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A question of origin – dioxin-like PCBs and their relevance in stock management of European eels

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Abstract

The stock of European Eel (*Anguilla anguilla* L.) has reached an all-time low in 2011. Spawner quality of mature eels in terms of health status and fitness is considered one of the key elements for successful migration and reproduction. Dioxin-like Polychlorinated Biphenyls (dl-PCBs) are known Persistent Organic Pollutants (POPs) potentially affecting the reproductive capability and health status of eels throughout their entire lifetime. In this study, muscle tissue samples of 192 European eels of all continental life stages from 6 different water bodies and 13 sampling sites were analyzed for contamination with lipophilic dl-PCBs to investigate the potential relevance of the respective habitat in light of eel stock management. Results of this study reveal habitat-dependent and life history stage-related accumulation of targeted PCBs. Sum concentrations of targeted PCBs differed significantly between life stages and inter-habitat variability in dl-PCB levels and -profiles was observed. Among all investigated life stages, migrant silver eels were found to be the most suitable life history stage to represent their particular water system due to habitat dwell-time and their terminal contamination status. With reference to a possible negative impact of dl-PCBs on health and the reproductive capability of eels, it was hypothesized that those growing up in less polluted habitats have a better chance to produce healthy offspring than those growing up in highly polluted habitats. We suggest that the contamination status of water systems is fundamental for the life cycle of eels and needs to be considered in stock management and restocking programs.

Keywords: Dioxin like PCB. Eel. Silver eel. Habitat quality. Spawner quality. Stock management

Introduction

The panmictic stock of the European Eel (*Anguilla anguilla L.*) has experienced a drastic decline since the early 1980s when recruitment numbers of arriving glass eels have dropped startlingly (Moriarty 1986, 1996; ICES 2010). Even though slight increases of arriving glass eels have been observed since, the stock is still considered as outside safe biological limits. Reasons for this decline are currently subject to ongoing comprehensive research on a global scale. Apart from natural phenomena such as oceanic factors and predation (Knights 2003; Friedland et al. 2007; Durif et al. 2010; Bevacqua et al. 2011; Wahlberg et al. 2014), a number of anthropogenic influences including habitat loss, overfishing, denaturation of water bodies, the introduction of parasites and pollution are suspected to be contributing factors (Robinet and Feunteun 2002; Dekker 2003; Palstra et al. 2006, 2007; Quadroni et al. 2013, Sühring et al. 2013, 2014; Barry et al. 2014). Their biology as sediment related, bottom dwelling predators with high body fat contents make eels in their growth phase (yellow eels) particularly vulnerable to chemical pollution by a variety of lipophilic bio-accumulating contaminants including metals (Maes et al. 2008), polycyclic aromatic hydrocarbons (Kammann et al. 2014), chlorinated and brominated flame retardants (CFRs & BFRs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and furans (PCDDs/Fs) (Geeraerts and Belpaire 2010; Tapie et al. 2011; Sühring et al. 2013, 2014; Szlinder-Richert et al. 2014). Some of these contaminants are known to cause a variety of adverse health effects including cancer, reproductive failure, nervous and endocrine system disorders, and others (Safe 1994; Robinet and Feunteun 2002; Ross 2004; Corsi et al. 2005). Due to their specific predisposition towards xenobiotics, the composition of chemical contamination in eels could be interpreted as a result of environmental imprinting by the local environment (Belpaire and Goemans 2007; Belpaire et al. 2008; Grabowska 2010; Byer et al. 2013). In a Portuguese study by Guimaráes et al. (2009), results indicated that yellow eels originating from stronger polluted habitats showed higher adverse physiological effects determinant for their survival and performance than yellow eels originating from a less polluted habitat. In another study on Japanese eels (*Anguilla japonica)* by Arai and Takeda (2012) from Japan, the authors state that the ecological risks of organochlorine compounds (OC) increase as the freshwater residence period in eel become longer. Therefore individual lipid contents and migratory histories directly affect the accumulation of those OCs in anguillid eels.

One of the probably most prominent groups among these xenobiotics with negative effects on aquatic organisms are dioxin-like polychlorinated biphenyls (dl-PCBs) since these lipophilic and mostly persistent compounds tend to accumulate through the trophic cascade (James et al. 2014). Some congeners, dependent on the number and position of chlorine atoms, have particularly been identified to be capable of causing severe health damages and to possibly influence the ovarian and embryonic development, as shown for different species (Gutleb et al. 1999; 2007; Daouk et al. 2011) as well as specifically for the European eel (Robinet and Feuteun 2002; Corsi et al. 2005; Palstra et al. 2006). The production of PCBs was stopped in the 1980’s and PCBs have been considered POPs and listed for a global ban in 2001 by the Stockholm Convention (Stockholm Convention 2001, Porta and Zumeta 2002). However, due to their persistence, they are still found in considerable concentrations in the atmosphere, in soils as well as in aquatic sediments (Nizzeto et al. 2010; Grabowska 2010; Wetzel et al. 2013) which, mainly after flood events or excavation works, play an important role as secondary sources also for the contamination of inland water bodies and flood plains (Stachel et al. 2004; Lake et al. 2014). It has been estimated that the total dioxin-like toxicity in the historically produced 1.3 million metric tons PCB (Breivik et al. 2002) were between 11 000 and 16 000 kg toxic equivalents (TEQ) (Weber et al. 2008). This can be compared to the current global PCDD/F emission inventory of approximately 58 kg TEQ for 68 countries, covering 50% of the world population (Fiedler et al. 2012). Therefore, dl-PCBs still account for the largest share of dioxin-like toxicity in European rivers, considerably higher than the toxic equivalence (TEQ^{2005}) contribution from PCDDs and PCDFs (Stachel et al. 2007; Blanchet-Letrouvé et al. 2014; Guhl et al. 2014). Furthermore, approximately 3

million metric tons of PCB-contaminated waste oils and contaminated equipment still need to be managed, globally contributing to ongoing environmental pollution (Stockholm Convention 2010; Weber et al. 2013).
The European eel is a species of high economic and ecological value, which is why a number of protection measures have been set up within the European Union, to counter-act against the decline of the stock. These actions include habitat restoration, fisheries and trade restrictions like keeping-size limits, closed seasons and a variety of accessory measures to increase the escapement of silver eels to a given target of 40% of the pristine biomass (Council Regulation (EC) 1100/2007). A common counter measure against locally dropping numbers of recruits is the catch and reallocation of glass eels for stocking purposes (ICES 2013). The aim of this practice (besides the support of local commercial fisheries) is to harvest individuals from water bodies exceeding their carrying capacity and to distribute them into less recruited habitats and thereby eventually reduce rates of natural mortality. However, until now, very few studies targeted the effectiveness of such management actions. Considering the negative impact of environmental contaminants such as dl-PCBs on the quality of eel spawners, it is vital to assess the contamination status of those water bodies selected for restocking measures as part of management plans that are explicitly aiming at the recovery of the eel stock. Until now, habitat adequacy for stocking programs in terms of the environmental status of targeted rivers has not been taken into account.
The aim of this study was to investigate the influence of different habitats on the quantity and patterns of dl-PCBs in eels throughout their different continental life history stages. Results are supposed to help identify quality indicators for the habitat selection with regards to restocking purposes as well as appropriate life stages for certain monitoring strategies. In addition, findings of this study may lead to an improvement of eel-related management and assessment in line with the European Data Collection Framework (EU DCF) in the future. The DCF is a Community framework for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy (CFP). Our results give an impression on decisive factors for the contamination of eels with dl-PCBs and why the selection of habitats for stock management measures should be influenced by their contamination status.

Material and methods

Samples

A total of 100 European glass eels were obtained from French Atlantic coast glass eel fisheries and 30 young-of-the-year elvers (young, recently ascended yellow eels) from an elver-monitoring site in the river Vidå at the German-Danish border (Fig. 1). In addition, 35 migrating female silver eels were purchased from commercial fishers situated in the potamal sections (lower stretch) of the rivers Elbe, Eider, Ems, the Schlei Fjord and the lower river Rhine close to the German/Dutch border in the frame of the German data collection according to the EU Data Collection Regulation (DCR) (European Commission 2008, 2010) (Fig. 1). In addition, 27 yellow eels were caught at six sampling sites along river Elbe by electrofishing, also in line with the EU DCR. A list with detailed biological parameters of the analyzed eels can be found in Table 1. Eels in this study were killed by decapitation after being anaesthetized with 2-Phenoxyethanol (ROTH, Karlsruhe, Germany). To eliminate sources of contamination, samples were strictly handled with cleaned equipment made of glass, aluminum or steel, preventing any contact with plastics, oils or other possible sources of cross-contamination. For further analyses, between 10 and 25 g muscle tissue of yellow and silver eels was excised from the skeletal muscle just behind the level of the anus. From elvers, whole filets of 3 randomly chosen individuals were pooled and homogenized. For glass eels, 10 randomly chosen individuals were each entirely combined to a pool-sample and homogenized. Age determination of yellow and silver eels was based on otolith readings following the cutting and burning method (Graynoth 1999) as recommended by ICES (2009, 2011). For better comparability, yellow eels were selected to fit in a certain age frame and maturation stage (between eight and twelve years old and growth- & pre-migrating silvering stages I, II or III after Durif (2005)). All silver eels were in the silvering stage V (migrating phase V after Durif (2005)). Due to low availability of stage V eels in the Schlei fjord, 2 Stage III specimens with similar biological characteristics according to length, weight and age and a Pankhurst stage higher than 7 (migrating stage, Pankhurst 1982) were included in the analysis of this sample group (Table1).

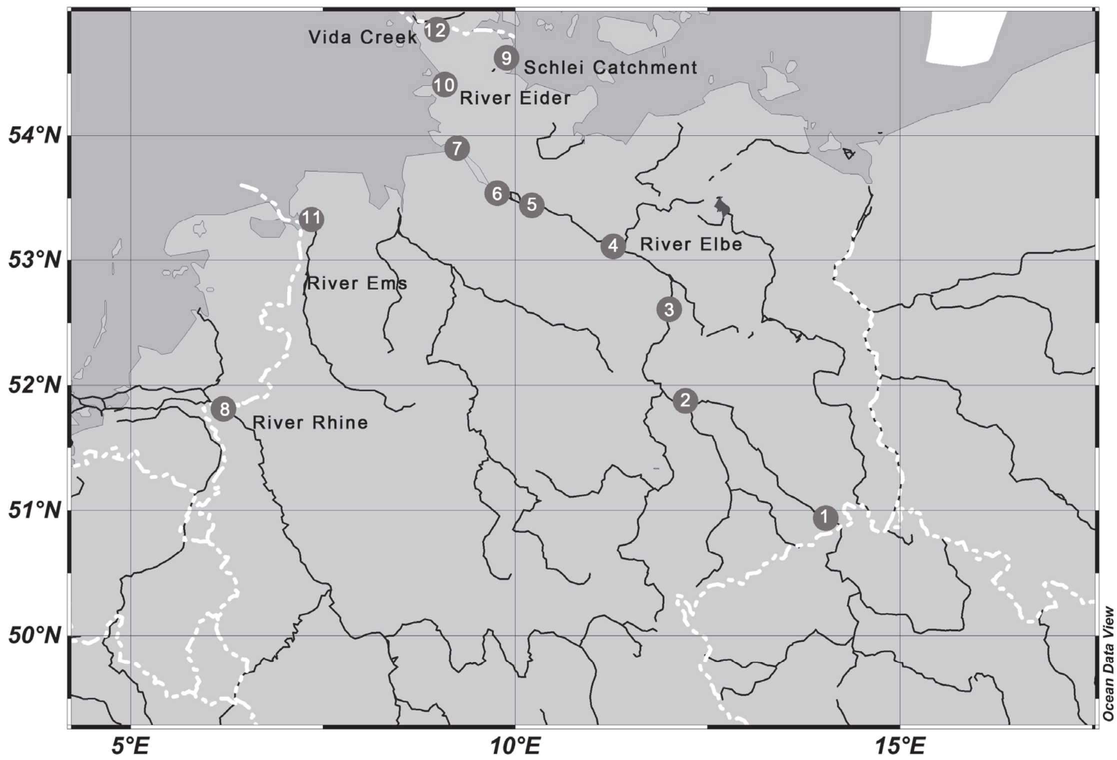

Figure 1
Sampling positions of all collected continental life stages in the German waterbodies Elbe (1: Bad Schandau, 2: Dessau, 3: Hohengören, 4: Gorleben, 5: Winsen, 6: Jork, 7: Cuxhaven), Rhine (8: Kalkar-Grieth), Schlei (9: Schleswig), Eider (10: Nordfeld), Ems (11: Emden), Vidå (12: Verlath).

Table 1 Summary of amalgamated data (mean ±standard deviation) for different life history stages of European eels collected from different sampling sites and water bodies.

Life Stage	Sample size (n)	River Basin	Sampling Location (pos. on map (Fig1))	Length (cm) ±sd	Weight (g) ±sd	Age (y) ±sd	Lipid (%) ±sd
Y	1	Elbe	Bad Schandau (1)	67,0 ±N/A	533,3 ±N/A	12 ±N/A	24,5 ±N/A
Y	7	Elbe	Dessau (2)	59,7 ±12,9	366,0 ±213,9	8,4 ±2,2	25,7 ±6,1
Y	5	Elbe	Hohengoeren (3)	62,4 ±5,4	406,6 ±149,4	9,4 ±1,5	28,4 ±11,0
Y	4	Elbe	Gorleben (4)	64,3 ±3,9	416,3 ±94,8	8,5 ±1,3	35,3 ±5,1
Y	5	Elbe	Winsen (5)	61,6 ±5,0	459,8 ±129,1	9,4 ±1,1	25,6 ±10,1
Y	5	Elbe	Jork (6)	58,6 ±10,4	362,4 ±204,2	8,4 ±0,9	22,0 ±16,6
S	10	Elbe	Lower stretch (6&7)	69,4 ±6,7	646,2 ±154,2	10,6 ±1,5	27,5 ±2,0
S	10	Rhine	Kalkar (8)	69,0 ±7,0	639,2 ±111,2	13,3 ±2,8	24,3 ±3,5
S	5	Eider	Nordfeld (10)	66,6 ±3,8	530,0 ±166,3	13,8 ±2,2	23,6 ±4,0
S	5	Ems	Emden (11)	70,6 ±4,5	683,8 ±129,4	14,6 ±2,1	26,4 ±4,9
S	5	Schlei	Schleswig (9)	66,8 ±4,0	624,8 ±109,3	10,0 ±3,2	21,8 ±4,1
ELV	10x3	Vidå	Verlath (12)	12,0 ±0,9	1,8 ±0,4	N/A	1-2 ±N/A
GE	10x10	Atlantic Coast	France	6,9 ±0,4	0,2 ±0,1	N/A	0-1 ±N/A

Data were grouped according to their life stages (Y= Yellow Eel, S=Silver Eel, ELV= Elver, GE= Glass Eel) and sampling locations respectively.

Extraction, clean-up and lipid content

Extraction and clean-up were conducted following the protocol as described by Sühring et al. (2013). Frozen yellow and silver eel muscle samples were homogenized with anhydrous Na_2SO_4 (2:1; w/w) for approximately 20 minutes using a 1 L stainless steel / glass laboratory blender (Rotorblender, neoLab, Heidelberg, Germany). The homogenized samples were extracted by accelerated solvent extraction (ASE-200, Dionex, Sunnyvale, USA) using dichloromethane (DCM, ROTH, Karlsruhe, Germany) at 100°C and 120 bar. All samples were spiked with 13C mass labeled internal standards (IS) analogous for each analyzed compound (WHO PCB+PCB-170+PCB-180 CLEAN-UP STANDARD (13C12, 99%), Cambridge Isotope Laboratories (CIL), Tewksbury, USA). Any remaining volume was filled with anhydrous Na_2SO_4 (ROTH, Karlsruhe, Germany). For extraction of the homogenized glass eel samples, a Na_2SO_4–eel–mixture (equal to 3 g eel tissue) for each pool was extracted by Soxhlet filled with 28 - 60 mm glass–fiber extraction thimbles with DCM at 55 °C for 24 h. After extraction, the samples were reduced to approx. 2 mL using rotary evaporators. For the first clean-up step, a gel permeation chromatography (GPC) was used with 30 g Bio-Beads SX-3 (Bio-Rad Laboratories, Hercules, USA) and DCM:Hexane (1:1; v:v) as eluent. The first fraction (75 mL) was discarded while the second fraction (110 mL), that contained the target substances, was reduced to about 2 mL and then transferred into hexane. A column with 2.5 g 10% H_2O deactivated silica gel (ROTH, Karlsruhe, Germany). was used as a second clean-up step. Analytes were eluted with 20 mL hexane and the volume concentrated to 150 µL before transferring them to measurement vials. Finally, 10 µL 13C PCB 141/PCB 208 (50 ng mL^{-1}) was added as injection standard to each sample. The lipid content of samples was determined gravimetrically from separate aliquots following a method described in Sühring et al. (2013).

2.4. Instrumental Analysis

The instrumental analyses were performed on a GC/MS-system (Agilent 6890 GC/5973 MSD, Agilent Technologies, Santa Clara, USA) fitted with a HP-5MS column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific, Agilent Technologies, Santa Clara, USA) in electron capture negative ionization mode (ECNI) using methane as ionization gas. The instrument was operated in selected ion monitoring mode. Samples were analyzed for dl-PCBs (IUPAC numbering) 77, -81, -105, -114, -118, -126, -156, -157, -167, -169, -189 as well as the non dioxin-like PCBs 170 and -180. PCB 170 and PCB 180 were included in the analysis because of their physiological relevance as active inducers of EROD activity and their quantitative significant presence in environmental samples and thus to provide for better comparability with studies involving non-dl and / or indicator PCBs.

QA/QC

Extraction and clean-up were conducted in a clean lab (class 10000). Recovery rates of IS were determined for each sample. Mean IS recoveries ranged from 59± 24 % for PCB 81 to 77 ± 31 % for PCB 169. A blank test, using Na_2SO_4 treated similar to real samples, was performed with every extraction batch (eleven samples). All blanks were either below MQL or otherwise 1-2 magnitudes lower than lowest samples concentrations. The limits of detection and quantification (LOD/LOQ) were calculated either from the blank or from a signal to noise ratio of 3 and 10. The LOD ranged from 0.003 – 0.012 ng/g wet weight (ww) for PCB 189 to 0.012 – 0.09 ng/g ww for PCB 81. The LOQ ranged from 0.004 – 0.04 ng/g ww for PCB 189 to 0.032 – 0.30 ng/g ww for PCB 180. For further quality control, a twofold measurement was conducted for samples from areas with low sample numbers and a threefold measurement was done for PCB 126 from randomly selected samples from remaining areas. Results for PCB 123 were excluded from our results due to incomplete chromatographic separation.

Data processing and statistical analyses

TEQ values were calculated under consideration of the WHO-2005 Toxicity Equivalent Factors (TEFs) (Van den Berg et al 2006). TEQ concentrations are reported for dl-PCB TEFs solely, thus not PCDD/Fs.
All statistical analyses were performed using R 3.1.2 (R Core Team 2014). Differences in accumulation quantities of targeted dl-PCBs between the respective sample groups were tested on sums of dl-PCB concentration. For more than 2 groups, the Kruskal-Wallis test was used and subsequent post-hoc tests were performed with Bonferroni corrected p-value adjustment using the package agricolae (Mendiburu 2014). When testing just 2 groups against each other, the Mann-Whitney *U* test was performed. Nonmetric multidimensional scaling (nMDS) was used to compare congener accumulation patterns of individuals between life-history-stages, intra habitat catch locations and inter habitats (river systems). To avoid zero values, a small constant was added to each congener measure and data was log-transformed subsequently. NMDS was performed using the function metaMDS in the package vegan (Oksanen et al. 2015). Euclidean distance was used to calculate the dissimilarity matrix. Number of dimensions was set to two. Maximum number of random starts was set to 100, and no automatic transformation was used. Permutational

multivariate analysis of variance (PERMANOVA) was performed to test whether groups differed significantly. Number of permutations was set to 10,000. Bonferroni correction was used for post hoc tests.

Results

dl-PCB accumulation & patterns in eels of different life history stages

Detailed concentrations for individual detected congeners as well as the resulting TEQs from the investigated compounds can be found in Table 2. Dl-PCB congener patterns of eels of different life history stages are displayed in Figure 2a. Congeners with highest overall concentrations among targeted dioxin-like PCBs were 118, 105 and 156, summing up for 84.8% (PCB 118 = 58.5%; PCB 105 = 13.5%; PCB 156 = 12.8%) of dl-PCBs in all samples. Considering the congener concentration patterns of the different stages, nMDS (stress = 1.92 %) led to a clear separation of both glass eels and elvers from all other groups (PERMANOVA, F-model = 147.23, df = 3, $p < 0.001$ (Fig. 3a)). Yellow eels and silver eels did not differ significantly from each other (PERMANOVA, F-model = 0.23, df = 1, $p > 0.05$).

Sum concentrations of the targeted PCBs in the respective sample groups (glass eels, elvers, yellow eels, silver eels) are displayed in Fig. 2b. Glass eels and elvers originating from the Atlantic Coast and the Vidå creek revealed low accumulated concentrations accounting for a median of 0.3 ng/g ww in glass eels and 0.2 ng/g ww in elvers. Yellow and silver eels from river Elbe showed dl-PCB concentrations summing up to a median of 51.4 ng/g ww and 74.1 ng/g ww respectively. Sum concentrations of measured dl-PCBs in yellow and silver eels showed high interindividual variability (Table 2). However, silver eels were tested to have accumulated significantly higher amounts of dl-PCBs than yellow eels (Mann-Whitney-U-Test W = 48, p = < 0.001). Median TEQs resulting from dl-PCB concentrations in glass eel and elver samples both were lower than 0.1 pg/g ww, while they ranged from 65 pg/g ww for for yellow eels to 71 pg/g ww for silver eels (Tab. 2). All targeted congeners except PCB 169 were detected in ng/g ww range in yellow and silver eels from river Elbe with some individual congeners ranging below or close to the detection or quantification limits.

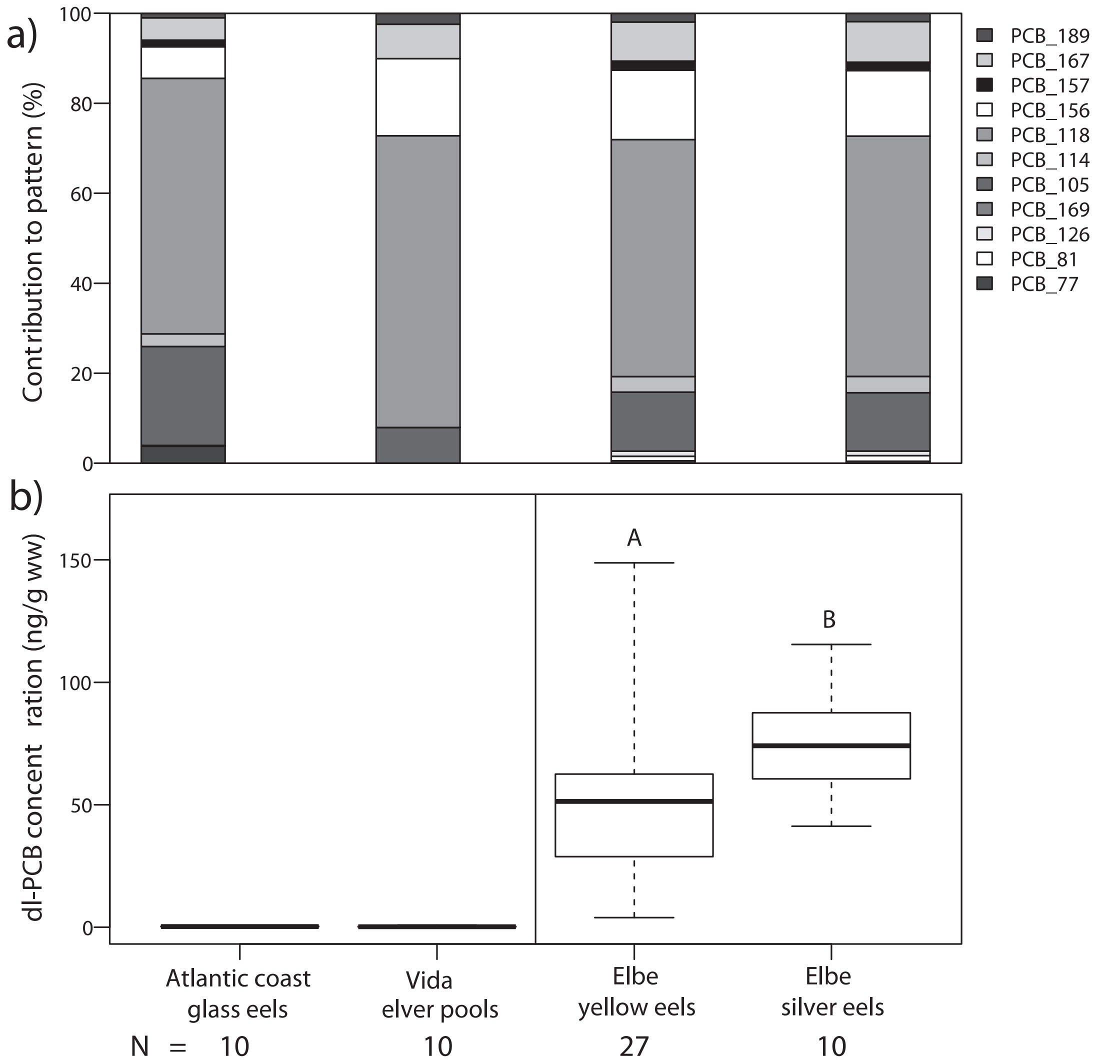

Figure 2

a) Median congener patterns of different life history stages (glass eel & elver each pooled (n=10), yellow eels (n=27) and silver eels (n=10)) from river Elbe in percent of total sum dl-PCB. Numbers of the most abundant congeners are given in boxes.

b) Mean sum PCBs in ng/g ww.. Whiskers represent medium and maximum values, boxes represent middle 50% of data set, bold line indicates median value. Significant differences (P<0.05) between groups from the same habitat are indicated by capital letters. N indicates the total number of individual eels in the respective groups.

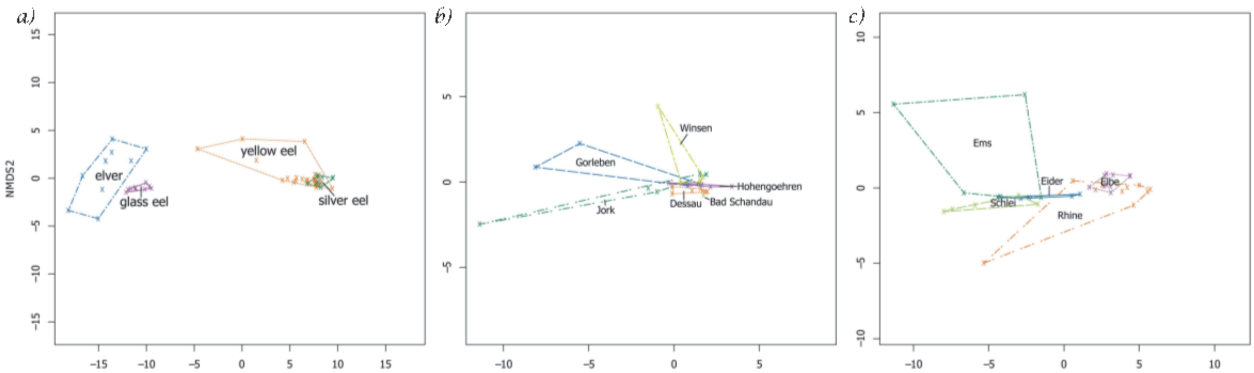

Figure 3

NMDS Plots of congener patterns displaying a) different life history stages with b) yellow eels sampled from different sampling locations along River Elbe c) silver eels from different German catchments.

PCB accumulation in yellow eels from different sampling locations along the river Elbe

Dl-PCB congener patterns of yellow eel samples from different sites along the river Elbe (Figure 4a) were dominated by congeners 118, 105 and 156 summing up for 80.9% (PCB 118 = 52.5%; 105 = 13.0%; 156 = 15.5%) of the targeted dl-PCBs in all samples (Fig. 4a). The nMDS (stress 2.41%) revealed similar dl-PCB congener patterns between individuals of the different locations and high variability between individuals for Jork, Gorleben and Winsen (Fig. 3b). No significant differences between the locations along the river Elbe were found (PERMANOVA, F-model = 1.58, df = 5, p > 0.05).

Sum concentrations of targeted dl-PCBs in analyzed eels sampled from the respective sampling locations are displayed in Figure 4b. Yellow eels sampled from different locations along the same habitat showed median concentrations of targeted dl-PCBs ranging from 22.9 ng/g ww in the most downstream sampling location Jork to 80.2 ng/g ww and 59.6 ng/g ww for the most upstream locations Bad Schandau (1 individual) and Dessau, respectively. Although no statistically significant differences were found (Kruskal-Wallis-Test H = 4.92, df = 4,p = >0.05), median sums of dl-PCBs indicated a slight decreasing trend from the Czech Boarder towards the Estuary of the Elbe River. Mean TEQs resulting from dl-PCBs in yellow eels ranged from 23 pg/g ww (Jork) to 77 pg/g ww (Bad Schandau) (Table 2).

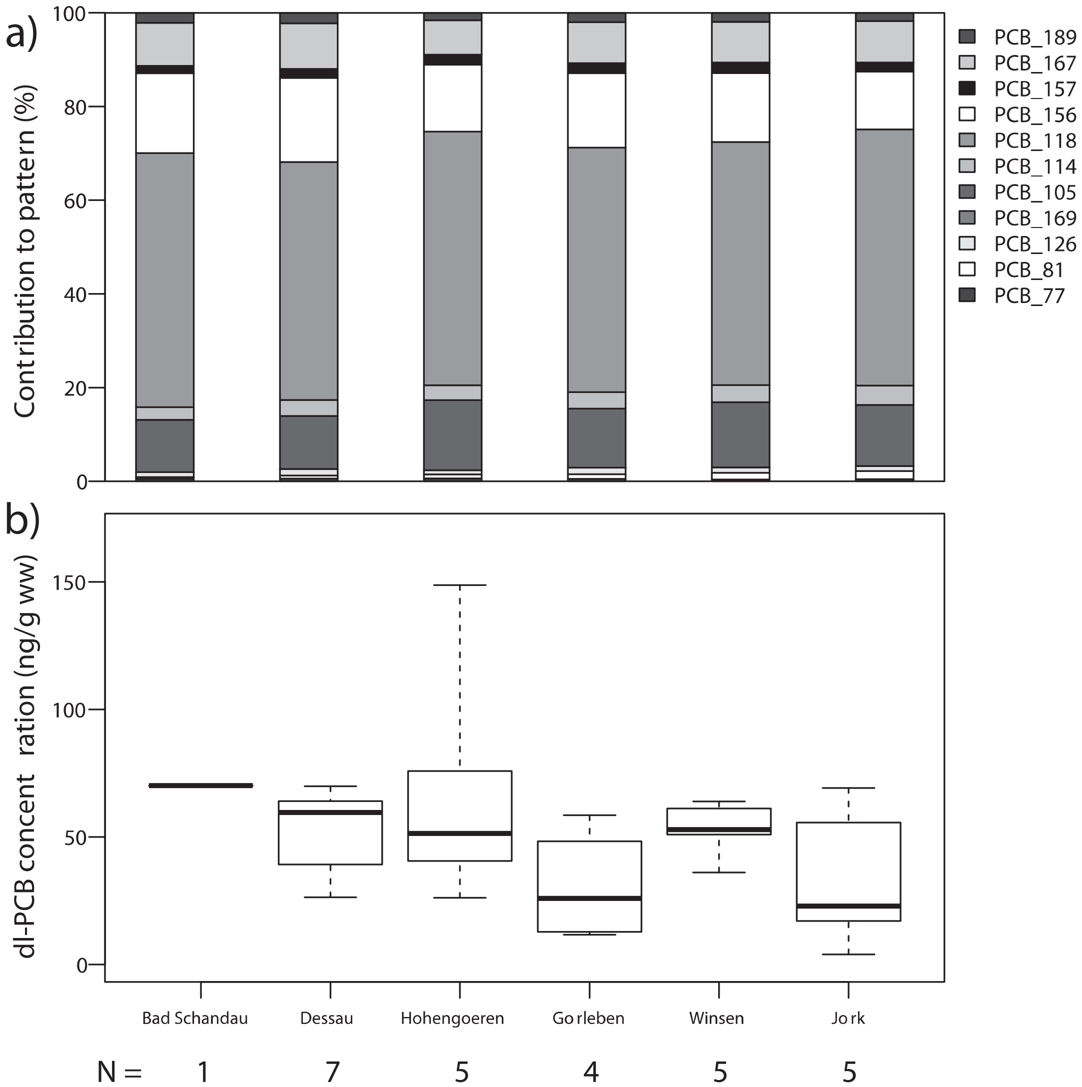

Figure 4
a)Median congener patterns and Sum d-PCB of yellow eels sampled along river Elbe in percent of total sum dl-PCB.
b)Sum dl-PCBs of sampled eels given as means arranged in order of distance from the estuary beginning with the location furthest away. N indicates the total number of individual eels in the respective groups (Bad Schandau: n=1, Dessau: n=7, Hohengoehren: n=5, Gorleben: n=4, Winsen: n=5, Jork: n=5).

PCB accumulation in silver eels from different German rivers

Congener patterns of silver eels from different German rivers are displayed in Figure 5a. Strongest represented congeners summing up highest overall concentrations among targeted dioxin-like PCBs in all examined silver eel samples were PCB 118, 105 and 156 accounting for a median sum of 79.5% (PCB 118 = 48.4%, 105 = 18.3%; PCB 156 =12.8%). All targeted congeners except PCB 169 were detected in silver eels in ng/g ww range from all investigated habitats.

Silver eels from different rivers could slightly be separated by nMDS (stress: 2.80%) using their congener concentration patterns (Fig. 3c). Silver eels from the river Elbe differed significantly in their congener patterns from all rivers other than the Rhine, while silver eels from the Rhine displayed congener patterns significantly different from the Schlei and Ems (PERMANOVA, F-model=11.39, df=4, $p < 0.05$). Dl-PCB sum concentrations measured in silver eels from different habitats are displayed in Figure 5b. Concentrations in silver eels from the rivers Rhine and Elbe were significantly higher than in silver eels from Eider, Ems or Schlei (Kruskal-Wallis-Test H = 24.35, df = 4, $p > 0.01$). The highest median sum was found in eels from river Rhine (127.2 ng/g ww) followed by samples from the rivers Elbe (74.1 ng/g ww), Eider (11.3 ng/g ww), Ems (6.0 ng/g ww) and Schlei (4.6 ng/g ww). Median TEQs resulting from dl-PCBs in silver eels summed up to 95 pg TEQ/g, 71 pg TEQ/g, 15 pg TEQ/g, 8 pg TEQ/g and 6 pg TEQ/g ww for silver eels from the rivers Rhine, Elbe, Eider, Ems and Schlei fjord, respectively.

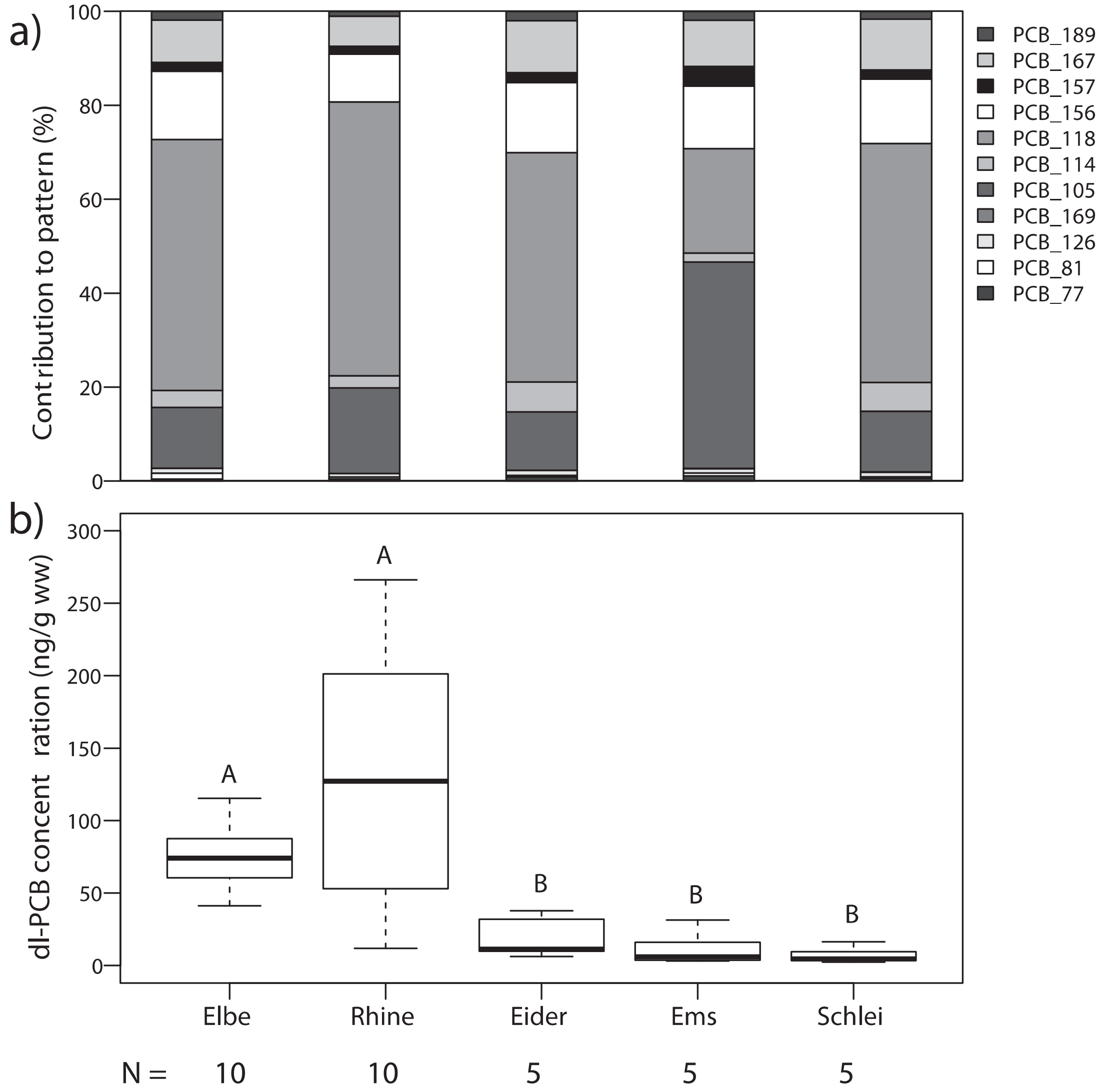

Figure 5

a)Median congener patterns of silver eels sampled in different German river bodies given in percent of total sum dl-PCB.

b)Sum dl-PCBs sampled eels in ng/g ww.. Whiskers represent maximum and minimum values, boxes the middle 50% of the datasets and the bold line indicates the median value of each data set. Significant differences in (P<0.05) between groups of fish from different habitats are indicated by capital letters. N indicates the total number of individual eels in the respective groups (Elbe: n=10, Rhine: n=10, Eider: n=5, Ems: n=5, Schlei: n=5)

Table 2 PCB concentrations (median values with maximums & minimums) for each targeted congener along with median sum concentrations (wet weight (ww) & lipid weight (lw)) and resulting TEQs (WHO 2005) of each group obtained in this study

Sampling Group	Sampling Location (Pos. on map (Fig1))	dl-PCBs											non dl-PCBs		Median Σ dl-PCBs (ng/g ww)	Median Σ dl-PCBs (ng/g lw)	Median Σ -WHO-PCB-TEQ (2005) (pg g^{-1} ww)
		PCB-81 (pg g^{1} ww)	PCB-77 (pg g^{-1} ww)	PCB-118 (pg g^{1} ww)	PCB-114 (pg/g^{-1} ww)	PCB-105 (pg g^{-1} ww)	PCB-126 (pg g^{-1} ww)	PCB-167 (pg g^{-1} ww)	PCB-156 (pg g^{-1} ww)	PCB-157 (pg g^{-1} ww)	PCB-169 (pg g^{-1} ww)	PCB-189 (pg g^{-1} ww)	PCB-170 (pg g^{-1} ww)	PCB-180 (pg g^{-1} ww)			
Elbe (Y)	**Bad Schandau (1)**	**273**	**341**	**38049**	**1915**	**7832**	**745**	**6460**	**11959**	**1093**	**0**	**1491**	**48496**	**109965**	**80.2**	**268.6**	**77**
	MIN / MAX	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A	±N/A
Elbe (Y)	**Dessau (2)**	**376**	**281**	**30782**	**1991**	**6856**	**737**	**5532**	**10150**	**1121**	**0**	**1224**	**36758**	**83533**	**59.6**	**208.9**	**76**
	MIN	172	135	12821	982	3432	379	2340	4615	540	0	623	18218	40073	26.3	131.6	39
	MAX	431	438	35936	2349	7766	1014	7113	13173	1280	0	1560	49576	109855	69.9	241.0	103
Elbe (Y)	**Hohengoeren (3)**	**598**	**353**	**25834**	**2081**	**5859**	**633**	**4917**	**8824**	**1095**	**0**	**1154**	**36764**	**73658**	**51.4**	**214.2**	**65**
	MIN	450	228	14285	1099	3097	330	1784	3843	538	0	480	14921	32318	26.1	144.8	34
	MAX	679	702	83709	3784	27253	827	9130	18091	3175	0	1585	41752	94734	148.7	338.0	85
Elbe (Y)	**Gorleben (4)**	**279**	**150**	**13528**	**916**	**3634**	**431**	**2110**	**3896**	**535**	**0**	**542**	**14550**	**31553**	**25.9**	**80.7**	**44**
	MIN	0	0	6091	421	1791	119	963	1730	264	0	145	4722	8598	11.7	32.4	12
	MAX	652	313	30602	2020	6327	745	5448	9945	1294	0	1214	35430	82655	58.6	139.4	76
Elbe (Y)	**Winsen (5)**	**756**	**180**	**27133**	**1963**	**7113**	**637**	**4604**	**8064**	**1128**	**0**	**1098**	**26766**	**59398**	**52.9**	**244.6**	**66**
	MIN	505	0	18519	1313	4903	402	3345	5437	779	0	698	19581	43313	36.1	100.2	41
	MAX	1017	367	33103	2438	9423	807	5782	9747	1521	0	1235	30123	68981	63.9	509.2	83
Elbe (Y)	**Jork (6)**	**317**	**134**	**11427**	**790**	**2928**	**229**	**2685**	**3677**	**448**	**0**	**574**	**15343**	**31995**	**22.9**	**227.9**	**23**
	MIN	0	0	2345	146	606	0	296	462	74	0	60	1741	3849	4.0	14.2	0
	MAX	1244	350	39945	2926	10025	755	5504	7408	1273	0	1108	28127	66391	69.2	660.5	78
Elbe (S)	**Cuxhaven (7)**	**785**	**342**	**38839**	**2486**	**10651**	**689**	**6324**	**10095**	**1317**	**0**	**1265**	**32153**	**68343**	**74.1**	**265.0**	**71**
	MIN	265	204	18697	1421	4585	574	5096	7759	1039	0	1011	26734	52808	41.3	137.6	59
	MAX	1815	451	63944	4852	14381	1347	9926	16518	2262	19	2116	68049	159318	115.4	450.4	138
Rhine (S)	**Kalkar (8)**	**533**	**505**	**71168**	**3603**	**22336**	**921**	**7456**	**13334**	**2186**	**0**	**1454**	**38553**	**74278**	**127.2**	**461.4**	**95**
	MIN	0	0	6542	292	2600	75	690	1205	225	0	119	3772	7413	11.7	51.1	8
	MAX	939	1483	164603	6580	50034	2097	17598	24687	4323	0	2261	74651	150920	266.0	1157.9	215
Schlei (S)	**Rendsburg (9)**	**15**	**19**	**2472**	**275**	**780**	**53**	**346**	**453**	**100**	**5**	**48**	**1025**	**1984**	**4.6**	**20.8**	**6**
	MIN	6	9	1187	132	475	24	195	212	53	0	25	698	1139	2.3	10.1	3
	MAX	36	117	8201	981	1425	137	2276	2633	287	9	309	16322	27920	16.3	70.9	14
Eider (S)	**Nordfeld (10)**	**35**	**80**	**5298**	**752**	**1355**	**146**	**1374**	**1693**	**295**	**10**	**267**	**8805**	**13953**	**11.3**	**66.8**	**15**
	MIN	26	56	3202	300	920	59	571	881	112	0	104	3782	5024	6.2	23.9	6
	MAX	112	331	18087	2298	4620	436	4371	5906	859	17	694	28138	43295	37.7	137.5	45
Ems (S)	**Emden (11)**	**24**	**71**	**1533**	**143**	**1501**	**74**	**640**	**929**	**464**	**6**	**146**	**5131**	**7023**	**6.0**	**29.8**	**8**
	MIN	21	52	311	0	505	19	11	0	59	0	39	1422	2090	3.0	11.2	2
	MAX	250	330	7564	640	21676	211	2746	3852	1086	25	422	18247	21039	31.4	138.6	22
ELV (pools)	**Verlath (12)**	**0**	**0**	**140**	**0**	**0**	**0**	**15**	**34**	**0**	**0**	**0**	**210**	**425**	**0.2**	**13.8**	**<1**
	MIN	0	0	74	0	0	0	0	0	0	0	0	0	0	0.1	4.9	0
	MAX	0	0	499	0	97	0	82	163	0	0	32	854	1558	0.9	58.1	0
GE (pools)	**French Atl. Coast**	**0**	**10**	**143**	**7**	**59**	**0**	**14**	**18**	**4**	**0**	**3**	**78**	**169**	**0.3**	**25.5**	**<1**
	MIN	0	7	90	5	36	0	7	11	3	0	1	42	91	0.2	161	0
	MAX	0	19	292	15	113	3	23	37	7	0	6	159	279	0.5	50.5	0

Data were grouped according to their life stages (Y= Yellow Eel, S=Silver Eel, ELV= Elver, GE= Glass Eel) and sampling locations respectively

Discussion

PCB congener patterns in eels

PCB patterns in eels analyzed in this study show distinct signs of environmental imprinting with life stage-specific differences among targeted congeners. This is well in line with findings of previous studies on eels from Canada, Belgium and France (Tapie et al. 2011; Byer et al. 2013) and should be regarded as crucial for eel management driven stocking measures. While glass eels and elvers represent life stages with no or only short termed influence by their freshwater habitat, yellow and silver eels have been dwelling in their growth habitats for several years resulting in a site-specific alteration of their dl-PCB profile. Possible reasons for occurring differences in congener patterns between life history stages may lie in the phenomenon that highly chlorinated congeners tend to remain in the body longer than less-chlorinated congeners due to their physiological character or they can be result of preferential metabolism (Steele et al. 1986; Hopf et al. 2013). Uptake of lipophilic xenobiotics in water by biota mainly follows three basic pathways: bioconcentration bioaccumulation and biomagnification. While bioconcentration describes the direct uptake from water by diffusion over the body surface (e.g. skin and gills), bioaccumulation is the increase in concentration of a substance in certain tissues within an organism's body due to absorbtion from food and the environment. Biomagnification however, is defined by the increase in concentration of a pollutant from one link in a food chain to another (Kwon et al. 2006; James and Kleinow 2014). This mode of steady and continuous uptake of dl-PCBs and other hazardous, biomagnifying xenobiotics over a longer period of time should be considered in stock management and possible inter-habitat comparisons for restocking measures.

Our results indicate glass eels to be mainly influenced by congeners taken up during their oceanic life, and in contrast, elvers to be already affected by continental pollution impacts, as expressed by higher loads of highly chlorinated dl-PCB congeners as well as higher concentrations of non-dl-PCBs 180 and 170 (Table 2). These congeners occur in higher concentrations in the continental environment due to their widespread anthropogenic use in technical mixtures, their high chlorination degree and resulting persistency. A similar shift in contamination patterns from oceanic to freshwater between glass eels and elvers has previously been reported for PCBs by Tapie et al. (2011), Blanchet-Letrouvé et al. (2014) as well as by Sühring et al. (2013) for brominated and chlorinated flame retardants. The differences in congener patterns despite the similarly low lipid content of glass eels and elvers indicate a rapid uptake of halogenated contaminants by eels as soon as they enter polluted freshwater habitats during their feeding and growth life history phases. Evidently the growth phase in continental freshwater and coastal systems is the decisive phase for the uptake of contaminants during the eel's life cycle. These findings are in agreement with results from similar studies (Tapie et al. 2011; Arai and Takeda 2012; Byer et al. 2013; Sühring et al. 2013; Blanchet-Letrouvé et al. 2014). Congener patterns of yellow and silver eels from the same habitat in this study showed no significant differences. In addition, congener patterns of targeted PCBs in yellow eels from different sampling sites along the same river system did not differ significantly. These findings indicate either evenly distributed sources for the contaminants or at least the same emission pathways within the same system. Significant differences in congener patterns found in silver eels sampled from different river systems in this study also suggest that sources along the same habitat may play a secondary role compared to the system as a source itself. Since the worldwide ban for PCBs in 2002, active point sources have become increasingly unlikely and recent contamination of biota apparently follows remobilization of PCBs deposited in sediments, soils or suspended particles (Stachel et al. 2004; Wetzel et al. 2013; Lake et al. 2014).

Accumulation and sum concentrations of targeted PCBs in eels

The here measured PCB levels and ranges are well in line with findings from previous studies. Sum concentrations of PCBs measured in glass eels are similar to comparable investigations along the French Atlantic coast (Blanchet-Letrouvé et al 2014). Results from yellow eels from river Elbe were close to results for yellow eels from the Elbe published by Stachel et al. in 2007 and comparable to yellow eels in similar size and age from the French river Loire (Blanchet-Letrouvé et al. 2014; Couderc et al. 2015). Silver eels from the Rhine and Elbe analyzed in this study however, showed very high concentrations of targeted PCBs, almost up to twice as high as found in silver eels from the Loire Estuary (Blanchet-Letrouvé et al. 2014; Couderc et al. 2015).

Previous studies by Belpaire et al. (2007; 2008), Byer et al. (2013) as well as Sühring et al. (2013) reported high intra-habitat variability in sum concentrations for halogenated contaminants such as PCBs and BFRs in yellow eels, concluding this life history stage to be most suitable for the detection of local point sources due to their sedentary lifestyle (Belpaire and Goemans 2007; Belpaire et al. 2008; Van Ael et al. 2014). In the here presented study, results of sum concentrations of targeted PCBs in yellow eels showed no significant differences along different sampling locations within the same habitat (river Elbe). Nevertheless, a slight decreasing tendency was observed from the upper river towards the tidal zone close to the river's mouth. This may be rooted either in age and lipid content of tested individuals or in local passive sources of the contaminants, which does match with the literature. A previous study by Stachel et al. (2004) reported sediments in the mouth of the Mulde river and a source in the Czech Republic to be the main historical entry paths of PCBs into the Elbe system.

Generally speaking, accumulated sum concentrations of targeted PCBs in eel samples used in this study were correlated to life history stage. Similar to findings concerning the congener patterns, glass eels and elvers show rather low alterations concerning concentrations of targeted PCBs compared to yellow and silver eels. Reasons for this lie in the habitat dwell time of each individual that defines the range and intensity of contamination stress it was exposed to. In a study by Tapie et al. (2011), the authors investigated PCB concentrations in eels and their data revealed a clear rise of accumulated PCBs with age and size of the fish as well. However, this accumulation effect is specifically critical for semelparous species like the European eel but has to be viewed at in context with the fish's life history stage. While studies on yellow eels may allow for a valuable snapshot of their current contamination status, silver eels on their downstream migration form the most representative life history stage to provide information on the health and fitness status of local populations from a certain habitat. Looking at our results of dl-PCB concentrations in muscle tissue of silver eels from different water bodies, it becomes evident that the respective origin of each eel is the most important driving factor for final lipophilic contaminant loads. Silver eels have a lower variation in fat content since they begin the migration to their spawning grounds (Larsson et al. 1990) and are not likely to experience any further influential events with strong impact on their complete and final contamination status. These final amounts of accumulated OCs may be important for future assessments of the contaminants potential risks for the eels offspring after possible maternal transfer (Palstra et al. 2006; Sühring et al. 2015) or for the lipid metabolism of the individual itself during its migration (Corsi et al 2005). Unfortunately effects of dioxin-like contaminants on eels are yet not entirely understood and (due to a lack of available data) it remains difficult to entirely assess the consequences for the health and reproductive capability of eel stocks caused by dioxin-like contaminants. One possible way to quantitatively facilitate risk assessment and regulatory control of the toxicity of these compounds is the use of Toxic Equivalents (TEQs).

TEQ-levels of silver eel samples out of nearly all sampling locations (except fish from the Schlei fjord and River Ems) in this study exceeded the minimum risk levels (MRLs) of 12 pg / g ww TEQ for human consumption (EC regulation No 1881/2006) and those (less than 4 pg / g ww TEQ), that were held responsible to have impaired normal embryonic development of eels in a study by Palstra et al. (2006). Generally speaking, total sums of PCBs and their resulting TEQs in analyzed silver eels from this study were highly dependent on their provenance and the respective urbanization: While Rhine or Elbe account for industrial rivers with historically higher anthropogenic influence and sediment contamination, more rural rivers such as the Eider, Ems or the Schlei fjord (as an example for Baltic-associated water body) tend to produce silver eels with lower loads of lipophilic contaminants and as a result form more suitable habitats for eels in terms of their contamination-related reproductive capacities. With regard to German and other European national eel management programs which contemplate restocking as a stock enhancement measure, it has to be considered whether restocking is meant to support local utilization and use for commercial interests or if it is done for stock enhancing purposes to increase the escapement of healthy and high-quality spawners.

Conclusions

This study strongly confirms that (dl-)PCB contamination of eels is mainly driven by uptake during their continental growth phase. Eels originating from the here analyzed German river systems differ significantly in their total sum PCB contamination and pollution patterns. The potential negative effects of dl-PCBs on the health and reproductive capability of eels make it crucial to evaluate designated habitats for restocking of eels. We conclude that concentrations of dl-PCBs found in muscle tissue of silver eels can be used along with other crucial indicators to describe the quality of their respective habitat. Considering the high impact of habitat combined with the continuous accumulation of PCBs up to the silver stage, stocking and reallocation of young eels as stock enhancement measures should only be performed in suitable habitats. For this, the contamination levels of the rivers and river sections should be assessed and only the most suitable water bodies or sections should be selected. This implies meeting requirements and conditions for eels to gain an improvement of living conditions in their continental phase and thus, to produce healthy and qualitatively generative silver eels and spawners, which is in accordance with the goals of the eel management plans of the European Union.

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