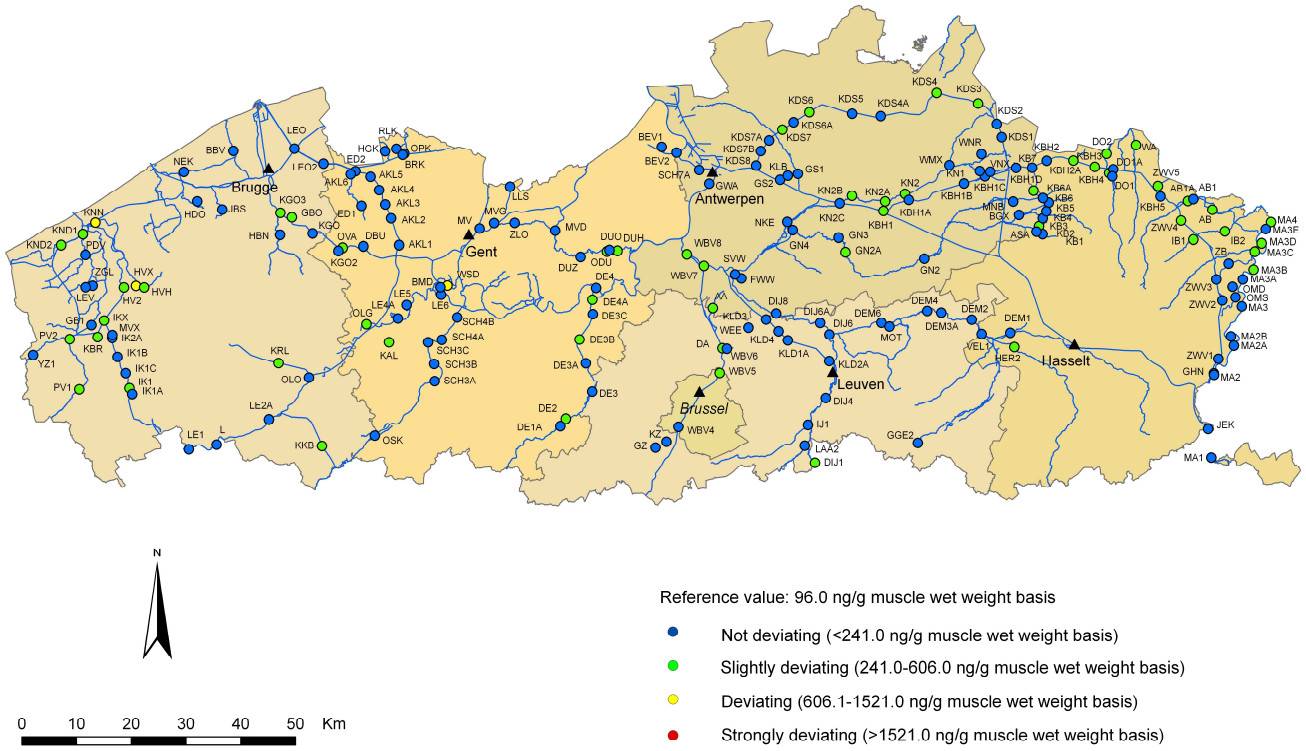
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXVIII: Heavy metals in eel (Flanders, 2002-2005): Chromium

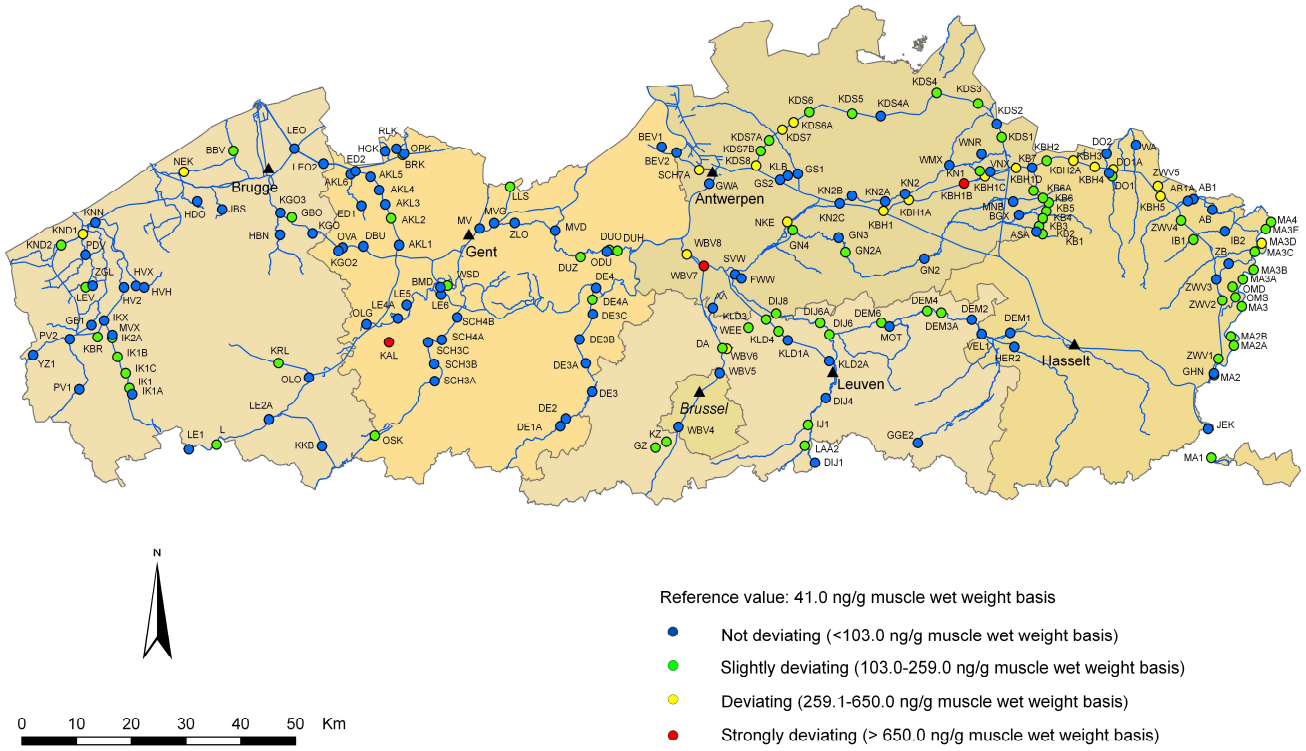
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXIX: Heavy metals in eel (Flanders, 2002-2005): Arsenic

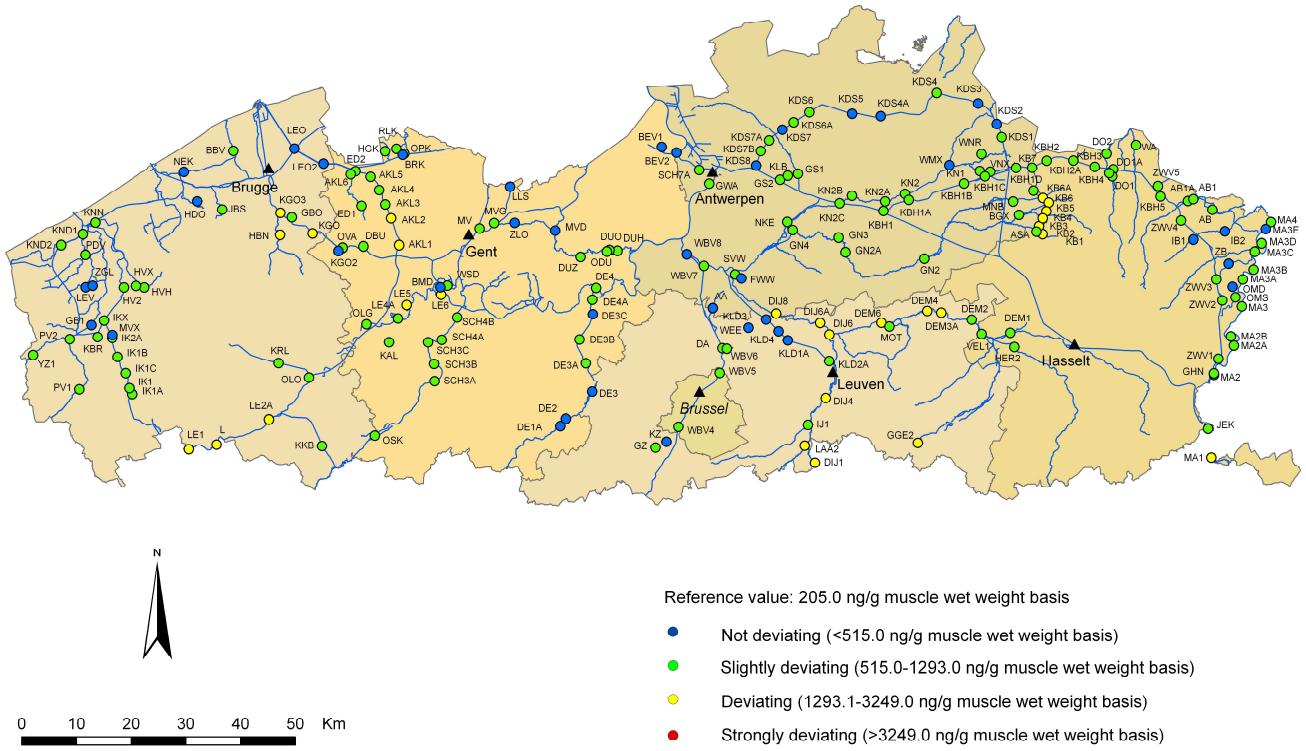
Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

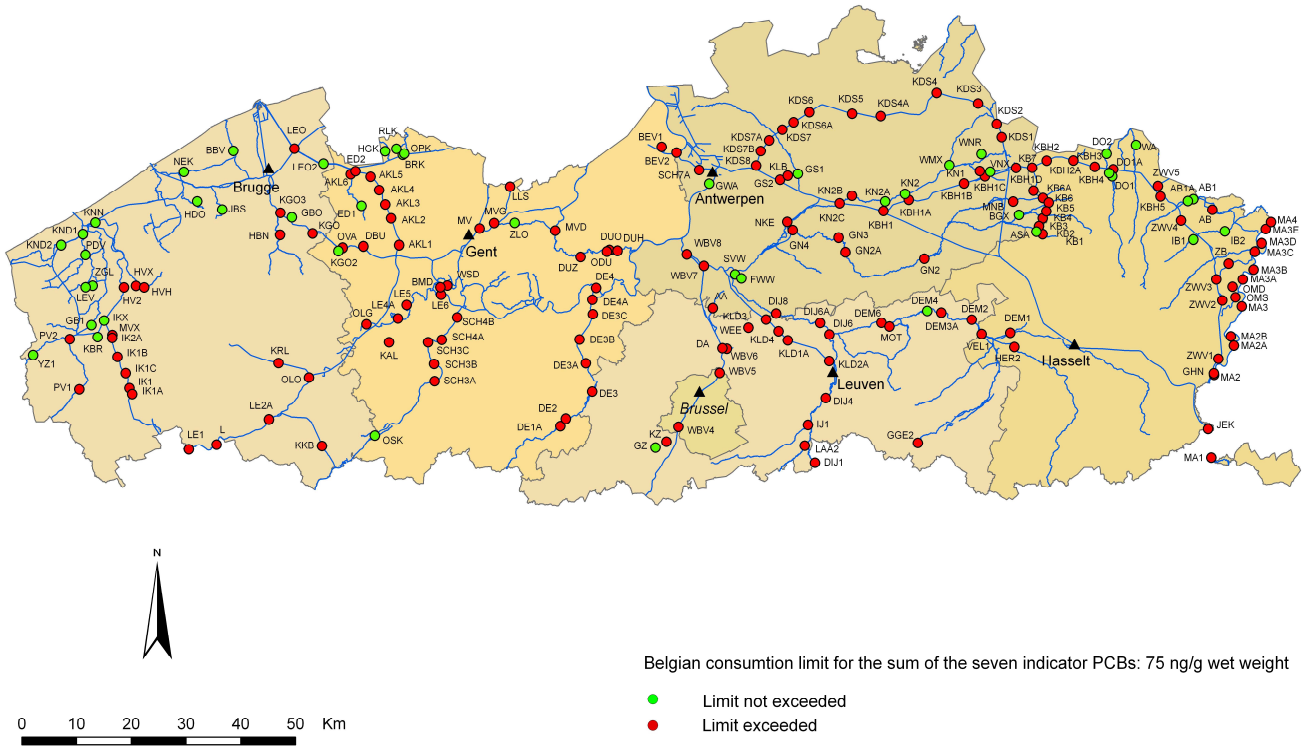
Figure XXX: Heavy metals in eel (Flanders, 2002-2005): Selenium

Means on muscle wet weight basis, classified following the deviation from the reference value



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Figure XXXI: Sites exceeding Belgian consumtion limit for PCBs
Means for Flanders, 2002-2005



Goemans *et al.*, 2008: The Eel Pollutant Monitoring Network: results for 2002-2005. Carthography.

Synthetic colorants like the carcinogenic Sudan red dyes are used to colour maggots and may accumulate in the eels' flesh. Above: two wild eels, a normal and a coloured one.

Photo: Gerlinde Van Thuyne, INBO

Annex II

The Eel Pollutant Monitoring Network: results for 1994-2005. Data tables.

Geert Goemans[1], Claude Belpaire[1], Koen Parmentier[2] and Ludwig De Temmerman [3]

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

[2] Institute for Agricultural and Fisheries Research (ILVO Fischeries), Ankerstraat 1, B-8400 Oostend, Belgium

[3] Veterinary and Agrochemical research Centre, Leuvensesteenweg 17, B-3080 Tervuren, Belgium

The data presented in this annex are also available via the VIS website.

Goemans, G., Belpaire, C., Parmentier , K. and De Temmerman, L., 2008 The Eel Pollutant Monitoring Network: results for 1994-2005. Data tables. Data available at http://vis.milieuinfo.be/.

Data tables

The chapters presented in this book are based on data generated through the Flemish Eel Pollutant Monitoring.

Goemans *et al.* (2003) published a report describing the results of the EPMN for the period 1994-2001 and included data tables with location and sample description, and means of the PCBs, OCPs and heavy metals measured in eel for each sampling site.

In this annex, we present in two tables an update for this report including new data from samples taken in the period 2002-2005. A first table (Table II.1) describes details about the sampling and location characteristics. The second table (Table II.2) gives the means per location and per sampling date for PCBs, OCPs and heavy metals, expressed on a wet weight basis. For individual data per eel, and for data on a lipid weight basis for PCBs and OCPs we refer to the authors or to the VIS website (http://vis.milieuinfo.be/).

References

Goemans, G., Belpaire, C., Raemaekers, M., Guns, M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollution monitoring network 1994-2001: polychlorinated biphenyls, organochlorine pesticides and heavy metals in eel]. Report of the Institute for Forestry and Game Management, IBW. Wb.V.R.2003.99., 169p.

VIS, 2008. Fish Information System. Flanders' database with fish-related data. World Wide Web electronic publication. Research Institute for Nature and Forest (INBO). http://vis.milieuinfo.be/, version (02/2008). Accessed February 2008.

Table II.1. Characteristics of eel samples taken for analyses in the framework of the EPMN during the period 1994-2005. Code refers to locations presented in the maps of Annex I. X and Y refer to geographical Lambert coordinates. The number of eel (N) and the length range (min-max, in cm) is indicated. Under basin the catchment is given (Y – Ijzer, L - Leie, BP – Brugse Polders, GK – Gentse Kanalen, BOS – Boven Schelde, BES – Beneden Schelde, Den – Dender, DZ – Dijle-Zenne, Dem – Demer, N – Nete, M – Maas).

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
A	199411	239599	Aa	Poppel	M	1996-10-22	3	40,4 - 45,2
A	199411	239599	Aa	Poppel	M	2001-06-20	1	53,7 - 53,7
AA	151497	189027	Aabeek	Hombeek, Mechelen	DZ	2002-04-24	1	53,2 - 53,2
AB	242769	206835	Abeek	Urlobroek	M	1998-04-15	3	46 - 52
AB	242769	206835	Abeek	Urlobroek	M	2004-05-03	6	32,6 - 58
AB1	239310	208800	Abeek	Bocholt	M	2000-05-24	5	34,2 - 47,1
AB1	239310	208800	Abeek	Bocholt	M	2004-05-04	1	63,3 - 63,3
AB1A	238284	208455	Abeek	Bocholt, thv molenkom Luisen	M	2004-05-04	3	45,5 - 52,7
AB2	242450	207430	Abeek	Kinrooi	M	2000-05-24	5	34,5 - 45
AK0	239785	169540	Albertkanaal	Vroenhoven	M	2000-08-07	5	40,4 - 47,8
AK1	238895	175215	Albertkanaal	Briegden	M	2000-08-07	5	35,3 - 44,9
AK2	229035	181190	Albertkanaal	Langerlo, sluis	DEM	2000-08-07	5	36,4 - 45
AK3	217860	181835	Albertkanaal	Hasselt, jachthaven	DEM	2000-08-07	5	34,8 - 46,7
AK4	206810	196195	Albertkanaal	Tervant, kolenhaven+kanaal	DEM	2000-08-08	5	32,6 - 45,6
AK5	192485	202070	Albertkanaal	Geel, Stelen	N	2000-08-08	5	37,9 - 52,5
AK6	176005	207840	Albertkanaal	Bouwel	N	2000-08-08	2	31 - 60,2
AK7	167260	210760	Albertkanaal	Oelegem, zwaaikom	N	2000-06-21	4	32,5 - 68,2
AKK	228840	182030	Albertkanaal	Langerlo, kolenhaven	DEM	1997-06-06	3	36,6 - 67,2
AKL1	94181	200468	Afleidingskanaal van de Leie	Zomergem (Overdam)	GK	1999-10-07	4	29,6 - 46,8
AKL1	94181	200468	Afleidingskanaal van de Leie	Zomergem (Overdam)	GK	2005-10-04	5	40,4 - 46,1
AKL2	92699	205280	Afleidingskanaal van de Leie	Oostwinkel	GK	1999-10-07	3	32,5 - 53,8
AKL2	92699	205280	Afleidingskanaal van de Leie	Oostwinkel	GK	2005-10-04	5	34 - 41,99
AKL3	91650	207828	Afleidingskanaal van de Leie	grens Eeklo-Maldegem	GK	1999-10-07	4	37,5 - 57
AKL3	91650	207828	Afleidingskanaal van de Leie	grens Eeklo-Maldegem	GK	2005-10-04	5	46 - 48,9
AKL4	90519	210428	Afleidingskanaal van de Leie	Adegem	BP	1999-10-07	3	38,3 - 54,3
AKL4	90519	210428	Afleidingskanaal van de Leie	Adegem	BP	2005-10-04	3	34,2 - 49,7
AKL5	88949	212882	Afleidingskanaal van de Leie	grens Maldegem-Sint-Laureins	BP	1999-10-07	2	31,9 - 39

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
AKL5	88949	212882	Afleidingskanaal van de Leie	grens Maldegem-Sint-Laureins	BP	2005-10-03	4	33 - 45,9
AKL6	86223	213911	Afleidingskanaal van de Leie	Maldegem	BP	1999-10-07	4	37,2 - 48
AKL6	86223	213911	Afleidingskanaal van de Leie	Maldegem	BP	2005-10-03	5	36,1 - 43,7
AKL7	71096	223668	Afleidingskanaal van de Leie	Ramskapelle	BP	1999-10-07	5	30,9 - 36,5
AKS	213876	184777	Albertkanaal	Hasselt, Stokrooi (brug)	DEM	1999-07-07	2	41 - 41,5
ASA	210633	202850	Asdonkbeek	Asdonk	N	2002-04-02	1	42,4 - 42,4
ATB	161520	221900	Antitankgracht	Brasschaat, t.h.v. stuw, Hof ter Mik	BES	2000-06-21	5	26,9 - 32,7
BB	124391	191512	Domeinbos Berlare Broek	Berlare	BOS	1994-09-12	1	29 - 29
BBO	241430	197075	Bosbeek	Opoeteren	M	2000-05-24	4	32 - 53,7
BBU	120200	190875	Bellebeek	Uitbergen	BOS	2000-08-02	5	35,1 - 40,6
BBV	63870	217620	Blankenbergse Vaart	Zuienkerke, Zielebrug	BP	2000-06-27	5	35,3 - 50
BBV	63870	217620	Blankenbergse Vaart	Zuienkerke, Zielebrug	BP	2003-06-04	10	34,8 - 39,2
BEM	245324	161469	Berwijn	Moelingen	M	2000-08-30	1	47,8 - 47,8
BEV1	142134	218341	Dokken	T.h.v. het noordelijk insteekdok-Waaslandkanaal	BES	2002-06-14	2	31 - 42
BEV2	144876	217309	Dokken	Beveren, t.h.v. Kallosluis	BES	2002-06-14	1	50,6 - 50,6
BGG	102330	194380	Oude Leie Bourgoyen	Gent	GK	2000-05-30	5	33,9 - 42,6
BGP	66523	170742	Bergeleput	Gullegem	L	2001-10-05	5	39,6 - 45
BGX	207422	205906	Balengracht	T.h.v. Mol Neet	N	2002-04-02	2	32,1 - 34,6
BK1	68737	222588	Boudewijnkanaal	Lissewege, ten N van de slibway	BP	1994-05-01	24	23,8 - 52,1
BK1	68737	222588	Boudewijnkanaal	Lissewege, ten N van de slibway	BP	1994-08-30	26	23,1 - 42,5
BK1	68737	222588	Boudewijnkanaal	Lissewege, ten N van de slibway	BP	1994-11-30	2	30 - 44
BK1	68737	222588	Boudewijnkanaal	Lissewege, ten N van de slibway	BP	1995-06-12	10	24 - 69
BK2	69282	218914	Boudewijnkanaal	Dudzele, zwaaikom Herdersbrug	BP	1994-05-01	44	19,2 - 36,9
BK2	69282	218914	Boudewijnkanaal	Dudzele, zwaaikom Herdersbrug	BP	1994-08-30	24	28,1 - 56,5
BK2	69282	218914	Boudewijnkanaal	Dudzele, zwaaikom Herdersbrug	BP	1994-11-30	9	30,5 - 61,1
BK2	69282	218914	Boudewijnkanaal	Dudzele, zwaaikom Herdersbrug	BP	1994-12-01	6	33,9 - 56,5
BK4	69549	213857	Boudewijnkanaal	Brugge	BP	1994-05-01	7	22 - 36,8
BK4	69549	213857	Boudewijnkanaal	Brugge	BP	1994-08-30	34	20,5 - 57,2

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
BK4	69549	213857	Boudewijnkanaal	Brugge	BP	1994-11-30	23	26 - 54,2
BK4	69549	213857	Boudewijnkanaal	Brugge	BP	1994-12-01	2	34 - 37,5
BK4	69549	213857	Boudewijnkanaal	Brugge	BP	1995-03-08	3	30,5 - 37
BK5	70522	222488	Boudewijnkanaal	Zeebrugge, haven	BP	1994-05-01	12	26,2 - 51,2
BK5	70522	222488	Boudewijnkanaal	Zeebrugge, haven	BP	1994-08-30	20	26,2 - 68
BK5	70522	222488	Boudewijnkanaal	Zeebrugge, haven	BP	1994-09-01	1	31,2 - 31,2
BK5	70522	222488	Boudewijnkanaal	Zeebrugge, haven	BP	1995-03-08	1	65,5 - 65,5
BL	43978	187089	Natuurreservaat De Blankaart	Woumen	Y	1997-12-04	3	32,7 - 43,7
BMD	101720	192850	Blaarmeersen	Drongen	GK	2000-05-30	5	37,4 - 44,4
BMD	101720	192850	Blaarmeersen	Drongen	GK	2004-10-11	10	37,6 - 46,9
BND	161260	199900	Beneden Nete	Duffel, Ter Elst	N	2000-06-15	5	28,8 - 51,2
BOK	221927	183651	Vijver Bokrijk	Bokrijk	DEM	1998-10-20	1	69,5 - 69,5
BRK	94825	216900	Boerekreek	St.-Jan-in-Eremo, Janspolderdijk	GK	2000-08-01	2	45,6 - 47,6
BRK	94825	216900	Boerekreek	St.-Jan-in-Eremo, Janspolderdijk	GK	2003-03-25	8	29,9 - 56,7
BVW	115900	203785	Bosdamvijver	Wachtebeke, Provinciaal Domein	GK	2000-05-29	5	34,5 - 44,8
BWK1	69736	214370	Boudewijnkanaal	Brugge	BP	2000-09-04	5	35,2 - 45,4
BWK2	69536	216874	Boudewijnkanaal	Brugge, RWZI	BP	1994-05-01	29	23,8 - 57,2
BWK2	69536	216874	Boudewijnkanaal	Brugge, RWZI	BP	1994-08-30	9	23,5 - 36,5
BWK2	69536	216874	Boudewijnkanaal	Brugge, RWZI	BP	1994-11-30	11	22,2 - 46,2
BWK2	69536	216874	Boudewijnkanaal	Brugge, RWZI	BP	1994-12-01	1	36,5 - 36,5
BWK2	69536	216874	Boudewijnkanaal	Brugge, RWZI	BP	2000-09-04	5	35,5 - 43
BWK3	68995	222850	Boudewijnkanaal	Zeebrugge	BP	2000-09-04	5	35,5 - 45,9
COM	200465	212535	Congovaart + lagune	Mol, t.h.v. het SCK en Electrabel	N	2001-08-10	21	32,2 - 58,6
DA	154050	181570	Darse	Vilvoorde	DZ	1994-11-29	18	30 - 51,2
DA	154050	181570	Darse	Vilvoorde	DZ	1997-09-18	3	35,9 - 47,3
DA	154050	181570	Darse	Vilvoorde	DZ	2003-06-07	4	37,4 - 48,2
DA1	145752	223242	Antwerpse dokken	Insteekdok 1, Kaai 629	BES	2000-10-03	5	35,9 - 51,1
DA2	147445	218245	Antwerpse dokken	Van Cauwelaertsluis, Kruisschansbrug	BES	2000-10-03	5	37,3 - 64,8

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
DAM	173960	235245	Den Aerd, E-10 put	Minderhout	M	2000-05-16	5	35,1 - 43,2
DAV	74500	217000	Damse Vaart	Damme	BP	1995-06-14	1	43,5 - 43,5
DAV1	72500	214420	Damse vaart	Apertje	BP	2000-05-25	5	36,3 - 45
DAV2	75745	218655	Damse vaart	Oostkerkebrug	BP	2000-05-25	5	37,4 - 44,7
DBR	141000	197733	Domein Breeven	Bornem	BES	1999-05-18	3	46 - 53,6
DBU	87643	200248	Driesbeek	Driesbeek, GPS102 - Aalter-Knesselare	BP	2002-05-28	1	47,2 - 47,2
DE1	118995	165485	Dender	Idegem, sluis	DEN	2000-10-13	2	36,8 - 40,3
DE1A	123640	167580	Dender	Pollaresluis, stroomafwaarts	DEN	2002-03-27	2	60,8 - 61
DE2	124635	168890	Dender	Ninove, sluis	DEN	2000-10-16	5	43,7 - 68
DE2	124635	168890	Dender	Ninove, sluis	DEN	2005-03-21	3	42 - 49,4
DE3	129460	173740	Dender	Liedekerke, sluis	DEN	2000-10-16	1	62,5 - 62,5
DE3	129460	173740	Dender	Liedekerke, sluis	DEN	2002-03-27	1	55,3 - 55,3
DE3	129460	173740	Dender	Liedekerke, sluis	DEN	2005-03-21	1	42,8 - 42,8
DE3	129460	173740	Dender	Liedekerke, sluis	DEN	2005-03-22	2	52 - 52,7
DE3A	128295	178927	Dender	Erembodegem, Denderhoutem	DEN	2002-03-27	3	38,2 - 46,6
DE3B	127140	183260	Dender	Hofstade	DEN	2002-03-28	2	43,3 - 57,6
DE3B	127140	183260	Dender	Hofstade	DEN	2005-03-22	5	35,6 - 46,3
DE3C	129580	187900	Dender	Getijdesluis, stroomafwaarts	DEN	2002-03-28	10	36,6 - 52,9
DE3C	129580	187900	Dender	Getijdesluis, stroomafwaarts	DEN	2003-10-02	10	32 - 49,9
DE4	129488	190545	Dender	Appels	DEN	2000-10-13	5	27,3 - 51,6
DE4	129488	190545	Dender	Appels	DEN	2005-03-22	2	42,8 - 46,4
DE4	129488	190545	Dender	Appels	DEN	2005-03-23	3	32,8 - 36,4
DE4A	130180	192700	Dender	Appels, Dendermonde	DEN	2002-03-28	8	35,2 - 48
DE4A	130180	192700	Dender	Appels, Dendermonde	DEN	2003-10-01	10	35 - 47,5
DEM1	205842	184488	Demer	Linkhout	DEM	1999-04-13	1	44,7 - 44,7
DEM1	205842	184488	Demer	Linkhout	DEM	2001-10-29	1	49,9 - 49,9
DEM1	205842	184488	Demer	Linkhout	DEM	2003-09-04	2	52 - 63,7
DEM2	198810	186907	Demer	Diest, Grote Steunbeer	DEM	1999-04-13	1	50 - 50
DEM2	198810	186907	Demer	Diest, Grote Steunbeer	DEM	2003-09-02	3	35,1 - 61,3

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
DEM3	196568	186924	Demer	Diest, waterzuiveringsstati	DEM	1999-04-13	1	56,9 - 56,9
DEM3A	193230	188176	Demer	stroomafwaarts de molen - Zichem	DEM	2003-04-09	2	25,2 - 59,5
DEM4	190683	188489	Demer	Testelt	DEM	1999-04-13	1	31,6 - 31,6
DEM4	190683	188489	Demer	Testelt	DEM	2003-04-09	1	44,8 - 44,8
DEM5	183218	186189	Demer	Aarschot (monding Motte)	DEM	1999-04-13	1	34 - 34
DEM6	182306	186335	Demer	Hertogenmolen	DEM	2003-09-02	2	54,1 - 56,1
DGH	76270	170800	Gavers	Harelbeke, Provinciaal Dome	L	2000-07-12	5	50,8 - 64,9
DIJ1	170169	160893	Dijle	grens Ottenburg-Archennes (Florival	DZ	1999-04-27	2	44,1 - 59
DIJ1	170169	160893	Dijle	grens Ottenburg-Archennes (Florival	DZ	2003-05-06	1	52,4 - 52,4
DIJ2	169334	169769	Dijle	Korbeek-Dijle	DZ	1999-04-28	2	46,8 - 47,6
DIJ3	171086	171118	Dijle	Oud-Heverlee onder E40	DZ	1999-04-27	3	45,1 - 47
DIJ4	172126	172534	Dijle	Heverlee (Arenbergmolen)	DZ	1999-04-28	1	49,7 - 49,7
DIJ4	172126	172534	Dijle	Heverlee (Arenbergmolen)	DZ	2003-05-07	1	47,5 - 47,5
DIJ5	173057	173910	Dijle	Leuven (Dijlemolens)	DZ	1999-04-28	1	58 - 58
DIJ6	172794	184168	Dijle	Werchter aan samenvloeiing met Demer	DZ	1999-04-27	2	31 - 53,5
DIJ6	172794	184168	Dijle	Werchter aan samenvloeiing met Demer	DZ	2003-05-07	1	48,8 - 48,8
DIJ6A	171090	186306	Dijle	a.d. monding van de Laak	DZ	2003-05-08	3	29,2 - 47,3
DIJ7	169185	186323	Dijle	grens Haacht-Keerbergen, Hansbrug	DZ	1999-04-27	2	45,5 - 53,5
DIJ8	163070	188039	Dijle	grens Bonheiden-Boortmeerbe	DZ	1999-04-27	4	31,7 - 38,9
DIJ8	163070	188039	Dijle	grens Bonheiden-Boortmeerbe	DZ	2003-05-08	1	47,2 - 47,2
DO1	224474	212989	Dommel	Overpelt, ziekenhuis, slagmolen	M	1998-05-07	3	36 - 53
DO1	224474	212989	Dommel	Overpelt, ziekenhuis, slagmolen	M	2000-05-23	5	32,7 - 44,9
DO1	224474	212989	Dommel	Overpelt, ziekenhuis, slagmolen	M	2004-04-21	10	30,8 - 50,6
DO1A	223907	213568	Dommel	Overpelt, brug, Eindergatloop	M	2004-04-22	1	31,2 - 31,2

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
DO2	223455	217115	Dommel	Neerpelt, Lommels Goor	M	2000-05-23	5	36,5 – 57
DO2	223455	217115	Dommel	Neerpelt, Lommels Goor	M	2004-04-21	1	44,5 - 44,5
DSS	119180	190465	Driessesloot	Schellebelle	BOS	2000-08-02	5	35,1 - 45,4
DUH	134094	199474	Durme	Hamme, Mirabrug	BES	2004-04-07	4	36,6 - 59,6
DUH	134094	199474	Durme	Hamme, Mirabrug	BES	2005-04-28	5	36 - 43,6
DUL	122940	199570	Durme	Lokeren, centrum	GK	2000-08-29	5	33,4 - 43
DUO	132609	199596	Durme	Ter hoogte van Oude Durme, Waasmunster	BES	2004-04-07	2	40 - 53,2
DUZ	127331	198365	Durme	Zele, SA sluis	BES	2004-04-08	4	27 - 42,8
DUZ	127331	198365	Durme	Zele, SA sluis	BES	2005-04-27	5	30,3 - 47,3
ED1	87293	207553	Ede	Ede, GPS:99 - Maldegem	BP	2002-05-28	1	43,6 - 43,6
ED2	85338	213356	Ede	Ede, GPS:98 - Maldegem	BP	2002-05-28	4	29,7 - 47,7
EEND						2005-10-26	1	58,5 - 58,5
FOO	167050	212820	Antitankgracht	Oelegem, Fort	BES	2000-05-16	5	35,2 - 43,8
FSA	148765	225820	Antitankgracht	Stabroek, Fort	BES	2000-06-21	5	35,4 - 42,8
FWW	156710	194480	Gracht rond Fort	Walem, Fort	N	2000-05-10	5	34,6 - 38,5
FWW	156710	194480	Gracht rond Fort	Walem, Fort	N	2003-09-30	10	34,6 - 43,8
GAG	117285	164915	De Gavers	Geraardsbergen, Provinciaal domein	DEN	2000-07-19	5	35,8 - 44,9
GB	40700	194500	Grote Beverdijk	Oostkerke, Pervijze	Y	1995-05-23	4	27,7 - 39,1
GB1	37961	185902	Grote Beverdijk	Lo-Reninge, Busbrug	Y	2000-07-10	5	35,4 - 44,1
GB1	37961	185902	Grote Beverdijk	Lo-Reninge, Busbrug	Y	2003-06-16	1	59 - 59
GB2	40523	197774	Grote Beverdijk	Stuivekenskerke, karpelbrug	Y	2000-06-28	5	34,5 - 46,5
GBO	74593	205486	Geuzenbeek	Oostkamp	BP	2002-06-05	4	30,6 - 45,7
GBR	61995	183000	Grote Bassin	Roeselare	L	2000-07-12	5	52,1 - 74,3
GGE2	188950	164500	Grote Gete	Tienen, Groot Overlaar ad Bellekomsemolen	DEM	2004-12-10	1	58,2 - 58,2
GGZ	49189	204432	Groot Geleed	Zevekote	Y	2000-08-28	5	35,8 - 41,7
GHN	243040	177070	Grindplassen Maasstreek Hochterbampt	Neerharen	M	2000-09-21	5	36,9 - 45,4
GHN	243040	177070	Grindplassen Maasstreek Hochterbampt	Neerharen	M	2005-09-27	5	33,9 - 45,4

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
GN1	201820	203700	Grote Nete	Meerhout, Hulsen	N	2000-06-14	5	36,4 - 44,2
GN2	190146	198075	Grote Nete	Westerlo, Zammelsebrug	N	2000-06-14	6	33,5 - 45,9
GN2	190146	198075	Grote Nete	Westerlo, Zammelsebrug	N	2003-03-19	5	32 - 36,2
GN2A	175729	199228	Grote Neet	Itegem, Krombeekweg (Hof ter Borcht)	N	2003-03-19	10	30,9 - 44,3
GN3	174473	201795	Grote Nete	Bevel, Hbg 't Schipke	N	2000-06-14	2	30,7 - 31,9
GN3	174473	201795	Grote Nete	Bevel, Hbg 't Schipke	N	2003-03-19	2	33,7 - 34
GN4	166110	203115	Grote Neet	SO spoorwegbrug - Lier	N	2003-03-18	6	28,6 - 49
GPG	154045	161495	Ganzepoot	Groenendaal-Hoeilaart	DZ	2000-05-05	5	35,4 - 48,5
GPG	154045	161495	Ganzepoot	Groenendaal-Hoeilaart	DZ	2000-10-27	5	40 - 52,5
GS1	167042	213446	Groot Schijn	Regenbooghoeve	BES	2002-05-23	2	35,6 - 47,8
GS2	163797	212362	Groot Schijn	Achter zuiveringsstation Albertkanaal	BES	2002-05-23	8	31,6 - 38,3
GSK	251945	203475	Grindplassen Maasstreek Steenberg, tss plas 1 & 2	Kessenich	M	2000-09-21	5	33 - 44
GVZ	110760	210625	Gemeentevijver	Zelzate	GK	2000-08-01	5	35,9 - 43,6
GW	144794	201022	Groene Wiel	Hingene	BES	1998-05-19	3	42,3 - 63,3
GWA	150850	211630	Galgenweel	A'pen, LO	BES	2000-05-17	5	36 - 42,8
GWA	150850	211630	Galgenweel	A'pen, LO	BES	2005-10-12	5	34,5 - 45,2
GZ	141010	163660	Groot Zuunbekken	St.-Pieters-Leeuw	DZ	1996-09-20	3	48,8 - 55,1
GZ	141010	163660	Groot Zuunbekken	St.-Pieters-Leeuw	DZ	2002-09-23	11	33,2 - 43,1
HBB1	151805	193870	Het Broek, vijver 1	Blaasveld-Heffen	BES	2000-05-08	3	36 - 77
HBB3	152530	194280	Het Broek, vijver 3	Blaasveld-Heffen	BES	2000-05-08	5	46,5 - 52,3
HBB4	151110	194330	Het Broek, vijver 4	Blaasveld-Heffen	BES	2000-05-08	2	63 - 70,5
HBN	72413	202297	Hertsbergebeek	Hertsberge-Nieuwenhove	BP	2002-05-29	1	34,3 - 34,3
HDO	57295	208390	Hoge dijken (Roksem put)	Oudenburg, Ettelgem	BP	2000-08-28	5	35,4 - 43,6
HDO	57295	208390	Hoge dijken (Roksem put)	Oudenburg, Ettelgem	BP	2003-09-23	6	31,7 - 49,8
HEL			Palingkwekerij	Helmond (NL)		2001-04-20	5	34,8 - 38
HER2	206582	181901	Herk	SA watermolen	DEM	2003-09-09	8	33,6 - 70,1
HGK	91620	217580	Hollandersgatkreek	St. Laureins, Kattenhoek	GK	2000-05-05	5	41,4 - 51,7

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
HGK	91620	217580	Hollandersgatkreek	St. Laureins, Kattenhoek	GK	2000-08-01	3	40,6 - 42,3
HGK	91620	217580	Hollandersgatkreek	St. Laureins, Kattenhoek	GK	2003-03-25	5	33,4 - 43,2
HO	160024	186243	Rijksdomein	Hofstade (Zemst), vijver	DZ	1997-09-18	5	27,2 - 65
HV2	46084	193094	Handzamevaart	Diksmuide, hoogspanningslijn	Y	2002-11-04	10	32 - 36,5
HVG	154325	161600	Hengelvijver	Hoeilaart	DZ	2000-05-05	5	36,6 - 46,6
HVH	47598	192802	Handzamevaart	Handzame	Y	2001-10-05	1	36,1 - 36,1
HVH	47598	192802	Handzamevaart	Handzame	Y	2002-11-04	10	30,9 - 35,2
HVX	43898	192823	Handzamevaart	Diksmuide (Beerst), monding	Y	2002-11-04	7	29,7 - 57,3
HZW	152440	194640	Hazewinkel, roeivijver	Willebroek	BES	2000-05-10	5	32,5 - 35,9
IB1	239296	201443	Itterbeek	Oppiter, Bree, aan de kasteelmolen	M	2001-06-07	5	34,6 - 41,4
IB1	239296	201443	Itterbeek	Oppiter, Bree, aan de kasteelmolen	M	2005-06-01	5	35,5 - 45,3
IB2	245054	202897	Itterbeek	Kinrooi, Maaseik	M	2001-06-06	3	36,6 - 48
IB2	245054	202897	Itterbeek	Kinrooi, Maaseik	M	2005-06-01	2	46,7 - 52,1
IBK	246575	204275	Itterbeek	Kinrooi	M	2000-05-24	5	34,6 - 49
IJ	161474	162267	Ijse	Overijse	DZ	1998-04-03	2	32 - 40
IJ1	168872	167795	Ijse	Huldenberg, Eigenstraat op 300m van monding in de Dijle	DZ	2005-03-10	2	55,4 - 67,4
IK1	44903	174360	Ieperkanaal	Ieper, brug	Y	2000-07-10	5	34,9 - 43,1
IK1	44903	174360	Ieperkanaal	Ieper, brug	Y	2002-09-09	9	32,6 - 42,9
IK1A	45396	173275	Ieperkanaal	Ieper, voor monding beek	Y	2002-09-09	10	34,8 - 43
IK1B	44224	177100	Ieperkanaal	Boezinge, SA Boezingebrug	Y	2002-09-09	10	34 - 43,8
IK1C	42712	180070	Ieperkanaal	Zuidschote-Bikschote, steenstratebrug	Y	2002-09-12	10	32,3 - 40,7
IK2	41938	182118	Ieperkanaal	5 km van de Yzer	Y	2001-05-14	5	34 - 38,1
IK2A	41745	183561	Ieperkanaal	Noordschote-Merkem	Y	2002-09-09	10	35,4 - 41,1
IKX	40267	186729	Ieperkanaal	Houthulst, monding Yzer	Y	2002-09-09	10	34,1 - 40,2
JBS	61840	206870	Jabbeekse Beek	Snellegem-Jabbeke - GPS:109	BP	2002-05-30	10	30,3 - 48,5
JEK	241980	167090	Jeker	Riemst	M	2002-03-12	3	57 - 66,4

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
KAL	92343	182757	Kallemoeie	Deinze	L	2003-06-15	7	70,3 - 102,3
KB1	211754	202401	Kanaal van Beverlo	Leopoldsburg	N	1997-10-15	4	29,8 - 59,2
KB1	211754	202401	Kanaal van Beverlo	Leopoldsburg	N	1999-11-03	2	32,3 - 65,1
KB1	211754	202401	Kanaal van Beverlo	Leopoldsburg	N	2005-10-25	6	39,5 - 52,7
KB2	211084	203852	Kanaal van Beverlo	Balen	N	1999-11-03	4	34,2 - 79,1
KB2	211084	203852	Kanaal van Beverlo	Balen	N	2000-06-19	5	33,7 - 48,3
KB2	211084	203852	Kanaal van Beverlo	Balen	N	2005-10-27	5	36,1 - 45
KB3	211787	205282	Kanaal van Beverlo	Balen, brug Balen-Zweiling	N	1999-11-03	6	36,1 - 64,6
KB3	211787	205282	Kanaal van Beverlo	Balen, brug Balen-Zweiling	N	2005-10-25	5	54 - 62,8
KB4	212442	206553	Kanaal van Beverlo	grens Antwerpen - Limburg	N	1999-11-03	2	43,2 - 51,5
KB4	212442	206553	Kanaal van Beverlo	grens Antwerpen - Limburg	N	2005-10-27	5	45,3 - 52,1
KB5	212872	208162	Kanaal van Beverlo	Lommel	N	1999-11-03	2	37,3 - 55,3
KB5	212872	208162	Kanaal van Beverlo	Lommel	N	2005-10-25	5	39,1 - 48,7
KB6	211810	208990	Kanaal van Beverlo	Mol (Wezel)	N	1999-11-03	10	31,5 - 55
KB6	211810	208990	Kanaal van Beverlo	Mol (Wezel)	N	2000-06-19	5	32,5 - 53
KB6	211810	208990	Kanaal van Beverlo	Mol (Wezel)	N	2005-10-27	5	42,8 - 48,8
KB6A	210100	210336	Kanaal van Beverlo	Balen, aan brug ter hoogte van Vielle Montagne	N	2005-10-25	5	37,3 - 56,4
KB7	209828	214533	Kanaal van Beverlo	Lommel (Blauwe Kei)	N	1999-11-03	9	35,9 - 83
KB7	209828	214533	Kanaal van Beverlo	Lommel (Blauwe Kei)	N	2005-10-26	4	30,7 - 34,7
KBH1	182685	206631	Kanaal Bocholt-Herentals	Herentals, sluis	N	1996-10-08	4	37,2 - 50
KBH1	182685	206631	Kanaal Bocholt-Herentals	Herentals, sluis	N	2002-10-07	11	31,3 - 48,8
KBH1A	187285	208653	Kanaal Bocholt-Herentals	Olen, grens Olen-Geel	N	2002-10-07	2	33,2 - 37,1
KBH1B	197408	211673	Kanaal Bocholt-Herentals	Mol	N	2002-10-07	10	35,8 - 45,3
KBH1C	201202	212977	Kanaal Bocholt-Herentals	Mol, Electrabel	N	2002-10-08	10	37,3 - 61
KBH1D	206896	214511	Kanaal Bocholt-Herentals	Mol, bij natuurreservaat 'De Maat'	N	2002-10-08	10	37,3 - 49
KBH2	212485	215835	Kanaal Bocholt-Herentals	Blekerheide	N	1996-10-08	1	51,5 - 51,5
KBH2	212485	215835	Kanaal Bocholt-Herentals	Blekerheide	N	2002-10-09	2	34,9 - 41,4
KBH2A	217386	215859	Kanaal Bocholt-Herentals	Lommel, Mortels	M	2002-10-07	1	41,2 - 41,2

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
KBH3	221299	214685	Kanaal Bocholt-Herentals	Overpelt, fabriek	M	1996-10-08	3	35 – 45
KBH3	221299	214685	Kanaal Bocholt-Herentals	Overpelt, fabriek	M	2002-10-10	4	29,5 - 34,8
KBH4	224673	214249	Kanaal Bocholt-Herentals	Neerpelt, zwaaikom	M	2002-10-07	3	41,3 - 48,5
KBH5	233279	209277	Kanaal Bocholt-Herentals	Bocholt, voor samenkomen ZWV	M	2002-10-07	10	34,9 - 45,4
KBL	210639	212382	Kanaal van Beverlo	Lommel	N	2001-04-09	10	35,2 - 50,9
KBL	210639	212382	Kanaal van Beverlo	Lommel	N	2001-10-05	11	34,9 - 87,6
KBR	39100	183700	Kemmelbeek	Reninge	Y	2002-05-07	3	31,7 - 33,5
KBR1	153561	179318	Kanaal Brussel-Rupel	Vilvoorde	DZ	1997-09-26	2	38,2 - 48,4
KBR2	153098	180093	Kanaal Brussel-Rupel	Grimbergen	DZ	1997-09-23	3	43 - 49,2
KBW	116200	204330	Klaverbladvijver	Wachtebeke, Provinciaal Domein	GK	2000-05-29	4	49,5 - 61,9
KDS1	204278	220135	Kanaal van Dessel naar Schoten	Retie (Reties Goor)	N	1999-09-10	2	47 - 50,1
KDS1	204278	220135	Kanaal van Dessel naar Schoten	Retie (Reties Goor)	N	2003-09-15	5	34,4 - 45,3
KDS2	203377	222531	Kanaal van Dessel naar Schoten	Arendonk, ten zuiden van Goorheide	N	1999-09-10	2	42 - 50,9
KDS2	203377	222531	Kanaal van Dessel naar Schoten	Arendonk, ten zuiden van Goorheide	N	2003-09-15	2	41,1 - 76
KDS3	199967	226308	Kanaal van Dessel naar Schoten	Ravels (Ravelse Hoek)	N	1998-11-20	3	40,8 - 58,8
KDS3	199967	226308	Kanaal van Dessel naar Schoten	Ravels (Ravelse Hoek)	N	2003-09-15	9	48,4 - 56,1
KDS4	192395	228234	Kanaal van Dessel naar Schoten	Turnhout, tssn kmp 23 en 24	N	1999-09-10	2	37,2 - 38,9
KDS4	192395	228234	Kanaal van Dessel naar Schoten	Turnhout, tssn kmp 23 en 24	N	2003-09-15	6	26,6 - 57
KDS4A	182185	223971	Kanaal van Dessel naar Schoten	Beerse, Hout	N	2003-09-15	10	31,5 - 45,6
KDS5	176954	224425	Kanaal van Dessel naar Schoten	Rijkevorsel, KMP 40	M	1999-09-10	5	32,4 - 64,1
KDS5	176954	224425	Kanaal van Dessel naar Schoten	Rijkevorsel, KMP 40	M	2003-09-15	8	35,6 - 60,3
KDS6	169126	224715	Kanaal van Dessel naar Schoten	Brecht, Eindhoven	M	1999-09-10	3	33,5 - 63
KDS6	169126	224715	Kanaal van Dessel naar Schoten	Brecht, Eindhoven	M	2003-09-17	10	34 - 48,2
KDS6A	166263	222862	Kanaal van Dessel naar Schoten	Brecht, Merel	N	2003-09-17	10	35,3 - 43,7
KDS7	164182	221507	Kanaal van Dessel naar Schoten	Sint-Job -in't-Goor	BES	1999-09-10	3	57,1 - 71,1
KDS7	164182	221507	Kanaal van Dessel naar Schoten	Sint-Job -in't-Goor	BES	2003-09-17	10	38,5 - 60,2
KDS7A	161790	219541	Kanaal van Dessel naar Schoten	Schoten, t.h.v. fort	BES	2003-09-15	5	32,3 - 53,8
KDS7B	160243	217595	Kanaal van Dessel naar Schoten	Schoten, Berkenrode	BES	2003-09-15	7	31,3 - 51

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
KDS8	159400	214956	Kanaal van Dessel naar Schoten	Schoten	BES	1998-11-20	3	44,5 – 56
KDS8	159400	214956	Kanaal van Dessel naar Schoten	Schoten	BES	2003-09-15	5	39 - 67,5
KG	50068	209851	Kamerlingsgeleed	Oudenburg	Y	1997-06-03	1	34,9 - 34,9
KGO	78351	202546	Kanaal Gent-Oostende	Beernem, Oude arm Miseriebocht	BP	1998-09-17	3	42,7 - 57,3
KGO	78351	202546	Kanaal Gent-Oostende	Beernem, Oude arm Miseriebocht	BP	2004-09-27	3	32,9 - 49,7
KGO	78351	202546	Kanaal Gent-Oostende	Beernem, Oude arm Miseriebocht	BP	2004-09-28	5	32,9 - 49,7
KGO	78351	202546	Kanaal Gent-Oostende	Beernem, Oude arm Miseriebocht	BP	2004-09-29	2	37,9 - 40,4
KGO1	103650	194325	Kanaal Gent-Oostende	Gent	GK	2000-05-17	5	47 - 56,8
KGO2	83870	199987	Kanaal Gent-Oostende	Aalter, nabij Hollebeek	BP	2004-09-27	6	33 - 47,8
KGO3	72472	206280	Kanaal Gent-Oostende	Oostkamp, ad Moerbruggebrug	BP	2004-09-28	5	35,6 - 38,3
KGT	105301	199304	Kanaal Gent-Terneuzen	Evergem, monding ringvaart	GK	1998-06-17	10	46,9 - 60,8
KKB	80071	163960	Kanaal Kortrijk-Bossuit	Moen, oude arm	BOS	1997-11-19	3	33,6 - 42,6
KKB	80071	163960	Kanaal Kortrijk-Bossuit	Moen, oude arm	BOS	2003-10-27	1	49 - 49
KLB	165203	213088	Kleinbeek	Schilde	BES	2002-05-23	5	33,9 - 38,4
KLD1	154782	192751	Kanaal van Leuven naar de Dijle	Battel	DZ	1994-11-28	10	33,8 - 54,5
KLD1A	165188	183128	Kanaal van Leuven naar de Dijle	Kampenhout, stroomafwaarts sas	DZ	2001-10-23	5	36,9 - 42,8
KLD1A	165188	183128	Kanaal van Leuven naar de Dijle	Kampenhout, stroomafwaarts sas	DZ	2002-06-17	4	46 - 57,6
KLD2	169147	181957	Kanaal van Leuven naar de Dijle	Tildonk	DZ	1996-10-11	3	50,2 - 58,5
KLD2	169147	181957	Kanaal van Leuven naar de Dijle	Tildonk	DZ	2001-10-23	5	38,1 - 45,3
KLD2A	172753	179338	Kanaal van Leuven naar de Dijle	Kanaal Leuven-Dijle, Wijgmaalbrug	DZ	2002-06-13	7	50,6 - 65
KLD3	163507	184780	Kanaal van Leuven naar de Dijle	Mechelen, t.h.v. de Barebeek	DZ	2002-02-07	1	57,5 - 57,5
KLD4	161212	186918	Kanaal van Leuven naar de Dijle	Boortmeerbeek, stroomopwaarts sas	DZ	2002-02-07	1	59,3 - 59,3
KLD4	161212	186918	Kanaal van Leuven naar de Dijle	Boortmeerbeek, stroomopwaarts sas	DZ	2002-05-23	10	37,6 - 50,1
KLD4	161212	186918	Kanaal van Leuven naar de Dijle	Boortmeerbeek, stroomopwaarts sas	DZ	2002-06-19	9	38,9 - 57,5
KLD4	161212	186918	Kanaal van Leuven naar de Dijle	Boortmeerbeek, stroomopwaarts sas	DZ	2002-06-26	10	40,7 - 62

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
KM	121300	191825	Kalkense Meersen	's Herenmeers	BOS	1995-06-12	27	30 – 65
KN1	200277	213939	Kleine (Witte) Nete	Dessel, Hoeve Boeretang	N	1996-10-02	3	36 - 62,5
KN1	200277	213939	Kleine (Witte) Nete	Dessel, Hoeve Boeretang	N	2000-06-13	4	42,2 - 59,6
KN1	200277	213939	Kleine (Witte) Nete	Dessel, Hoeve Boeretang	N	2002-04-04	9	29,5 - 47,2
KN2	186620	209750	Kleine Neet	Olen, Steenovenloop	N	2000-06-13	5	33,4 - 46,2
KN2	186620	209750	Kleine Neet	Olen, Steenovenloop	N	2003-03-19	10	30,9 - 44,3
KN2A	182974	208385	Kleine Neet	thv stuw	N	2003-09-18	10	33,2 - 43,9
KN2B	176905	209400	Kleine Neet	Grobbendonk, thv stuw	N	2003-09-16	5	32,9 - 40,7
KN2B	176905	209400	Kleine Neet	Grobbendonk, thv stuw	N	2004-06-09	5	36,1 - 40,3
KN2C	174679	208027	Kleine Neet	Bouwel, SA sifon Albertkanaal	N	2003-09-25	10	34,2 - 49,4
KN3	173905	207570	Kleine Neet	Bouwel, derde Sas	N	2000-06-13	5	35,1 - 46
KND1	36388	202401	Kanaal van Nieuwpoort naar Duinkerke	grens Koksijde -Nieuwpoort	Y	1999-06-17	3	45,3 - 51,7
KND1	36388	202401	Kanaal van Nieuwpoort naar Duinkerke	grens Koksijde -Nieuwpoort	Y	2005-09-14	5	34,8 - 45,2
KND2	32438	200455	Kanaal van Nieuwpoort naar Duinkerke	Wulpen, Koksijde	Y	1999-06-17	2	37 - 47,2
KND2	32438	200455	Kanaal van Nieuwpoort naar Duinkerke	Wulpen, Koksijde	Y	2005-09-12	5	37,5 - 45,2
KNN	38690	204510	Kreek van Nieuwendamme	Nieuwpoort	Y	2000-10-24	3	30,7 - 49,8
KNN	38690	204510	Kreek van Nieuwendamme	Nieuwpoort	Y	2002-11-06	10	32,4 - 42,6
KOO	37131	202361	Koolhofput	Nieuwpoort	Y	1999-06-10	3	32,3 - 38,5
KRL	72125	178971	Kanaal van Roeselare naar de Leie	Ingelmunster, Zwaaikom	L	1998-10-13	3	45 - 50,8
KRL	72125	178971	Kanaal van Roeselare naar de Leie	Ingelmunster, Zwaaikom	L	2004-09-13	2	38 - 38,3
KRO	134408	194193	Kleine Roggeman	Hamme	BES	1999-09-15	5	32 - 50,8
KSE	124091	206985	Kanaal van Stekene naar Eksaarde	grens Sinaai-Stekene	GK	1998-11-06	3	42,4 - 57,7
KVK	118625	191000	Kalkenvaart	Kalken, Broekmeers	BOS	2000-08-02	5	32,8 - 33,7
KZ	143010	164740	klein Zuunbekken	St. Pietersleeuw, wachtbekken	DZ	2002-09-24	10	35,1 - 43,6
L	60811	164207	Leie	Menen, sluis	L	1996-07-04	2	46,5 - 64,9
L	60811	164207	Leie	Menen, sluis	L	2003-06-23	10	32,6 - 70,2
LAA	166651	162020	Laan	Terlanen (aan de molen)	DZ	1998-05-25	3	44 - 50
LAA	166651	162020	Laan	Terlanen (aan de molen)	DZ	1999-05-03	1	30 - 30
LAA2	168275	164024	Laan	St. Agatha Rode	DZ	2002-04-25	1	29,9 - 29,9
LAN	33009	200815	Langeleed	Wulpen	Y	1999-06-07	2	34,2 - 50,1

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
LE1	55750	163360	Leie	Wervik	L	2000-09-19	5	39,4 - 64,3
LE1	55750	163360	Leie	Wervik	L	2001-06-01	6	41,1 - 76,5
LE1	55750	163360	Leie	Wervik	L	2001-10-26	5	29,5 - 67,2
LE1	55750	163360	Leie	Wervik	L	2003-06-23	5	28,5 - 60,2
LE2	65170	165830	Leie	Wevelgem	L	2000-09-19	5	39,3 - 56
LE2A	70387	168771	Leie	Bissegem	L	2003-06-23	4	32,8 - 63,2
LE3	74045	171300	Leie	Kuurne, aan de Heulebeek	L	2000-09-19	2	49 - 60,6
LE4	87785	184400	Leie	Oeselgem, machelenbrug	L	2000-09-26	3	54,5 - 68,3
LE4A	93970	187125	Leie	Deinze, Leiehoek	L	2003-06-25	9	35 - 45,5
LE5	95550	189575	Leie	St.-Martens Leerne, jachthaven	L	2000-09-26	5	36,6 - 45
LE5	95550	189575	Leie	St.-Martens Leerne, jachthaven	L	2003-06-25	10	35,6 - 49,2
LE6	101854	191462	Leie	monding Ringvaart, Kromelei Gent	L	2003-06-23	8	33,7 - 51
LEO	75043	218050	Leopoldkanaal	Damme	BP	1997-10-03	3	34,9 - 38
LEO	75043	218050	Leopoldkanaal	Damme	BP	2003-10-06	4	23,9 - 54,7
LEO1	97590	217250	Leopoldkanaal	Watervliet, Mariapolder	GK	2000-10-03	5	34,9 - 44,3
LEO2	80360	215300	Leopoldkanaal	Moerkerke, Damme kmp 15	BP	2000-10-03	5	33,3 - 40,7
LEO2	80360	215300	Leopoldkanaal	Moerkerke, Damme kmp 15	BP	2003-10-06	10	30,8 - 42,5
LEV	36889	192853	Leerzevaart	Lampernisse	Y	1999-06-10	3	27,6 - 35
LEV	36889	192853	Leerzevaart	Lampernisse	Y	2003-05-14	5	22,9 - 67,5
LEV	36889	192853	Leerzevaart	Lampernisse	Y	2003-07-08	5	32,2 - 46,8
LEY	194074	237180	Leyloop	T.h.v. de Nederlandse grens	M	2001-06-21	1	55,4 - 55,4
LIB	74540	201270	Lippensgoed-Bulskampveld	Hertsberge, Provinciaal Domein	BP	2000-08-31	4	26,2 - 40,3
LLS	114457	211072	Langelede	Stekene	GK	2002-03-18	6	35,4 - 43,5
LO	111611	194058	Loopsloot	Destelbergen	BOS	1998-04-08	1	48 - 48
LWV	68385	220435	Lissewege Vaart	Lissewege, Monnikenwerve	BP	2000-06-27	1	50,8 - 50,8
MA1	242590	161825	Grensmaas	Hof Navangne, Lixhe	M	2000-08-30	5	31 - 49,7
MA1	242590	161825	Grensmaas	Hof Navangne, Lixhe	M	2002-05-15	7	40,2 - 69,2
MA1	242590	161825	Grensmaas	Hof Navangne, Lixhe	M	2005-06-02	3	32 - 37,1
MA2	243070	176785	Grensmaas	Itteren, Hooge Maas	M	2000-06-20	5	34,8 - 45,1

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
MA2	243070	176785	Grensmaas	Itteren, Hooge Maas	M	2002-05-15	10	35,6 - 63,3
MA2	243070	176785	Grensmaas	Itteren, Hooge Maas	M	2005-05-19	7	35,3 - 66,7
MA2A	246705	182145	Grensmaas	KMP 27	M	2002-05-15	6	38,7 - 59,7
MA2B	246154	183842	Grensmaas	Kotem	M	2002-05-14	10	38,3 - 64
MA2B	246154	183842	Grensmaas	Kotem	M	2005-05-19	10	35 - 46
MA3	248135	189345	Grensmaas	Meeswijk, veerpont	M	2000-06-22	5	34,7 - 44,7
MA3	248135	189345	Grensmaas	Meeswijk, veerpont	M	2002-05-14	4	45,4 - 57,8
MA3	248135	189345	Grensmaas	Meeswijk, veerpont	M	2005-05-18	7	43,8 - 53,5
MA3A	248328	194292	Grensmaas	KMP 45	M	2002-05-14	10	43,6 - 57,6
MA3A	248328	194292	Grensmaas	KMP 45	M	2005-05-18	10	38 - 50
MA3B	250306	196068	Grensmaas	Damiaan	M	2002-05-14	5	48,6 - 53,6
MA3B	250306	196068	Grensmaas	Damiaan	M	2005-05-18	10	32 - 53
MA3C	250514	199306	Grensmaas	Schansberg	M	2002-05-13	9	40,5 - 56,6
MA3C	250514	199306	Grensmaas	Schansberg	M	2005-05-17	9	36,8 - 51,8
MA3D	251787	200766	Grensmaas	Maas, a/d jachthaven	M	2002-05-13	10	42,3 - 54,7
MA3D	251787	200766	Grensmaas	Maas, a/d jachthaven	M	2005-05-17	10	35,7 - 50,5
MA3E	252525	203303	Grensmaas	Kinrooi	M	2002-05-13	10	41,1 - 48,7
MA4	253485	204590	Grensmaas	Stevensweert, Molensteen	M	1997-10-16	3	46,5 - 67,4
MA4	253485	204590	Grensmaas	Stevensweert, Molensteen	M	2000-06-22	5	36,8 - 44,7
MA4	253485	204590	Grensmaas	Stevensweert, Molensteen	M	2005-05-17	10	35,5 - 48,7
MBE	152585	219355	Kleine put	Ekeren, recreatiegebied Mui	BES	2000-05-17	5	36,3 - 44,8
MNB	206375	208350	Mol Neet	Balen	N	2002-04-03	6	32,3 - 51,8
MOT	183779	185662	Motte	Aarschot (achter molen Schoonhoven)	DEM	1999-09-28	2	40,2 - 41,3
MOT	183779	185662	Motte	Aarschot (achter molen Schoonhoven)	DEM	2003-03-04	2	55,5 - 56,7
MSG	102845	193750	stadsvijvers Malem	Gent	GK	2000-05-30	5	35,4 - 42,5
MV	108882	203405	Moervaart	Rodenhuize	GK	1996-06-04	3	39 - 56
MV	108882	203405	Moervaart	Rodenhuize	GK	2003-04-24	3	38,7 - 45,2
MVD	122685	203020	Moervaart	Daknam	GK	2000-05-29	5	36,5 - 44,8

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
MVD	122685	203020	Moervaart	Daknam	GK	2003-04-24	10	34 – 48
MVG	111550	204401	Moervaart	Gent	BOS	2003-04-23	9	37,5 - 43,4
MVX	41773	184071	Martjevaart	Kerkom, net voor uitmonding	Y	2002-05-08	2	32,3 - 33,6
NB	37889	204427	Nieuw Bedelf	Lombardsijde	Y	1997-06-04	3	29,1 - 37,3
NEK	54850	213780	Noord-Ede	Klemskerke, De Haan	BP	2000-06-27	5	33,2 - 45,2
NEK	54850	213780	Noord-Ede	Klemskerke, De Haan	BP	2003-06-04	10	35,6 - 44,3
NGO	54965	211915	Noordgeleed	Oudenburg, 150 m O van Plassendalebrug	BP	2000-06-27	5	41,3 - 49,4
NKE	165110	204620	Netekanaal	Lier, Het Alliers	N	2000-06-13	5	35 - 44,7
NKE	165110	204620	Netekanaal	Lier, Het Alliers	N	2003-03-20	10	34,7 - 45,2
NP0	54734	211099	Kanaal Nieuwpoort-Plassendale	Oudenburg, tss spoorwegbrug en brug autostrade	Y	2001-09-19	5	32,4 - 40,8
NP1	54640	210130	Kanaal Nieuwpoort-Plassendale	Oudenburg	Y	2000-10-24	4	30,9 - 43,8
NP1A	54002	209521	Kanaal Nieuwpoort-Plassendale	Oudenburg, t.h.v. zandvoordebrug	Y	2001-09-19	5	30,5 - 38,7
NP1B	51638	208886	Kanaal Nieuwpoort-Plassendale	Grens Gistel Oudenburg, t.h.v. Gistelbrug	Y	2001-09-20	4	33,5 - 37,8
NP1C	42436	207150	Kanaal Nieuwpoort-Plassendale	Middelkerke, t.h.v. slijpebrug	Y	2001-09-20	3	35,8 - 42,6
NP2	38405	204380	Kanaal Nieuwpoort-Plassendale	Nieuwpoort	Y	2000-10-24	5	38,1 - 44,2
NP2	38405	204380	Kanaal Nieuwpoort-Plassendale	Nieuwpoort	Y	2001-09-20	5	39,8 - 44,8
OAV	38057	195959	Oude A-vaart	Pervijse (Rousdamme)	Y	1999-06-07	3	40,6 - 45,5
OAV	38057	195959	Oude A-vaart	Pervijse (Rousdamme)	Y	2001-04-25	10	31,6 - 46,8
OAV	38057	195959	Oude A-vaart	Pervijse (Rousdamme)	Y	2001-10-24	9	26,5 - 46,8
ODU	132149	199337	Oude Durme	Hamme	BES	1999-05-10	3	41,7 - 49,2
ODU	132149	199337	Oude Durme	Hamme	BES	2002-10-24	10	31 - 47,9
OLA	93000	187950	Oude Leie Astene	Deinze, Astene	L	1999-08-16	10	30 - 49,8
OLA	93000	187950	Oude Leie Astene	Deinze, Astene	L	2000-04-18	5	43,1 - 51
OLBH	77010	175115	Oude Leie	Bavikhove	L	2000-07-05	5	36,3 - 45
OLBV	80855	178160	Oude Leie	Sint-Baafs-Vijve	L	2000-07-04	5	33,5 - 44,7
OLD	86670	183615	Oude Leie	Deinze, Gottem	L	2000-08-22	5	34,5 - 43,7

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
OLEV	82995	178200	Oude Leie Sisput	Sint-Eloois-Vijve	L	2000-07-04	5	36,6 - 44,3
OLG	88205	186058	Oude Leiearm Grammene	Grammene	GK	1999-05-10	3	41,5 - 60,1
OLG	88205	186058	Oude Leiearm Grammene	Grammene	GK	2005-09-19	5	34,3 - 43,9
OLLW	68320	167090	Oude Leie Leiebos	Wevelgem	L	1997-10-14	3	48,4 - 57,3
OLLW	68320	167090	Oude Leie Leiebos	Wevelgem	L	2000-07-05	5	39,8 - 53,8
OLM	87875	183560	Oude Leie	Machelen	L	2000-08-22	5	35 - 41,8
OLO	77700	176310	Oude Leie	Ooigem	L	1997-05-30	3	41,2 - 62,2
OLO	77700	176310	Oude Leie	Ooigem	L	2004-06-16	5	36 - 43
OLOE	84920	181840	Oude Leie	Oeselgem	L	1999-08-16	8	32,2 - 51,1
OLSW	80490	176605	Oude Leie Schoendaelebocht	Wielsbeke	L	2000-07-05	2	33,3 - 34,4
OMD	246467	192924	Oude Maas	Dilsen	M	2002-09-16	20	33,2 - 43,6
OMS	247000	191000	Oude Maas	Stokkem	M	1997-09-29	6	33 - 63
OMS	247000	191000	Oude Maas	Stokkem	M	2002-09-02	10	36,9 - 44
OOS	51000	214000	Spuikom van Oostende	Oostende	BP	1998-09-23	3	36,6 - 70
OPK	95125	217140	Oostpolderkreek	St.-Jan-in-Eremo, Oostpolderdijk	GK	2000-08-01	5	33,4 - 37,2
OPK	95125	217140	Oostpolderkreek	St.-Jan-in-Eremo, Oostpolderdijk	GK	2003-03-25	5	33,6 - 49,2
OSA	92213	168373	Oude Scheldearm Het Anker	Wortegem-Petegem	BOS	1998-03-16	3	37,1 - 79,5
OSB	101625	175960	Oude Schelde Blarewater	Zwalm, Nederzwalm	BOS	2000-07-24	5	35,5 - 43
OSD	102085	184325	Oude Schelde Doornhammetje	De Pinte, Zevergem	BOS	2000-08-21	5	36,6 - 40,3
OSE	91930	167700	Oude Schelde Elsegem	Wortegem-Petegem	BOS	2000-07-24	5	36,3 - 51,8
OSG	101825	176940	Oude Schelde Mesureput	Zingem	BOS	2000-07-26	5	35,2 - 44,7
OSH	98660	175260	Oude Schelde Den Heuvel	Heurne, Heuvel	BOS	2000-07-24	5	34,8 - 42,5
OSK	89745	165845	Oude Schelde	Kerkhove	BOS	2000-07-24	5	35,1 - 42
OSK	89745	165845	Oude Schelde	Kerkhove	BOS	2002-10-23	9	29,7 - 45,6
OSM	91890	166790	Oude Schelde Meerseput	Meerse	BOS	2000-07-19	5	34,3 - 45,1
OSME	102245	176965	Oude Schelde	Meilegem, Zwalm	BOS	2000-07-26	5	34,3 - 47
OSME	102245	176965	Oude Schelde	Meilegem, Zwalm	BOS	2000-10-21	5	42,7 - 81
OSN	97795	172705	Oude Schelde Nederename	Oudenaarde, Nederename	BOS	2000-07-24	5	35,6 - 43,1
OSS	97870	174695	Oude Schelde Eine De Sterre	Oudenaarde	BOS	2000-04-26	5	46,3 - 58,8
OSSK	100955	182670	Oude Schelde Kriephoek	Gavere, Semmerzake	BOS	2000-07-26	5	35,5 - 42

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
OSSZ	101114	176125	Oude Scheldearm Spettekraai	Zingem	BOS	1998-03-16	3	41,7 – 72
OST	102040	183150	Oude Schelde Teirlinckput	Merelbeke, Melsen	BOS	2000-07-26	5	35 - 41,4
OSV	94000	167940	Oude Schelde Het Veer	Oudenaarde, Melden	BOS	2000-07-19	5	35,3 - 45,6
OSZ	104495	187190	Oude Schelde Zonneput	Gent, Zwijnaarde	BOS	2000-08-21	5	32,2 - 45,9
OSZ	104495	187190	Oude Schelde Zonneput	Gent, Zwijnaarde	BOS	2001-10-18	5	33,8 - 43,9
OVA	83073	199422	Oude Vaart	Aalter, Oude Vaart (in verbinding met KGO)	BP	2003-09-30	5	31,6 - 43,8
PAL	48604	167837	Palingbeek, Kanaal van Ieper naar Komen	Ieper, Zillebeke	L	1999-09-22	5	38,4 - 45
PDV	36877	198756	Slijkvaart	Booitshoeke	Y	2000-06-28	5	35,7 - 39,9
PDV	36877	198756	Slijkvaart	Booitshoeke	Y	2003-05-15	8	28,5 - 37
PN	148480	199350	Putten van Niel	Niel	BES	1997-09-19	3	45,2 - 51,5
PO	163100	163240	Ijsebroeken	Overijse	DZ	1998-06-10	2	65 - 86,5
PRI	226486	219962	Prinsenloop	Achel	M	2000-02-04	4	35,5 - 49,5
PV1	35730	174130	Poperingevaart	Poperinge	Y	2002-05-06	1	34,2 - 34,2
PV2	33988	183320	Poperingevaart	Alveringem, Vleteren	Y	2002-05-06	2	31,7 - 34,7
RHD	157085	211340	Grote hengelvijver	Deurne, Provinciaal Domein rivierenhof	BES	2000-05-16	5	37,7 - 44,4
RLK	93690	217995	Roeselarekreek	St.-Jan-in-Eremo	GK	2000-08-01	1	30,7 - 30,7
RLK	93690	217995	Roeselarekreek	St.-Jan-in-Eremo	GK	2003-03-27	4	30,8 - 35
RM	175263	183379	Rotselaar meer	Rotselaar	DEM	2001-09-28	7	27,6 - 62
ROG	134148	194693	De Roggeman	Hamme	BES	1999-09-15	5	37,8 - 54
S1	121500	190400	Schelde	Schellebelle, aan bemaling	BOS	1995-06-01	3	38 - 53,7
S2	142856	223540	Schelde	Doel	BES	1998-04-29	3	29 - 56
SC	144800	229500	Schelde-Rijnkanaal	Antwerpen	BES	1995-01-10	3	40 - 62,4
SCH	90539	166271	Scheyteput	Berchem Kluisbergen	BOS	1998-04-29	3	39 - 66
SCH1	80825	158035	Schelde	Pottes, brug van Pottes	BOS	2000-09-28	1	73 - 73
SCH3	97330	171550	Schelde	Oudenaarde, stroomafwaarts	BOS	2000-09-28	4	37,8 - 57,4
SCH3A	100645	175649	Schelde	Schelde, monding Zwalm - Zingem	BOS	2002-06-04	5	35 - 58

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
SCH3B	100576	178795	Schelde	Schelde, SA sluis - Asper-Gavere	BOS	2002-06-04	1	53,6 - 53,6
SCH3C	99483	182782	Schelde	Schelde, afw. Moerbeek - Eke-Gavere	BOS	2002-06-04	10	34,5 - 50
SCH4A	101971	183223	Schelde	Schelde, afw. Molenbeek - Melsen-De Pinte	BOS	2002-06-06	10	37,2 - 48,2
SCH4B	104770	187300	Schelde	Schelde, SA stuw - Merelbeke	BOS	2002-06-04	2	40,7 - 41,9
SCH6	137585	193345	Schelde	Kastel	BES	2000-10-10	3	29,9 - 66,3
SCH7	150050	210800	Schelde	Antwerpen, Kennedytunnel	BES	2000-10-10	5	35,1 - 43,5
SCH7A	150050	214150	Schelde	net voor St.-Anneke	BES	2004-08-24	5	35,1 - 43,8
SCH8	145750	220000	Schelde	Doel, Liefkenshoek	BES	2000-09-26	5	35,5 - 41,5
SCH9	140310	227175	Schelde	Doel, grens Land van Saeftinge	BES	2000-09-26	5	37,1 - 48,8
SGS	32570	195770	Steengracht	Steenkerke	Y	2000-06-28	2	50,8 - 51,7
SK	103842	204847	Waterwinningsput Kluizen	Kluizen	GK	2001-10-01	2	50 - 79
SM	204421	183507	Schulensmeer - Linkhout	Schulensmeer - Linkhout	DEM	1999-10-12	17	33,8 - 43,8
SVW	155555	195190	Spildoornvijver, kleine vijver	Walem	N	2000-05-10	5	35,5 - 45,5
SVW	155555	195190	Spildoornvijver, kleine vijver	Walem	N	2003-10-02	10	33 - 48,7
TB	137240	199690	Tielrode broek	Tielrode	BES	1997-06-26	3	32 - 46
VAM	70050	183960	't Veld	Ardooie, Provinciaal Domein	L	2000-07-12	5	33,6 - 44,7
VEL1	200624	184274	Velpe	Halen, zelkermolen	DEM	2000-04-11	3	31,6 - 42,6
VEL1	200624	184274	Velpe	Halen, zelkermolen	DEM	2004-03-02	1	72,5 - 72,5
VEL2	190979	171941	Velpe	Glabbeek, molen van Bunsbeek	DEM	2000-04-10	2	58,6 - 59
VNX	202170	213834	Voorste Neet	Dessel, uitmonding Witte Neet	N	2002-04-03	2	32,5 - 38,8
VR	42500	197300	Visconia kleiputten	Stuivekenskerke, reservaat	Y	1995-05-01	2	41 - 44,3
VVK	44205	197785	Vladslovaart	Keiem, Molenbrug	Y	2000-10-24	2	47,7 - 53,7
WA	228829	218690	Warmbeek	Achel	M	1997-10-15	3	50,5 - 53,4
WA	228829	218690	Warmbeek	Achel	M	2004-04-19	5	25,2 - 59,3
WBA	227900	221480	Warmbeek	Achel, kluis	M	2000-05-23	5	36,7 - 45,3
WBV4	145221	167447	Willebroekse vaart	Anderlecht, ad ring waar kanaal uitkomt	DZ	2004-10-05	2	36,9 - 50,3
WBV5	152751	177153	Willebroekse vaart	Vilvoorde, Budabrug	DZ	2004-10-05	3	30,2 - 40,2

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
WBV6	153256	181684	Willebroekse vaart	Vilvoorde, industriegebied 'verbrande brug'	DZ	2002-10-14	15	32,1 - 45,4
WBV6	153256	181684	Willebroekse vaart	Vilvoorde, industriegebied 'verbrande brug'	DZ	2002-12-02	10	36,2 - 50,6
WBV6	153256	181684	Willebroekse vaart	Vilvoorde, industriegebied 'verbrande brug'	DZ	2003-05-13	10	40,5 - 56,3
WBV7	149864	196761	Willebroekse vaart	Klein Willebroek, sluis naar Rupel	BES	2002-10-14	3	36,2 - 40,9
WBV8	146745	198844	Willebroekse vaart	Niel, oude arm, sluis Wintham	BES	2002-10-17	10	32,2 - 41,6
WEE	157993	185430	Meer van Weerde	Weerde	DZ	1997-09-18	3	34,3 - 59,9
WEE	157993	185430	Meer van Weerde	Weerde	DZ	1998-10-01	8	40,8 - 69,5
WEE	157993	185430	Meer van Weerde	Weerde	DZ	1999-05-03	8	45,3 - 62,4
WEE	157993	185430	Meer van Weerde	Weerde	DZ	2000-10-18	10	38 - 47,4
WEE	157993	185430	Meer van Weerde	Weerde	DZ	2001-04-10	10	34,3 - 46,7
WEE	157993	185430	Meer van Weerde	Weerde	DZ	2001-10-09	11	35,5 - 43,1
WEE	157993	185430	Meer van Weerde	Weerde	DZ	2002-12-09	10	31,4 - 46,2
WEE	157993	185430	Meer van Weerde	Weerde	DZ	2003-10-13	10	31,4 - 44,9
WEE	157993	185430	Meer van Weerde	Weerde	DZ	2004-10-12	5	32,8 - 43,1
WEE	157993	185430	Meer van Weerde	Weerde	DZ	2005-09-05	5	31,8 - 50,3
WIK	252310	205770	Witbeek	Kessenich	M	2001-06-06	2	56,6 - 60,4
WIN1	180501	181008	Winge	Blauwmolen	DEM	1999-09-29	1	51 - 51
WIN2	176575	180572	Winge	Rotselaar, nabij A2	DEM	1999-09-29	1	61,8 - 61,8
WL	219237	217184	Wateringen Lommel, Grote Fossé	Grote Fossé	M	1997-04-21	3	41,2 - 63
WLL	184073	186338	Weerderlaak	Langdorp, net voor monding in Demer	DEM	2001-05-16	5	38,3 - 50,7
WMX	194718	215017	Wamp	Kasterlee, uitmonding Kleine Neet	N	2002-04-04	7	33,4 - 58,5
WNR	200572	217050	Witte Nete	Retie	N	2003-08-26	10	32,6 - 47,4
WSD	103000	193145	Watersportbaan	Drongen	GK	2000-05-30	5	36,3 - 43,7
WSD	103000	193145	Watersportbaan	Drongen	GK	2002-09-30	10	38,7 - 43,8

Code	X	Y	Water	Locality	Basin	Sampling date	N	L
WWB	67725	212545	Waggelwater	Brugge	BP	2000-06-27	5	36 - 42,9
YZ1	27300	180440	Ijzer	Roesbrugge	Y	2000-07-10	5	32,4 - 36,6
YZ1	27300	180440	Ijzer	Roesbrugge	Y	2005-06-14	5	32,3 - 39,2
YZ2	43695	191560	Ijzer	Diksmuide, Heernisse	Y	2000-07-10	5	30,5 - 60,8
YZ3	40275	203200	Ijzer	Nieuwpoort, Uniebrug	Y	2000-07-10	5	32,8 - 52,3
ZB	245753	197139	Zanderbeek	Dilsen-Stokkem	M	2002-03-14	1	48 - 48
ZBR	147117	156959	Zevenbronnen	Dworp-Beersel	DZ	2001-05-11	3	87 - 101,5
ZGL	38185	193113	Zaadgracht	Lampernisse	Y	2002-05-08	2	30,7 - 47
ZLM	112335	204240	Zuidlede	Mendonk	GK	2000-05-29	5	34,7 - 46,3
ZLO	115290	204428	Zuidlede	Oostdonk	GK	2002-03-21	1	38 - 38
ZWL	203633	183423	Zwart water	Linkhout, stroomafwaarts duiker	DEM	2001-05-17	5	39,4 - 60
ZWV1	243875	179730	Zuid-Willemsvaart	Rekem, jachthaven	M	2000-05-09	5	36 - 42
ZWV1	243875	179730	Zuid-Willemsvaart	Rekem, jachthaven	M	2001-04-09	10	35,1 - 44,4
ZWV1	243875	179730	Zuid-Willemsvaart	Rekem, jachthaven	M	2001-10-03	10	33,3 - 49,5
ZWV1	243875	179730	Zuid-Willemsvaart	Rekem, jachthaven	M	2004-09-06	1	39,2 - 39,2
ZWV2	244580	190445	Zuid-Willemsvaart	Lanklaar, jachthaven	M	2000-05-09	5	32,5 - 48
ZWV2	244580	190445	Zuid-Willemsvaart	Lanklaar, jachthaven	M	2004-09-06	5	36,4 - 44,1
ZWV3	243475	194270	Zuid-Willemsvaart	Rotem, Dilsen	M	2000-05-09	5	36,5 - 40
ZWV3	243475	194270	Zuid-Willemsvaart	Rotem, Dilsen	M	2004-09-08	1	39,5 - 39,5
ZWV4	237057	204875	Zuid-Willemsvaart	Bree, jachthaven	M	2000-05-09	5	36 - 42,1
ZWV4	237057	204875	Zuid-Willemsvaart	Bree, jachthaven	M	2004-09-06	5	36,6 - 43,1
ZWV4	237057	204875	Zuid-Willemsvaart	Bree, jachthaven	M	2004-09-09	1	30,2 - 30,2
ZWV5	232825	211180	Zuid-Willemsvaart	Bocholt, sluis van Lozen	M	2000-05-23	5	35,7 - 44,9
ZWV5	232825	211180	Zuid-Willemsvaart	Bocholt, sluis van Lozen	M	2004-09-09	1	40,8 - 40,8

Table II.2a. Means for PCBs and some OCPs (α-HCH, γ-HCH, Dieldrin (DIE), Endrin (End) and HCB) for eels from certain sites at specific sampling date sampled in the framework of the EPMN during the period 1994-2005. Concentrations are expressed in ng/g wet weight.

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
A	22/10/1996	22,1	9,76	4,64	9,05	4,62	15,12	15,08	22,02	2,05	8,5	96,51	0,09	34,43	35,1	15,44	6,01
A	20/06/2001	0,85	0,46	2,36	9,8	3,09	5,56	28,49	44,81	1,87	27,55	119,42	0,28	13,7	23,17	0,29	6,84
AA	24/04/2002	1,36	1,36	8,69	22,28	6,73	20,47	45,24	49,09	3,27	18,33	165,46	0,63	5,58	30,5	0,13	6,96
AB	15/04/1998	2,18	3,12	5,12	13,83	8,25	20,99	113,89	111,89	10,4	75,69	343,59	3,91	17,6	76,04	0,61	10,1
AB	3/05/2004	0,42	0,19	3,69	6,92	3,54	11,13	50,52	93,68	7,84	66,67	233,05	0,11	0,19	0,14	0,08	2,02
AB1	24/05/2000	0,3	0,47	3,18	6,19	2,59	7,7	51,69	65,74	3,68	45,44	180,23	0,22	15,82	4,54	6,73	3,47
AB1	4/05/2004	0,54	0,33	0,29	3,51	1,16	3,2	9,39	16,35	0,44	8,83	42,11	0,46	0,36	0,29	0,29	2,45
AB1A	4/05/2004	0,47	0	3,54	3,96	1,07	3,65	8,23	13,23	0,5	5,23	38,31	0	1,49	0	0	2,19
AB2	24/05/2000	0,15	0,1	2,99	5,45	2,44	7,33	30,88	41,17	1,96	24,09	112,05	0,13	14,56	3,87	0,05	2,57
AK0	7/08/2000	3,31	2,12	70,56	163,4	40,33	116,1	390,46	575,49	29,3	268,32	1587,7	0,18	6,08	5,68	0,07	12,2
AK1	7/08/2000	2,1	1,61	53,17	119,7	27,92	77,16	289,25	392,07	20,14	184,81	1118,26	0,27	8,71	6,83	0,1	11,7
AK2	7/08/2000	2,27	1,67	40,93	145,5	34,2	102,4	342,03	576,47	19,06	154,64	1364,23	0,25	7,01	4,59	0,08	8,04
AK3	7/08/2000	0,53	0,58	16,02	55,04	18,5	47,84	178,5	287,46	11,33	82,18	667,57	0,1	3,51	2,51	0,04	2,85
AK4	8/08/2000	0,78	0,44	9,3	26,39	7,5	20,1	108,13	178,76	4,14	44,07	351,59	0,04	3,18	1,77	0,03	1,14
AK5	8/08/2000	0,23	0,22	2,99	7,65	4,11	9,88	40,15	63,91	2,78	27,57	152,39	0,05	2,34	1,18	0,02	0,5
AK6	8/08/2000	0,43	0,48	7,09	23,87	5,98	16,42	93,27	126,28	5,09	54,3	321,66	0,23	9,45	4,71	0,06	2,63
AK7	21/06/2000	0,52	0,4	7,91	27,71	8,46	22,96	169,19	237,14	7,69	100,49	565,93	0,26	15,64	5,5	0,06	3,36
AKK	6/06/1997	29,88	13,24	84,4	182,7	71,84	273,8	233,78	400,48	41,29	213,79	1418,82	11,21	132,62	13,65	20,27	15,5
AKL1	7/10/1999	5,93	2,61	69,65	110,9	23,64	77,58	253,84	377,98	22,28	176	1071,9	0,82	15,73	21,81	0,33	9,28
AKL1	4/10/2005	4,7	2,86	37,25	26,83	9,69	21,87	36,99	47,04	3,69	22,23	196,91	0,46	0,55	0,17	0,17	6,31
AKL2	7/10/1999	7,3	3,11	61,47	60,25	17,59	45,13	83,92	90,79	6,28	38,08	386,93	1,18	17,86	35,39	0,69	5,52
AKL2	4/10/2005	4,99	2,71	31,17	27,76	8,77	24,95	39,51	56,04	3,64	23,85	208,28	0,44	0,48	0,17	0,17	5,42
AKL3	7/10/1999	14,42	6,64	80,14	75,46	26,92	64,35	114,46	116,08	8,55	49,01	513,91	1,76	19,82	47,13	0,78	9,7
AKL3	4/10/2005	4,45	2,28	32,4	20,82	12,2	29,87	43,25	59,85	3,55	20,48	211,12	0,47	0,58	0,19	0,19	4,86

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
AKL4	7/10/1999	19,76	9,29	127,1	123,7	37,33	103,3	198,58	221,99	13,98	92,61	886,97	1,71	17,37	63,68	1,21	10,5
AKL4	4/10/2005	5,93	2,74	23,72	19,47	7,21	21,07	30,75	44,07	2,06	18,47	163,48	0,44	1,29	0,2	0,2	4,65
AKL5	7/10/1999	5,77	3,22	80,05	84,38	23,96	52,92	151,21	147,1	10,9	74,16	595,58	1,32	10	43,93	1,05	7,87
AKL5	3/10/2005	4,46	2,41	30,78	34,81	8,16	27,32	67,31	84,86	6,72	50,77	300,31	0,33	0,38	0,15	0,15	5,41
AKL6	7/10/1999	8,98	4,34	106,2	118,8	33,45	82,39	218,71	202,53	14,55	89,38	826,94	1,33	11,94	93,11	1,63	9,83
AKL6	3/10/2005	3,96	2,18	27,92	30,02	6,73	22,45	59,32	77,72	4,55	39,02	260,41	0,33	2,68	0,16	0,16	5,52
AKL7	7/10/1999	6,76	3,12	82,74	67,72	27,23	58,22	120,57	106,99	8,72	46,41	489,42	1,35	14,22	61,59	1,18	9,45
AKS	7/07/1999	0,75	0,42	15,62	46,98	14,68	42,46	220,56	369,81	22,17	69,23	765,42	0,31	13,45	5,09	0,11	2,46
ASA	2/04/2002	0,32	0,62	1,04	3,98	1,34	3,92	11,74	12,68	0,75	5,77	39,46	0,11	0,83	4,83	0,06	3,73
ATB	21/06/2000	0,65	0,09	2,99	5,74	2,49	4,8	22,37	27,48	2,09	12,27	76,31	0,12	4,9	2,62	0,04	0,83
BB	12/09/1994	0,59	0,18	1,07	0,92	0,58	2,77	4,85	7,35	0,42	3,05	20,6	0,57	0,69	0,22	0,01	0,15
BBO	24/05/2000	0,3	0,24	4,4	15,79	5,93	15,74	72	84,48	6,04	50,29	242,99	0,06	5,03	2,91	0,06	5,44
BBU	2/08/2000	0,47	0,79	2,54	4,68	2,63	5,35	10,46	12,81	1,05	4,69	41,01	0,28	2,93	3,36	0,11	1,15
BBV	27/06/2000	0,63	0,68	2,73	5,58	1,33	5,13	11,24	13,25	0,89	6,03	44,6	0,1	12,2	2,42	0,06	1,43
BBV	4/06/2003	0,6	0,54	0,53	3,01	1,38	4,24	5,98	9,83	0,58	3,27	27,45	1,25	16,7	5,51	0,12	0,8
BEM	30/08/2000	0,53	0,44	3,19	8,12	4,7	12,18	49,7	64,81	4,37	38,94	177,47	0,14	2,27	0,44	0,05	2,9
BEV1	14/06/2002	2,87	1,17	10,69	8,61	18,25	44,53	90,4	132	9,55	62,33	351,43	0,09	3,08	9,05	0,03	1,21
BEV2	14/06/2002	7,6	3,3	91,99	132,4	51,98	149,3	275,59	370,8	45,16	0,12	1027,79	0,42	22,67	35	0,12	10,4
BGG	30/05/2000	5,42	1,51	65,47	68,3	20,74	64,52	153,47	189,8	7,75	71,11	618,09	0,24	10,91	8,59	0,08	3,53
BGP	5/10/2001	5,28	4,49	40,48	68,78	25,77	106,8	239,63	337,44	19,35	105,08	903,53	4,67	6,78	12,47	0,08	15,7
BGX	2/04/2002	0,26	0,3	0,56	1,5	0,69	2,03	7,37	9,94	0,58	5,93	27,59	0,02	0,09	1,23	0,02	0,59
BK1	1/05/1994																
BK1	30/08/1994																
BK1	30/11/1994																
BK1	12/06/1995	1,02	0,87	12,25	22,09	5,79	21,88	35,4	63,06	2,58	22,68	178,38	3,82	32,95	15,33	2,36	1,48
BK2	1/05/1994																

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
BK2	30/08/1994																
BK2	30/11/1994																
BK2	1/12/1994	1,74	1,87	14,46	20,46	6,1	24,72	31,57	49,68	2,5	14,56	157,2	5,22	17,41	18,48	1,71	3
BK4	1/05/1994																
BK4	30/08/1994																
BK4	30/11/1994																
BK4	1/12/1994	5,66	2,57	38,28	76,92	33,72	221,9	125,86	319,05	10,71	387,32	1174,96	3,5	12,49	13,27	2,73	1,34
BK4	8/03/1995																
BK5	1/05/1994																
BK5	30/08/1994																
BK5	1/09/1994	8,36	4,38	21,77	26,63	11,14	39,51	44,25	66,77	3,56	18,89	226,19	3,92	17,79	16,37	0,07	2,72
BK5	8/03/1995																
BL	4/12/1997	28,97	12,8	3,68	9,77	7,69	26,59	30,16	41,38	4,51	18,98	159,53	6,18	11,35	16,36	28,39	5,76
BMD	30/05/2000	0,2	0,21	3,07	7,04	5,99	16,76	40,96	48,61	2,66	18,77	135,41	0,75	12,41	0,23	0,06	0,94
BMD	11/10/2004	0,45	0,13	2,76	6,16	3,54	11,57	20,68	33,17	0,79	14,14	88,92	0,06	1,29	0,08	0,06	0,49
BND	15/06/2000	1,47	1,43	21,1	57,88	16,37	44,58	120,21	128,69	8,33	41,27	415,2	0,16	9,05	11,43	0,05	11,9
BOK	20/10/1998	0,79	0,41	2,15	7,39	2,68	8,18	17,72	22,2	1,16	8,9	67,34	0,64	7,1	1,13	1,69	1,66
BRK	1/08/2000	0,22	0,11	0,66	1,98	1,26	2,61	4,28	5,49	0,8	2,08	17,31	0,11	1,87	3,9	0,05	0,51
BRK	25/03/2003	0,48	0,44	0,37	2,68	0,87	2,79	3,74	6,55	0,51	2,36	18,97	0,09	2,52	14,13	0,09	0,63
BVW	29/05/2000	0,1	0,07	1,66	2,38	0,79	2,66	5,98	7,04	0,4	3,1	22,93	0,12	6,07	9,08	0,03	0,25
BWK1	4/09/2000	2,73	1,78	38,13	92,28	32,19	137,8	126,36	199,97	12,27	35,99	633,24	0,41	8,09	14,87	0,06	1,07
BWK2	1/05/1994																
BWK2	30/08/1994																
BWK2	30/11/1994																
BWK2	1/12/1994	2,1	2,39	15,91	35,57	9,92	33,78	44,55	64,19	3,94	24,75	220,85	5,06	41,66	9,77	0,09	8,89
BWK2	4/09/2000	3,54	1,83	22,12	37,98	17,68	58,61	91,21	132,24	8,4	28,75	374,44	0,43	8,3	15,42	0,07	3,55

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
BWK3	4/09/2000	1,77	1,09	10,87	20,12	6,12	20,52	32,43	45,09	2,61	9,19	140	0,3	5,82	11,99	0,06	1,34
COM	10/08/2001	4,62	1,27	57,11	413	38,78	214	1552,6	2195,2	113	816,03	5252,47	0,14	3,39	12,49	0,06	3,63
DA	29/11/1994	59,55	18,35	213,9	202,2	62,07	169,5	245,56	337,15	20,99	143,48	1371,41	3,44	49,34	32,15	2,94	5,65
DA	18/09/1997	32,24	14,18	153,3	136,6	77,87	311	224,52	329	33,37	117,29	1303,89	3,51	11,63	7,34	30,47	3,83
DA	7/06/2003	43,31	19,19	139,1	167,6	46,76	130	217,66	332,82	27,17	118,86	1149,35	0,12	24,24	23,39	0,12	5,03
DA1	3/10/2000	2,1	0,41	12,26	17,27	11,59	38,53	106,15	157,85	8,66	68,07	402,23	0,14	3,66	9,97	0,05	11,6
DA2	3/10/2000	2,82	0,7	24,78	38,32	19,63	64,4	123,31	160,64	15,2	84,84	499,11	0,13	4,89	4,39	0,11	7,37
DAM	16/05/2000	0,09	0,21	1,48	3,19	1,23	4,55	11,83	15,21	0,59	6,93	43,27	0,15	3,84	8,55	0,02	0,6
DAV	14/06/1995	6,27	1,97	99,95	92,04	29,77	127,3	155,3	244,38	10,93	78,04	803,29	6,84	82,03	15,61	5,14	4,95
DAV1	25/05/2000	5,55	2,43	65,61	62,23	19	83,03	128,98	179,33	8,26	59,84	584,55	2,03	30,03	12,8	0,17	2,88
DAV2	25/05/2000	2,25	0,9	6,54	4,81	2,68	9,52	18,13	24,83	1,31	12,26	78,34	1,68	15,01	1,74	0,07	0,54
DBR	18/05/1999	1,42	0,48	4,92	12,41	9,45	35,51	59,57	81,1	5,2	31,28	226,22	0,67	19,08	16,45	6,56	3,77
DBU	28/05/2002	0,93	0,85	2,54	10,33	3,46	10,55	23,57	24,24	1,91	8,47	80,62	0,27	9,15	73,43	0,1	4,15
DE1	13/10/2000	0,28	0,24	1,86	2,87	2,63	8,07	18,23	25,62	1,91	10,95	67,88	0,35	8,04	16,62	0,02	2,65
DE1A	27/03/2002	1,31	1,48	8,27	17,91	6,14	19,59	46,44	58,02	2,87	17,23	168,77	0,37	13,29	36,44	0,12	13
DE2	16/10/2000	1,36	0,85	10,54	23,47	8,81	30,8	86,92	119,83	7,39	57,14	330,06	0,46	23,45	35,08	0,1	16
DE2	21/03/2005	0,97	0	4,77	5,12	3,38	11,97	28,4	48,08	1,53	15,04	114,35	0,11	0,96	0	0	4,02
DE3	16/10/2000	2,31	2,79	19,78	26,7	14,59	42,85	74,7	93,76	10,15	39,8	299,9	0,81	17,99	30,89	0,1	23,2
DE3	27/03/2002	14,03	8,26	14,5	37,4	7,25	27,39	91,13	127,72	6,6	57,35	369,52	0,1	3,61	12,79	0,06	5,69
DE3	21/03/2005	19,8	9,91	32,33	72,08	15,51	46,9	123,56	206,19	9,8	107,09	607,96	0	0	0	0	7,85
DE3	22/03/2005	53,03	25,85	126,6	47,52	21,68	49,42	57,72	87,12	4,3	30,26	451,7	0,24	0,62	0,25	0,25	6,21
DE3A	27/03/2002	15,59	12,55	21,32	39,23	6,48	23,39	85,13	110,4	5,93	52,92	347,98	0,15	3,29	16,98	0,07	6,97
DE3B	28/03/2002	19,58	15,58	23,3	42,03	8,37	27,8	88,96	114,64	6,56	58,22	374,53	0,19	4,92	14,63	0,08	8,93
DE3B	22/03/2005	6,89	2,14	26,04	19,73	7,97	19,96	52,48	72,94	3,57	34,36	232,4	0	1,14	0,15	0	2,52
DE3C	28/03/2002	27,69	19,53	26,74	38,62	8,3	26,83	92,48	120,61	6,65	57,98	390,96	0,11	2,03	14,48	0,05	5,94
DE3C	2/10/2003	18,19	9,59	26,14	31,49	7,56	20,22	72,37	120,14	7,65	60,35	348,9	0,07	2,45	0,07	0,07	4,27

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
DE4	13/10/2000	1,5	0,78	18,93	33,18	10,16	31,36	94,76	120	10,31	46,68	346,42	0,63	19,79	44,23	0,06	10,7
DE4	22/03/2005	4,2	1,33	35,7	70,31	14,59	44,16	108,68	153,7	9,07	77,03	493,78	0,23	3,6	0,59	0	3,95
DE4	23/03/2005	6,93	3,59	24,64	28,55	11,33	21,34	61,35	88,26	5,4	40,43	271,49	0,22	3,18	0,22	0	3,69
DE4A	28/03/2002	9,91	7,73	17,29	36,38	6,54	23,05	88,53	123,67	6,14	60,92	359,74	0,1	2,17	15,51	0,05	4,89
DE4A	1/10/2003	2,98	1,3	21,37	31,26	7,47	22,9	45,86	65,62	4,64	30,52	220,51	0,15	0,79	0,07	0,07	2,48
DEM1	13/04/1999	4,53	1,61	29,72	56,68	14,48	41,86	80,3	82,93	6,41	34,1	330,12	0,58	86,62	14,27	3,06	10,7
DEM1	29/10/2001	4,95	2,89	27,4	42,95	13,33	33,41	59,28	62,27	4,79	23,67	253,93	2,77	9,25	11,28	0,1	12,5
DEM1	4/09/2003	2,67	1,07	10,65	22,44	6,52	18,11	31,74	44,68	3,87	15,84	146,12	0,61	2,69	6,55	0,06	6,64
DEM2	13/04/1999	3,21	2,63	24,74	46,24	13,46	34,85	107,9	88,3	5,49	40,06	345,31	0,44	449,05	28,09	8,25	10,8
DEM2	2/09/2003	1,64	0,67	6,71	12,83	4,94	14,5	23,97	33,29	2,61	10,95	103,89	0,72	31,11	32,02	0,05	4,1
DEM3	13/04/1999	13,27	6,33	49,04	98,87	19,42	75,26	136,14	175,67	11,23	71,5	619,75	1	319,82	50,79	14,09	23,7
DEM3A	9/04/2003	2,34	1,35	12,4	26,38	8,84	24,11	44,14	60,35	5,5	27,63	197,36	0,87	45,24	35,15	0,08	7,27
DEM4	13/04/1999	0,89	0,75	10,82	21,89	5,06	16,12	35,69	43,85	2,36	20,54	149,81	0,41	179	18,06	7,31	5,51
DEM4	9/04/2003	0,47	0,37	5,06	9,16	2,8	7,8	13,12	19,38	1,18	5,31	60,31	0,78	27,63	12,66	0,03	4,33
DEM5	13/04/1999	0,79	0,54	7,5	15,91	3,1	9,27	28,48	25,69	1,8	12,21	99,85	0,32	114,28	22,38	6,96	4,81
DEM6	2/09/2003	1,88	0,93	11,72	22,6	8,06	21,71	37,09	52,56	4,34	18,25	165,81	1,01	37,87	51,23	0,06	5,7
DGH	12/07/2000	0,59	0,42	25,89	19,81	13,66	39,51	129,94	148,87	10,49	63,88	428,49	0,34	18,76	7,57	0,06	1,12
DIJ1	27/04/1999	2,29	1,64	36,87	67,71	20,08	67,1	132,68	155,09	7,22	42,38	504,13	1,46	180,91	22,48	0,59	10,7
DIJ1	6/05/2003	1,52	1,39	16,66	25,19	8,8	25,57	37,1	56,27	3,36	12,11	174,42	0,42	52,18	22,35	0,1	5,76
DIJ2	28/04/1999	2,32	1,11	41,22	94,03	27,64	80,12	142,36	163,8	10,08	48,15	572	0,83	143,58	23,89	0,48	11
DIJ3	27/04/1999	1,11	0,97	26,66	35,69	12,75	36,18	77,43	79,27	4,47	26,47	282,8	0,6	108,28	12,07	0,15	5,89
DIJ4	28/04/1999	3,36	1,76	47,05	81,51	19,34	57,04	110,57	140,4	6,11	47,6	487,52	1,26	196,14	33,16	0,15	14,7
DIJ4	7/05/2003	0,57	0,79	11,08	17,09	6,13	17,78	27,77	47,39	2,43	13,07	134,75	0,45	34,15	37,42	0,07	3,56
DIJ5	28/04/1999	3,23	1,5	53,22	71,13	28,84	81,05	125,4	144,42	5,91	24,93	503,38	1,34	197,22	31,75	0,16	13,6
DIJ6	27/04/1999	4,74	2,79	28,2	49,71	13,87	43,44	112,11	131,16	9,28	64,07	433,43	1,3	445,52	50,67	8,47	9,28
DIJ6	7/05/2003	0,78	0,66	12,95	23,41	6,11	17,43	27,98	43,93	2,49	8,95	135,43	0,35	37,45	29,48	0,08	4,51

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
DIJ6A	8/05/2003	1,01	0,66	10,23	21,47	7,64	18,94	45,69	75,01	8,13	32,6	204,95	0,7	27,76	19,54	0,04	3,23
DIJ7	27/04/1999	3,53	2,14	42,63	86,41	25,05	78,75	190,11	200,68	13,65	88,72	690,83	0,81	201,08	22,44	4,77	12,3
DIJ8	27/04/1999	1,85	1,31	29,3	63,11	15,23	41,48	132,26	141,1	9,77	68,33	477,43	0,57	158,39	17,47	1,2	7,86
DIJ8	8/05/2003	0,8	0,73	9,54	14,73	6,04	16,85	31,79	45,89	2,98	12,09	131,7	0,06	14,6	0,06	0,06	4,44
DO1	7/05/1998	1,89	0,52	8,77	11,53	5,49	13,42	46,28	46,01	3,3	22,56	150,47	1,05	44,37	0,28	0,14	10,4
DO1	23/05/2000	0,39	0,06	10,24	19,23	4,71	13,07	27,78	29,31	1,47	10,59	110,6	0,26	20,96	5,38	0,06	8,62
DO1	21/04/2004	0,81	0,47	1,4	3	1,52	4,27	7,26	11	0,65	4,5	32,24	0,06	1,61	0,24	0,06	1,9
DO1A	22/04/2004	0	0	1,2	1,75	0,82	2,47	4,92	6,88	0,39	2,58	19,8	0	0,09	0	0	1,4
DO2	23/05/2000	1,76	1,06	15,01	20,23	7,35	21,09	41,61	45,35	3,05	18,27	163,32	0,29	61,17	7,43	0,09	10,1
DO2	21/04/2004	0,47	0,44	2,85	4,55	2,31	6,04	9,25	12,74	0,58	3,31	39,2	0,07	0,9	0,07	0,07	3,12
DSS	2/08/2000	2,55	0,37	9,39	12,43	7,12	11,04	27,11	33,59	5,58	17,44	113,56	0,6	12,55	17,26	0,09	5,35
DUH	7/04/2004	1,79	0,05	30,83	48,83	8,86	33,73	68,2	109,12	6,21	47,34	339,84	0	0	0,3	0	2,8
DUH	28/04/2005	2,72	1,13	33,66	56,45	9,34	34,66	74,22	113,96	7,31	51,13	366,81	0,3	9,46	0,16	0,16	3,14
DUL	29/08/2000	0,92	0,43	9,24	15,97	7,49	28,93	74,2	106,03	6,38	50,59	285,87	0,17	2,21	5,42	0,06	3,93
DUO	7/04/2004	3,37	1,55	40,5	62,37	12,46	43,97	94,4	141,96	9,91	62,57	449,14	0,19	3,75	0,15	0,15	3,55
DUZ	8/04/2004	3	0,77	26,2	49,8	9,37	34,36	78,45	124,22	8,48	55,09	371,13	0,24	0,86	0,15	0,15	2,82
DUZ	27/04/2005	0,72	0	21,41	44,87	8,97	29,82	70,72	110,19	6,31	46,67	324,4	0	4	0,13	0	2,65
ED1	28/05/2002	0,54	1,29	0,88	3,34	0,92	3,51	9,6	10,35	0,62	3,04	31,26	0,23	6,66	16,11	0,09	2,25
ED2	28/05/2002	2,02	1,67	59,66	127,1	29,69	97,52	269,52	310,57	22,29	117,37	983,77	0,24	12,11	21,77	0,12	7,39
EEND	26/10/2005	0,18	0,08	0,67	0,72	0,89	2,18	5,14	9,26	0,67	4,04	22,19	0,14	0	0	0	0,34
FOO	16/05/2000	0,24	0,19	5,44	8,34	6,44	22,97	27,47	31,94	2,04	8,65	105,05	0,19	5,87	0,09	0,04	0,41
FSA	21/06/2000	0,45	1,05	3,2	6,57	2,2	11,02	17,71	23,12	1,74	7,6	69,68	0,23	9,83	8,1	0,09	2,67
FWW	10/05/2000	0,01	0,01	1,39	2,44	2,71	8,11	28,76	39,54	1,81	20,23	100,48	0,04	1,87	5,95	0,01	0,16
FWW	30/09/2003	0,22	0,11	0,65	2,11	1,65	5,3	10,25	19,19	1,66	10,53	48,26	0,07	0,13	1,85	0,01	0,12
GAG	19/07/2000	0,06	0,03	0,2	1,59	1,33	6,11	18,2	24,66	1,25	10,26	61,08	0,04	1,66	1,12	0,01	0,3
GB	23/05/1995	0,33	0,69	1,22	3,75	0,82	6,67	9,73	16,93	0,81	7,55	46,18	2,4	66,38	14,29	5,76	1,93
GB1	10/07/2000	0,93	0,62	2,35	4,18	1,87	4,24	7,88	10,11	1,02	5,09	34,77	0,82	68,17	29,36	0,09	2,44

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
GB1	16/06/2003	0,27	0,02	0,07	0,71	0,55	1,77	2,54	4,24	0,33	1,96	11,55	0,15	5,7	0,02	0,02	0,35
GB2	28/06/2000	0,24	0,2	2,58	3,27	1,04	3,13	7,32	8,63	0,5	3,58	28,76	2,42	119,49	0,79	0,08	2,85
GBO	5/06/2002	0,05	0,05	0,72	2,91	1,67	4,48	9,26	13,98	1,23	5,25	36,64	1,16	2,96	17,81	0,05	0,84
GBR	12/07/2000	0,84	0,74	10,74	33,76	9,9	33,06	77,89	92,64	6,41	49,47	298,4	0,55	52,05	130,83	0,1	8,93
GGE2	10/12/2004	0,44	0,26	5,88	27,61	9,26	27,98	44,79	64,52	4,37	37,23	208,45	0,28	1,7	0,2	0,2	4,67
GGZ	28/08/2000	4	2,83	6,62	12,81	7,67	19	38,47	52,92	3,33	19,64	153,46	0,67	41,89	12,42	0,15	5,56
GHN	21/09/2000	6,9	2,59	31,41	84,59	22,27	66,44	285,96	412,66	23,3	274,3	1162,27	0,09	1,42	2,6	0,04	12,8
GHN	27/09/2005	1,41	0,42	8,24	6,59	5,56	19,93	92,72	196,39	7,6	133,28	458,57	0	0,17	0,18	0	2,44
GN1	14/06/2000	0,14	0,66	2,32	6,95	1,99	7,18	17,35	20,5	1,26	9,05	63,49	0,1	10,19	7,38	0,08	5,24
GN2	14/06/2000	0,12	1,21	32,26	70,39	21,24	46,25	111,67	104,42	10,12	43,06	408,17	0,9	18,97	13,71	0,12	72,7
GN2	19/03/2003	3,7	2,29	20,5	47,79	12,97	32,13	64,18	93,65	9,3	42,57	305,38	1,44	9,56	15,31	0,1	61,6
GN2A	19/03/2003	1,25	0,81	8,38	19,47	6,94	15,75	31,59	42,37	5,49	17,18	136	0,06	3,89	18,61	0,06	29,7
GN3	14/06/2000	0,27	1,1	11,72	30,13	9,51	21,62	51,73	55,61	5,93	23,57	194,65	0,34	13,74	12,16	0,06	26,7
GN3	19/03/2003	1,01	1,42	6,17	24,38	8,54	19,7	40,41	57,91	5,68	23,38	172,97	0,06	4,94	13,51	0,06	27,6
GN4	18/03/2003	1,17	1,41	13,78	38,65	13,01	31,37	75,9	104,37	11,59	46,63	311,87	0,05	4,46	15,94	0,05	21
GPG	5/05/2000	0,02	0,05	0,58	1,63	0,76	2,93	7,06	9,14	0,45	4,31	25,67	0,14	3,91	0,25	0,02	0,11
GPG	27/10/2000	0,43	0,53	1,56	3,19	2,96	8,15	21,06	27	2,04	16,6	77,98	0,17	1,21	0,98	0,05	0,77
GS1	23/05/2002	1,06	0,8	2,74	8,15	3,28	9,04	19,84	20,31	2,05	10,67	71,8	0,11	2,59	6,31	0,1	2,27
GS2	23/05/2002	1,41	1,68	3,65	13,05	4,69	13,58	28,53	30,72	2,61	10,93	101,87	0,2	7,14	10,16	0,13	4,02
GSK	21/09/2000	1,18	0,3	8,66	25,63	10,74	31,8	119,79	180,71	10,9	95,16	462,93	0,12	5,16	2,98	0,03	2,28
GVZ	1/08/2000	0,32	0,09	6,28	12,4	8,37	31,92	76,31	105,06	7,36	41,49	273,79	0,25	3,61	0,91	0,07	3,31
GW	19/05/1998	1,82	0,84	24,86	70,71	12,6	65,25	187,86	261,79	11,16	83,15	695,44	0,08	28,51	2,49	0,34	10,8
GWA	17/05/2000	0,52	0,28	9,44	19,48	7,67	26,29	65,26	81,05	3,73	33,31	235,35	0,52	12,6	0,43	0,07	1,66
GWA	12/10/2005	0,19	0,08	1,33	3,24	1,56	6,41	16,03	26,67	1,6	12,21	66,07	0,06	0,18	0,04	0,04	0,13
GZ	20/09/1996	7,9	3,49	8,75	17,77	13,25	38,54	45,45	59,28	6,41	26,1	203,78	1,83	3,26	2,07	13,96	1,78
GZ	23/09/2002	1,18	0,71	0,46	3,05	2,17	9,2	16,41	25,52	2,62	12,09	67,9	0,29	0,79	7,96	0,04	1,62

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
HBB1	8/05/2000	1,55	0,57	6,33	11,49	3,52	14,64	27,84	37,29	1,67	12,75	111,88	0,32	15,17	4,41	0,13	1,02
HBB3	8/05/2000	2,87	2,06	8,55	14,21	6,07	25,92	45,58	60,85	4,46	24,14	182,12	0,19	9,77	8,02	0,14	2,68
HBB4	8/05/2000	0,91	0,54	3,52	10,53	3,53	11,16	24,89	31,11	1,83	12,92	95,04	0,26	6,59	2,48	0,09	0,75
HBN	29/05/2002	0,48	0,42	25,32	13,81	11,32	30,61	51,74	60,53	5,13	25,48	207,97	0,04	2,09	7,76	0,03	1,5
HDO	28/08/2000	0,14	0,25	0,6	1,57	1,63	3,74	7,65	10,33	0,73	7,95	31,98	0,08	2,18	1,2	0,03	0,38
HDO	23/09/2003	0,4	0,37	0,59	2,03	1,34	5,05	11,51	19,58	1,78	16,91	56,07	0,08	0,34	0,03	0,03	0,11
HEL	20/04/2001	0,54	0,99	4,5	9,98	6,34	11,27	15,9	20,7	2,02	6,49	69,38	1,9	3,28	9,29	3,02	1,75
HER2	9/09/2003	0,75	0,69	3,43	10,67	4,26	11,44	22,34	30,99	3,33	16,05	95,67	0,43	4,87	15,41	0,06	3,52
HGK	5/05/2000	0,61	0,71	1,44	3,8	2,64	4,85	8,57	10,75	0,69	3,91	33,93	0,45	13,48	17,83	0,13	0,93
HGK	1/08/2000	0,14	0,03	0,4	0,79	0,92	2,09	4,13	5,74	0,49	2,48	15,76	0,08	1,18	4,02	0,03	0,26
HGK	25/03/2003	0,34	0,38	0,17	1,09	1,01	1,56	2,49	4,58	0,26	1,56	11,8	0,05	1,1	16,57	0,05	0,37
HO	18/09/1997	16,13	7,12	11,83	23,67	17,42	63,05	76,87	133,6	9,96	50,02	375,15	1,45	6,34	6,98	18,85	2,99
HV2	4/11/2002	2,17	2,34	9,14	16,19	5,42	17,65	24,85	33,5	4,16	14,67	118,15	0,9	23,21	148,07	0,11	7,17
HVG	5/05/2000	0,67	0,47	4,23	12,85	4,36	16,45	36,08	48,08	3,62	25,25	143,62	0,28	8,37	1,57	0,09	0,91
HVH	5/10/2001	0,55	0,5	6,87	11,7	4,62	10,63	17,75	18,53	1,32	7,79	73,81	0,19	2,17	71,06	0,05	5
HVH	4/11/2002	1,59	1,74	1,1	11,96	3,61	15,11	18,9	23,98	2,28	9,82	82,46	0,83	20,5	132,91	0,1	6,03
HVX	4/11/2002	1,26	1,27	2,16	10,06	1,25	17,25	19,79	33,54	3,18	13,88	97,94	0,73	34,89	110,59	0,09	6,19
HZW	10/05/2000	0,89	0,37	2,24	5,41	4,61	9,37	16,04	18,85	2,1	7,86	60,65	0,41	11,99	9,15	0,08	1,25
IB1	7/06/2001	0,21	0,24	1,33	3,19	1,82	3,54	8,95	11,68	0,7	4,57	33,47	0,06	1,38	2,47	0,16	1,59
IB1	1/06/2005	0,07	0,11	0,17	1,36	0,67	2,4	3,67	7,29	0,11	1,92	16,89	0,14	0,61	0,11	0,11	0,94
IB2	6/06/2001	0,27	0,3	1,95	5,19	2,62	4,7	14,86	21,11	1,02	9,76	57,83	0,1	3,6	2,95	0,11	2,78
IB2	1/06/2005	0,13	0,08	0,11	1,63	0,52	2,64	10,39	17,81	0,82	10,01	42,71	0,12	0,12	0,11	0,11	0,91
IBK	24/05/2000	0,32	0,41	2,08	5,91	3,03	8,44	36,03	50,55	2,9	31,04	134,38	0,04	5,82	2,64	0,05	3,26
IJ	3/04/1998	0,91	0,49	2,97	5,8	2,03	4,82	14,97	15,12	1,26	5,52	50,12	0,1	24,89	11,76	0,26	4,16
IJ1	10/03/2005	0,46	0,12	3,36	11,83	2,7	8,53	19,18	30,02	0,63	10,9	84,28	0,24	0,73	0,25	0,25	4,4
IK1	10/07/2000	6,55	3,11	27,21	40,88	13,36	42,65	83,08	126,31	6,55	39,13	365,81	0,83	94,19	63,01	0,07	13,6

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
IK1	9/09/2002	50,63	26,91	46,89	21,22	11,77	35,26	37,42	49,89	4,43	21,32	262,63	0,48	8,01	51,31	0,05	4,99
IK1A	9/09/2002	19,47	10,26	17,69	17,5	9,34	34,66	38,68	50,22	5,19	19,94	198,16	0,71	21,91	83,13	0,07	8,01
IK1B	9/09/2002	18,59	8,12	11,47	11,42	5,59	26,75	37,4	62,18	4,3	22,66	190,48	0,38	8,26	89,85	0,07	5,2
IK1C	12/09/2002	14,02	6,48	11,86	11,14	3,92	22,27	21,06	25,52	2,9	8,18	114,05	0,51	6,11	56,4	0,1	3,96
IK2	14/05/2001	1,31	0,56	12,67	18,23	10,09	26,75	35,97	40,16	3,74	13,7	148,78	0,4	15,45	86,37	0,08	4,18
IK2A	9/09/2002	17,76	9,77	31,94	60,84	28,2	81,77	69,12	80,7	10,42	20,83	362,96	0,5	7,69	76,53	0,11	5,64
IKX	9/09/2002	0,14	0,78	7,36	8,9	0,9	2,98	6,1	7,58	1,35	4,25	37,31	0,99	15,64	132,22	0,14	0,18
JBS	30/05/2002	1,27	1,67	2,44	6,56	2,08	6,07	14,04	13,69	1,13	5,15	49,22	0,23	9,98	10,82	0,11	5,31
JEK	12/03/2002	1,81	2,08	18,97	48,96	12,29	34,35	108,7	129,36	4,37	45,57	387,72	0,32	24,87	13	2,23	18,8
KAL	15/06/2003	2,18	1,52	2,51	8,69	4,89	15,34	27,57	61,07	6,6	62,32	179,7	0,75	4,82	21,73	0,12	1,68
KB1	15/10/1997	13,87	6,12	7,43	11,91	6,9	25,12	28,64	48,14	3,72	23,21	158,34	3,16	8,32	4,35	12,26	3,52
KB1	3/11/1999	1,6	0,54	17,71	39,1	15,53	47,25	112,71	179,76	9,35	74,46	472,59	0,47	5,53	4,47	0,08	2,93
KB1	25/10/2005	0,31	0,05	4,09	6,49	3,45	11,89	30,69	52,1	3,06	22,17	127,74	0,07	0,15	0,06	0,06	0,74
KB2	3/11/1999	2,73	0,45	44,24	97,6	27,14	88,52	282,37	494,28	14,75	196,36	1206,11	0,47	6,57	6,37	0,08	7,58
KB2	19/06/2000	0,24	0,17	3,81	6,76	2,88	6,98	17,93	21,64	1,33	9,12	66,47	0,15	12,28	3,18	0,04	1,73
KB2	27/10/2005	0,39	0,12	2,66	6,11	1,82	6,85	17,69	31,36	1,62	11,54	76,61	0,16	0,61	0,11	0,11	1,45
KB3	3/11/1999	2,78	0,84	32,11	52,34	13,01	38,84	118,49	164,04	9,58	74,37	482,97	0,45	11,07	9,1	0,1	7,6
KB3	25/10/2005	0,7	0	7,44	18,28	4,08	15,11	48,8	92,84	2,66	41,4	224,58	0,26	0,5	0,36	0	2,49
KB4	3/11/1999	1,56	0,53	17,06	22,8	7,45	24,77	76,93	121,42	5,87	42,5	307,04	0,09	3,65	4,05	0,03	2,13
KB4	27/10/2005	0,77	0,09	7,4	17,76	3,7	12,91	40,01	75,43	2,04	30,46	184,74	0	0,42	0,22	0	1,5
KB5	3/11/1999	2,42	0,95	32,25	60,12	10,45	34,06	115,47	161,02	10,54	69,11	474,45	0,27	5,36	6,67	0,13	5,64
KB5	25/10/2005	1,23	0	13,71	25,69	4,53	17,68	51,19	100,46	2,72	35,76	245,72	0	1,09	0,25	0	3,2
KB6	3/11/1999	3,31	1,05	29,8	47,55	13,13	37,25	104,52	99,49	7,77	52,54	374,48	0,41	7,64	7,99	0,15	6,08
KB6	19/06/2000	2,51	1,02	27,5	45,85	11,37	31,9	91,38	124,42	6,01	52,71	376,27	0,18	16,17	4,11	0,06	6,45
KB6	27/10/2005	2,44	0,59	20,58	48,56	9,55	33,34	83,28	147,44	7,12	57,51	393,15	0,28	0,44	0,15	0,15	4,77
KB6A	25/10/2005	2,67	1,17	27,86	41,38	10,45	34,56	89,5	161,3	5,87	65,95	423,23	0	1,73	0,77	0	5,58

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
KB7	3/11/1999	3,57	0,91	65,66	117,3	28,82	95,76	402,93	597,68	29,91	271,46	1554,36	0,61	8,04	7,66	0,25	11,4
KB7	26/10/2005	0,46	0,13	8,96	18,06	5,29	21,8	61,02	127,05	4,7	34,3	271,65	0,06	0,19	0,16	0,04	1,53
KBH1	8/10/1996	6,38	2,87	63,99	81,53	42,11	141,3	149,13	267,63	21,94	111,35	821,26	3,04	24,49	6,6	11,99	9,66
KBH1	7/10/2002	1,42	0,88	11,94	94,48	10,57	52,51	297,99	582,56	21,61	141,17	1182,06	0,3	2,33	9,18	0,09	3,09
KBH1A	7/10/2002	1,25	0,83	13,98	98,38	10,71	61,3	346,88	673,9	24,51	170,49	1366,18	0,34	2,79	11,75	0,09	4,37
KBH1B	7/10/2002	2,59	0,69	27,88	203,9	16,49	117,1	530,21	1086	51,14	258,85	2226,45	0,32	2,12	7	0,06	3,13
KBH1C	8/10/2002	2,44	0,77	33,52	224,2	23,12	135	611,96	1126,1	84,53	326,94	2460,07	0,23	2,2	3,97	0,05	3,79
KBH1D	8/10/2002	4,17	1,73	34,48	111,7	20,96	81,62	248,63	395,18	22,33	181,53	1057,27	0,4	3,97	5,77	0,08	14,1
KBH2	8/10/1996	40,99	18,09	234,2	256,4	85,09	355,9	322,93	824,32	58,27	319,48	2354,17	0,15	57,2	7,34	33,59	39,6
KBH2	9/10/2002	2,65	0,91	14,48	26,08	9,57	31,45	103,87	165,26	8,06	57,1	400,89	0,18	2,14	3,33	0,04	7,87
KBH2A	7/10/2002	3,66	1,16	23,18	50,11	12,47	43,53	139,14	184,39	11,41	76,49	520,52	0,29	3,54	4,18	0,07	10,6
KBH3	8/10/1996	18,79	8,3	52,96	45,39	29,2	125,8	88,13	132,09	11,45	55,5	518,7	2,5	25,4	4,65	10,17	9,93
KBH3	10/10/2002	3,82	1,72	11,24	48,88	12,73	44,78	136,29	223,27	10,97	88,19	556,46	0,27	3,32	3,99	0,06	8,67
KBH4	7/10/2002	8,91	3,28	81,27	189,6	25,43	107,8	301,21	500,83	21,3	171,61	1361,21	0,63	5,81	8,4	0,1	20,9
KBH5	7/10/2002	7,71	3,19	55,08	115,8	24,89	95,56	201,34	374,84	20	160,51	1010,8	0,4	4,27	6,06	0,07	14,7
KBL	9/04/2001	2,77	1,08	30,78	52,96	14,52	39,09	109,1	141,83	7,12	62,31	438,85	0,52	7,53	8,79	0,29	9,27
KBL	5/10/2001	3,92	1,05	54,65	112	31,89	87,54	234,06	318,76	14,94	145,39	956,32	0,34	6,31	9,4	0,1	13,2
KBR	7/05/2002	0,99	1,32	1,47	4,33	1,82	4,57	11,44	11,12	1,01	4,45	38,37	1,46	1704,2	80,5	0,09	6,17
KBR1	26/09/1997	32,4	14,28	109,4	141,3	49,4	190,5	155,33	251,63	22,46	79,28	959,88	4,71	16,1	7,55	16,48	5,65
KBR2	23/09/1997	54,24	20,56	131	145,9	33,85	100,7	204,15	202,57	13,88	91,57	930,07	0,74	23,9	22,81	3,21	8,94
KBW	29/05/2000	0,31	0,3	1,52	4,08	1,39	5,31	11,4	14	0,74	5,53	42,16	0,19	7,6	1,31	0,09	0,45
KDS1	10/09/1999	2,74	1,7	66,35	90,12	28,22	75,07	255,26	248,9	25,19	97,43	835,86	0,51	11,35	15,7	0,37	18,8
KDS1	15/09/2003	1,89	0,75	22,77	66,68	10,53	47,92	192,26	390,82	14,84	108,69	831,03	0,14	0,25	0,11	0,11	5,81
KDS2	10/09/1999	1,84	0,82	89,23	138	35,74	110,9	463,98	635,28	41,92	266,58	1705,78	1,51	12,87	13,93	0,15	18,7
KDS2	15/09/2003	1,62	0,58	11,4	30,32	6,61	28,55	116,87	119,64	9,26	100,06	408,47	0,23	1,26	2,99		3,66
KDS3	20/11/1998	1,83	0,39	29,32	78,46	15,75	54,06	112,42	417,64	12,47	182,65	876,38	0,58	13,79	6,44	1,6	10,2

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
KDS3	15/09/2003	1,43	0,73	8,68	33,57	6,38	28,46	121,56	167,44	10,62	103,36	464,5	0,18	1,7	5,59		4,84
KDS4	10/09/1999	0,5	0,12	9,26	26,2	7,8	24,33	160,04	252,46	14,53	116,77	589,55	0,09	1,12	2,7	0,02	1,19
KDS4	15/09/2003	1,44	0,85	14,59	45,85	11,26	44,43	124,97	222,11	20,19	115,82	569,21	0,27	1,34	5,73		3,84
KDS4A	15/09/2003	1,05	0,56	4,54	24,64	7,4	27,86	111,26	198,48	31,72	167,79	535,61	0,12	0,56	2,92		1,83
KDS5	10/09/1999	9,14	3,69	116,9	326,9	74,38	224,4	1903	2818,7	162,6	1334,3	6733,34	0,31	5,81	23,57	0,14	18,7
KDS5	15/09/2003	4,46	1,71	36,35	108,9	15,35	59,19	220,34	418,47	44,29	218,5	1066,19	0,22	0,8	3,71		5,68
KDS6	10/09/1999	1,68	0,68	33,04	112,2	21,37	66,09	716,04	1235,5	67,97	570,39	2734,89	0,23	6,01	10,95	0,19	3,15
KDS6	17/09/2003	1,08	0,43	7,78	29,57	5,17	20,19	91,11	197,56	17,33	90,68	437,98	0,1	0,27	1,9		0,96
KDS6A	17/09/2003	1,53	0,74	11,57	51,96	9,41	37,17	206,99	421,89	38,51	243,59	974,71	0,1	0,47	3		1,93
KDS7	10/09/1999	3,22	1,1	82,87	314,2	50,23	106,5	1164,1	1999,5	106,6	902,77	4573,21	0,52	13,18	12,21	0,21	17,2
KDS7	17/09/2003	4,04	1,85	45,69	150,7	31,51	124,9	288,08	561,98	59,27	287,29	1462,65	0,2	1,27	9,38		9,24
KDS7A	15/09/2003	0,58	0,27	5,49	21,22	5,3	24,3	107,87	240,61	26,64	119,11	519,18	0,05	0,2	1,18		0,69
KDS7B	15/09/2003	2,62	1,13	27,36	54,8	13,2	37,86	121,05	266,15	20,38	95,72	605,56	0,17	0,63	4,7		7,76
KDS8	20/11/1998	1,26	0,19	31,12	81,43	23,91	84,94	673,66	1194,6	27,11	490,89	2557,88	0,34	7,34	4,72	0,06	6,48
KDS8	15/09/2003	2,06	1,13	14,29	83,59	16,85	67,49	228,95	513,66	41,53	196,26	1106,31	0,26	1,62	8,46		4,35
KG	3/06/1997	32,42	14,34	8,24	18,38	9,42	30,84	26,88	38,47	4,28	18,26	173,48	13,76	256,66	11,13	14,8	5,84
KGO	17/09/1998	15,81	7,53	118,5	121,9	26,93	77,72	137,16	141,84	9,57	51,53	664,39	1,92	30,96	31,24	3,34	14,5
KGO	27/09/2004	6,53	2,6	54,33	35,23	11,71	26,22	33,94	42,81	2,48	16,6	215,66	0,36	1,86	0,21	0	3,08
KGO	28/09/2004	11,12	4,39	90,89	49,73	19,21	42,11	49,97	52,87	4,51	18,61	315,3	0,19	0,45	0,19	0	4,37
KGO	29/09/2004	5,52	1,4	44,42	39,56	10,75	30,17	42,2	60,16	3,29	22,93	244,97	0	0,92	0,57	0	2,16
KGO1	17/05/2000	11,44	4,64	127,7	129,8	44,07	135,1	302,18	343,39	17,34	129,26	1178,84	0,66	37,64	32,12	0,1	9,56
KGO2	27/09/2004	7,8	2,66	75,58	52,08	18,96	41,62	55,92	71,75	4,52	25,89	330,64	0,47	2,39	0,09	0	4,67
KGO3	28/09/2004	11,12	4,39	90,89	49,73	19,21	42,11	49,97	52,87	4,51	18,61	315,3	0,19	0,45	0,19	0	4,37
KGT	17/06/1998	13,39	7,8	120,6	121,6	24,78	81,85	156,73	166,67	8,9	60,01	720,89	0,15	141,99	15,79	10,97	8,7
KKB	19/11/1997	15,72	6,96	58,79	52,71	33,28	110,8	105,47	131,47	13,03	48,02	522,96	4,21	64,5	89,51	94,35	6,91
KKB	27/10/2003	5,9	4,37	21,27	48,94	25,3	82,09	107,62	152,23	12,43	72,48	490,53	0,73	13,68	0,15	0,15	18

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
KLB	23/05/2002	1,24	1,67	4,24	10,42	3,29	9,35	23,25	23,92	1,58	7,97	80,39	0,33	7,77	11,76	0,14	6,78
KLD1	28/11/1994	11,35	24,98	40,86	41,8	25,94	82	107,05	163,76	25,89	99	545,82	6,26	27,36	10,69	6,8	3,6
KLD1A	23/10/2001	6,12	2,49	24,98	31,96	11,81	31,13	69,21	76,51	5,71	33,87	273,8	0,31	20,25	5,08	0,08	2,68
KLD1A	17/06/2002	6,87	3,7	29,82	36,79	12,43	44	62,69	72,72	5,4	30,56	283,44	0,25	17,46	7,01	0,1	4,07
KLD2	11/10/1996	34,43	15,2	41,37	53,5	25,04	81,73	64,97	89,35	9,2	41,76	407,11	6,55	132,22	11,55	19,67	7,29
KLD2	23/10/2001	4,58	2,66	21,21	25,03	10,8	26,96	50,95	59,78	3,89	24,5	213,02	0,31	19,32	8,28	0,08	6,91
KLD2A	13/06/2002	4,57	3,48	23,99	26,53	10,53	28,89	42,66	46,93	3,99	19,63	193,19	0,39	38,22	9,26	0,12	8,37
KLD3	7/02/2002	7,07	2,24	46,34	34,83	22,76	72,26	127,38	136,49	10,7	60,15	484,51	0,38	18,05	11,92	0,13	5,01
KLD4	7/02/2002	13,67	6,13	72,52	94,35	26,27	89,46	129,45	161,94	10,52	67,46	628,85	0,61	30,16	20,84	0,15	13,6
KLD4	23/05/2002	2,79	1,81	16,54	21,5	7,96	27,37	44,23	51,97	3,48	19,41	183,8	0,19	14,55	3,26	0,35	1,94
KLD4	19/06/2002	4,23	2,43	20,68	22,84	9,87	32	54,5	65,15	4,51	27,04	226,43	0,2	16,9	4,26	0,08	2,03
KLD4	26/06/2002	4,59	2,83	21,68	24,34	10,13	30,47	50,1	59,35	4,13	23,24	213,77	0,22	14,77	4,29	0,23	2,82
KM	12/06/1995	0,55	0,82	2,98	7,78	2,67	11,9	21,64	34,58	1,9	17,28	96,7	2,5	8,87	2,86	1,07	0,83
KN1	2/10/1996	16,92	7,48	9,67	19,98	9,49	33,95	48,54	86,41	6,24	32,9	248,37	2,12	20,8	7,09	16,02	3
KN1	13/06/2000	1,66	0,76	34,02	60,75	26,66	54,54	77,28	78,89	9,53	29,98	337,12	0,11	6,83	7,53	0,05	3,45
KN1	4/04/2002	0,55	0,52	5,95	11,96	9,81	22,52	36,66	42,26	3,87	17,1	137,01	0,06	0,26	4,01	0,03	1,06
KN2	13/06/2000	0,4	0,43	4,2	6,22	2,54	6,95	15,45	17,36	1,08	6,37	56,97	0,18	10,11	12,17	0,07	2,62
KN2	19/03/2003	0,36	0,35	3,12	3,75	2,02	5,13	9,08	13,8	1,41	5,56	40,82	0,55	1,82	19,23	0,06	1,64
KN2A	18/09/2003	0,58	0,49	1,81	5,52	2,73	7,85	14,89	22,38	1,78	8,55	61,58	0,54	0,81	0,1	0,08	3,09
KN2B	16/09/2003	0,77	0	8,01	10,29	4,27	11,04	22,81	31,38	1,55	11,63	95,92	0	0	0	0	4,76
KN2B	9/06/2004	0,89	0	8,35	12,33	4,25	11,77	24,75	35,88	1,88	13,19	107,16	0	0,03	0	0	7,73
KN2C	25/09/2003	1,2	0,87	2,83	12,15	6,28	15,71	27,22	37,53	3,5	15,72	112,37	0,45	1,65	0,11	0,11	4,95
KN3	13/06/2000	0,38	0,62	5,06	9,71	4	11,27	20,29	22,11	1,87	8,69	77,51	0,21	13,15	11,49	0,08	10,4
KND1	17/06/1999	1,74	0,61	5,74	8,66	5,39	17,65	33,72	44,63	2,33	16,91	129,05	1,14	281,69	38,34	3,9	5,63
KND1	14/09/2005	0,92	0,49	4,9	3,95	5,2	11,82	17,4	24,41	1,98	8,59	71,99	0,25	3,43	0,19	0,19	4,85

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
KND2	17/06/1999	0,27	0,07	2,82	27,54	3,51	10,95	22,07	32,55	1,38	13,3	109,51	0,14	16,03	8,21	0,01	0,75
KND2	12/09/2005	0,75	0,35	1,3	2,52	2	6,7	11,59	18,9	0,81	6,68	48,45	0,31	0,39	0,17	0,17	2,08
KNN	24/10/2000	0,46	0,44	1,73	2,73	1,74	5,55	9,77	12,81	0,86	5,23	38,28	0,15	10,98	4,37	0,05	1,77
KNN	6/11/2002	0,91	1,18	4,33	2,46	0,72	5,36	6,85	10,09	1,29	3,89	33,9	0,65	41,61	13	0,08	1,5
KOO	10/06/1999	0,44	0,44	1,92	4,28	1,15	4,98	9,74	12,39	0,74	5,18	38,93	2,4	791,02	28,82	2,41	2,35
KRL	13/10/1998	1,88	0,29	39,42	47,77	22,3	58,54	142,69	165,69	9,42	55,75	511,74	0,64	9,03	13,09	1,45	6,55
KRL	13/09/2004	2,79	0,84	17,87	29,06	9,89	35,84	58,6	86,74	7,12	32,12	263,02	0,16	0,5	0,07	0,07	1,27
KRO	15/09/1999	0,95	0,17	6,59	8,51	4,83	16,49	42,72	58,91	3,33	28,33	162,49	0,07	0,69	2,07	0,05	0,47
KSE	6/11/1998	3,37	1,48	24,47	42,18	11,55	43,44	120,58	178,61	7,17	62,35	475	0,68	16,61	71,21	0,8	9,38
KVK	2/08/2000	0,81	0,75	3,84	8,27	3,75	9,47	26,59	32,91	2,91	16,48	98,37	0,29	13,41	29,6	0,18	3,03
KZ	24/09/2002	3,84	1,63	14,15	58,78	9,76	59,32	126,62	185,02	15,57	99,85	547,58	0,88	3,25	5,17	0,11	2,93
L	4/07/1996	45,62	20,12	185,3	132,8	77,75	216,7	173,62	247,26	21,89	102,82	1104,18	4,04	144,6	32,79	44,08	12,4
L	23/06/2003	12,6	5,69	116,8	142,1	52,89	130,2	159,84	244,1	23,03	100,53	906,2	1,17	20,97	27,14	0,09	19,8
LAA	25/05/1998	4,33	1,35	65,73	229,8	34,13	126,2	894,74	781,55	54,04	455,23	2557,5	1,15	30,4	0,35	0,15	28
LAA	3/05/1999	0,33	0,29	6,71	14,07	4,24	12,06	24,5	24,48	2,1	11,63	93,78	0,07	5,84	3,19	0,55	1,35
LAA2	25/04/2002	1,26	1,43	9,4	12,06	5,02	12,28	36,35	37,35	2,3	16,3	124,99	0,17	19,35	6,76	0,07	4,52
LAN	7/06/1999	0,48	0,24	4	8,01	3,25	11,52	20,51	25,85	1,86	10,74	81,1	0,6	152,77	11,06	1,79	2,34
LE1	19/09/2000	8,45	5,2	119	103,9	54,77	128,6	190,58	227,49	17,53	79,35	857,25	1,8	31,36	34,73	0,13	26
LE1	1/06/2001	6,43	3,1	69,02	77,81	25,67	70,78	107,96	128,35	8,88	53,33	513,68	0,72	40,41	30,08	0,41	22,6
LE1	26/10/2001	6,73	4,22	80,3	93,65	33,13	86,51	155,96	186,03	13,71	81,44	690,61	0,64	17,23	17,66	0,11	20,9
LE1	23/06/2003	3,8	1,61	52,22	72,14	27,83	74,34	102,25	137,54	13,35	59,29	501,58	0,46	11,85	24,93	0,05	13,6
LE2	19/09/2000	15,71	7,81	135,3	127,2	60,96	141,6	190,09	218,65	18,81	86,73	915,26	2,13	38,94	39,95	0,15	28
LE2A	23/06/2003	6,43	3,49	52,03	79,96	20,31	58,89	101,25	158,34	11,16	69,21	526,12	0,7	1,67	0,11	0,1	11,1
LE3	19/09/2000	17,63	9,46	155,4	95,78	58,21	134,3	142,81	152,62	18,03	63,49	762,11	1,74	39,66	35,64	0,14	24
LE4	26/09/2000	8,91	5,74	88,76	73,46	34,86	82,87	94,32	102,98	9,72	31,22	482,53	1,23	27,34	37,05	0,14	24,8
LE4A	25/06/2003	5,86	3,34	59,11	58,29	16,09	50,54	78,91	110,05	8,69	51,23	413,98	0,51	1,62	0,13	0,08	6,8

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
LE5	26/09/2000	5,38	3,35	44,48	45,26	16,16	38,91	62,74	62,11	6,68	23,75	282,63	1,21	38,55	40,93	0,06	8,94
LE5	25/06/2003	4,52	2,2	28,45	26,51	11,18	28,43	40,69	55,42	5,51	24,58	208,59	0,51	10,64	33,61	0,04	3,72
LE6	23/06/2003	7,26	3,53	50	45,94	13,37	41,08	54,94	76,17	7,05	31,92	307,32	0,88	18	41,78	0,06	4,6
LEO	3/10/1997	37,66	16,64	3,48	16,82	9,01	34,29	39,62	56,48	5,55	25,41	213,75	4,71	14,01	18,08	28,57	6,87
LEO	6/10/2003	0,62	1,14	2,05	5,74	3,24	9,22	21,62	35,46	2,88	20,96	95,69	0,27	1,75	0,06	0,06	1,11
LEO1	3/10/2000	0,48	0,3	2,98	7,33	4,35	9,72	15,19	17,07	1,61	6,24	59,01	0,5	9,67	8,64	1,02	2,95
LEO2	3/10/2000	1	0,63	4,56	8,89	4,69	12,29	25,25	32,5	2,19	15,2	99,69	0,56	13,86	19,77	0,14	3,36
LEO2	6/10/2003	1,6	0,73	2,15	5,46	2,27	7,03	9,86	15,42	0,78	6,31	47,82	0,34	1,85	0,09	0,09	1,35
LEV	10/06/1999	0,13	0,14	1,44	3,02	0,97	2,8	5,99	8,45	0,28	5,28	27,1	1,42	424,13	5,94	1,72	1,2
LEV	14/05/2003	0,16	0,07	0,4	1,25	0,54	1,31	2,82	4,99	0,39	2,63	13,56	0,48	25,53	6,01	0,03	0,71
LEV	8/07/2003	0,1	0,09	0,06	0,63	0,42	1,12	2,34	3,78	0,13	1,18	9,21	0,05	0,88	0,06	0,06	1
LEY	21/06/2001	5,22	3,53	18,39	266,2	114,7	50,34	736,59	1194,1	53,73	800,01	3070,88	0,16	13,31	7,78	0,23	7,99
LIB	31/08/2000	0,09	0,09	0,23	0,58	0,57	1,35	4,72	7,66	0,37	7,42	22,05	0,02	0,14	0,11	0,01	0,11
LLS	18/03/2002	0,64	0,41	7,8	24,06	5,87	25,89	69,7	98,84	4,74	43,02	269,96	0,19	0,87	3,68	0,05	2,92
LO	8/04/1998	0,78	0,56	5,24	11,8	7,15	21,1	72,87	92,92	5,21	38,57	243,29	3,04	45,05	88,03	0,13	2,58
LWV	27/06/2000	1,16	0,88	9,66	11,06	3,79	10,7	18,49	22,73	1,55	8,55	82,35	0,26	15,74	10,15	0,07	3,07
MA1	30/08/2000	3,4	1,59	21,04	101,4	19,54	54,09	423,98	486,2	29,13	334,77	1424,91	0,11	2,08	2,71	0,03	5,07
MA1	15/05/2002	1,57	0,96	19,4	55,22	15,09	44,15	226,28	291,88	15,95	164,04	802,55	0,14	6,56	2,53	0,04	7,52
MA1	2/06/2005	0,63	0,28	5,49	9,98	4,29	12,9	46,03	85,05	7,34	53,84	213,91	0,05	1,21	0,06	0,06	1,6
MA2	20/06/2000	1,91	1,24	25,82	110,2	21,95	62,5	591,67	571,89	28,37	280,22	1644,23	0,07	4,7	3,18	0,03	8,11
MA2	15/05/2002	1,96	1,5	25,24	92	21,66	61,38	490,83	571,77	25,92	367,92	1611,11	0,15	13,46	3,25	0,06	10,5
MA2	19/05/2005	1,77	0,7	27,66	60,52	13,13	47,35	174,1	332,76	23,17	182,48	826,65	0,22	1,41	0,8	0,09	7,15
MA2A	15/05/2002	3,85	2,45	36,84	145,6	32,21	87,34	653,28	732,49	36,12	482,47	2141,87	0,22	19,09	4,45	0,1	15,7
MA2B	14/05/2002	2,91	2,17	29,67	86,9	23,94	70,51	369,72	441,59	24,54	246,29	1247,6	0,15	11,4	3,68	0,09	14,9
MA2B	19/05/2005	1,26	0,61	14,76	29,03	10,89	34,67	135,88	247,07	20,09	157,21	619,89	0,16	0,47	0,58	0,07	6,02
MA3	22/06/2000	2,68	2,03	43,59	161,4	22,82	77,8	769,44	847,96	26,3	465,49	2368,34	0,37	22,09	6,98	0,08	17,4

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
MA3	14/05/2002	3,34	1,91	30,85	108,2	25,75	76,9	422,26	506,25	28,55	293,61	1441,4	0,18	10,5	1,88	0,1	12,5
MA3	18/05/2005	1,12	0,52	13,41	26,19	10,34	32,55	133,58	250,47	21,02	153,64	610,97	0,13	1,13	0,13	0,07	5,79
MA3A	14/05/2002	3,14	1,91	35,36	131,3	29,44	87	530,16	596,5	33,35	355,24	1738,7	0,25	18,76	4,93	0,13	20,7
MA3A	18/05/2005	0,74	0,35	9,51	20,71	8,54	26,94	115,57	206,45	18,53	129,57	509,5	0,03	1,93	0,38	0,05	4,17
MA3B	14/05/2002	2,65	2,45	32,04	101,7	22,45	69,1	395,98	400,37	23,56	238,64	1240,52	0,29	17,28	6,61	0,14	19,7
MA3B	18/05/2005	1,38	0,7	19,17	48,2	12,84	41,02	159,23	280,43	22,9	162,52	711,94	0,17	1,55	0,43	0,1	9,4
MA3C	13/05/2002	3,32	1,76	33,3	106,1	26,37	77	365,74	434,38	26,21	268,36	1288,25	0,18	13,17	4,75	0,09	16,3
MA3C	17/05/2005	0,91	0,49	11,47	15,67	10,28	31,72	110,96	201,63	14,05	104,31	476,67	0,12	0,41	0,21	0,06	4,72
MA3D	13/05/2002	2,18	1,42	23,23	63,49	16,26	48,67	198,01	245,84	13,71	140,27	721,7	0,15	11,55	3,25	0,08	11,8
MA3D	17/05/2005	1,2	0,64	17,44	32,22	11,66	37,73	132,94	238,94	17,77	146,22	606,68	0,2	0,81	0,28	0,09	7,61
MA3E	13/05/2002	4,5	4,64	22,95	56,79	17,19	47,23	199,47	242,56	15,69	148,09	721,59	0,15	11,57	3,24	0,08	13,7
MA4	16/10/1997	33,31	14,72	100,8	144,4	59,29	227,6	241,37	514,57	50,66	237,71	1499,72	7,58	31,2	9,26	15,87	9,16
MA4	22/06/2000	3,08	1,57	34,11	87,45	24,69	64,39	342,05	432,15	24,34	234,68	1197,9	0,17	14,71	4,91	0,07	13,1
MA4	17/05/2005	1,02	0,42	13,14	24,12	10,01	32,31	100,37	178,09	13,56	105,57	454,61	0,05	2,78	0,85	0,07	5,08
MBE	17/05/2000	0,13	0,05	0,89	2,27	2,37	6,42	16,81	21,05	1,34	9,29	56,87	0,04	1,42	1,66	0,01	0,27
MNB	3/04/2002	3,6	2	25,57	51,46	18,71	59,63	163,23	221,23	11,67	110,99	635,71	0,59	5,69	12,17	0,12	12,1
MOT	28/09/1999	1,62	1,11	11,3	16,45	8,73	21,31	41,09	41,45	2,33	15,86	149,07	0,26	6,3	235,24	1,35	12,3
MOT	4/03/2003	2,03	1,46	8,95	15,76	9,11	23,92	35,22	49,73	4,98	18,62	154,23	0,11	7,36	237,65	0,11	10,5
MSG	30/05/2000	0,25	0,26	9,04	18,46	5,77	24,47	93,19	179,38	4,08	69,58	394,38	0,25	9,61	5,08	0,06	0,9
MV	4/06/1996	34,72	15,32	161,1	151,4	61,72	209,5	152,11	216,9	19,2	80,43	1006,16	5,01	118,37	25,03	52,9	7,51
MV	24/04/2003	5,92	2,4	42,33	40,89	17,25	49,86	77,73	112,35	8,63	42,9	371,98	0,18	5,84	0,04	0,04	2,87
MVD	29/05/2000	1,52	0,56	15	23,16	7,7	28,79	68,47	100,34	5,78	36,1	273,39	0,39	18,94	25,42	0,07	3,86
MVD	24/04/2003	1,48	0,7	5,93	8,73	3,6	13,52	27,58	45,92	2,36	22,99	126,16	0,19	3,47	0,08	0,06	1,88
MVG	23/04/2003	3,79	1,88	19,24	22,22	12,8	30,11	47,69	66,32	5,42	30,66	220,04	0,2	2,52	0,55	0,06	1,96
MVX	8/05/2002	3,87	3,25	104	149,6	97,61	223,1	167,69	125,62	21,99	19,12	793,02	2,06	1857,4	141,46	0,15	9,43
NB	4/06/1997	31,56	13,95	4,45	6,59	5,27	16,78	14,41	19,77	2,51	6,72	100,28	2,79	114,32	10,22	11,75	5,21

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
NEK	27/06/2000	0,41	0,42	2,71	6,39	1,19	5,31	11,97	16,87	0,73	6,61	50,27	0,29	63,46	4,68	0,08	1,25
NEK	4/06/2003	0,09	0,19	0,44	2,11	0,94	3,23	6,43	10,59	0,71	4,17	27,06	0,73	16,05	3,09	0,07	0,64
NGO	27/06/2000	0,11	0,14	1,93	3,74	1,16	3,68	10,41	15,48	0,58	6,68	42,02	0,19	7,43	3,58	0,08	1,44
NKE	13/06/2000	0,67	0,28	8,79	22,55	10,68	23,77	112,23	131,48	6,44	66,71	366,2	0,8	12,61	3,7	0,06	2,24
NKE	20/03/2003	0,3	0,21	2,36	13,72	5,81	17,02	78	157,78	8,55	59,95	329,12	0,64	2,5	7,19	0,07	2,11
NP0	19/09/2001	38,74	24,84	66,4	47,56	24,47	54,29	60,15	63,9	5,67	24,3	355,33	0,89	15	31,91	0,13	7,21
NP1	24/10/2000	10,97	6,81	40,93	36,38	20,85	50,08	68,17	75,8	6,41	29,43	311,77	2,12	17,79	40,07	0,13	8,49
NP1A	19/09/2001	19,52	9,33	59,16	42,08	26,63	56,14	71,98	73,71	6,69	30,32	352,92	0,84	14,85	35,08	0,13	6,7
NP1B	20/09/2001	27,3	13,98	58,44	47,42	25,66	51,12	58,28	58,64	6	23,2	324,41	0,76	13,29	25,98	0,11	5,56
NP1C	20/09/2001	2,93	1,37	34,34	47,01	23,34	51,76	55,6	53,64	6,62	16,41	261,69	0,56	23,33	21,5	0,12	2,69
NP2	24/10/2000	3,23	1,12	16,85	31,02	12,59	37,32	59,52	78,73	5,65	25,81	252,48	0,88	25,63	15,03	0,09	3,53
NP2	20/09/2001	2,05	0,67	9,16	14,69	7,93	21,11	33,31	41,01	2,94	17,98	139,32	0,23	3,63	8,9	0,04	1,14
OAV	7/06/1999	0,57	0,7	5,5	6,73	2,41	8,57	14,41	17,67	1	8,23	61,68	4,03	1286,3	20,4	3,81	4,72
OAV	25/04/2001	0,13	0,41	1,44	4,07	3,01	6,28	9,43	11,4	0,9	6,76	39,51	0,48	183,38	15,55	0,08	2,58
OAV	24/10/2001	0,24	0,29	0,69	2,38	1,11	3,44	5,47	6,67	0,46	2,86	21,74	0,29	33,55	10,29	0,05	1,78
ODU	10/05/1999	3,06	1,1	24,09	29,62	10,74	42,82	83,43	111,2	6,48	50,77	345	0,48	13,51	7,75	4,57	2,13
ODU	24/10/2002	0,74	0,66	3,21	5,3	3,17	16,57	33,85	50,41	3,33	21,83	131,92	0,33	0,71	3,71	0,07	0,8
OLA	16/08/1999	1,31	0,7	22,39	37,85	8,5	34,6	141,39	224,18	13,38	100,09	561,8	0,16	1,5	3,31	0,08	4,98
OLA	18/04/2000	0,18	0,23	3,05	3,78	1,35	7,07	14,61	18,74	1,06	7,61	55,05	0,25	5,31	5,15	0,07	0,95
OLBH	5/07/2000	1,46	0,59	25,93	24,63	8,34	44,51	114,06	162,68	4,64	50,69	423,97	0,06	1,75	3,98	0,02	0,83
OLBV	4/07/2000	4,03	2,06	38,61	35,55	15,02	50,11	80,72	99,47	6,37	49,18	357,67	0,12	7,28	5,39	0,2	1,28
OLD	22/08/2000	2,18	0,9	22,93	27,17	11,25	43,39	118,58	171,95	7,97	87,93	474,13	0,44	4,33	15,01	0,04	2,77
OLEV	4/07/2000	0,49	0,64	7,26	8,04	4,68	16,07	31,03	37,25	3,05	17,15	117,28	0,19	6,86	4,34	0,05	1,61
OLG	10/05/1999	2,22	0,44	27,71	31,23	18,56	72,76	134,81	217,67	9,07	94,71	581,11	0,13	4,8	9,4	0,83	0,58
OLG	19/09/2005	8,17	4,4	49,25	29,14	15,17	59,24	86,66	143,88	9,18	64,04	440,38	0,26	0,4	0,16	0,16	0,11
OLLW	14/10/1997	14,92	6,66	58,6	49,22	43,39	128,7	112,18	152,91	12,26	52,02	568,58	4,2	35,19	47,09	43,35	9,23

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
OLLW	5/07/2000	0,49	0,38	5,69	7,64	7,13	11,18	21,54	17,81	2,41	8,55	72,9	0,08	4,04	8,27	0,04	2,24
OLM	22/08/2000	0,97	0,68	5,07	9,07	7,24	19,63	53,7	73,51	3,88	36,74	198,69	0,33	19,09	99,54	0,09	3,07
OLO	30/05/1997	29,76	13,14	36,01	44,97	32,03	111,8	118,89	196,8	17,83	88,52	626,71	1,98	64,02	36,62	29,1	6,03
OLO	16/06/2004	0,72	0,17	2,42	5,3	2,81	10,1	22,72	36,55	2,62	16,5	94,31	0,21	1,37	0,16	0,16	0,75
OLOE	16/08/1999	1,45	0,64	12,18	16,48	7,31	26,03	80,58	112,82	6,57	55,38	304,92	0,09	3,51	2,8	0,03	0,38
OLSW	5/07/2000	0,38	0,27	5,39	15,6	5,04	21,45	61,15	86	4,3	34,39	224,35	0,07	2,05	0,84	0,03	1,18
OMD	16/09/2002	1,95	1,4	5,27	21,86	3,03	25,95	68,6	118,94	7,84	62,23	304,8	0,41	1,9	1,9	0,08	2,84
OMS	29/09/1997	12,78	5,15	39,98	58,76	15,17	54,87	188,57	239,83	16,28	133,14	727,93	2,29	5,33	7,58	10,34	7,41
OMS	2/09/2002	7,45	1,97	6,93	27,68	16,98	81,15	205,27	500,47	41,93	267,21	1096,16	0,18	0,81	1,52	0,04	2,9
OOS	23/09/1998	1,66	0,34	3,83	10,4	5,75	19,84	46,03	66,8	2,51	15,84	164,41	0,91	8,07	5,84	4,39	0,69
OPK	1/08/2000	0,63	0,62	1,8	2,94	2,37	4,91	10,57	14,2	1,73	7,57	42,63	0,19	1,31	21,98	0,1	3,25
OPK	25/03/2003	0,41	0,36	0,4	1,43	0,83	2,5	3,92	6,54	0,43	2,4	17,61	0,08	1,94	38,84	0,08	1,2
OSA	16/03/1998	0,5	0,32	8,28	20,03	17,1	484,3	131,16	203,38	7,84	84,98	932,65	0,93	23,22	61,35	1,36	11,1
OSB	24/07/2000	0,01	0,07	0,32	0,76	0,65	1,21	2,6	2,95	0,25	1,42	9,27	0,11	1,29	0,59	0,01	0,11
OSD	21/08/2000	0,26	0,52	1,36	4,51	2,93	4,93	16,26	22,79	1,16	10,83	60,94	0,27	2,45	6,74	0,06	0,85
OSE	24/07/2000	0,27	0,45	0,65	1,44	0,91	2,69	5,61	7,12	0,43	3,09	20,88	0,12	7,3	2,74	0,03	0,82
OSG	26/07/2000	0,36	0,36	2,41	2,75	2,67	5,3	10,09	12,39	1,6	5,08	38,37	0,16	4,41	3,16	0,07	0,84
OSH	24/07/2000	0,06	0,06	1,75	5,18	2,57	5,42	13,35	21,47	1,88	9,46	56,69	0,36	5,11	4,38	0,04	0,84
OSK	24/07/2000	0,44	0,53	1,92	6,63	4,11	10,66	27,84	37,81	2,17	14,93	100,23	0,34	27,3	25,24	0,18	4,78
OSK	23/10/2002	0,33	0,55	0,79	3,85	0,89	8,23	17,97	27,83	1,97	11,74	70,74	0,14	0,91	11,85	0,05	1,67
OSM	19/07/2000	0,4	0,18	0,38	1,05	0,66	2,19	9,77	16,31	0,76	9,02	39,13	0,07	0,53	0,22	0,01	0,05
OSME	26/07/2000	0,11	0,07	0,62	1,48	1,21	2,61	6,18	8,16	0,58	3,64	22,81	0,06	1,2	13,95	0,02	1,68
OSME	21/10/2000	0,16	0,13	1,88	5,81	3,6	9,86	20,16	28,71	1,84	11,4	77,97	0,17	2,17	12,18	0,05	3,65
OSN	24/07/2000	0,26	0,26	7,68	35,99	7,46	28,56	72,51	99,48	5,49	35,97	280,46	0,09	2,66	3,17	0,04	3,4
OSS	26/04/2000	0,74	0,74	10,32	9,25	2,71	9,35	20,54	25,14	1,23	11,26	86,6	0,3	9,79	6,97	0,1	1,54
OSSK	26/07/2000	0,97	0,76	8,37	14,1	6,54	19,87	45,07	65,35	3,58	33,17	186,92	0,36	9,88	9,96	0,11	2,35

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
OSSZ	16/03/1998	0,55	0,21	2,77	4,13	3,63	11,84	24,1	34,41	1,6	12,97	90,78	0,42	7,14	0,39	0,89	0,55
OST	26/07/2000	0,18	0,24	1,2	2,34	2,26	5,22	13,56	17,64	0,99	8,56	48,71	0,13	1,73	20,23	0,04	0,88
OSV	19/07/2000	2,01	1,07	20,21	37,85	11,26	40,69	87,09	118,55	7,6	55,45	361,84	0,31	30,47	8,23	0,09	6,83
OSZ	21/08/2000	0,84	0,54	7,08	15,4	9,88	26,99	56,6	95,75	4,69	66,86	269,52	0,2	3,28	14,43	0,08	2,45
OSZ	18/10/2001	3,86	4,51	9,28	13,75	9,23	25,82	45,33	76,13	5,14	57,83	232	3,61	5,29	21,74	0,07	2,81
OVA	30/09/2003	0,09	0	1,45	1,55	0,94	2,17	5,92	8,58	0,37	3,44	23,19	0	0	0	0	0,61
PAL	22/09/1999	0,7	0,58	3,82	9,27	4,22	15,88	43,5	58,53	4,39	27,93	159,64	0,25	4,65	31,4	0,16	1,3
PDV	28/06/2000	0,57	0,9	5,46	8,92	2,15	6,21	15,49	20,03	0,97	9,75	66,43	2,08	488,76	15,87	0,14	3,88
PDV	15/05/2003	0,13	0,34	1,05	1,92	0,83	1,24	2,48	4,73	0,26	2,16	13,72	0,88	152,43	8,6	0,04	1,01
PN	19/09/1997	17,79	7,86	2,87	5,67	10,74	36,87	45	76,85	6,27	24,93	209,98	1,63	9,44	5,47	6,8	2,91
PO	10/06/1998	1,57	0,93	6,77	12,21	11,79	41,49	100,01	150,33	6,96	103,66	416,04	0,08	19,75	1,06	0,49	1,9
PRI	4/02/2000	0,56	0,6	9,03	12,92	5,06	15,62	59,77	79,69	5,56	37,59	215,16	0,19	24,34	9,95	1,55	3,31
PV1	6/05/2002	3,57	3,14	9,54	15,76	5,28	11,93	29,49	25,61	1,96	11,03	106,95	2,35	2076,4	56,12	0,16	11,5
PV2	6/05/2002	2,93	2,9	8,31	17,91	9,18	20,29	49,21	47,17	3,89	22,04	167,86	2,39	1922	133,71	0,16	5,97
RHD	16/05/2000	1,49	0,18	9,21	13,17	7,24	26,17	57,79	83,45	3,67	41,77	233,06	0,33	7,19	0,36	0,07	1,05
RLK	1/08/2000	0,09	0,26	0,51	2,55	1,82	2,79	5,69	7,1	0,45	2,81	21,54	0,25	1,81	13,77	0,09	1,76
RLK	27/03/2003	0,15	0,14	0,22	0,48	0,39	0,99	1,64	2,78	0,14	0,94	7,2	0,03	0,83	11,74	0,03	0,45
RM	28/09/2001	0,26	0,3	1,46	3,14	2,17	5,99	12,05	15,26	1,13	7,1	45,27	0,14	1,93	2,84	0,04	0,44
ROG	15/09/1999	0,7	0,46	3,53	7,4	2,75	7,87	39,23	47,44	3,84	28,93	135,1	0,16	0,69	1,62	0,04	0,59
S1	1/06/1995																
S2	29/04/1998	8,36	3,34	89,78	135,5	28,3	128	284,8	329,46	21,72	182,97	1158,84	0,11	90,59	21,39	0,74	13,7
SC	10/01/1995	1,7	3,44	31,18	36,31	21,31	89,31	178,64	318,79	15,35	143,24	799,16	9,94	23,58	53,39	8,34	4,64
SCH	29/04/1998	2,18	0,71	13,62	20,25	16,35	62,33	152,88	201,14	9,11	80,72	533,12	0,14	50,52	13,62	0,41	5,43
SCH1	28/09/2000	2,25	1,03	78,08	111,6	36,75	107,4	146,99	175,05	10,48	45,01	666,32	0,51	19,28	27,57	0,15	20,3
SCH3	28/09/2000	7,35	4,21	39,12	56,45	18,45	54,75	111,01	141,49	46,13	56,99	467,18	0,63	15,14	11,05	1,39	12,2
SCH3A	4/06/2002	7,11	3,04	35,86	52,86	14,11	47,16	93,09	107,52	20,53	41,54	385,13	1,55	196,64	96,41	3,55	7,25

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
SCH3B	4/06/2002	9,29	5,65	62,92	65,95	19,53	56,68	94,3	102,53	41,43	45,09	436,76	0,47	36,8	60,75	0,12	13,4
SCH3C	4/06/2002	16,27	4,54	43,38	47,26	12,92	38,34	65,16	70,22	15,73	29,82	310,45	0,51	49,29	47,65	0,34	7,86
SCH4A	6/06/2002	30	4,76	45,21	48,67	12,9	37,45	65,73	72,55	20,69	33,61	333,21	0,43	35,71	52,76	0,87	9,89
SCH4B	4/06/2002	28,68	5,44	39,51	54	11,14	39,56	97,92	120,82	14,45	51,09	431,57	0,27	22,08	21,89	0,37	4,15
SCH6	10/10/2000	2,47	1,25	14,85	29,59	9,39	28,98	65,75	88,41	8,79	45,59	275,65	0,8	34,76	46,7	0,1	10,7
SCH7	10/10/2000	3,67	1,85	48,32	95,47	23,76	85,82	197,82	265,95	23,27	106,66	803,71	0,38	7,94	17,39	0,08	9,48
SCH7A	24/08/2004	1,57	0,39	17,84	27,22	7,54	29,52	57,31	92,19	7,75	44,01	269,65	0,12	0,89	0,08	0,08	1,58
SCH8	26/09/2000	3,6	0,7	47,26	102,7	29,59	101,9	232,49	321,56	29,71	153,13	962,69	0,33	11,79	39,16	0,08	6,24
SCH9	26/09/2000	1,95	0,52	29	63,42	21,23	71,63	180,11	242,44	21,81	117,52	706,07	0,18	5,57	15,1	0,05	5,35
SGS	28/06/2000	0,07	0,15	0,93	4,07	1,67	4,11	10,83	14,42	1,36	7,48	41,91	0,44	78,28	9	0,51	2,91
SK	1/10/2001	0,6	0,6	2,51	4,13	3,11	5,84	13,38	14,36	1,02	5,46	46,27	0,28	4,11	20,86	0,09	1,34
SM	12/10/1999	0,84	0,35	7,67	12,22	7,06	22,24	48,85	58,53	3,33	24,76	175,1	0,1	1,81	1,98	0,05	0,8
SVW	10/05/2000	0,27	0,35	2,05	9,48	7,23	19,88	41,59	55,3	3,84	23,69	152,28	0,36	6,93	5,46	0,06	1,37
SVW	2/10/2003	0,29	0,16	0,17	2,61	3,09	9,28	18,33	30,1	2,63	13,5	74,27	0,14	0,96	0,05	0,04	0,4
TB	26/06/1997	27,85	12,3	78,14	121,8	43,37	175,7	137,19	245,28	24,13	100,9	886,87	4,53	49,6	7,56	22,88	8,7
VAM	12/07/2000	0,14	0,04	2,75	4,54	5,69	17,77	48,26	65,57	3,92	25,81	164,83	0,06	1,79	2,26	0,02	0,74
VEL1	11/04/2000	4,23	4,89	12,8	18,68	8,56	21,1	66,16	75,5	4,83	46,19	244,66	3,69	21,5	26,32	4,08	8
VEL1	2/03/2004	0,62	0,43	4,82	20,4	5,93	19,8	46,67	77,02	6,9	60,05	229,36	0,13	3,62	0,25	0,25	5,7
VEL2	10/04/2000	7,01	8,44	12,92	34,87	13,79	36,02	108,43	154,19	9,44	111,69	465,13	5,86	98,25	49,48	0,11	15,8
VNX	3/04/2002	0,41	0,64	2,67	6,83	1,81	5,69	18,34	23,12	1,3	8,75	65,81	0,13	0,64	11,8	0,07	5,3
VR	1/05/1995	1,48	0,98	4,27	2,22	2,16	5,02	10,39	14,42	0,97	4,88	42,69	8,53	56,85	24,69	5,89	5,16
VVK	24/10/2000	0,15	0,14	0,45	1,01	0,99	1,39	2,77	3,02	0,39	1,26	10,06	0,1	9,66	13,82	0,03	1,34
WA	15/10/1997	23,86	10,54	23,45	40,02	27,09	78,88	82,77	100,48	10,71	35,37	384,83	5,6	17,28	8,84	9,65	5,75
WA	19/04/2004	0,09	0,07	0,86	2,16	1	3,28	8,37	14,49	1,08	6,03	35,3	0,12	0,28	0,06	0,06	0,76
WBA	23/05/2000	0,54	0,32	10,23	33,9	13,34	33,96	86,34	101,12	10,4	42,55	308,63	0,15	13,28	6,03	0,73	2,92
WBV4	5/10/2004	142	104,2	227,8	75,72	25,33	61,57	120,59	162,12	10,28	58,49	848,34	0	0,87	0,83	0	4,34

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
WBV5	5/10/2004	55,76	28,36	195,2	168	44,89	102,1	173,62	221,63	17,56	94,15	1010,47	0,97	0,74	0,75	0	3,86
WBV6	14/10/2002	55,35	22,21	132,9	138,8	43,37	107,8	187,16	244,47	21,35	98,39	964,91	0,53	3,65	14,6	0,09	6,84
WBV6	2/12/2002	45,49	19,72	156,6	143,6	46,92	113	180,16	227,84	21,59	95,89	962,61	0,67	7,65	19,79	0,11	6,82
WBV6	13/05/2003	63,83	31,52	165,1	159,7	35,07	114,8	196,82	283,09	21,52	122,29	1105,53	0,44	1,99	0,11	0,11	7,13
WBV7	14/10/2002	102,6	46,05	224,7	181,9	59,87	157,7	227,76	333,38	24,04	115,48	1343,49	1,28	9,13	33,64	0,18	11,9
WBV8	17/10/2002	55,31	20,08	97,62	95,47	38,6	102,7	140,68	194,22	16,64	80,86	766,87	0,65	5,73	23,78	0,09	4,75
WEE	18/09/1997	23,96	10,54	136,2	391,5	159,4	775,3	925,64	1955,1	72,8	542,31	4749,93	2,15	17,11	76,6	183,9	7,23
WEE	1/10/1998	55,07	18,53	348,2	832,2	240,2	786,3	2044,8	3201,2	114,1	1221,7	8489,49	1,01	29,77	63,3	2,51	16
WEE	3/05/1999	49,62	13,78	276,3	593,3	208,3	684,2	1739,1	2334,7	116,3	957	6634,1	1,18	23,96	85,52	4,7	19,9
WEE	18/10/2000	8,9	3,35	38,37	58,14	43,2	132,5	247,55	291,27	25,62	117,99	894,68	0,05	0,51	0,38	0,02	1,03
WEE	10/04/2001	6,47	2,27	47,36	66,49	46,03	123,5	255,3	308,7	24,87	131,88	939,7	0,23	1,07	4,81	0,03	1,97
WEE	9/10/2001	11,65	3,47	57,94	93,33	66,3	190,7	374,2	429,5	35,16	171,41	1328,72	0,11	0,51	3,26	0,02	1,34
WEE	9/12/2002	9,58	3,41	48,31	85,09	62,44	169,7	179,94	242,27	36,89	108,35	843,18	0,26	0,99	4,04	0,04	1,72
WEE	13/10/2003	6,3	2,33	18,79	30,63	22,23	54,92	95,77	128,38	17,94	55,58	390,36	0,18	0,18	1,35	0,02	0,43
WEE	12/10/2004	2,32	0,79	13,39	22,34	17,95	61,98	119,48	179,51	12,01	73,43	472,45	0	0,14	0,88	0	0,25
WEE	5/09/2005	3,32	1,27	13,82	22,93	18,24	61,56	124,26	178,15	12,31	79,95	484	0	0	0,41	0	0,29
WIK	6/06/2001	1,45	0,65	15,03	62,01	16,43	46,1	168,67	243,6	11,55	141,94	678,8	0,63	119,83	16,92	3,24	19
WIN1	29/09/1999	0,86	0,07	13	34,86	10,9	35,27	50,88	62,75	2,58	22,28	219,9	0,21	6,12	47,37	2,19	3,07
WIN2	29/09/1999	2,02	0,75	35,05	79,97	26,8	85,14	179,35	216,33	11,26	134,65	732,49	0,47	19,11	126,21	9,04	9,32
WL	21/04/1997	15,41	6,85	111,4	148	52,63	208,1	244,94	521,13	46,32	255,74	1504,66	1,54	56,88	10,11	23,35	13,7
WLL	16/05/2001	1,11	0,71	12,1	22,24	6,99	19,39	45,56	55,7	3,22	28,97	185,07	0,82	570,56	46,36	1,35	7,62
WMX	4/04/2002	1,79	1,42	5,13	7,89	3,42	8,76	18,7	20,75	1,52	7,83	70,86	0,19	1,54	18,1	0,1	4,36
WNR	26/08/2003	0,15	0,11	0,76	1,87	1,25	2,89	4,56	6,54	0,68	1,91	18,68	0,11	0,81	0,15	0,06	1,14
WSD	30/05/2000	4,38	1,09	49,13	51,97	23,48	70,86	123,28	154,32	10,78	57,29	511,23	0,32	10,97	4,35	0,07	2,83
WSD	30/09/2002	4,35	1,97	30,2	36,77	13,66	54,45	89,89	131,9	9,17	61,17	408,73	0,28	1,34	3,26	0,08	1,96
WWB	27/06/2000	0,74	0,77	2,7	5,74	6,01	9,74	17,3	21,12	3,66	9,33	66,67	0,57	15,3	2,38	0,13	1,6

Code	Date	CB 18	CB 32	CB 52	CB 101	CB 105	CB 118	CB 138	CB 153	CB 156	CB 180	Sum 7PCB	α HCH	γ HCH	DIE	END	HCB
YZ1	10/07/2000	0,98	1,36	9,4	21,13	6,93	22,01	46,6	50,57	3,85	26,45	177,13	2,19	193,68	88,34	0,14	8,79
YZ1	14/06/2005	0,89	0,31	1,65	5,47	2,44	6,29	12,78	19,62	1,15	8,79	55,5	0,38	10,98	0,2	0,2	3,83
YZ2	10/07/2000	1,83	1,4	11,77	16,57	8,62	22,72	30,25	33,81	3,03	15,86	132,81	1,66	289,33	67,41	0,08	6,59
YZ3	10/07/2000	0,61	0,47	4,85	7,07	3,2	10,33	17,32	20,75	1,65	8,29	69,2	0,59	118,19	38,68	0,04	2,91
ZB	14/03/2002	0,92	1,16	9,06	22,24	5,21	14,26	44,68	69,56	3,15	32,28	192,99	0,24	2,84	8,19	1,14	6,77
ZBR	11/05/2001	1,39	0,45	9,23	26,76	12,27	37,47	95,96	137,16	9,33	60,81	368,77	0,53	3,94	4,51	0,17	1,21
ZGL	8/05/2002	0,11	0,27	0,33	1,34	0,62	1,63	3,71	4,78	0,29	2,16	14,06	0,2	116,86	4,79	0,02	0,67
ZLM	29/05/2000	5,08	3,19	32,81	32,98	14,68	34,39	57,19	60,03	5,9	26,1	248,58	0,61	36,01	58,45	0,08	8,87
ZLO	21/03/2002	0,17	0,21	1,85	4,83	2,5	7,17	15,71	19,14	1,49	9,46	58,32	0,04	0,18	7,53	0,02	0,57
ZWL	17/05/2001	1,36	0,56	11,92	22,25	13,27	37,06	70,74	77,48	4,98	29,38	250,2	0,27	10,49	6,34	0,34	3,24
ZWV1	9/05/2000	4,91	2,01	61,04	122,2	21,93	70,54	303,59	465,97	13,98	220,31	1248,54	1,01	48,34	0,89	0,08	18,7
ZWV1	9/04/2001	4,89	2,82	61,29	135,1	26,58	78,04	322,18	435,83	21,58	227,05	1264,43	0,43	9,31	10,07	0,9	24
ZWV1	3/10/2001	4,93	2,26	38,1	92,76	21,57	58,68	223,86	289,41	15,74	148,88	856,6	0,22	3,97	4,5	0,07	22,8
ZWV1	6/09/2004	0,79	0,3	7,37	9,85	5,05	15,07	37,56	68,11	3,42	24,3	163,05	0,07	0,09	0,04	0,04	2,54
ZWV2	9/05/2000	4,67	1,99	56,01	93,93	19,68	64,98	280,96	402,35	10,82	163,6	1066,5	0,41	28,6	4,81	0,06	13,1
ZWV2	6/09/2004	7,36	3,82	44,55	75,03	14,97	58,11	128,59	231,78	12,19	99,94	645,36	0,18	1,61	0,17	0,17	14,6
ZWV3	9/05/2000	6,82	4,31	70,68	96,45	29,52	79,17	217,79	292,26	17,8	125,15	888,31	0,57	31,06	6,87	0,07	13,6
ZWV3	8/09/2004	5,18	2,98	33,81	35,53	8,84	24,77	62,19	105,96	6,62	54,97	322,41	0,25	0,35	0,17	0,17	7,18
ZWV4	9/05/2000	3,55	2,12	61,04	83,63	24,56	64,95	213,05	279,26	16,15	130,99	836,47	0,42	21,48	6,13	0,05	9,77
ZWV4	6/09/2004	6,09	3,2	44,52	73,56	16,41	60,75	122,28	223,31	11,21	79,6	610,1	0,17	1,25	0,14	0,14	6,7
ZWV4	9/09/2004	8,91	3,54	57,17	110,3	16,63	69,93	155,4	278,21	9,95	110,47	790,36	0	3,15	0	0	7,94
ZWV5	23/05/2000	3,55	2,15	71,82	128	20,61	80,99	364,36	527	11,69	178,28	1354,05	0,63	25,72	0,38	6,06	10,7
ZWV5	9/09/2004	8,31	4,61	78,28	138,6	21,84	99,42	221,53	486,23	17,56	139,76	1172,13	0,21	0,73	0,18	0,18	7,34

Table II.2b. Means for some OCPs and heavy metals for eels from certain sites at specific sampling date sampled in the framework of the EPMN during the period 1994-2005. Concentrations are expressed in ng/g wet weight or - for contaminants marked with * - in µg/g wet weight.

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
A	22/10/1996	0,09	0,09	43,87	44,05	0,92	80	34	11,67						
A	20/06/2001	0,08	13,28	48,51	61,87	0,08	166	4,2	10	0,31	14,1	26	142		
AA	24/04/2002	0,13	25,69	165,3	191,12	0,13	371	18	27	0,76	15,2	230,9	310,2	68	360,7
AB	15/04/1998	0,85	93,21	33,03	127,09	0,9	33,33	82,67	44,83						
AB	3/05/2004	5,65	1,26	16	22,91	1,62	168,5	27,33	102,5	0,73	30,52	33,58	366,33	66	869,67
AB1	24/05/2000	0,07	7,25	48,51	55,83	0,07	107	4,78	42,4	0,37	21,62	126,4	233,8		
AB1	4/05/2004	10,1	1,42	26,05	37,52	1,11	115	54	6,5	0,38	18,04	17,5	45,5	57	777
AB1A	4/05/2004	6,21	0,22	27,32	33,75	0,45	75,33	8,08	12,78	0,35	16,52	108,52	395,7	52,61	794,53
AB2	24/05/2000	0,07	12,14	37,18	49,39	0,05	63,2	3,62	65,4	0,48	21,96	159	606,4		
AK0	7/08/2000	0,07	12,17	40,53	52,77	0,07	67,6	2	31,6	0,33	21,44	15,6	247,8	190	513
AK1	7/08/2000	0,1	12,45	37,49	50,04	0,1	48,4	2,3	19,2	0,27	22,3	14,6	142,2	377	322
AK2	7/08/2000	0,08	12,11	28,82	41,01	0,12	71,8	3,72	38,4	0,32	18,72	14	138,8	369	321
AK3	7/08/2000	0,04	6,49	16,7	23,23	0,04	33,6	2,36	254,8	0,44	18,9	17,4	191	324	300
AK4	8/08/2000	0,03	5,08	10,97	16,08	0,03	38,2	2,82	110	0,45	24,9	21	304,2	414	259
AK5	8/08/2000	0,02	1,46	5,46	6,95	0,02	44	12,5	121	0,32	21,52	28	356	305	263
AK6	8/08/2000	0,06	9,76	8,12	17,93	0,06	59,5	2	72	0,36	25,35	35	228	395	264
AK7	21/06/2000	0,06	13,02	16,49	29,57	0,06	47	2,2	57,5	0,43	26,6	47,75	149	198	790
AKK	6/06/1997	0,16	4,4	65,75	70,31	2,97	116,7	18,33	49,33						
AKL1	7/10/1999	0,08	33,31	75,42	108,81	1,12	48,75	10,58	106,15						
AKL1	4/10/2005	2,9	3,25	22,38	28,53	2,92	64,72	4	55,9	0,66	25,17	17,5	113,2	77,2	1526,6
AKL2	7/10/1999	0,05	31,54	46,23	77,83	1,74	68,53	2,03	45,5						
AKL2	4/10/2005	2,2	1,88	21,75	25,82	2,44	51,94	1,5	17,5	0,45	17,25	17,5	58	108,2	1303,6
AKL3	7/10/1999	0,1	46,7	97,35	144,15	1,01	117,2	2	24,8						
AKL3	4/10/2005	5,29	4,33	37,92	47,54	2,5	65,8	2	13,5	0,36	20,86	17,5	192,3	73,6	901,2

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
AKL4	7/10/1999	0,15	66,97	125	192,14	1,69	175,7	2,57	44,83						
AKL4	4/10/2005	2,57	10,98	33,32	46,87	1,83	77,03	1,5	6,5	0,4	22,94	17,5	155	57,67	697,33
AKL5	7/10/1999	0,08	34,13	51,64	85,85	0,79	64,05	2,85	16,3						
AKL5	3/10/2005	3,41	5,08	28,08	36,56	2,98	41,95	2,5	16,75	0,45	22,06	17,5	164,75	50,5	957
AKL6	7/10/1999	0,09	60,8	80,83	141,73	1,06	88	14,28	98,72						
AKL6	3/10/2005	2,59	21,1	23,17	46,86	2,62	60,98	3,1	13,4	0,46	21,95	17,5	144	50,8	936,4
AKL7	7/10/1999	0,1	50,42	67,77	118,28	0,54	106,5	11,76	49,38						
AKS	7/07/1999	0,06	10,91	19,59	30,56	0,06	43,45	5,6	67,5						
ASA	2/04/2002	0,06	13,91	28,53	42,51	0,06	63	34	12	0,41	16,1	86,92	81,86	41	601,3
ATB	21/06/2000	0,26	5,46	8,34	14,06	0,16	79,4	2,62	32,4	0,37	25,34	43,6	248,6	252	743
BB	12/09/1994	0,01	9,51	16,63	26,15	0,16	45	2,5	75						
BBO	24/05/2000	0,05	4,36	27,44	31,85	0,06	139	17,12	10	0,42	23,62	73,5	441,5		
BBU	2/08/2000	0,11	6,71	26,59	33,41	0,23	102,2	2,16	19,2	0,24	22,64	26	139		
BBV	27/06/2000	0,06	6,11	19,26	25,43	0,06	128,4	2	21,4	0,29	30,48	38,2	200,6		
BBV	4/06/2003	1,56	8,7	19,12	29,38	1,81	90,23	1,5	33,5	0,35	13,87	35,8	112,2	155,9	554,3
BEM	30/08/2000	0,77	5,95	19,56	26,27	0,81	95	12,8	10	0,69	20,1	50	146		
BEV1	14/06/2002	0,03	9,82	20,77	30,62	0,03									
BEV2	14/06/2002	0,12	39,31	85,47	124,9	0,12									
BGG	30/05/2000	0,08	58,75	177,7	236,54	0,08	149,4	2	10	0,34	24,18	75,6	574		
BGP	5/10/2001	0,08	46,07	97,92	144,08	0,08	174,3	2	10,6	0,31	26	29,6	146,4	358,4	373
BGX	2/04/2002	0,02	5,53	21,47	27,01	0,02	84	19,5	20	0,52	16,8	196,48	144,95	48	599,15
BK1	1/05/1994						89,92	40,7	214,74						
BK1	30/08/1994						55,83	21,17	146,35						
BK1	30/11/1994						70	15	269,25						
BK1	12/06/1995	2,39	14,93	27,2	44,51	0,85	52,3	5,05	134,3						
BK2	1/05/1994						58,73	38,98	230,43						
BK2	30/08/1994						57,5	12,42	91,44						

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
BK2	30/11/1994						54,25	17,67	132						
BK2	1/12/1994	3,07	17,21	29,34	49,63	1,2									
BK4	1/05/1994						48,86	37,43	371,29						
BK4	30/08/1994						45,18	9,53	135,65						
BK4	30/11/1994						39,14	5,65	148,96						
BK4	1/12/1994	4,5	16,53	22,16	43,18	0,8									
BK4	8/03/1995							8	63						
BK5	1/05/1994						51,17	28,6	343						
BK5	30/08/1994						98,85	8,58	162,16						
BK5	1/09/1994	5,31	19,96	26,11	51,38	1,06									
BK5	8/03/1995							2,5	50						
BL	4/12/1997	1,16	1,32	78,25	80,73	1,26	43,33	2,33	65,67						
BMD	30/05/2000	0,06	2,5	31,13	33,68	0,06	184,4	2,46	17,6	0,25	19,52	24,4	194,4		
BMD	11/10/2004	0,87	7,03	19,15	27,05	1,02	185,6	1,5	19,86	0,45	23,04	27,7	55,41	96,94	363,86
BND	15/06/2000	0,06	19,71	36,29	56,06	0,05	63,8	2,24	74,8	0,49	24,02	90,6	188,8	146	713
BOK	20/10/1998	0,15	3,93	13,68	17,75	0,15	80	3	12,5						
BRK	1/08/2000	0,05	2,36	18,14	20,54	0,05	49	2	68	0,24	17,15	20,5	209		
BRK	25/03/2003	2,05	16,31	24	42,36	1,18	72,06	5,31	29,69	0,32	15,9	53,25	135,75	116,38	372,88
BVW	29/05/2000	0,03	7,02	21,5	28,55	0,03	87,2	2	58,6	0,38	20,22	20,6	847,6	206	234
BWK1	4/09/2000	0,06	14,35	15,24	29,64	0,06	104,6	2	11,4	0,41	23,56	36	206,8		
BWK2	1/05/1994						47,1	40,32	298,89						
BWK2	30/08/1994						35,67	11,61	184,56						
BWK2	30/11/1994						49,38	12,64	134,45						
BWK2	1/12/1994	6,33	29,41	47,25	82,99	1,29									
BWK2	4/09/2000	0,07	23,58	27,79	51,44	0,07	23,4	2	123	0,43	27,54	37	245,8		
BWK3	4/09/2000	0,06	8,31	13,65	22,02	0,06	49,4	2,6	49,2	0,66	25,12	23,8	381,2		
COM	10/08/2001	0,56	9,6	33,46	43,63	0,06	114,5	4,85	73,9	0,4	20,15	34,71	190,67	169,19	205,62

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
DA	29/11/1994	4,66	55,43	111,3	171,35	5,33	123,3	2,72	31,06						
DA	18/09/1997	0,4	6,33	76,95	83,69	1,43	66,67	72	10,83						
DA	7/06/2003	4,49	49,58	54,92	108,99	11,6	50,1	3,38	106,75	0,4	16,77	49	143,5	384,75	674,25
DA1	3/10/2000	0,23	15,89	19,74	35,86	0,75	66,2	6,98	87,6	0,57	28,9	90	174,6		
DA2	3/10/2000	0,27	21,77	40,19	62,23	0,41	66	2	57,6	0,83	24,46	10	200,2		
DAM	16/05/2000	0,02	4,45	19,48	23,95	0,02	83,6	2,26	12,6	0,26	25,64	25,6	164		
DAV	14/06/1995	9,1	87,03	212	308,1	1,37	130	5	170						
DAV1	25/05/2000	0,13	61,19	116,7	177,97	0,13	95,6	2	10	0,26	23,38	45	121,2		
DAV2	25/05/2000	0,07	7,41	21,45	28,92	0,07	49	5,34	32,2	0,32	20,44	25,4	246,8		
DBR	18/05/1999	0,16	25,54	142,1	167,74	0,16	73,33	4	39,67						
DBU	28/05/2002	0,1	39,34	172	211,45	0,1	386	6,2	50,98	0,45	18,92	120,9	88,07	45,24	652,1
DE1	13/10/2000	0,02	19,42	32,26	51,7	0,02	69,5	2	46	0,88	31,8	58	695,5	227	195
DE1A	27/03/2002	0,12	42,44	106,3	148,87	0,3	95,5	12	16	0,3	30,35	109,08	73,78	33	411,9
DE2	16/10/2000	0,1	37,82	147,3	185,25	0,21	196,2	2	15	0,81	25,32	69,4	279,4	135	535
DE2	21/03/2005	5,07	1,4	62,98	69,45	7,49	92,14	9,55	15,66	0,38	21,55	16,44	248,66	43,52	435,28
DE3	16/10/2000	0,1	84,76	154,5	239,38	0,1	66	2	48	0,48	23,9	87	230	157	354
DE3	27/03/2002	0,06	25,58	67,36	93	0,06	75	16	19	0,83	22,3	433,2	95,46	33	404,2
DE3	21/03/2005	6,15	2,84	143,7	152,65	3,79	256,4	3,59	5,14	0,86	30,1	11,3	287,2	59,67	385,2
DE3	22/03/2005	17,5	4,97	104,3	126,72	9,76	77,2	5,5	11,25	0,43	34,9	17,5	189,5	56,5	563,5
DE3A	27/03/2002	0,22	30,24	46,24	76,7	1,18	20	10,33	30	0,63	26,83	446,57	177,13	72	530,97
DE3B	28/03/2002	0,08	38,14	67,3	105,51	2,83	123,5	8	24,5	0,63	24,2	440,65	106,26	48	439,05
DE3B	22/03/2005	3,92	2,81	55,66	62,38	3,06	99,43	6,45	12,43	0,57	21,07	31,82	451,76	86,07	623,97
DE3C	28/03/2002	0,12	30,92	75,69	106,74	0,35	80	9,4	21	0,42	26,02	386,98	103,85	50,5	463,41
DE3C	2/10/2003	6,28	22,13	53,82	82,23	4,85	72,35	4,46	39,56	0,3	16,56	29,31	50,13	63,81	381,59
DE4	13/10/2000	0,06	49,19	51,88	101,12	0,06	62,6	4,52	15,4	0,62	28,64	46,2	305,6	41	398
DE4	22/03/2005	0	4,17	43,14	47,31	0,68	27,43	1,17	10,81	0,8	20,76	9,25	487,68	171,11	780,55
DE4	23/03/2005	5,04	7,66	59,02	71,73	4,03	83,42	3,83	18,45	0,6	22,5	26,26	362,98	85,35	712,17

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
DE4A	28/03/2002	0,06	29,74	65,1	94,9	0,07	75,38	15	28,5	0,76	30,29	674,88	163,42	67,62	545,81
DE4A	1/10/2003	1,7	1,6	23,22	26,51	2,91	38,61	1,73	7,77	0,61	19,81	21,34	79,37	84,84	718,89
DEM1	13/04/1999	0,12	22,08	78,18	100,38	0,12	20	2	60						
DEM1	29/10/2001	0,1	24,78	69,18	94,06	0,1	129	2	10	0,41	33	10	126	833	548
DEM1	4/09/2003	7,74	17,07	28,99	53,8	2,21	68,45	8,5	30	0,6	14,7	9	157	92,5	897
DEM2	13/04/1999	0,12	42,64	75,59	118,34	0,12	51	9,4	52						
DEM2	2/09/2003	36,5	68,4	48,31	153,17	21,55	97,9	4,67	8,67	0,65	25,13	10,33	130,67	84	939
DEM3	13/04/1999	0,18	75,62	204,6	280,4	0,18	40	6	27						
DEM3A	9/04/2003	22,3	63,92	85,23	171,44	16,58	116,3	6	19,75	0,56	14,85	28,5	207,5	132,5	2419
DEM4	13/04/1999	0,05	16,98	51,08	68,11	0,1	90	4	71						
DEM4	9/04/2003	14,1	26,6	27,89	68,62	0,03	81,8	12	15	0,82	25	18	107	130	1670
DEM5	13/04/1999	0,05	9,79	32,55	42,39	0,05	50	4	33						
DEM6	2/09/2003	15,1	56,67	50,73	122,51	8,17	117,9	5,5	41,25	0,62	28,45	12	152	142,5	1414,5
DGH	12/07/2000	0,46	6,15	30,7	37,31	0,77	184,2	2	10	0,31	35,28	238,6	174,2		
DIJ1	27/04/1999	0,14	35,28	67,57	102,99	0,14	122,5	6,2	63,5						
DIJ1	6/05/2003	16,7	30,6	37,77	85,09	22,54	107	7	17	0,55	13,1	13	338	89	1610
DIJ2	28/04/1999	0,1	31,25	79,51	110,87	0,1	475	2	30						
DIJ3	27/04/1999	0,08	21,87	56,19	78,14	0,08	311,3	5,37	38,87						
DIJ4	28/04/1999	0,15	22,77	65,19	88,11	0,15	280	2	78						
DIJ4	7/05/2003	12,2	46,76	26,03	85,01	14,45	253	10	79	0,65	28	5	114	83	1345
DIJ5	28/04/1999	0,16	31,68	86,6	118,45	0,39	340	2	59						
DIJ6	27/04/1999	0,14	35,93	139,2	175,31	0,14	190,5	11,15	22,05						
DIJ6	7/05/2003	12,2	44,38	27,45	84,01	11,17	113	6	67	0,54	20,9	5	195	187	1873
DIJ6A	8/05/2003	12,9	35,29	30,53	78,68	7,31	131,3	14,33	35	0,57	15,2	25,33	135,67	192	2414
DIJ7	27/04/1999	0,1	35,65	65,81	101,56	0,1	436	10,15	53,45						
DIJ8	27/04/1999	0,05	30,14	82,27	112,47	0,07	108,3	29,35	29,98						
DIJ8	8/05/2003	3,66	14,68	32,65	50,98	3,24	82,4	8	6,5	0,34	1,2	5	135	122	1519

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
DO1	7/05/1998	0,57	16,45	68,76	85,78	0,11	53,33	33,67	47,83						
DO1	23/05/2000	0,06	7,07	40,43	47,56	0,06	58,2	2,04	12,4	0,34	26,4	105	274,2	260	470
DO1	21/04/2004	4,77	4,36	18,22	27,36	0,68	72,99	10,82	45,86	0,79	23,69	97,04	164,09	120,44	692,16
DO1A	22/04/2004	5,25	0,67	11,9	17,82	0	69,83	9,43	13,06	0,41	17,1	225,3	282,7	71,98	649,8
DO2	23/05/2000	0,17	19,24	59,11	78,51	0,09	307,4	50,86	33,6	0,4	31,12	56	280,8	167	857
DO2	21/04/2004	3,08	0,07	16,42	19,57	0,77	166	23	21	0,47	17,12	41	593	102	1155
DSS	2/08/2000	0,18	24,18	49,15	73,52	0,73	113	2	92	0,35	22,98	27	184,6		
DUH	7/04/2004	0	0	28,96	28,96	1,24	48,16	2,03	35,79	0,43	18,53	30,62	235,06	119,11	1313,2
DUH	28/04/2005	1,53	19,63	25,68	46,84	3,78	34,48	2,4	24,6	0,63	14,86	17,5	320,8	183,2	1248
DUL	29/08/2000	0,48	49,51	95,15	145,14	0,06	217	2,54	126,2	0,4	22,82	201,4	194,8		
DUO	7/04/2004	1,95	19,07	30,94	51,96	4,94	42,15	10	16,25	0,6	21,82	17,5	227,5	223,5	1106,5
DUZ	8/04/2004	2,97	10,09	31,04	44,1	3,34	82,02	20,25	51,58	0,51	16,14	22,23	152,25	178,6	878,25
DUZ	27/04/2005	3,08	11,19	38,31	52,58	0,7	64,13	10,07	31,14	0,42	17,26	44,69	206,15	134,43	1026,9
ED1	28/05/2002	0,09	19,59	73,24	92,92	0,09	259	9,32	62,84	0,69	19,56	523,1	149,2	60,28	730,9
ED2	28/05/2002	0,12	25,07	82,23	107,42	0,12	129,8	10,95	143,65	1,7	28,88	216,8	103,84	55,19	672,68
EEND	26/10/2005	1,18	0,13	21,3	22,61	0,43	450,3	11,81	17,85	0,41	27	6,76	191,9	38,54	529,35
FOO	16/05/2000	0,04	9,3	64,69	74,03	0,04	148	2,14	10	0,32	26,74	66,4	353,8	225	209
FSA	21/06/2000	0,09	10,62	22,24	32,95	0,09	104	2	57,4	0,31	24,52	49,4	218	249	76
FWW	10/05/2000	0,01	4,24	33,92	38,17	0,01	258,2	5,1	36,6	0,5	28,6	40,8	415,8	225	24
FWW	30/09/2003	0,57	2,91	9,28	12,76	0,25	148,7	1,5	47,2	0,37	19,26	14,4	222,7	69,4	151
GAG	19/07/2000	0,11	1,76	9,68	11,55	0,17	110	2,34	60,8	0,29	18,96	23	814,4	292	316
GB	23/05/1995	0,04	26,63	101,4	128,04	2,33	88	4,38	69,5						
GB1	10/07/2000	0,12	14,57	20,77	35,47	0,09	59,6	2,08	75,4	1,2	19,2	77,2	632,4	135	329
GB1	16/06/2003	0,66	3,34	10,27	14,27	0,84	130	4	6,5	0,41	23,5	5	189	69	265
GB2	28/06/2000	0,08	5,2	28,01	33,28	0,14	93,6	2,4	27,6	0,32	20,52	63,6	255,2	198	218
GBO	5/06/2002	8,51	14,59	32,46	55,56	1,54	139	68,24	69,7	0,67	15,91	237,55	290,83	219,95	816,65
GBR	12/07/2000	0,15	64,03	90,15	154,33	0,1	31,6	2	49	0,4	22,22	24	228,6		

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
GGE2	10/12/2004	15,2	2,95	41,45	59,63	13,93	319	7	6,5	0,47	13,72	17,5	228	78	2141
GGZ	28/08/2000	1,06	16,73	42,31	60,09	1,07	99	5,06	55	0,48	24,9	54,4	99,2		
GHN	21/09/2000	0,19	11,52	30,72	42,44	0,81	124,6	14,72	42,8	0,57	30	112,4	168,4		
GHN	27/09/2005	0	0,34	11,02	11,36	0,88	187,5	21,04	27,26	0,58	29,72	14,97	132,26	91,03	531,86
GN1	14/06/2000	0,15	17,18	59,48	76,81	0,08	58,6	20,4	11,2	0,36	22,46	146,8	1238	239	305
GN2	14/06/2000	0,12	36,02	61,85	97,98	0,12	194,6	3,56	14,2	0,51	32,9	35,8	977,4	180	957
GN2	19/03/2003	12,8	67,11	77,35	157,26	2,58	126,1	7,4	15,3	0,46	15,7	27,6	201	61,8	1085,6
GN2A	19/03/2003	22,6	38,92	56,96	118,49	1,87	208,7	10	9,55	0,43	21,52	25,2	272,9	107,3	1248
GN3	14/06/2000	0,06	20,52	48,81	69,39	0,06	122,5	3,5	60	0,42	30,8	540	252	159	811
GN3	19/03/2003	16,7	27,16	73,34	117,22	1,49	172,5	8,5	22,5	0,45	18,3	23	187	86,5	1091,5
GN4	18/03/2003	14,9	33,53	54,74	103,12	2,29	177,5	13,17	35,17	0,52	22,08	52,17	163,83	104,83	1232,8
GPG	5/05/2000	0,03	1,25	7,22	8,51	0,02	66,2	2	38,2	0,33	19,74	41,4	217,2		
GPG	27/10/2000	0,05	10,96	21,84	32,86	0,45	76,4	2	52,4	0,79	25,94	110	564,2		
GS1	23/05/2002	0,1	17,63	35,89	53,63	0,1	62,5	14,37	43,1	0,49	15,95	74,36	128,45	87,88	698,2
GS2	23/05/2002	0,13	25,92	45,67	71,72	0,13	77,12	8,56	17,73	0,51	16	140,16	133,99	78,41	796,39
GSK	21/09/2000	0,56	5,4	19,28	25,24	1,12	93,4	2	90,8	0,51	25,58	213,4	1636		
GVZ	1/08/2000	0,07	15,54	32,04	47,65	0,07	535,4	2	75,2	0,32	23,74	11,6	168,8	120	126
GW	19/05/1998	0,18	29,7	87,78	117,66	0,08	350	17	22						
GWA	17/05/2000	0,07	3,72	20,41	24,2	0,07	117,2	3,16	24,2	0,47	31,12	42	254,6		
GWA	12/10/2005	0,34	2,15	5,21	7,7	0,33	62,58	2	22,7	0,5	25,99	21,4	221,4	98,2	790,8
GZ	20/09/1996	0,03	1,75	103,1	104,9	1,43	103,3	4	29,83						
GZ	23/09/2002	1,8	9,99	28,9	40,69	1,91	35,36	7,04	31,43	0,99	47,93	385,08	87,08	105,64	736,53
HBB1	8/05/2000	0,13	20,71	45,72	66,56	0,13	49,67	2	10	0,28	20,73	62	409		
HBB3	8/05/2000	0,14	19,62	75,3	95,07	0,14	77	2	74,6	0,32	27,3	40	189,6		
HBB4	8/05/2000	0,17	56,51	42,09	98,77	0,09	102,5	4,1	17,5	0,14	13,25	10	83		
HBN	29/05/2002	0,03	19,08	37,86	56,96	0,03	98	10,08	75,72	0,79	31,27	274,7	218,2	87,21	1335
HDO	28/08/2000	0,05	5,8	20,68	26,52	0,06	221	2,26	71,6	0,47	25,34	54,4	302,4		

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
HDO	23/09/2003	0,47	9,94	32,28	42,69	0,47	149,6	1,5	17,83	0,3	26,67	50,5	77,53	19	110
HEL	20/04/2001	4,36	14,39	20,87	39,62	2,01	49,64	42,42	67	0,42	10,63	75,2	97,4		
HER2	9/09/2003	15,6	58,87	123,4	208,05	11,46	135,5	3,56	37,44	0,58	25,86	45,25	242	40,75	1198
HGK	5/05/2000	0,34	19,94	44,64	64,92	0,19	126,8	2,86	16,4	0,21	18,58	50,2	232,8		
HGK	1/08/2000	0,13	5,18	21,52	26,83	0,18	112	4,83	14,67	0,39	24,17	20	192		
HGK	25/03/2003	2,41	14,41	19,74	36,57	0,93	111,1	4,5	26,5	0,39	18,82	21,6	192,4	79,4	667,2
HO	18/09/1997	0,06	1,56	80,31	81,93	0,75	134	2,6	17,8						
HV2	4/11/2002	11,3	60,79	52,89	125,02	2,68	47,46	10,09	38,82	4,16	25,17	1080,9	1277,9	50,09	663,34
HVG	5/05/2000	0,09	8,94	22,13	31,16	0,09	76,4	2	15,6	0,37	26,8	45	218,4		
HVH	5/10/2001	0,05	29,2	32,23	61,48	0,05	63,5	2	10	0,34	17,1	21	150	453	370
HVH	4/11/2002	6,5	54,84	49,19	110,52	2,15	40,82	10,82	38,46	1,29	23	321,28	423,63	48,12	711,76
HVX	4/11/2002	30,8	62,75	17,65	111,18	3,22	93,69	16,2	28,46	2,11	28,3	1058,2	478,44	71,09	691,33
HZW	10/05/2000	0,3	3,49	23,68	27,47	0,11	94	2	53,2	0,36	34,1	28,4	160,8		
IB1	7/06/2001	0,09	5,68	26,67	32,43	0,04	65,82	3,24	26,8	0,32	12,86	45,8	206,8		
IB1	1/06/2005	10,2	6,64	21,79	38,64	0,84	117,6	11,12	5,8	0,65	17,71	47,08	261,1	119,73	464,22
IB2	6/06/2001	0,2	9,62	35,79	45,62	0,06	58,3	9,4	78,67	0,36	13,13	39,67	176,67		
IB2	1/06/2005	3,35	0,45	21,28	25,08	0,56	121	8	6,5	0,53	18,49	17,5	365,5	63,5	378,5
IBK	24/05/2000	0,07	4,32	31,01	35,39	0,05	95,4	7,62	35,6	0,46	22,22	37,4	458,6	165	496
IJ	3/04/1998	1,66	14,55	16,76	32,97	0,1	150	45	49,25						
IJ1	10/03/2005	11,5	1,12	29,05	41,7	3,65	109,3	2,25	6,5	0,61	27,13	17,5	225,5	172	766,5
IK1	10/07/2000	0,1	31,55	59,73	91,37	0,07	139,4	2,58	26,8	0,39	23,78	38,8	285,6		
IK1	9/09/2002	9,58	23,5	47,89	80,96	3,8	112,7	71,35	78,7	1,02	43,6	511,46	270,19	163,45	590,21
IK1A	9/09/2002	5,39	31,4	56,35	93,14	3,78	116	25,05	47,63	1,11	54,58	332,77	180,89	67,27	614,75
IK1B	9/09/2002	17,9	27,96	57,43	103,33	4,18	165,5	33,02	64,17	1,32	131,7	493,26	130,76	137,73	881,62
IK1C	12/09/2002	1,56	23,01	37,91	62,48	2,92	112,7	16,22	37,38	0,84	55,08	138,9	135,18	138,06	572,85
IK2	14/05/2001	0,08	29,25	52,83	82,16	0,08	150,9	2,98	76,4	0,32	14,13	28,8	185,8		
IK2A	9/09/2002	3,35	41,51	78,15	123,02	6,04	109,3	27,82	52,86	1,18	102,7	349,15	155,17	117,2	885,74

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
IKX	9/09/2002	6,35	36,53	2,97	45,85	4,57	103,6	24,8	31,81	1,12	58,44	254,22	312,32	94,61	711,19
JBS	30/05/2002	0,13	14,5	36,2	50,83	0,11	72,7	12,31	44,34	0,52	17,8	111,14	114,78	76,73	671,54
JEK	12/03/2002	0,15	21,89	60,42	82,45	0,15	57,67	4,33	10,67	0,52	21,27	151,56	71,86	34,33	849,17
KAL	15/06/2003	9,8	100	205,7	315,46	1,74	135,5	5,45	45,39	1,84	38,11	203,79	351,81	656,47	976,43
KB1	15/10/1997	0,05	1,77	45,75	47,57	0,8	45	128	179						
KB1	3/11/1999	0,08	55,47	101,8	157,32	0,08	40,6	122,2	39,4						
KB1	25/10/2005	1,52	1,11	24,97	27,59	0,78	45,25	27,12	78,52	0,8	28,94	28,28	214,83	187,92	2444,9
KB2	3/11/1999	0,08	92,41	334,8	427,26	0,1	86,93	656,3	111,58						
KB2	19/06/2000	0,04	12,34	30,95	43,33	0,04	27,8	68,4	22,6	0,43	23,2	145,8	391,6	235	504
KB2	27/10/2005	4,34	8,96	26,25	39,54	0,75	40,4	93,4	64,2	0,44	25,42	17,5	382,2	250,6	1961,8
KB3	3/11/1999	0,18	42,95	120	163,1	0,1	41,55	151,4	279,33						
KB3	25/10/2005	3,47	1,12	50,05	54,63	0,74	114,3	36,77	38,21	0,5	15,97	3,57	161,8	161,1	1304,7
KB4	3/11/1999	0,12	30,63	111,7	142,42	0,03	188,2	33,1	172,5						
KB4	27/10/2005	1,39	1,06	29,21	31,66	0,37	70,26	74,88	80,17	0,43	19,97	12,49	123,89	202,28	1684,7
KB5	3/11/1999	0,07	25,52	92,37	117,96	0,04	59,1	63,25	352						
KB5	25/10/2005	3,21	3,64	29,32	36,16	0,56	92,15	50,43	258,47	0,49	19,37	18,13	207,79	185,16	1708,4
KB6	3/11/1999	0,08	27,69	66,73	94,5	0,08	90,87	284,9	516,3						
KB6	19/06/2000	0,06	17,4	44,37	61,83	0,06	42,8	61,84	327,8	1,37	24,54	148,4	172,6	320	739
KB6	27/10/2005	6,61	3,33	38,81	48,75	3,62	145,2	83,6	439,2	0,42	26,52	17,5	183,6	151	2029,4
KB6A	25/10/2005	2,62	4,02	33,27	39,92	1,4	152,5	33,57	57,75	0,59	30,14	22,17	243,2	211,06	1143,9
KB7	3/11/1999	0,18	29,58	90,58	120,34	0,12	220,8	20,78	86,04						
KB7	26/10/2005	6,11	1,77	18,16	26,04	1,77	146,8	12,75	39,25	0,48	23,56	17,5	184,5	78,25	1068,5
KBH1	8/10/1996	0,08	3,95	41,38	45,4	0,92	97,5	52,75	54,38						
KBH1	7/10/2002	21,3	22,51	36,01	79,86	1,89	55,25	70,13	70,4	0,57	26,85	184,75	249,69	416,87	659,21
KBH1A	7/10/2002	16,7	32,73	39,68	89,11	2,75	41,35	29,44	29,58	0,66	38,88	62,28	121,8	514,35	723,85
KBH1B	7/10/2002	11,4	12,29	38,49	62,19	2,72	58,31	20,99	97,36	1,64	64,02	268,23	141,7	866,83	946,93
KBH1C	8/10/2002	11,6	11,7	26,77	50,03	4,13	59	14,59	52,85	0,84	42,56	239,59	151,48	512,99	589,35

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
KBH1D	8/10/2002	5,24	17,93	47,54	70,71	3,43	94,63	13,26	99,69	1,3	38,96	268,6	176,86	349,44	758,17
KBH2	8/10/1996	0,15	9,59	76,06	85,8	3,18	50	7	12,5						
KBH2	9/10/2002	6,78	10,17	26,01	42,97	2,19	146	89,06	93,68	0,45	20,9	162,9	222,7	173	730
KBH2A	7/10/2002	3,11	8,89	25,69	37,69	2,01	85,1	36,17	36,84	1,3	33,54	396,2	274,3	311,1	830,5
KBH3	8/10/1996	0,08	4,26	33,57	37,91	1,71	136,7	44,67	95,17						
KBH3	10/10/2002	12,7	12,15	28,96	53,8	2,31	99,42	87,04	94,64	0,57	27,94	191,02	254,85	268,35	656
KBH4	7/10/2002	10,6	23,71	51,61	85,89	5,25	93,77	24,21	65,51	1,61	71	326,7	179,57	479,6	1045,4
KBH5	7/10/2002	13,2	19,65	59,42	92,28	4,35	104,2	11,44	73,78	2,03	53,15	485,32	198,04	356,93	752,02
KBL	9/04/2001	0,25	35,9	81,06	117,21	0,1	113,6	29,93	1272,4	0,29	17,99	34	159,4	97	1790
KBL	5/10/2001	0,1	49,9	112,6	162,55	0,1	142,2	31,45	1744,2	3,4	21,62	151,45	123,55	249	1581
KBR	7/05/2002	0,09	40,5	63,42	104,01	0,09	102,7	22,67	27,67	1,15	17,7	1209,7	339,7	123	966,47
KBR1	26/09/1997	0,1	1	56	57,09	0,1	45	2,5	73						
KBR2	23/09/1997	0,13	49,36	77,66	127,15	0,13	16,67	2	77,67						
KBW	29/05/2000	0,09	2,04	11	13,13	0,09	55,25	2	10,75	146,3	21,05	2139	575,75	127	270
KDS1	10/09/1999	0,16	48,79	72,83	121,78	0,14	283,5	42,75	133						
KDS1	15/09/2003	29,3	6,74	52,62	88,65	5,37	175,9	23,2	26,2	0,55	28,6	17,5	194,4	242,4	645,4
KDS2	10/09/1999	0,15	60,46	109,6	170,17	0,15	205	10,3	41,1						
KDS2	15/09/2003	4,73	13,27	21,2	39,21	2,7	83,25	5,95	36,5	0,57	16,75	82,5	148	70,5	341
KDS3	20/11/1998	0,11	29,09	51,9	81,1	0,1	320	10,33	56,17						
KDS3	15/09/2003	8,41	23,37	21,44	53,22	3,22	80,78	6,17	41,67	0,45	16,91	34,78	297,22	153,22	500,56
KDS4	10/09/1999	0,02	18,2	29,03	47,25	0,02	161,5	8,8	47,8						
KDS4	15/09/2003	6,8	35,46	31,23	73,49	2,72	122,2	6,53	43	0,47	18,02	29,83	403,33	246,33	536
KDS4A	15/09/2003	27,8	21,02	23,21	72,07	1,35	120,2	10,59	26,4	0,39	16,8	39	217,8	99	339,2
KDS5	10/09/1999	0,13	113,2	176,2	289,52	0,11	333,8	17,06	108,82						
KDS5	15/09/2003	6,85	19,57	19,14	45,57	3,19	79,01	7,04	39,38	0,54	23,41	46,12	238,88	195,38	349,62
KDS6	10/09/1999	0,1	66,07	58,96	125,13	0,07	127,3	6,63	85,07						
KDS6	17/09/2003	35,1	19,98	24,05	79,1	1,36	95,45	11,57	86	0,38	25,51	92,9	295	148,6	558,5

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
KDS6A	17/09/2003	5,34	25,63	21,96	52,93	2,24	87,53	5,98	32,9	0,46	19,47	77,8	93,1	289,6	766,6
KDS7	10/09/1999	0,26	50,8	92,84	143,9	0,14	355	13,1	57,27						
KDS7	17/09/2003	10,3	48,85	56,47	115,6	7,24	114,2	9,85	78,8	0,4	21,92	97	383	312,4	489,5
KDS7A	15/09/2003	2,87	12,8	14,98	30,65	1,08	92,88	8,12	19,8	0,16	16,5	115,6	73,8	142,8	674
KDS7B	15/09/2003	8,77	21,07	28,65	58,49	5,95	109,4	11,99	98,57	0,25	24,97	90,14	114,29	215,14	584,14
KDS8	20/11/1998	0,06	46,97	95,12	142,15	0,06	183,3	7,33	32,5						
KDS8	15/09/2003	19,6	62,05	57,37	139	6,57	87	15,44	48	0,53	30,22	73,2	147	299,6	471
KG	3/06/1997	0,14	0,14	31,04	31,33	0,14	10	2	12,5						
KGO	17/09/1998	0,13	44,91	89,77	134,81	1,04	26,67	2	61,67						
KGO	27/09/2004	5,07	5,12	44,95	55,15	1,06	47,19	0,85	11,52	0,52	22,92	22,13	194,13	93,62	1583
KGO	28/09/2004	1,46	2,87	68,26	72,58	0									
KGO	29/09/2004	3,39	4,95	45,5	53,84	0,78	39,2	0,99	10,16	0,45	18,07	3,08	161,82	82,78	1491,1
KGO1	17/05/2000	0,1	67,34	138,2	205,6	0,26	74	7,72	12,8	0,38	21,42	65,6	109,4		
KGO2	27/09/2004	1,87	8,08	56,32	66,26	1,47	51,6	2,9	14,28	0,51	20,24	24,29	334,71	46,19	949,44
KGO3	28/09/2004	1,46	2,87	68,26	72,58	0	53,93	2,05	31,06	0,3	14,36	51,71	464,86	61,34	1415,4
KGT	17/06/1998	0,74	50,48	67,94	119,15	0,13	50	7,6	172,1						
KKB	19/11/1997	0,08	1,02	152,9	154	2,28	110	21,67	28,5						
KKB	27/10/2003	3,31	161,1	221	385,41	9,48	85,7	15	41	0,41	25,4	40	261	57	1195
KLB	23/05/2002	0,14	46,78	56,19	103,11	0,18	79,2	10,45	17,88	0,59	17,03	189,93	115,1	67,26	615,16
KLD1	28/11/1994	24	23,58	72,45	120,03	2,03	104,3	2,95	32,9						
KLD1A	23/10/2001	0,08	7,52	51,4	59	0,08	94,44	2,46	11	0,27	21,94	39,8	138,2	215,6	630,6
KLD1A	17/06/2002	0,1	13,66	70,73	84,49	0,16	78,35	9,26	30,1	0,86	26,03	2086,9	162,37	65,62	384,55
KLD2	11/10/1996	0,13	0,13	65,19	65,46	3,77	56,67	2,33	12,5						
KLD2	23/10/2001	0,08	10,87	54,77	65,72	0,08	63,14	2	18,6	0,34	24,94	19,4	137,2	288,6	607,4
KLD2A	13/06/2002	0,12	13,74	55,72	69,58	0,2	86,77	11,82	74,99	0,84	22,56	1565,7	153,39	60,58	515,71
KLD3	7/02/2002	0,13	23,12	107,6	130,89	0,83	150	6	33	0,92	34,7	291,2	94,34	119	412,4
KLD4	7/02/2002	0,15	47,25	152,9	200,26	0,15	66	9	56	1,51	76	616,5	157,9	37	446,9

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
KLD4	23/05/2002	0,27	8,59	42,99	51,85	0,81	83,34	24,77	235,99	0,7	25,68	1241,6	177,85	212,31	544,25
KLD4	19/06/2002	0,08	7,72	54,65	62,45	0,16	91,23	8,63	127,68	1,14	19,89	3887,6	337,74	199,26	446,5
KLD4	26/06/2002	0,08	8,35	55,5	63,93	0,18	91,39	11,73	65,5	0,79	20,64	1950,2	182,03	93,55	454,01
KM	12/06/1995	2,86	10,52	58,53	71,91	0,44	63,59	2,7	106,85						
KN1	2/10/1996	0,07	2,57	45,11	47,76	1,22	96,67	2,33	33,17						
KN1	13/06/2000	0,25	27,35	67,65	95,25	0,08	130,5	13,58	10	0,36	23,62	316,5	933	243	676
KN1	4/04/2002	0,15	15,44	45,6	61,18	0,03	127,1	12,67	63,89	0,76	16,62	211,47	150,85	107	774,2
KN2	13/06/2000	0,09	21,13	47,34	68,57	0,07	83,4	6,06	20,6	0,43	24,44	71,4	329,2	189	571
KN2	19/03/2003	14,7	24,97	35,05	74,68	1,51	72,94	9,4	31,98	0,53	23,4	75,6	248,8	80,69	957,4
KN2A	18/09/2003	10,4	16,42	39,83	66,65	1,02	98,59	25,2	19,85	0,49	19,16	112,2	251,8	50,8	1137,6
KN2B	16/09/2003	22	0,67	58,81	81,53	0,1	116,8	10,52	23,65	0,32	16,91	140,75	311,62	50,65	648,36
KN2B	9/06/2004	16,6	0,96	59,67	77,27	0,07	109,2	9,39	12,36	0,3	15,96	118,23	303,12	81,78	669,52
KN2C	25/09/2003	16,6	23,78	58,89	99,27	1,4	116,2	9,1	15,9	0,38	19	59,3	226,2	77,2	814,2
KN3	13/06/2000	0,08	15,95	39,25	55,28	0,08	85,8	22	10	0,34	21,72	73	549,8	132	584
KND1	17/06/1999	0,1	21,9	47,2	69,21	0,1	36,67	9,33	24,67						
KND1	14/09/2005	0,92	10,34	16,57	27,83	1,39	51,1	1,5	13,5	0,45	24,14	45,9	273	264	595,4
KND2	17/06/1999	0,01	10,65	23,67	34,32	0,06	182	7,75	34,25						
KND2	12/09/2005	4,97	0,52	22,57	28,06	1,29	83,42	3,7	43,8	0,77	34,26	39,3	246,2	255,6	596,8
KNN	24/10/2000	0,1	13,46	31,87	45,43	0,29	146,7	2	82,33	0,5	24,5	40,33	1397,3		
KNN	6/11/2002	1,25	13,15	24,3	38,7	0,92	75,82	6,06	20,25	1,91	26,31	708,53	867,08	91,38	684,51
KOO	10/06/1999	0,67	3,39	12,73	16,79	1,39	50	4,67	34						
KRL	13/10/1998	0,07	21,79	90	111,86	0,07	96,67	2	21,33						
KRL	13/09/2004	2,1	21,05	59,41	82,56	2,5	66,35	3,75	70	0,73	37,51	73,5	242,5	114	1275
KRO	15/09/1999	0,02	45,41	171,4	216,78	0,04	64,86	2,06	59,46						
KSE	6/11/1998	0,08	48,67	113,6	162,31	0,08	160	2	25,17						
KVK	2/08/2000	2,77	43,22	98,68	144,68	0,44	157,2	5,92	38,2	2,13	23,08	114	298,4		
KZ	24/09/2002	1,07	12,17	45,85	59,09	2,6	22,12	6,89	25,14	0,82	40,33	69,07	176,59	126,75	502,55

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
L	4/07/1996	0,17	5,79	71,59	77,55	3,62	60	2	40,25						
L	23/06/2003	4,52	33,6	36,66	74,78	7,02	63,02	6,1	161,25	0,52	32,03	140	166,8	142,3	2382,8
LAA	25/05/1998	0,19	9,51	37,65	47,35	0,14	110	177	25,17						
LAA	3/05/1999	0,01	24,32	25,73	50,07	0,01	230	90	125						
LAA2	25/04/2002	0,07	27,24	22,9	50,21	0,07	192	9	17	0,56	11,6	209,4	192,1	128	1468
LAN	7/06/1999	0,14	8,4	68,48	77,02	0,08	100	2,5	37						
LE1	19/09/2000	0,13	92,57	119	211,71	0,13	58,8	9	78,6	0,69	23,84	74	136	176	1647
LE1	1/06/2001	0,12	24,48	49,12	73,72	0,12	140,4	2,93	19,33	0,42	22,38	169,83	128,5	50	1075
LE1	26/10/2001	0,11	32,23	49,66	82	0,11	143	6,52	209,4	0,97	25,12	150	133	424	1707
LE1	23/06/2003	5,28	28,93	27,29	61,5	4,01	83,16	7,4	94,6	0,69	26,94	32,2	185,4	92	3031,8
LE2	19/09/2000	0,15	37,12	50,29	87,56	0,15	81,2	6,28	233,8	0,57	26,96	35,4	160,4	139	1865
LE2A	23/06/2003	8,44	42,17	30,88	81,49	11,01	52,42	5,1	28,08	0,85	33,33	52,6	189,68	90,05	1351,5
LE3	19/09/2000	0,14	39,2	46,11	85,45	0,14	211	9,75	313	0,74	32,85	62	135	153	2590
LE4	26/09/2000	0,14	33,43	54,75	88,32	0,14	58,33	2	34	0,64	28,73	37,67	121	165	1752
LE4A	25/06/2003	10,6	6,13	57,1	73,8	3,35	44,16	5,56	17,63	0,61	26,57	17,5	165,99	85,63	1265,2
LE5	26/09/2000	0,06	31,41	31,78	63,25	0,06	41,8	2	60,4	0,67	28,22	10	266,6	730	925
LE5	25/06/2003	2,56	24,08	20,17	46,81	2,22	60,26	4,85	94,2	0,47	27,27	111,4	170,7	92,8	2334,1
LE6	23/06/2003	4,13	41,63	33,72	79,49	4,81	115,3	5,31	109,25	0,48	24,89	24,38	178,75	100,88	2215,3
LEO	3/10/1997	0,16	3,4	127,2	130,78	0,16	56,67	2,33	61,83						
LEO	6/10/2003	1,46	10,25	26,59	38,3	0,66	104,3	2,25	34,5	0,31	19,8	57	164	96	382,75
LEO1	3/10/2000	0,11	20,09	76,02	96,23	0,34	88,2	7,4	10	0,65	24,1	58,4	140,6		
LEO2	3/10/2000	0,14	21,28	59,9	81,32	0,14	85,2	2	100,2	0,47	25,8	63	106,4		
LEO2	6/10/2003	3,69	21,99	50,77	76,45	0,87	136,2	2,95	13,7	0,28	19,73	18,9	95,3	58,1	274,1
LEV	10/06/1999	0,05	2,11	8,1	10,26	0,05	106,7	7	27,33						
LEV	14/05/2003	0,96	5,76	9,54	16,26	2,76	176,9	20,8	25,4	0,32	17,62	67	121	105,8	448
LEV	8/07/2003	0,51	0,06	9,31	9,88	1,86	155,8	5,9	9,9	0,59	21,16	17,5	116,5	117,22	484,88
LEY	21/06/2001	0,07	4,28	17,06	21,41	0,07	101	2	15	0,3	22,6	45	180		

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
LIB	31/08/2000	0,01	0,87	5,74	6,62	0,14	67,5	2	10	0,53	16,27	38,5	226		
LLS	18/03/2002	0,05	19,28	55,37	74,7	0,05	260,8	5,83	17,17	0,55	19,52	147,41	120,3	144,67	204,67
LO	8/04/1998	5,73	167,1	66,98	239,77	0,13	60	26	12,5						
LWV	27/06/2000	0,6	6,63	34,55	41,78	0,28	166	2	74	0,31	16,5	39	177		
MA1	30/08/2000	0,04	2,13	14,97	17,14	0,03	140,6	16,4	10	0,71	25,94	42,8	949,4	263	1081
MA1	15/05/2002	0,04	4,43	15,86	20,33	0,04	147,9	19,86	16,57	0,59	27,26	114,95	171,83	71,86	619,81
MA1	2/06/2005	2,63	1,74	6,38	10,75	1,09	124,5	24	199,67	0,67	33,56	82,33	223	236,33	1774,7
MA2	20/06/2000	0,08	6,42	27,86	34,36	0,03	227,6	31,12	39,2	0,59	33,02	114,2	153,2	168	743
MA2	15/05/2002	0,06	6,92	21,31	28,28	0,17	198,8	27,3	47,8	0,8	24,95	218,9	180,67	100,4	764,71
MA2	19/05/2005	12,2	6,81	18,17	37,2	6,36	162	26,41	20,31	0,87	28,26	57,21	52,04	268,99	1178,9
MA2A	15/05/2002	0,13	9,71	31,48	41,32	0,1	138,2	34,2	75,42	1,25	34,08	2944,7	183,95	118,38	1193,3
MA2B	14/05/2002	0,09	8,84	28,52	37,45	0,1	182,2	23,6	58,9	0,58	30,9	684,71	257,54	85	649,49
MA2B	19/05/2005	11	3,69	17,43	32,09	3,68	158,9	16,74	33,83	0,6	20,87	27,65	127,83	119,51	1042,3
MA3	22/06/2000	0,08	8,32	31,41	39,82	0,08	182	25,36	27,6	0,32	22,46	78,8	146,2	733	488
MA3	14/05/2002	0,1	7,56	26,7	34,35	0,1	143,8	17,75	31,5	0,53	24,28	167,83	144,83	91,75	464,38
MA3	18/05/2005	9	0,07	16,03	25,1	3,75	158,6	29,83	18,36	0,61	25,67	35,54	147,3	185,86	798,01
MA3A	14/05/2002	0,13	11,17	32,59	43,89	0,13	135,9	20,2	14,9	0,64	23,13	339,49	210,45	128,4	593,43
MA3A	18/05/2005	7,06	4,76	14,2	26,02	2,61	176,8	32,41	13,38	0,7	30,65	39,57	106,35	177,46	1138,9
MA3B	14/05/2002	0,14	10,51	31,08	41,72	0,14	133	24,6	25,2	0,42	33,28	176	158,44	139,8	493,78
MA3B	18/05/2005	9,4	0,1	20,13	29,64	5,24	149,8	44,77	43,72	0,71	27,51	31,15	398,76	196,91	1089,3
MA3C	13/05/2002	0,09	7,95	28,29	36,33	0,1	187,2	42,67	89,89	0,5	32,17	182,39	168,7	101,78	597,1
MA3C	17/05/2005	5,52	0,06	17,26	22,84	2,63	154,1	22,33	46,22	0,88	33,22	35	336,56	166,78	1067,2
MA3D	13/05/2002	0,08	7,57	23,76	31,42	0,08	104,9	21,7	140,5	0,52	32,45	441,79	167,28	144,1	487,02
MA3D	17/05/2005	5,98	0,09	18,47	24,54	3,99	146,7	12,9	33	0,63	29,87	39	311,3	391,1	864,1
MA3E	13/05/2002	0,08	6,52	20,31	26,91	0,13	172,5	19,3	75,5	0,43	32,79	376,53	195,06	158,2	507,67
MA4	16/10/1997	1,14	7,23	47,83	56,2	2,31	56,67	2	31,5						
MA4	22/06/2000	0,08	8,59	32,98	41,65	0,07	144,2	5,36	83,6	0,36	23,84	28	197,2	321	342

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
MA4	17/05/2005	6,36	6,07	16,54	28,97	3,46	157,1	15,9	79,4	0,7	29,84	44,45	302,7	234	992,6
MBE	17/05/2000	0,06	10,85	43,57	54,48	0,02	157,4	2,02	71	0,31	21,86	66,8	293		
MNB	3/04/2002	0,66	143,1	152,8	296,58	0,17	84,5	24,67	39,83	0,54	15,25	222,93	179,7	97,17	857,93
MOT	28/09/1999	0,11	70,62	301,8	372,54	0,06	84,75	3,6	28,4						
MOT	4/03/2003	62,6	80,13	222,8	365,56	10,58	138	9,5	32,5	0,36	25,25	46	137	44,5	565
MSG	30/05/2000	0,06	26,03	51,13	77,23	0,06	151,4	2,02	13	0,32	25,72	94,4	243,6		
MV	4/06/1996	0,13	0,13	71,43	71,68	2,86	36,67	5,33	138,67						
MV	24/04/2003	4,25	22,9	33,84	60,98	1,8	72,73	32,33	214,67	0,76	26,57	50	116,33	61,33	1086,7
MVD	29/05/2000	0,07	26,14	66,68	92,89	0,07	144,6	13,38	33,8	0,35	25,46	47,8	1554,2	140	204
MVD	24/04/2003	2,88	30,25	56,4	89,52	0,66	110,1	7,85	99,5	0,31	23,59	52,3	158,9	102,7	385,4
MVG	23/04/2003	6,93	25,58	56,81	89,33	1,14	115,4	1,67	41,44	0,4	20,22	39	90,11	63,11	655,22
MVX	8/05/2002	0,15	51,86	71,04	123,05	0,15	62	17,5	24	0,61	13,95	131,54	150,85	74,5	404,9
NB	4/06/1997	0,14	0,14	18,01	18,28	2,66	33,33	3	29,5						
NEK	27/06/2000	0,08	4,8	12,81	17,69	0,08	98,6	2,4	10,8	0,35	25,4	65	199,8		
NEK	4/06/2003	0,93	5,83	10,7	17,45	1,1	103,1	1,9	15,35	0,3	25,39	65	143,5	264,8	448,2
NGO	27/06/2000	0,08	1,96	8,3	10,34	0,08	71	2,52	31,8	0,31	26,46	24,8	165,4		
NKE	13/06/2000	0,14	9,36	13,2	22,71	0,09	49,8	2,08	10	0,4	32,16	22,4	350,2	182	718
NKE	20/03/2003	6,1	13,72	12,53	32,34	2,39	66,5	9,9	85,6	0,23	19,34	83,5	172,4	286,1	992,4
NP0	19/09/2001	0,13	27,67	65,89	93,69	0,13	49,28	2,16	13,2	0,44	19,68	31,6	150,4	318	521,8
NP1	24/10/2000	0,13	43,35	94,74	138,23	0,13	59	3,92	10	0,53	26,17	25,25	330		
NP1A	19/09/2001	0,73	34,52	83,93	119,18	0,13	45,44	2,64	10	0,37	22,14	15,4	188,6	382	577,4
NP1B	20/09/2001	0,11	32,63	63,85	96,59	0,11	36,15	2	14	0,35	22,08	13,75	184,25	147,5	655,25
NP1C	20/09/2001	0,12	16,77	48,86	65,75	0,12	87,73	3,3	15	0,4	16,9	17	189,67	129	424
NP2	24/10/2000	0,91	44,89	60,54	106,34	0,43	52,4	7,16	11,8	0,49	23,38	37	200,8		
NP2	20/09/2001	0,04	29,72	36,6	66,37	0,04	42,1	2	10	0,33	19,98	33,2	238,2	171,8	473,8
OAV	7/06/1999	0,15	12,88	43,17	56,2	0,15	96,67	5,33	33,33						
OAV	25/04/2001	0,08	9,14	24,2	33,42	0,1	85,97	8,26	70	0,29	16,45	53,9	140,2	50	210

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
OAV	24/10/2001	0,05	7,07	27,94	35,05	0,05	122,4	2,38	31,89	0,5	18,27	214,33	226,67	548	195
ODU	10/05/1999	0,1	138,6	402,5	541,18	0,1	80	4	65,33						
ODU	24/10/2002	2,38	76,3	190,7	269,43	0,36	149	4,22	101,39	2,02	22,13	1275	342,54	96,37	709,28
OLA	16/08/1999	0,05	28,08	61,03	89,16	0,06	174,6	13,16	28,99						
OLA	18/04/2000	0,07	24,49	34,47	59,04	0,07	91,2	3,62	10	3,37	22,7	26,6	140,6		
OLBH	5/07/2000	0,03	11,05	42,06	53,15	0,02	146,2	8,06	13,6	0,46	26,04	62,2	278,8		
OLBV	4/07/2000	0,1	25,79	74,35	100,24	0,08	105,2	5,34	38,2	0,3	19,78	51,2	200		
OLD	22/08/2000	2,01	54,76	54,75	111,51	0,47	121,8	2,66	10	0,47	26,52	49,2	363,2		
OLEV	4/07/2000	0,05	36,39	57,96	94,4	0,05	197	6,6	57,8	0,32	21,74	74	240,6		
OLG	10/05/1999	0,02	10,49	61,11	71,63	0,02	163,3	4,67	24						
OLG	19/09/2005	2,55	6,7	42,98	52,23	2,43	146,3	4,32	130,25	0,37	21,74	45,72	253	100,84	672
OLLW	14/10/1997	11,8	4,11	97,6	113,48	0,12	60	4,5	49,5						
OLLW	5/07/2000	0,04	4,81	12,77	17,61	0,04	139,6	7,4	11,2	0,46	20,12	39	262,6		
OLM	22/08/2000	14,9	55,03	127,1	196,99	0,75	50	2,4	16,2	0,58	25,16	23,6	179,4		
OLO	30/05/1997	0,12	5,38	111,4	116,92	1,87	133,3	4,33	24,17						
OLO	16/06/2004	2,03	5,93	24,17	32,14	0,73	190,5	2,85	64,3	0,27	16,56	26,48	219,2	46,35	529,2
OLOE	16/08/1999	0,03	20,42	49,74	70,19	0,03	86,54	18,91	45,48						
OLSW	5/07/2000	0,03	32,19	53,65	85,87	0,09	104	6,8	10	0,42	25,3	73,5	137		
OMD	16/09/2002	1,28	7,4	20,21	28,9	0,67	69,17	70,08	99,45	0,54	23,62	243,76	113,79	161,35	498,67
OMS	29/09/1997	1,54	8,88	43,92	54,34	0,07	80	8,67	40,42						
OMS	2/09/2002	6,61	3,99	45,36	55,96	3,16	113,4	74,93	80,09	0,44	23,17	129,75	78,99	131,26	949,44
OOS	23/09/1998	0,09	2,54	12,12	14,75	0,09	100	2	12,5						
OPK	1/08/2000	0,19	20,56	41,08	61,84	0,55	45,8	4,38	71,8	0,35	25,66	96	156,2		
OPK	25/03/2003	3,46	30,19	34,68	68,34	2,91	56,02	15	29,4	0,31	25,36	83,8	193,8	53,8	570,6
OSA	16/03/1998	0,09	55,26	274,7	330,04	0,09	286,7	8,67	36,33						
OSB	24/07/2000	0,31	19,09	27,42	46,82	0,01	83,8	2,2	95,8	0,43	22,28	29	310,6		
OSD	21/08/2000	4,18	192,3	483,7	680,25	1,15	39	3,16	55,8	0,46	22,72	78,2	222		

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
OSE	24/07/2000	0,06	5,84	11,36	17,27	0,9	316,8	4,38	40,6	0,34	26,48	44,6	226,6		
OSG	26/07/2000	0,07	3,69	21,08	24,84	0,07	154,4	2,58	13,6	0,3	19,2	22	144,6		
OSH	24/07/2000	0,11	52,55	97,39	150,05	1,13	145,6	2	100,6	0,25	20,68	19,4	201,2		
OSK	24/07/2000	8,62	74,54	423	506,11	1,97	109,6	46,08	115,4	0,41	27,84	50,8	218,2		
OSK	23/10/2002	6,62	24,77	84,1	115,49	3,66	260,4	79,63	78,99	0,62	27,41	177,28	237,66	227,7	598,28
OSM	19/07/2000	0,19	2,95	17,92	21,06	0,01	176,4	2	10	0,25	28,08	20	159,4		
OSME	26/07/2000	2,12	45,19	170,2	217,51	0,31	100,6	2	99,6	0,32	16,58	10	147,8		
OSME	21/10/2000	1,37	164,1	937,3	1102,74	0,58	214	2	14,6	0,89	24	46,2	183,8		
OSN	24/07/2000	0,88	28,48	62,28	91,64	0,2	337,6	4,74	22,2	0,26	23	14,2	206,6		
OSS	26/04/2000	0,1	51,14	243,6	294,82	0,1	63	2	19,8	0,46	22,52	139,8	181,8		
OSSK	26/07/2000	0,8	53,45	143,7	197,9	0,37	75	6,72	82	0,46	19,5	50,6	144,8		
OSSZ	16/03/1998	0,2	11,14	98,66	110	1,22	296,7	2	24,83						
OST	26/07/2000	0,46	31,54	76,28	108,28	0,14	145,4	18,56	58,6	0,44	20,32	37,6	230,4		
OSV	19/07/2000	0,28	42,31	123	165,61	0,7	205,6	3,72	67,8	0,31	21,64	178,6	305,4		
OSZ	21/08/2000	2,03	16,66	51,24	69,93	0,76	90,4	7,78	23,2	0,56	23,72	63,6	173,4		
OSZ	18/10/2001	0,07	14,16	54,06	68,29	0,07	72,62	2,84	20	0,31	20,8	119,4	148,8	359	339
OVA	30/09/2003	3,85	0,77	21,45	26,07	0	196,9	2,73	23,81	0,39	17,09	75,95	185,2	40,27	337,72
PAL	22/09/1999	0,11	14,34	51,14	65,59	0,11	110,8	7,14	27,7						
PDV	28/06/2000	0,14	8,96	23,27	32,38	0,14	60,8	17	15	0,38	18,54	37,4	132,4		
PDV	15/05/2003	0,49	7,66	8,77	16,92	2,6	73,09	3,88	25	0,4	18,88	52,75	196,25	57,62	543,25
PN	19/09/1997	1,11	0,32	45,74	47,16	0,71	340	2	12,5						
PO	10/06/1998	0,14	155,9	158,9	314,89	0,08	115	17,5	12,5						
PRI	4/02/2000	0,05	25,28	72,11	97,44	0,04	170,3	12,92	46,58						
PV1	6/05/2002	0,16	45,01	61,48	106,64	0,16	122	15	28	0,6	10,5	529,4	261,2	101	531,8
PV2	6/05/2002	0,16	68,09	120,1	188,3	0,16	121,5	24	56,5	2,4	14,1	2885,5	599,9	85	560,5
RHD	16/05/2000	0,07	28,41	67,2	95,68	0,07	81,4	6,88	33,6	0,3	26,18	20,6	330,2		
RLK	1/08/2000	0,09	8,53	32,27	40,89	0,09	114	2	170	0,27	24,8	10	123		

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
RLK	27/03/2003	2,29	8,71	19,66	30,66	2,23	77,15	8,25	30,25	0,41	17,7	50,25	152	67,5	643
RM	28/09/2001	0,04	5,84	20,7	26,57	0,04	112,3	2,44	10	0,33	16,14	377	167,14	394,43	557,86
ROG	15/09/1999	0,04	34,19	85,14	119,37	0,04	46,38	3,16	20,58						
S1	1/06/1995						120	4,33	75,33						
S2	29/04/1998	0,19	36,87	65,09	102,16	0,11	70	9	21						
SC	10/01/1995	3,75	42,36	51,67	97,79	2,9	77,67	2,5	78,33						
SCH	29/04/1998	0,44	54,97	246,7	302,11	0,3	293,3	4,67	35,83						
SCH1	28/09/2000	0,15	38,56	62,5	101,21	0,15	147	2	11	0,69	35,2	91	134	165	771
SCH3	28/09/2000	0,1	30,8	76,95	107,85	0,13	59,25	2	78,5	0,8	33,72	76,25	211,75	243	667
SCH3A	4/06/2002	0,13	94,64	50,47	145,23	0,93	39,42	9,75	48,31	0,62	33,65	709,9	170,81	37,16	687,68
SCH3B	4/06/2002	0,12	84,83	214,9	299,82	0,12	159	11,56	41,19	0,48	70,71	191,3	71,19	67,59	746,4
SCH3C	4/06/2002	0,45	57,25	62,97	120,67	0,73	73,89	10,31	62,13	0,6	30,89	686,46	162,28	43,45	670,28
SCH4A	6/06/2002	0,12	34,33	58,78	93,22	0,76	44,81	11,77	40,4	0,67	29,09	520,93	148,56	33,02	670,54
SCH4B	4/06/2002	0,08	32,47	62,56	95,11	0,08	233	15,68	38,4	0,77	30,45	769,8	146,6	61,52	624,4
SCH6	10/10/2000	0,53	54,66	149,4	204,63	1,49	142	2	95,33	0,7	23,83	37,33	135,33	257	913
SCH7	10/10/2000	0,08	32,21	48,62	80,9	0,08	91,2	3,96	10	0,58	19,74	28	139	229	1064
SCH7A	24/08/2004	1,07	8,32	16,79	26,17	2,21	74,18	4,07	44,1	0,48	16,38	25,11	143,6	474,3	1187
SCH8	26/09/2000	0,08	39,43	57,94	97,45	0,08	114	3,72	17,4	0,41	19,22	29,8	180,6	254	1166
SCH9	26/09/2000	0,07	22,04	42,64	64,75	0,05	118,2	2	65,6	0,65	25,3	54,8	179	704	1556
SGS	28/06/2000	0,1	8,22	19,73	28,06	0,63	93,5	2	70,5	0,36	18,5	19	253,5		
SK	1/10/2001	0,09	3,51	37,12	40,72	0,09	176,5	2	10	0,32	17,7	22	162,5	196	262,5
SM	12/10/1999	0,03	6,37	33,33	39,72	0,03	75,78	15,38	22,24						
SVW	10/05/2000	0,36	2,94	20,22	23,95	0,49	164,6	2,86	29,8	0,36	21,84	65,2	161,6		
SVW	2/10/2003	0,95	1,2	8,74	10,89	0,67	165,5	2	34,3	0,29	28,6	61,8	198,3	93,3	629,2
TB	26/06/1997	0,11	2,58	64,94	67,64	2,18	43,33	4,33	28,17						
VAM	12/07/2000	0,74	4,85	20,57	26,16	0,16	204,2	2	10	0,46	28,04	48,4	353,6		
VEL1	11/04/2000	1,13	37,3	120,7	159,15	2,47	65,23	5,93	48	0,59	17,93	40,33	158,67	104,67	688,33

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
VEL1	2/03/2004	16,4	69,8	231,6	317,85	12,96	90,3	4,97	18,2	0,59	34,74	17,5	200	58,04	553
VEL2	10/04/2000	1,45	63,07	261,6	326,13	0,11	140,5	2,3	10	0,62	23,35	80,5	120	103	497
VNX	3/04/2002	0,46	29,6	102,4	132,51	0,07	83,5	4	21	0,38	12,45	101,04	118,4	67,5	663,05
VR	1/05/1995	0,16	26,32	78,02	104,51	2,67	190,5	2,5	93						
VVK	24/10/2000	0,13	7,3	10,53	17,96	0,32	117	2	67,5	0,64	23,25	172,5	867,5		
WA	15/10/1997	0,1	4,11	39,09	43,3	0,69	50	4,33	20,33						
WA	19/04/2004	2,68	2,18	9,55	14,41	0,46	155,2	24,16	20,16	0,62	18,76	144,03	257,6	70,64	740
WBA	23/05/2000	0,1	5,53	30,28	35,92	0,06	121,6	2,04	13,2	0,4	28,44	90,4	239,4		
WBV4	5/10/2004	1,85	2,47	39,74	44,07	6,84	79,13	3,92	22,12	0,73	34,9	29,74	233,75	75,78	726,55
WBV5	5/10/2004	2,42	5,9	70,37	78,68	4,11	39,58	3,66	33,66	0,82	21,53	16,43	311,48	66,76	1096,8
WBV6	14/10/2002	5,17	27,33	56,45	88,96	6,15	46,34	7,79	146,79	1,49	67,23	357,38	230,9	175,89	871,41
WBV6	2/12/2002	4,25	34,45	61,75	100,44	7,18	50,41	4,9	32,34	1,49	23,53	2166,3	1957,5	233,07	878,07
WBV6	13/05/2003	6,04	4,16	55,04	65,24	9,31	99,83	4,7	26,45	0,73	40,98	17,5	296,15	129	890,9
WBV7	14/10/2002	8,58	53,44	88,42	150,45	8,03	66,7	9,07	87,06	3,18	34,6	1391,8	350,23	1142,4	543,17
WBV8	17/10/2002	4,61	33,4	74,05	112,06	4,13	80,51	6,26	172,26	2,73	16,68	1609,1	300,79	575,36	486,85
WEE	18/09/1997	0,06	14,57	310,5	325,13	1,66	283,3	7,67	40,5						
WEE	1/10/1998	0,18	287,6	741,1	1028,91	0,11	538,8	9,75	46						
WEE	3/05/1999	0,57	306,1	635,4	942,11	0,44	430	8,75	108,14						
WEE	18/10/2000	0,22	31,22	55,95	87,39	0,13	360,1	2	89,2	0,42	18,26	42,6	256,7	355	432
WEE	10/04/2001	0,03	36,01	79,8	115,85	0,03	330,3	9,21	159,8	0,27	18,77	50,2	233,2	180	345
WEE	9/10/2001	0,02	34,33	82,45	116,79	0,02	278,4	3,95	131,91	0,81	17,85	138,36	216,73	272	177
WEE	9/12/2002	5,45	30,76	59,4	95,61	1,52	293,8	3,82	37,73	1,12	22,05	1274,8	2133,9	99,64	563,73
WEE	13/10/2003	1,25	22,85	27,04	51,14	2,01	277,8	1,75	149,4	0,28	23,12	45,8	224,2	55,5	145,6
WEE	12/10/2004	0	2,34	31,71	34,05	0,11	245,3	1,81	22,34	0,44	21,08	20,58	167,24	53,56	405,56
WEE	5/09/2005	0	1,27	34,57	35,84	0,35	195,5	1,5	28,09	0,55	26	19,01	159,82	137,36	338,61
WIK	6/06/2001	0,14	25,61	93,57	119,32	0,14	71,2	2	40	0,33	16,65	13	132,5		
WIN1	29/09/1999	0,07	32,24	94,31	126,62	0,07	56,7	7,9	215						

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
WIN2	29/09/1999	0,14	106,4	447,3	553,89	0,14	104	4,1	21,2						
WL	21/04/1997	0,1	7,19	61,96	69,25	10,43	273,3	73	52,17						
WLL	16/05/2001	0,08	30,08	78,88	109,03	0,08	64,04	2,38	46	0,89	11,44	91,8	144		
WMX	4/04/2002	0,23	19,27	51,97	71,47	0,1	77,57	5,14	14,29	0,57	15,96	169,38	177,66	52,29	376,69
WNR	26/08/2003	6,17	3,19	19,8	29,16	0,64	140,8	7,9	11	0,46	27,55	28,55	215,1	74,9	785,9
WSD	30/05/2000	0,08	34,72	93,87	128,66	0,07	130,4	2,06	16,8	0,29	32,66	17,6	165,8		
WSD	30/09/2002	3,16	32,85	68,82	104,83	1,05	53,37	61,93	80,85	0,56	31,46	435,27	816,33	252,69	935,98
WWB	27/06/2000	0,13	6,74	12,88	19,75	0,27	97,6	2	28,6	0,28	27,9	43,4	135,4		
YZ1	10/07/2000	0,23	29,07	53,61	82,9	0,14	245,2	2,9	51,8	0,37	25,76	46	171		
YZ1	14/06/2005	6,83	13,96	38,3	59,09	2,79	161,3	6,34	6,5	0,5	14,45	17,5	201,8	100,58	1073,6
YZ2	10/07/2000	0,1	29,12	56,89	86,11	0,08	194,6	2,78	22	0,31	27,36	19,2	146,6		
YZ3	10/07/2000	0,05	21,86	56,67	78,57	0,04	112,8	3,3	40,4	0,33	23,3	54,4	242,8		
ZB	14/03/2002	0,1	6,5	34,87	41,46	0,1	113	13	7	0,4	16,2	130,1	87,72	20	413,7
ZBR	11/05/2001	0,17	10,53	51,2	61,9	0,17	80,23	2	82	0,36	19,9	11	144,33		
ZGL	8/05/2002	0,02	2,76	8,98	11,76	0,02	227	9	29,5	0,51	17,3	111,74	189,85	42,5	376,25
ZLM	29/05/2000	0,08	27,57	55,6	83,26	0,25	95,2	2,74	42,4	0,65	26,52	49,2	363,8		
ZLO	21/03/2002	0,02	12,47	37,4	49,88	0,08	100	11	13	0,49	17,3	121,8	59,55	60	493
ZWL	17/05/2001	0,24	15,04	56,58	71,86	0,14	83,48	2,2	43,8	0,3	14	22,4	172,2		
ZWV1	9/05/2000	0,08	6,94	40,26	47,28	0,12	149,6	2	10	0,42	26,34	42,4	268,6	263	600
ZWV1	9/04/2001	0,58	17,7	44,28	62,56	0,43	100,9	2,3	39,9	0,47	15,85	54,3	131	125	161
ZWV1	3/10/2001	0,07	9,09	31,78	40,95	0,07	121,9	8,47	48,1	0,29	20,66	44,2	176,1	107	283
ZWV1	6/09/2004	1,47	0,79	10,54	12,8	1,78	201	1,5	6,5	0,47	13,1	17,5	139	135,2	1077
ZWV2	9/05/2000	0,06	10,32	37,56	47,93	0,06	145	2,96	40,4	0,53	27,42	41,8	204,4	214	332
ZWV2	6/09/2004	4,59	10,22	25,84	40,65	7	72,96	3,17	14,14	0,43	17,19	17,5	177,4	195,66	624,8
ZWV3	9/05/2000	0,07	11,39	40,3	51,76	0,07	119,8	8,14	108,6	0,38	22,76	61,4	360,4	191	519
ZWV3	8/09/2004	1,61	1,53	14,63	17,78	2,98	66,8	4,06	21,91	0,3	12,73	17,5	198	89,4	786
ZWV4	9/05/2000	0,06	12,77	35,37	48,2	0,05	131,6	6,72	172,4	0,44	31,3	19,6	210,6	70	974

Code	Date	DDT	TDE	DDE	Sum DDT's	T-NONA	Hg	Cd	Pb	Cu*	Zn*	Ni	Cr	As	Se
ZWV4	6/09/2004	3,82	9,06	25,59	38,48	5,9	81,04	8,15	17,59	0,71	15,72	51,06	452,6	196,29	750,73
ZWV4	9/09/2004	2,44	10,25	45,62	58,31	0	74,4	5,32	40,28	0,34	14,73	17,5	172	152,2	813
ZWV5	23/05/2000	0,26	6,28	43,46	50	0,08	120,6	4,32	40,6	0,33	26,66	70,2	227,6	243	587
ZWV5	9/09/2004	9,45	12,02	46,55	68,02	9,71	76,7	13,46	21,79	0,5	20,72	17,5	346	529,7	541

Annex III

List of publications

Peer reviewed papers in international journals/books

Audenaert, V., Huyse, T., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2003. Spatio-temporal dynamics of the nematode *Anguillicola crassus* in Flanders, Belgium. *Diseases of Aquatic Organisms* 56, 223-233.

Belpaire, C., Ollevier, F., 1987. L'anguille, son histoire et son élevage*. Cahiers d'Ethologie appliquée* 7, 85-106.

Belpaire, C., De Charleroy, D., Thomas, K., Van Damme, P., Ollevier, F., 1989. Effects of eel restocking on the distribution of the swimbladder nematode *Anguillicola crassus* in Flanders, Belgium*. Journal of Applied Ichthyology* 5, 151-153.

Belpaire, C., Ollevier, F., 1990. The European eel (*Anguilla anguilla* L.): an endangered species in Flanders? *Belgian Journal of Zoology*, 2, 6-7.

Belpaire, C., Van Driessche , H., Gao, F.Y., Ollevier, F., 1992. Food and feeding activity of glasseel *Anguilla anguilla* (L.) stocked in earthen ponds. *Irish Fisheries Investigations*, series A (Freshwater) 36 (1992), 43-54

Belpaire, C., Smolders, R., Vanden Auweele, I., Ercken, D., Breine, J., Van Thuyne, G., Ollevier, F., 2000. An Index of Biotic Integrity characterising fish populations and ecological quality of Flandrian water bodies. *Hydrobiologia* 434, 17-33.

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 : Proceedings of the International Symposium 'Advances in Eel Biology' (Aida et al., Eds), University of Tokyo, 28-30 September 2001, 170-174

Belpaire, C., Goemans, G., 2007. Eels: contaminant cocktails pinpointing environmental contamination. *ICES Journal of Marine Science* 64, 1423-1436

Belpaire, C., Goemans, G., 2007. The European eel (*Anguilla anguilla*) a rapporteur of the chemical status for the Water Framework Directive? *Vie et Milieu – Life and Environment* 57 (4), 235-252.

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,* in press.

Belpaire, C., Goemans, G., Geeraerts, C., Quataert, P., Parmentier, K., Hagel, P., De Boer, J., submitted. Decreasing eel stocks: The Survival of the Fattest? *Ecology of Freshwater Fish*

Belpaire, C., Geeraerts, C., Evans, D., Ciccotti, E., Poole, R., in prep. The European Eel Quality Database: towards a pan-European monitoring of eel quality.

Bilau, M., Sioen, I., Matthys, C., De Vocht, A., Goemans, G., Belpaire, C., Willems, J.L., De Henauw, S., 2007. Probabilistic approach to polychlorinated biphenyl (PCB) exposure through eel consumption in recreational fishermen vs. the general population. *Food Additives & Contaminants* 24 (12), 1386-1393.

Breine, J., Simoens, I., Goethals, P., Quataert, P., Ercken, D., Van Liefferinghe, C., Belpaire C., 2004. A fish-based index of biotic integrity for upstream brooks in Flanders (Belgium). *Hydrobiologia* 522, 133-148

Breine, J.J., Maes, J., Quataert, P., Van den Bergh, E., Simoens, I., Van Thuyne, G., Belpaire, C., 2007. A fish-based assessment tool for the ecological quality of the brackish Schelde estuary in Flanders (Belgium). *Hydrobiologia* 575, 141-159

De Charleroy, D., Thomas, K., Belpaire, C., Ollevier, F., 1989. The viability of the free living larvae of *Anguillicola crassus*. *Journal of Applied Ichthyology* 5, 154-156.

De Charleroy, D., Grizez, L., Thomas, K., Belpaire, C., Ollevier, F., 1990. The life cycle of *Anguillicola crassus*. *Diseases of Aquatic Organisms* 8, 77-84.

De Schepper, N., Laceur, S., Christiaens, J., Van Liefferinge, C., Herrel, A., Goemans, G., Meire, P., Belpaire, C., Adriaens, D., 2007 submitted. Variation in cranial morphology of the European eel: broad- and narrow-headedness. *Biological Journal of the Linnean Society*

Denayer B., Belpaire C., 1996. Bottle-necks for restoration of the eel population, *Anguilla anguilla* (L.) of the river Yser basin (Flanders). *Archives of Polish Fisheries* 4, 175 - 186.

Fain, A., Belpaire, C., 1985. A new mite (Acari, Anoetidae) parasitizing the gills of young eels *Anguilla anguilla* (L.). *Bulletin et Annales de la Société royale belge d'Entomologie* 121, 285-292

Geeraerts, C., Ovidio, M., Verbiest, H., Buysse, D., Coeck, J., Belpaire, C., Philippart, J-C., 2007. Mobility of individual roach *Rutilus rutilus* (L.) in three weir-fragmented Belgian rivers. *Hydrobiologia* 582, 143-153

Geeraerts, G., Belpaire, C. in prep. A review of the effects of contaminants on European eel.

Goemans, G., Belpaire, C., 2003. The Flemish eel monitoring network : PCB-concentrations in eel from the Meuse catchment area, 2003. In : First International Scientific Symposium on the River Meuse, Proceedings, 58-62.

Goemans, G., Belpaire, C., 2004. The eel pollutant monitoring network in Flanders, Belgium. Results of 10 years monitoring. *Organohalogen Compounds* 66, 1834-1840.

Goemans, G., Belpaire, C., 2005. Congener profiles in European eel (Anguilla anguilla L.) as a method of tracing the origin of PCB contamination. *Organohalogen Compounds* 67, 1304-1307.

Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C., Ollevier, F., 1989. Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Drancunculoidea) and subsequent migration of larvae. *Diseases of Aquatic Organisms* 7, 97-101

Hoff, P.T., Van Campenhout, K., Van de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., Goemans, G., Belpaire, C., Blust, R., De Coen, W., 2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium) : relationships with biochemical and organismal effects. *Environmental pollution* 137, 324-333.

Ide, C., De Schepper, N., Christiaens, J., Van Liefferinge, C., Herrel, A., Goemans, G., Belpaire, C., Adriaens, D., 2007. A non-invasive analysis of head shape dimorphism in a European eel population (*Anguilla anguilla*). *Journal of Morphology* 268, 1088.

Maes, G.E., Raeymaekers, J.A.M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., Volckaert, F.A.M., 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology : Relationship between heavy metal bioaccumulation, condition and genetic variability. *Aquatic Toxicology* 73, 99-114.

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.

Morris, S., Allchin, C., Zegers, B., Haftka, J., Boon, J., Belpaire, C., Leonards, P., Van Leeuwen, S., De Boer, J., 2004. Distribution and Fate of HBCD and TBBPA Brominated Flame Retardants in North Sea Estuaries and Aquatic Food Webs. *Environmental Science & Technology* 38, 5497-5504.

Roose, P., Van Thuyne, G., Belpaire, C., Raemaekers, M., Brinkman, U., 2003. Determination of VOCs in yellow eel from various inland water bodies in Flanders (Belgium). *Journal of Environmental Monitoring* 5, 876-884 .

Roosens, L., Dirtu, A., Goemans, G., Belpaire, C., Gheorghe, A., Neels, H., Blust, R., Covaci, A., 2008. Brominated flame retardants and polychlorinated biphenyls in fish from the River Scheldt, Belgium. *Environment International,* in press.

Schabuss, M., Konecny, R., Belpaire, C., Schiemer, F., 1997. Endoparasitic helminths of the European eel, *Anguilla anguilla*, from our disconnected meanders from the rivers Leie and Scheldt in western Flanders, Belgium. *Folia Parasitologica* 44, 12-18.

Simoens, I., Breine, J., Verreycken, H., Belpaire, C., 2002. Fish stock assessment of Lake Schulen, Flanders: a comparison between 1988 and 1999. In: Cowx, I., *et al.* (eds.), Management and ecology of lake and reservoir fisheries. Fishing News Books, Oxford, 46-57.

Van Campenhout, K., Goenaga Infante, H., Goemans, G., Belpaire, C., Adams, F., Blust, R., Bervoets, L., 2008 in press. A field survey of metal binding to metallothionein and other cytosolic ligands in liver of eels using an on-line isotope dilution method in combination with size exclusion (SE) high pressure liquid chromatography (HPLC) coupled to Inductively Coupled Plasma time-of-flight Mass Spectrometry (ICP-TOFMS). *The Science of the Total Environment*, 2008, doi:10.1016/j.scitotenv.2008.01.026.

Verreycken, H., Anseeuw, D., Van Thuyne, G., Quataert, P., Belpaire, C., 2007. The non-indigenous freshwater fishes of Flanders (Belgium): review, status and trends over the last decade. *Journal of Fish Biology* 71 (Supplement D), 160–172

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium. *Environmental Pollution* 128, 363–371.

Peer reviewed papers in national journals/books

Belpaire, C., Van Thuyne, G., Maes, J., Denayer, B., Beyens, J., 1998. De visstand van de grote rivieren. In : Atlas van de Vlaamse beek- en riviervissen. Vandelannoote *et al*. (Eds), WEL, Wijnegem, 1998, 213-228.

Belpaire, C., Van Thuyne, G., Maes, J., 1998. De ecologie en verspreiding van enkele brakwater - en de anadrome vissoorten. In : Atlas van de Vlaamse beek- en riviervissen. Vandelannoote *et al.* (Eds), WEL, Wijnegem, 1998, 229-254.

Belpaire, C. 2002. Aanhoudende terugval van de glasaalintrek leidt naar een gecoördineerd Europees meetnet voor monitoring. In : Vissen in de openbare waters. Provinciale Visserijcommissie van Oost-Vlaanderen, 2001-2002, 6-15.

Belpaire, C., Goemans, G., de Boer, J., Van Hooste, H., 2003. Verspreiding van gebromeerde vlamvertragers. In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, p. 387-395.

Belpaire, C., Goemans, G., 2004. Monitoring en normering van milieugevaarlijke stoffen in paling: bruikbaarheid en relevantie voor het milieubeleid. *Water* 2004, 1-14.

Belpaire, C., De Cooman, W., Goemans, G., Onkelinx, T., Quataert, P., 2007. Waterbodem- en palingpolluentenmeetnet: een tandem voor de waterbodemsanering, *Water* 2007, 1-8

Bervoets, L., H. Reynders, D. Knapen, W. De Cooman, G. Goemans, C. Belpaire, G. Van Thuyne, R. Blust, 2007. Is de visindex bruikbaar voor de evaluatie van effecten van metalen op vislevensgemeenschappen? *Water* 27, 52-59.

Block, C., Vandecasteele, C., Andriessens, S., Goemans, G., Belpaire, C., Roose, P., Van Hooste, H., 2003. Verspreiding van vluchtige organische stoffen (VOS). In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, 137-146

Maes, J., Belpaire, C., Breine, J., Goemans, G., 2005. Vissen als gezondheidsindicatoren voor de toestand van het Zeeschelde-ecosysteem. *Water*, 2005, 1-7.

MIRA, 2007. Milieurapport Vlaanderen, MIRA Achtergronddocument 2007, Kwaliteit oppervlaktewater, De Cooman W., Peeters B., Theuns I., Vos G., Lammens S., Debbaudt, W., Timmermans G., Meers, B., Van Erdeghem M., Van Wauwe P., Callebaut R., Barrez I., Van den Broeck S., Emery J., Van Volsem S., Bursens K., Van Hoof K., D'Heygere T., Soetaert H., Martens K., Baten I., Goris M., Haustraete K., Breine J., Van Thuyne G., Belpaire C., Smis A., Vlaamse Milieumaatschappij, http://www. milieurapport.be.

MIRA, 2007. Milieurapport Vlaanderen, MIRA Achtergronddocument 2007, Verspreiding van bestrijdingsmiddelen, Claeys, S., Steurbaut, W., Theuns, I., De Cooman, W., De Wulf, E., Eppinger, R., D'hont, D., Dierckxens, C., Goemans, G., Belpaire, C., Wustenberghs, H., den Hond, E., Peeters, B., Overloop, S., Vlaamse Milieumaatschappij, http://www. milieurapport.be.

MIRA, 2007. Milieurapport Vlaanderen, Achtergronddocument 2007, Verspreiding van PCB's, M. Wevers, M., Voorspoels, S., Covaci, A., Schepens, P., D'Havé, H., De Coen, W., Van Gerven, T., Vandecasteele, C., Goemans, G., Belpaire, C., Van Hooste, H., MIRA H. Van Hooste, Vlaamse Milieumaatschappij, http://www. milieurapport.be.

Nouwen, J., Geuzens, P., Goemans, G., Van Thuyne, G., Belpaire, C., Guns, M., Schneiders, A., Van Hooste, H., 2001. Verspreiding van zware metalen in Mira-T 2001; Milieu- en Natuurrapport Vlaanderen, 177-191.

Nouwen, J., Geuzens, P., Goemans, G., Van Thuyne, G., Belpaire, C., Schneiders, A., Van Hooste, H., 2002. Verspreiding van zware metalen. In : MIRA-T 2002. Milieu-en natuurrapport Vlaanderen : thema's. VMM, 139-148.

Nouwen, J., Geuzens, P., Den Hond, E., Nawrot, T., Staessen, J., Goemans, G., Belpaire, C., Van Hooste, H., 2003. Verspreiding van zware metalen. In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, 157-170.

Overloop, S., Steurbaut, W., De Smet, B., Theuns, I., De Cooman, W., Goemans, G., Belpaire, C., 2003. Verspreiding van bestrijdingsmiddelen. In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, 171-181.

Peeters, B., De Cooman, W., Vos, G., Gabriels, W., Martens, K., Timmermans, G., Van Hoof, K., Breine, J., Van Thuyne, G., Goemans, G., Belpaire, C., Geeraerts, C., 2006. Oppervlaktewater: Biologische kwaliteit nog steeds

ondermaats. In: Van Steertegem, M., (ed.), Mira-T 2006 Milieurapport Vlaanderen: Focusrapport. Leuven Lannoo Campus, 89-104.

Simoens, I., J. Breine, C. Van Liefferinge, M. Stevens, C. Belpaire, 2007. Het belang van het Lippenbroek als habitat voor vissen in de Zeeschelde. *Water* 2007, 68-71.

Steurbaut, W., De Smet, B., Vercruysse, F., Belpaire, C., Goemans, G., Overloop S., 2001.Verspreiding van bestrijdingsmiddelen. In Mira-T 2001; Milieu- en Natuurrapport Vlaanderen, 193-206.

Van Gerven, T., Vandecasteele, C., Goemans, G., Belpaire, C., Van Hooste, H., 2002. Verspreiding van PCB's. In : MIRA-T 2002. Milieu- en natuurrapport Vlaanderen : thema's. VMM, 327-335.

Van Gerven, T., Vandecasteele, C., Goemans, G., Belpaire, C., Van Hooste, H., 2003. Verspreiding van PCB's. In : Mira-T 2003; Milieu- en Natuurrapport Vlaanderen, 379-385.

Vandecasteele, C., Van Gerven, T., Geysen, D., Belpaire, C., Goemans, G., Raemaekers, M., Van Hooste, H., 2001. Verspreiding van producten van onvolledige verbranding (POV's) en PCB's, deel PCB's. In Mira-T 2001; Milieu- en Natuurrapport Vlaanderen, 167-175.

Weltens, R, Goemans, G. Huyskens, G., Belpaire, C., Witters, H., 2003. Evaluatie van de kwaliteitsnormering voor oppervlaktewater in Vlaanderen: een praktijkstudie. *Water* 2003, 1-9.

A selection of research reports

Belpaire, C., 2002. Monitoring of glass eel recruitment in Belgium. In : Dekker W. (Ed) Monitoring of glass eel recruitment. Netherlands Institute of Fisheries research, report C007/02-WD, Volume 2B, pp.169-180

Belpaire, C., 2002. Monitoring van de glasaalrekrutering in België – April 2002. Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.V.R.2002.87.

Belpaire, C., 2003. Report on eel stock and fishery in Belgium, 2003. ICES/EIFAC Working Group on Eels, San Sebastian, Spain, October 2003. Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.V.BR.2003.102

Belpaire, C., 2005. Data collection for the European Eel in Belgium, Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.BR.2005.144

Belpaire, C., De Charleroy, D., Coeck, J., Janssens, L , Monden, S. 2002. Flemish plans for hydropower turbines : additional loss for eel spawning stock? ICES/EIFAC – Working Group on Eel (Nantes, 2-6 September 2002)

Belpaire, C., Goemans, G., 2002. Hoge meetwaarden van vlamvertragers in paling en sediment van waterlopen in het Scheldebekken. Nota voor Vera Dua, Vlaams minister van Leefmilieu. Instituut voor Bosbouw en Wildbeheer, 2002, IBW.Wb.V.Adv.2002.092.

Belpaire, C., Van Thuyne, G., Cooreman, K., 2000. PCB metingen in consumptiepaling uit de handel in Vlaanderen. Instituut voor Bosbouw en Wildbeheer, Departement Zeevisserij (Centrum voor Landbouwkundig Onderzoek), Nota voor de Hoge Gezondheidsraad, 22 november 2000. IBW.Wb.BR.2002.75

Belpaire, C., Verreycken, H., 2007. Determinatiesleutel voor de zoetwatervissen van Vlaanderen. Mededelingen van het Instituut voor Natuur- en Bosonderzoek 2007, INBO.M.2007.7. Instituut voor Natuur- en Bosonderzoek, 47p.

Berckmans, P., Witters, H., Goemans, G., Maes, J., Belpaire, C., 2007. Ondersteunend studiewerk en verdere karakterisatie van de Vlaamse toestand inzake hormoonverstoring: vraagstelling inzake ecologische relevantie. Studie uitgevoerd in opdracht van VMM door Vlaamse Instelling voor Technologisch Onderzoek, Mol, VITO rapport nr. 2007/TOX/R071 - Instituut voor Natuur- en Bosonderzoek, Brussel, INBO report nr. R.2007.37.

Bervoets, L., Goemans, G., Belpaire, C., Van den Boeck, H., De Boeck, G., De Jonge, M., Van Thuyne, G., Breine, J., Joosen, S., Van De Vijver, B., De Temmerman, L., De Cooman, W., 2007. Ecologische en ecotoxicologische toestand van de Dommel vóór de sanering van de waterbodem. University Antwerp and INBO, Report for Flanders Environmental Agency.

Geeraerts, C., Goemans, G., Quataert, P., Belpaire, C., 2007. Ecologische en ecotoxicologische betekenis van verontreinigende stoffen in paling. Studie uitgevoerd in opdracht van de Vlaamse Milieumaatschappij, MIRA, MIRA/ 2007/05, INBO/R/2007/40. Instituut voor Natuur- en Bosonderzoek. p. 207.

Goemans, G., Belpaire, C., Raemaekers, M., Guns M., 2003. Het Vlaamse palingpolluentenmeetnet, 1994-2001: gehalten aan polychloorbifenylen, organochloorpesticiden en zware metalen in paling. [The Flemish eel pollution monitoring network 1994-2001: polychlorine biphenyls, organochlorine pesticides and heavy metals in eel]. Reports of the Institute for Forestry and Game Management, IBW.Wb.V.R.2003.99.169 p.

Simoens, I., Goemans, G., Verreycken H., Belpaire, C., 2004. Visserijbiologisch onderzoek van de Kallemoeie-Papelenvijver te Nazareth in functie van de berging van baggerspecie uit het Leiebekken en met nabestemming natuurontwikkeling. Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.V.R.2005.135.

Van Thuyne, G., Belpaire, C., Guns, M., 1995. Onderzoek van gehalten aan zware metalen in vis van enkele Vlaamse binnenwateren. Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.V.R.95.033, 23p.

Van Thuyne, G., Belpaire, C., Guns, M., Denayer, B., 1995. Monitoring van de vispopulaties en de visvleeskwaliteit op het Boudewijnkanaal. Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.V.R.94.031, 87p.

Van Thuyne, G., Belpaire, C., 1999. Zware metalen in paling als indicator voor de immissietoestand in het aquatisch milieu. MIRA-T 1999. Milieu-en natuurrapport Vlaanderen : Wetenschappelijke rapporten.

Weltens, R., Goemans, G., Huyskens, G., Witters, H., Belpaire, C., 2002. Wetenschappelijke onderbouwing voor de normering van polluenten in Vlaamse oppervlaktewateren en selectie van ecologische en exotoxicologische indicatoren voor de waterkwaliteit. Reports of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.VR.2002.88, 120p.

A selection of advices

Belpaire, C., 2003. Advies van het Instituut voor Bosbouw en Wildbeheer aan de afdeling Bos en Groen van AMINAL naar aanleiding van een EG-plan voor het palingbeheer. Advice of the Institute for Forestry and Game Management, IBW.Wb.V.Adv.2003.104

Belpaire, C., Coussement, M., 2000. Nota omtrent het uitzetten van paling in de Vlaamse openbare waters - Advies voor de Vlaamse Hoge Raad voor de Riviervisserij (20 maart 2000). Advice of the Institute for Forestry and Game Management, IBW.Wb.V.Adv.2000.070

Belpaire, C., Goemans, G., De Cooman, W., 2002. Hoge meetwaarden van vlamvertragers in paling en sediment van waterlopen in het Scheldebekken . Nota voor Vera Dua, Vlaams minister van Leefmilieu. Instituut voor Bosbouw en Wildbeheer en Vlaamse Milieumaatschappij. September 2002. Advice of the Institute for Forestry and Game Management, IBW.Wb.V.Adv.2002.092

Belpaire, C., Goemans, G., Van Slycken, J., 2004. Antwoord van het Instituut voor Bosbouw en Wildbeheer naar aanleiding van de parlementaire vraag van de heer Jan Verfaillie omtrent de problematiek van het meeneemverbod voor paling (Vraag nr. 164 van 10 december 2004). Advice of the Institute for Forestry and Game Management, IBW.Wb.V.Adv.2004.114

Belpaire, C., Goemans, G., Van Slycken, J., 2005. Advies van het Instituut voor Bosbouw en Wildbeheer over het meeneemverbod voor paling. Advice of the Institute for Forestry and Game Management, Groenendaal, IBW.Wb.V.Adv.2005.116, 7p.

Belpaire, C., Goemans, G., Van Slycken, J., 2005. Advies van het Instituut voor Bosbouw en Wildbeheer rond het meeneemverbod voor paling (26 mei 2005). Advice of the Institute for Forestry and Game Management, IBW.Wb.V.Adv.2005.116

Belpaire, C., Verreycken, H., Denayer, B., 1997. Advies van het IBW naar aanleiding van de parlementaire vraag van de heer Johan Malcorps over de palingstand en het palingbeheer. Advice of the Institute for Forestry and Game Management, IBW.Wb.Adv.97.054

Coeck, J., Buysse, D., Van Thuyne, G., Belpaire, C., 2007. Vissen. In Decleer, K. (Ed). Europees beschermde natuur in Vlaanderen en het Belgisch deel van de Noordzee. Habitattypen. Dier- en plantensoorten. Mededelingen van het Instituut voor Natuur- en Bosonderzoek, INBO.M.2007.01, Brussel, 375-384.

Goemans, G., Belpaire, C., 2002. Antwoord van het Instituut voor Bosbouw en Wildbeheer naar aanleiding van de parlementaire vraag van de heer Jan Verfaillie naar het meeneemverbod voor paling in de Westhoek (Vraag nr 305 van 20 september 2002). 7 oktober 2002. Advice of the Institute for Forestry and Game Management, IBW.Wb.V.Adv.2002.093

Goemans, G., Belpaire, C., 2003. Antwoord van het Instituut voor Bosbouw en Wildbeheer naar aanleiding van de parlementaire vraag van de heer Jacky Maes naar het meeneemverbod voor paling in de Westhoek en afwijking voor hengelwedstrijden (vraag nr 151 van 28 maart 2003). Advice of the Institute for Forestry and Game Management, IBW.Wb.Adv.2003.098

Goemans, G., Belpaire, C., Vlietinck, K., 2002. Antwoord van het Instituut voor Bosbouw en Wildbeheer en de Afdeling Bos en Groen naar aanleiding van de parlementaire vraag van mevrouw Marleen Van den Eynde omtrent de PCB-vervuiling in paling (Vraag nr 187 van 28 maart 2002). 11 april 2002. Advice of the Institute for Forestry and Game Management, IBW.Wb.V.Adv.2002.089

Sioen, I., Bilau, M., Goemans, G., Belpaire, C., Willems, J., 2005. Schatting van de inname van PCB's door sportvissers en het hieraan gebonden gezondheidsrisico. Hoge Gezondheidsraad (2005) Advice to the “Hoge Gezondheidsraad” (Advice No HGR 7747 – brought out on February 23, 2005 and validated on March 9, 2005)