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Maternal transfer of dioxin-like compounds in artificially matured European eels

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Abstract

Several eel species of the genus *Anguilla* are considered endangered due to a severe decline in recruitment. Up to now, the reasons for this threatening development are not fully understood. The eel's highly specialized biology can lead to explicitly high accumulation of globally distributed organic lipophilic contaminants during its continental life. Because of this and due the particular toxicological sensitivity of early life stages of oviparous organisms towards dioxin-like compounds, it is crucial to improve our understanding concerning toxicokinetics and maternal transfer of organic contaminants in eels.

This study presents analytical data on maternal transfer of dioxin-like (dl) compounds in relevant tissue samples taken from artificially matured and non-matured European silver eels (*Anguilla anguilla*) from German inland waters using gas chromatography coupled with mass spectrometry (GC/MS) and high-resolution mass spectrometry (GC/HRMS). Detected concentrations revealed a lipid-driven transfer of targeted compounds from muscle-fat-reserves to gonads and eggs respectively, with no distinct preferences concerning the chlorination degree of targeted compounds. Dl-PCBs were shown to contribute the major share of toxicity equivalents found in analysed eel tissues. Maternal muscle tissue to egg concentration ratios in wet weight–based samples

had a mean of 6.95±1.49 in accordance with the differences in total lipid content in the respective body matrices. Dioxins and furans in analysed samples were (from a toxicological point of view) of less relevance. Furthermore it was shown that muscle concentrations in silver eels could be used in future assessments to make conservative predictions for expected egg concentrations in female eels.

This work provides novel analytical data on maternally transferred dioxin-like contaminants measured in European eel eggs.

Introduction

Since the 1980s, the three of the temperate freshwater eel species European eel (*Anguilla anguilla*), American eel (*Anguilla rostrata*) and Japanese eel (*Anguilla japonica*) have experienced severe declines in glass eel recruitment (Dekker, 2003; ICES, 2016). As a consequence, the affected species have been rated as critically endangered (*Anguilla anguilla*) and endangered (*Anguilla rostrata* and *Anguilla japonica*) by the International Union for Conservation of Nature (IUCN*)*. A number of different hypotheses on possible causes have been raised including habitat loss and degradation, overfishing, oceanic changes, parasitism and pollution (Knights, 2003; Geeraerts & Belpaire, 2010; Wysujack et al., 2014; Miller et al., 2015). It is more than likely that only a combination of these impacts has led to the recruitment declines and it is important to identify and evaluate the major drivers in this combination of influential factors.

Anthropogenically introduced chemical pollution especially by halogenated lipophilic persistent organic pollutants (POPs) is believed to be capable of severely impairing the reproductive success of European eels (Palstra 2006, Geeraerts & Belpaire, 2010; Geeraerts et al., 2011; Sühring et al., 2015, Foekema et al. 2016). Dioxin-like compounds such as polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are considered to be among the most toxic manmade substances in the world and constitute a frequently discussed group of hazardous contaminants in scientific literature. Dioxin-like compounds have been shown to cause several health effects on animals including endocrine disruption, terato- & mutagenesis, hepatic damage and impaired reproduction (Cook et al. 2003; Mandal 2005; Palstra 2006; King-Heiden et al. 2012; Foekema et al. 2014; Rigaud et al. 2016).

The eel's specific predisposition towards lipophilic contamination as semelparous, bottom-dwelling predators with naturally high body fat contents in combination with the chemical properties of dioxin-like substances and their high concentration in sediments and biota of many continental water bodies can lead to comparably high accumulation in muscle tissue of this species (Stachel et al., 2007; Byer et al., 2013; Blanchet-Letrouvé et al., 2014; Freese et al., 2016). A number of studies have made clear that different chemical profiles as well as different concentration ranges of contaminants in eel samples are related to the respective habitat (Belpaire et al., 2008; Sühring et al., 2013; Van Ael et al., 2013; Kammann et al., 2014; Blanchet-Letrouvé et al., 2014; Freese et al., 2016). Nevertheless, with exception of modeled scenarios (Foekema et al., 2016) and a single experimental work by Palstra et al. in 2006, no scientific studies are available in literature, in which the actual transfer of dioxin-like substances from the maternal tissue to eggs or larvae was investigated in eels.

In their study, Palstra et al. (2006) put the survivability of eel embryos in relation with Toxicity Equivalents (TEQs) of dioxin-like compounds (DLCs) determined by the DR-CALUX test in gonad and muscle tissue of artificially matured eels as well as in a control group. Even though the maternal transfer of dioxin-like substances and other POPs have already been described in many other species (Henriksen et al., 1996; Russell et al., 1999; Sühring et al., 2015), a lot of uncertainty about the involved mechanisms and effects of DLCs and their physiological relevance in eels remains. Reason for this may be that large parts of the eel's natural reproduction cycle are still considered a mystery and it is yet not entirely possible to artificially reproduce European and American eels. However, progress on the protocols in the artificial maturation and hatchery design made it possible to shed some light on the reproduction biology of these highly specialized species (*A. anguilla* : Tomkiewicz (2012); *A. rostrata* : Oliveira et al., 2010).

The major aim of this study was to get detailed insights into the extent of maternal transfer from body lipid reservoirs into ovarian tissues of dioxin-like substances during maturation of eels from European water bodies and also to gather information on the biological mechanisms and driving factors involved. As a consequence, this study was intended to enable the estimation of the total DLC TEQ-concentrations per egg-mass deriving from dl-PCB contamination in muscle tissue from female silver eels representing *in situ* occurring contamination histories.

Material & Methods

105

106 Samples

107 In this study we used female, migrating silver eels caught with fyke nets by commercial
108 fishermen in the potamal sections (lower stretch) of the river Ems and the Schlei fjord in
109 February 2013. From each sampled water body, we bought complete commercial hauls
110 of fish in line with samplings done for the European Data Collection Framework (DCF),
111 as defined by the European Commission (2008, 2010). After acclimatization in flow-
112 through freshwater tanks for seven days, eels were sacrificed and their otoliths excluded
113 for age estimations following an expert protocol for age determination in eels (ICES
114 2009, 2011). For possible later use, samples of white muscle and gonadal tissues were
115 taken from each specimen. For this, between 10 and 25 grams of fresh gonad- and skin-
116 free muscle tissue taken from the filet between anus and tip of the tail of the eels were
117 sampled and stored at -20°C until usage. To eliminate sources of contamination, samples
118 were strictly handled with clean equipment made of glass, aluminum or steel,
119 preventing possible sources of cross-contamination. After age reading, samples from five
120 female eels of comparable length, weight, age and migration stage (Durif et al., 2005)
121 from each water body were selected to determine their dioxin-related contamination
122 (See supplement information S1 for a detailed list of individual variables).

123

124 For artificial maturation, five fish from each batch were acclimatised to saltwater
125 (20±1°C; 35±0.5 practical salinity units (PSU)) and held under constant water flow in a
126 round recirculation system equipped with aeration stones and a trickle filter for
127 mechanical filtration and denitrification. All artificially maturated fish were held in the
128 experiment for a timespan of 17 to 19 weeks until final gonadal maturation. As under
129 natural conditions migrating and maturing silver eels are believed not to feed anymore,
130 maturing eels in this experiment were constantly moving against gentle water flow and
131 received no food. The eels were hormone-treated by one weekly intramuscular injection
132 (20 mg/kg into the dorsal muscle, close to the dorsal fin) of aqueous salmon pituitary
133 extract (SPE, Argent Aquaculture, Redmond, USA) to induce maturation and egg
134 development. With a final injection of 17α,20β-Dihydroxy-4-pregnen-3-one (DHP,
135 Sigma-Aldrich, Taufkirchen, Germany) ovulation was induced and after stripping the
136 eels were sacrificed and dissected for further analyses. Only entirely matured (visually
137 evaluated during dissection) eels (two from Schlei and three from Ems) were selected
138 for chemical analyses. Tissue samples of gonads, eggs and muscle from hormone-treated

fish were taken according to the sampling described for the untreated fish. All eels in this study were killed by decapitation after being anaesthetized with 2-Phenoxyethanol (ROTH, Karlsruhe, Germany).

Total lipid content in organs and tissue groups

Total extractable lipid levels in analysed tissue were determined as described by Smedes (1999) along with methodological alterations introduced by Schlechtriem et al. (2012). Briefly, approximately 100 mg of homogenized, freeze-dried tissue sample was used for lipid extraction with a mixture of cyclohexane (2.50 mL), propan-2-ol (2.00 mL) and water (2.75 mL), followed by a second extraction with cyclohexane (2.175 mL) and propan-2-ol (0.325 mL). The organic phase was collected after each extraction and the solvents were evaporated prior to gravimetric determination of the fat content. All samples were analysed in duplicates.

Extraction and clean-up

All analysed compounds were prepared the same way before extraction by pressurised liquid extraction: Frozen silver eel tissue 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). Then, separate aliquots for analyses of dl-PCBs and PCDDs/PCDFs were spiked with ^{13}C mass labeled surrogate standards analogous for each analysed compound respectively. (PCBs: WHO PCB+PCB-170+PCB-180 CLEAN-UP STANDARD ($^{13}C_{12}$, 99%), Cambridge Isotope Laboratories (CIL), Tewksbury, USA; PCDD & PCDFS: EPA1613 LCS, Wellington Laboratories, Guelph, Canada). Any remaining volume in the extraction cartridges was filled with anhydrous Na_2SO_4 (ROTH, Karlsruhe, Germany). Spiked, 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, following the method described in Sühring et al. (2013).

PCDD & PCDFs clean-up & Analyses

For PCDD and PCDF clean-up, CAPE technology acid silica columns (Cape Technologies L.L.C., South Portland, ME, USA) with carbon mini-columns were used. Each of these columns was conditioned using 10 ml each of acetone and hexane while the carbon mini-column was conditioned with 10 ml each hexane and toluene. The carbon mini-

column was attached to the outlet of the acid silica column and the extracts were then applied onto the acid silica column using the CAPE glass-syringe funnel.

First, the targeted analytes were eluted onto the activated carbon mini-column using ten ml of hexane. Subsequently, 20 ml of hexane were used to elute the dl-PCBs from the column. Following that, the mini-column was detached from the acid silica column and connected with a clean, empty CAPE column. Afterwards, five mL of a toluene-n-hexane (v/v 1:1) mixture was used to extract any remaining dl- PCBs from the column. The carbon mini-column was then reversed and the PCDDs/PCDFs were eluted with 30 mL of toluene. Analysis of PCDDs/PCDFs was conducted in accordance with the method previously published by Byer et al. (2013). Briefly, gas chromatography/high-resolution mass spectrometry (GC-HRMS) analyses were performed using a Micromass AutoSpec mass spectrometer (Micromass, Manchester, UK) in electron ionization (EI) and selected ion-monitoring (SIM) modes. The mass spectrometer was coupled with a Hewlett–Packard 6890 gas chromatograph (Hewlett Packard, Palo Alta, CF, USA) fitted with a Restek Dioxin-2, 60 m × 0.25 mm × 0.25 μm column (Restek, Bellefonte, PA, USA) and an CTC A200S autosampler (Leap Technologies, Chapel Hill, NC, USA). Following settings were used: Helium as carrier gas: 1.5 mL min^{-1}; source temp: 280°C; front Inlet temp: 280°C; transfer line temp: 280°C; splitless injection: 1.5 min at 30 mL min^{-1}. The system was tuned using perfluorokerosene as a reference (10,000 resolution at 5% peak height definition) over the mass range of the PCDD/F. To ensure stable conditions, the instrument was calibrated after every batch of five samples and the instrument was re-tuned and re-calibrated daily.

<u>Dl-PCB clean-up & Analyses</u>

As described in Sühring et al (2013), clean-up of the samples was done by gel permeation chromatography (GPC), using 30 g Bio-Beads SX-3 (Bio-Rad Laboratories, Hercules, USA) and dichlormethane:hexane (1:1; v:v) as eluent. While discarding the first fraction (75 mL), the second fraction (110 mL) containing the target compounds, was reduced to about 2 mL before its transfer into hexane. As a second step, we used a column with 2.5 g 10% H_2O deactivated silica gel (ROTH, Karlsruhe, Germany) and 20 mL of hexane as an eluent, before the samples were narrowed down to a volume of 150 µL and transferred to measurement vials. Finally, 10 µL ^{13}C-PCB 141 and ^{13}C-PCB 208 (50 ng mL^{-1}) was added as injection standard to each sample.

Analyses of targeted PCBs were conducted using a GC/MS-system (Agilent 6890

GC/5973 MSD, Agilent Technologies, Santa Clara, USA) equipped 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) operating in electron capture negative ionization mode (ECNI) with methane as ionization gas. Samples in our study were analysed for dl-PCBs (IUPAC numbering) 77, -81, -105, -114, -118, -126, -156, -157, -167, -169 and -189.

QA/QC:

All samples were handled in a clean-lab class 10000 (United States federal standard 209E).

PCDD & PCDFs

Analysis was performed in batches of five, in combination with two blanks and one certified reference material (CRM) sample (CARP-2, National Research Council of Canada) per batch. CARP-2 reference values then were compared to the measured results with a paired t-test (mean values from five replicates). 1,2,3,7,8-PeCDF and 1,2,3,4,6,7,8-HpCDF were up to 15% lower than the reference values (Student's t-test), while the remaining PCDD/F congeners were statistically indistinguishable from the reference values. Blank values were generally low with "not detected" for most analysed compounds. LODs ranged from 0.55 pg/g wet weight (ww) (1,2,3,4,7,8-HxCDD) to 2.4 pg/g ww (2,3,7,8-TCDF). LOQs ranged from 1.5 pg/g ww (1,2,3,4,7,8-HxCDD) to 5.2 pg/g ww (2,3,4,7,8-PeCDF). Recoveries of ^{13}C isotope marked surrogate standards were good and ranged between 77% and 128% with an average of 101%. For statistical analyses, concentrations below LOD were assigned a value of 1/2 of the LOD (mid-bound), concentrations below LOQ (one sample) were included in calculations.

PCBs

Recovery rates of isotope labelled (^{13}C) internal standards (IS) were determined for each sample. Mean IS recoveries ranged from 57± 26% for PCB 81 to 96 ± 34% 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 the method quantification limit (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 plus 3 times or 10 times blank standard deviation, or from a signal to noise ratio of 3 or 10, respectively. LODs ranged from 0.99 ± 0.5 pg/g ww (PCB 189) to 30.9 ± 29.7 pg/g ww (PCB 77). LOQs ranged from 3 ± 1.4 pg/g ww (PCB 189) to 102.3 ± 99.9 pg/g ww (PCB 77). For further quality control, a twofold measurement was

conducted for each sample. Results for PCB 123 were entirely excluded from our results due to incomplete chromatographic separation. For statistical analyses, concentrations below LOD were assigned a value of 1/2 of the LOD (mid-bound), concentrations below LOQ were included in calculations.

Data processing and statistical analyses

To assess toxicological relevance and provide for good comparability of results from our study with literature, World Health Organization Toxic Equivalent values (WHO_{2005} TEQs) were calculated based on re-evaluated Toxic Equivalent Factors (WHO_{2005} TEFs) (Van den Berg et al., 2006). All statistical analyses were performed using GraphPad Prism (Prism 6.0h, October 2015, GraphPad Software Inc., Ca, USA). Differences in accumulation quantities of targeted dl-PCBs between the respective sample groups were tested using the sum concentrations of individuals in each group. When testing two groups against each other, the Mann-Whitney test was performed. When testing more than two groups against each other, a Kruskal-Wallis-Test was performed. After investigating a possible influence of habitat on relevant characteristics in sampled untreated silver eels from Ems (N=5) and Schlei (N=5), we combined all untreated fish to one group (N=10) to compare them against the group of hormone-treated (N=5) fish.

Results and Discussion

Influence of sampling origin

Eels used in this study were caught in 2 German catchments. Length, weight and muscle lipid content of fish were not statistically different between the untreated groups from the two catchments (Mann-Whitney test of unpaired t-test; length: P=0.73; start weight: P=0.73; muscle lipid content: P=0.55) (Table 1). The origin of the sampled individuals also showed no statistical influence on the total concentration of targeted compounds detected in the sampled (untreated) fish (Mann-Whitney test of unpaired t-test; P=0.55).(See supplement information S1 for a detailed list of individual variables and concentrations)

Lipids and body composition in eels during maturation

Along with the metabolic reallocation of lipid stores from muscle to reproductive tissues, analytical data from our study confirm a transfer of dioxin-like contaminants

from maternal somatic to reproductive tissues in European eels. Total extractable lipid content in wet muscle tissue did not differ significantly between hormone-treated and untreated eels (Table 1) (Mann-Whitney test of unpaired t-test; P=0.49). This is well in line with observations made for other artificially matured European eels in studies by Palstra et al. in 2010 or Nowosad et al. in 2014 and Japanese eels by Ozaki et al. in 2008, in which artificially matured eels maintained their muscle lipid content and general body composition at the same levels as untreated eels. Nevertheless, total muscle-mass was reduced which indicates, as also suggested by Ozaki et al (2008) that in addition to lipids, other macronutrients such as proteins / amino acids are being metabolized in maternal muscle tissue during starvation and maturation. In line with these depletions of energy reserves in muscle tissue, gonadal mass in hormone-treated eels multiplied, making up to 51.6±6.1% of total pre spawning body weight compared to 1.4±0.3% in untreated eels. (See supplement information S1 for a detailed list of individual variables).

Tissue concentrations

DLC concentrations measured in eel muscle tissue in this study are in similar ranges as found in previous studies on European eels. Total WHO_{2005} TEQ concentrations for ΣPCDD/F ranged between 2 and 9 pg WHO_{2005} TEQ /g ww, including estimated middle-bound LOD concentrations for non-detected congeners. These results are in a comparable range as found in other studies on eel from European water bodies (Bordajandi et al., 2003; Stachel et al., 2007; Szlinder-Richert et al., 2010; Byer et al., 2013; Blanchet-Letrouvé, 2014).

TEQ concentrations for dl-PCBs in eel muscle tissue ranged from 8.35 to 75.56 pg WHO_{2005} TEQ /g ww in hormonally treated eels and from 1.98 to 40.35 pg WHO_{2005} TEQ /g ww in untreated eels with (by far) highest contribution of congener 126 to total WHO_{2005} dl-PCB TEQs. Also these results were comparable to concentrations found in previous studies for eels muscle from some European water bodies in Belgium, Germany and France (Stachel et al., 2007; Geeraerts et al., 2011; Blanchet-Letrouvé, 2014). The high individual variability in tissue concentrations (also from fish within the same water body) reflects the difficulties associated with field studies on fish contamination. Eels obviously are mobile throughout their continental life, which may lead to different contamination ranges due to local differences of pollution within different parts of the habitat (Freese et al., 2016). Concerning dl-PCBs TEQ concentrations in gonads and eggs,

we are not aware of many available publications with data on these matrices. Concentrations in eel eggs derived from indirect measurements using DR CALUX bioassay in a study by Palstra et al (2006) predicted similar concentrations in eel eggs as measured in this study.

Tissue burden calculations:

To depict the physical transfer of muscle (lipid)-bound POPs into the egg mass, we calculated the amounts of total dl-PCBs bound in entire reproductive tissue and put them in contrast to the absolute amount of these compounds calculated in total muscle tissue of the same individuals per group (Fig 1).

$$B\ REP = m\ (egg) * c_{\Sigma dl\text{-}PCB}\ (egg) + m\ (gon) * c_{\Sigma dl\text{-}PCB}\ (gon)$$

$$B\ MUS = (m\ (carc) - m\ (rest)) * c_{\Sigma dl\text{-}PCB}\ (mus)$$

Where B REP is the total mass of hydrated eggs (*m (egg)*) and the mass of remaining gonadal tissue (*m (gon)*) multiplied with measured dl-PCB concentrations found in respective reproductive tissues ($c_{\Sigma dl\text{-}PCB}\ (egg) + c_{\Sigma dl\text{-}PCB}\ (gon)$) and B MUS is the dl-PCB concentration found in total muscle tissue ($c_{\Sigma dl\text{-}PCB}\ (mus)$) calculated by the mass of the eels carcass (*m (carc)*) minus the combined mass of remaining tissue types (m (rest)) including reproductive tissues, intestines, skeletal bones and skin multiplied with measured dl-PCB concentrations found in muscle tissue samples. (See supplement information S1 for a detailed list of individual variables).

Total amounts of dl-PCBs bound in muscle and reproductive tissue of hormone-treated fish compared to amounts bound in gonad-tissue of untreated fish differed significantly (Kruskal-Wallis-Test H=18.63, p = 0.0003), with gonads of untreated fish having significantly less dl-PCBs bound than any other tested tissues. This finding reflects the change in mass of gonadal products in relation to total body mass between fully matured and non-mature silver eels during maturation. Although no statistically significant difference was found between muscle tissue of untreated fish compared to muscle tissue of hormone-treated fish (Mann Whitney test of unpaired t-test; P=0.86), it is noteworthy that tissue burdens in muscle of untreated fish showed a wider range than concentrations found in muscle tissue of treated fish. As a result, combined muscle and gonad / gonad&egg burdens of both groups sum up to similar concentration ranges

(treated=2661-11944 ng dl-PCB; untreated=1072-12930 ng dl-PCB) with no significant differences (Mann-Whitney test of unpaired t-test; P=0.24), underlining a statement made in our previous study (Freese et al., 2016), that escaping silver eels have reached their "final contamination status" before spawning migration and at the same time, giving further indication that elimination of higher chlorinated PCBs during starvation and migration is negligible (de Boer et al., 1994).

Maternal transfer of PCDDs & PCDFs

PCDDs and PCDFs were analysed in a subsample of n=3 artificially matured and n=4 non-matured individuals used in this study (Table 2).

Results revealed that total concentrations of PCDDs/PCDFs compared to those of dl-PCBs in eels from the sampled habitats play a secondary role, as most PCDDs/PCDFs congeners were not detected in any of our analysed samples. As a result, non-ortho and mono-ortho PCBs constituted the vast majority in both, total concentration and TEQs of analysed dioxin-like compounds in this study (Figure 2). Detected PCDDs & PCDFs in all samples had detection frequencies of less than 5% compared to 100% for most analysed dl-PCB congeners. Concentrations of total dl-PCBs were in ng g^{-1} ww range while quantified concentrations of dioxins and furans were much lower, with maximum total PCDD concentrations of 6.5 pg/g ww in gonads of the comparison group fish and maximum total PCDF of 31 pg/g ww in eggs from one hormone treated eel from river Ems. Congruent with the results reported by Byer et al. (2013) for eels from Belgium, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF were the highest concentrated PCDD/PCDFs in muscle and gonad tissue of the European eel comparison group, rather than TCDD reported as the predominant PCDD/PCDF in American eels from the Great Lakes region (Byer et al. 2013). The overall detection frequencies were too small to derive any statistically significant conclusions.

It is interesting to note that in hormone-treated eels, eggs were the only tested matrix in which 1,2,3,4,6,7,8-HpCDF was detected. This is especially noteworthy since lipid content in eggs was overall lower than in muscle tissue (Table 1). With respect to the small number of tested individuals, these findings could eventually be an indication for a selected transfer, changes in uptake, distribution or metabolism during the artificial maturation process, as we have previously observed for flame retardants (Sühring et al. 2015). Another influential factor could be the composition of the eggs, including

379 different lipid classes as well as vitellogenin, an egg yolk precursor protein for the
380 lipoproteins and phosphoproteins present in the protein content of yolk. Vitellogenin
381 has been suggested to associate with 2,3,7,8-TCDD and therefore may play an important
382 role as a vector in maternal transfer of dioxin-like substances. Its structure with both,
383 phosphate–rich regions and large non-polar lipid moieties makes it well suited to
384 function as a vessel or vector for maternal transfer of a variety of compounds
385 (Monterverdi et al. 2000). Apart from percental lipid content, also lipid composition
386 should be regarded as of importance in the kinetics of lipophilic POPs. The group of
387 lipids is constituted mainly of two slightly different classes: polar and non-polar lipids.
388 While the group of polar lipids consists primarily of phospholipids, the neutral and non-
389 polar lipids are formed essentially by triacylglycerols (TAGs), cholesterol and wax esters
390 (Tocher, 2003, Elskus et al., 2005). While TAGs are the most abundant among the non-
391 polar tissue lipids that are mainly used as energy reserves and storage depots, primarily
392 in liver, muscle and mesenteric fat, phospholipids are the main lipids in cellular
393 membranes and form one of the major fractions of egg yolk (Johnson, 2009) and thus
394 can be found in higher proportions in reproductive glands than in muscle tissue of fish
395 (Kammann et al, 1990, Jobling et al. 1998, Sutharshiny et al. 2013). Different lipid classes
396 may have different binding affinities to lipophilic compounds dependent partly on their
397 octanol-water partitioning coefficient (K_{ow}). Nevertheless, chemical partitioning solely
398 based on log K_{ow} values must be considered with caution, since octanol used as a
399 surrogate for biological lipid cannot simulate barriers to uptake such as molecular
400 configuration or steric hindrance by membranes and functions instead of simple linear
401 partitioning (Elskus et al, 2005). It is therefore likely that the composition of lipid
402 classes as well as the amount of generated and incorporated vitellogenin in the different
403 analysed matrices (muscle, gonads, eggs) has an impact on the concentration as well as
404 the composition of distinctive halogenated congeners.
405
406 Maternal transfer of dioxin-like PCBs
407 Congener patterns of dl-PCBs did not differ between hormone-treated and untreated
408 fish in our setup (Figure 3). Different from previous observations made in our study on
409 flame retardants (Sühring et al., 2015), where metabolites from halogenated flame
410 retardants seemed to increase after maternal transfer, the here targeted PCB congeners
411 remain stable and patterns in reproductive glands (gonads and eggs) did not change
412 noteworthy. In future approaches on this topic, it would be interesting to add lower

chlorinated PCBs to the targeted compounds to see if the chlorination degree then would have an effect on the found congener patterns.

Induced by hormonal treatment, energy reserves stored in muscle tissue are being reduced by catabolic processes and re-assembled in gonadal tissue during sexual maturation. Generally, the redistribution of lipophilic contaminants in altering body compartments is assumed to be limited by blood flow and diffusion (Nichols et al., 1990). It seems likely that the transportation of stored lipids from the muscle tissue follows a physiological pathway over the liver (Nichols et al., 1998). This suggestion is supported by results of Ozaki at al (2008) in which lipid content in livers of artificially matured Japanese eels increased along with maturation. Moreover, in a study by Okumura et al (2001), histological examinations showed that size and number of oil droplets in livers of Japanese eels increased during artificial maturation. As a result, it would be interesting to include samples of liver tissue in analyses of future investigations.

In our study, lipid normalized total-concentrations of dl-PCBs showed no significant differences (Kruskal-Wallis-Test $H=7.625$, $p = 0.1063$) among groups or tissue types (Figure 4). This is well in line with findings by Russell et al. (1999) who confirmed in a number of different fish that transport of hydrophobic organic compounds from maternal tissues to eggs results in equilibrium in concentration, after following a number of passive transport processes.

Transfer rates

Transfer rates of dl-PCBs from muscle to gonad tissue in treated and untreated fish were heterogeneous (Table 2) and ranged between 0.85 to 6.69 in untreated silver eels compared to 1.89 to 5.16 in treated silver eels. Reasons for this very likely lie in the differences in lipid concentration found in unripe, non-ovulated gonadal tissue as well as in growth dilution as a factor in still growing gonadal tissue of untreated silver eels.

In contrast, the transfer from muscle to eggs in our sampled eels followed a fairly stable ratio (Table 2). After egg release, total dl-PCB concentration based on wet weight in remaining muscle tissue of artificially matured fish was between 5.27- and 9.92- fold higher (average 6.95±1.49) than found in egg tissue. For the most part, this reflects the lipid contents of the matrices, as for lipid-normalized data; concentrations found in the three sampled tissue types were not significantly different (Figure 4) (although not perfectly even). This observation is in line with findings of a study by Russell et al.

(1999), in which the authors investigated the maternal transfer of hydrophobic organic chemicals in 14 different fish and snapping turtle species. One of their central results was that lipid normalization of most of the tested egg and maternal concentrations was not significantly different from 1.0. Mean values of untreated fish compared to the artificially matured individuals however, revealed slightly more balanced concentrations in muscle and gonad tissue. These observations could be explained by expectable differences in the earlier mentioned lipid-composition and vitellogenin content in each matrix along with the toxicokinetics of lipophilic compounds. The kinetics of lipophilic compounds in fish bodies during metabolic changes are believed to be rapid (Nichols et al., 1990) but still require time defined by blood flow, catabolic depletion of reserves and gonadal growth during maturation to reach equilibrium between body compartments.

Predictions of egg-TEQs based on muscle concentrations and implications for stock management

To help build a better understanding of consequences caused by contamination with dioxin-like substances for reproduction in eels, we used the mean muscle-egg concentration ratio of our hormonally matured silver eels and estimated the same ratio to be applicable for all migrating silver eels. Projected concentrations based on the muscle-egg ratio and measured concentrations in the muscle tissue alone were very close to actually measured concentrations in egg tissues due to the relatively low variability in calculated muscle-egg ratios (Figure 5, black and white circles). If this ratio of concentration transfer in artificially matured eels is similar to concentration ratios during the eel's natural migration, it can be used to predict the expectable TEQ concentration in eggs from migrating wild silver eels. As a consequence, we estimated expectable egg WHO_{2005} TEQ concentrations derived from silver eel muscle concentrations from different German water bodies (Freese et al., 2016), and put them in relation to threshold values for eel and different fish species, taken from literature. Even though our limited data set has to be regarded with caution, this approach may help to get an idea whether reproduction of eels from German river systems is likely to be impaired through contamination by dioxin-like contaminants and as a consequence, successfully contribute to the European eels spawning stock (Figure 5). More than 50% of the projected estimates led to values exceeding the threshold of 4 pg WHO_{2005} TEQ/g ww for developmental disruption in eel embryos suggested by Palstra et al. (2006) with

some of the examined water bodies being more affected than others. One of our projected concentrations even exceeded a value of 29 pg TEQ/g egg, representing the beginning of direct egg mortality measured in lake trout by King-Heiden et al. (2012). In a different study but also for lake trout, the lethal dose concentration (LD50) for maternally transferred 2,3,7,8-TCDD in eggs was determined at 65 pg/g ww (Walker et al., 1994). In a work on maternal transfer of dioxin in brook trout (*Salvelinus fontinalis*) by Johnson et al. (1998), the authors found that median lethal residue (LR50) values for 2,3,7,8-TCDD were as high as 127 pg/g ww in eggs. For several other fish species, even higher concentrations were needed to reach LR50. Embryos exposed to water concentration of TCDD of the, comparably, non-sensitive zebrafish (*Danio rerio*) or shovelnose sturgeon (*Scaphirhynchus platorynchus*) exhibited a much higher tolerance towards dioxin-like contaminants compared to the previously mentioned salmonid species with LD50s of 2610 and 13000 pg of TCDD/g ww of egg, respectively (Elonen et al., 1998, Buckler et al. 2015). Nevertheless, elevated incidences of malformations in embryos in other sturgeon species have been reported at concentrations as low as 50 pg of TCDD/g egg (Chambers et al., 2012). Some of the here mentioned concentrations are considerably higher than expectable concentrations in reproductive tissues from contaminated fish in the wild. In our study, even eels from waters, that have produced eels with comparably high DLC contamination in the past (e.g. Elbe, Rhine), would not reach concentrations of several hundred pg TCDD TEQ, even if TEQ-calculations were not limited on dl-PCBs values alone. However, due to the differing sensitivity among investigated species to the various dioxin-like compounds, there remains uncertainty regarding the risk assessment of DLCs in fishes.

Our here used approach can be regarded as rather conservative, since our predictions are based on dl-PCBs only and do not include TEQs deriving from PCDDs and PCDFs since in the current study as well as other studies from German & European water bodies showed that PCDDs and PCDFs contribute considerably smaller shares of TEQs compared to those driven by dl- PCBs (Stachel et al., 2007; Blanchet-Letrouvé et al., 2014). Also, under a natural scenario it has to be considered that the higher energy costs of locomotion during spawning migration would additionally alter the final contaminant concentrations in lipid rich tissues. In our study, we did not quantify the energetic difference between locomotion of our artificially matured eels during the experiment and the energy needs for locomotion occurring during natural migration. This gives our

projections another level of uncertainty that has to be considered for future experimental works on this topic.

For spawning, eels have to migrate several thousand kilometers and rely on their energy stores, formed mainly by muscle-lipids. In an early work by Böetius & Böetius (1980), the authors estimated that eels would use 75% of their total lipid reserves for spawning activities and their journey, of which 18% are used for gonad development, 27% for basic metabolism and an additional 30% depleted for locomotion. In contrast, Van Thillart et al. (2004) calculated that 60% of the total fat reserves of silver eels is required for swimming and basic metabolism and concluded in another study (Van den Thillart et al. 2007) around 36% for incorporation in the eggs. Palstra et al. (2010) estimated that 67% of the total energy stores in eels are spent on spawning migration and oocyte maturation. Since in our experimental setup, fish did not perform similar amounts of locomotion as under natural circumstances, less than the required 60% of their lipid reserves were presumably used for this partial aspect. As a consequence, this could lead to clearly elevated concentrations of lipophilic contaminants in muscle, gonads and eggs at the end of their journey in the field compared to those found in our experiment.

Metabolic elimination as an influential factor on the redistribution and thus concentration ratio of dioxin-like compounds in (newly built) reproductive tissue compared to respective muscle tissue can be disregarded in our case since elimination rates of higher chlorinated PCBs and other organochlorine contaminants in eels have been shown to be very low to not existent at all (De Boer et al., 1994). Also, differences in timespan as an influential factor can be neglected. Depending on the distance from the spawning area, modeled estimations for the duration of natural migration based on average dates of escapement and timing of estimated peak spawning in the Sargasso Sea lie between 63 and 209 days (Righton et al., 2016). This timeframe is well in the range of the time used for the here-applied artificial maturation of the fish (119-135 days).

Conclusions & outlook

Results of our study deliver analytical proof of maternal transfer of DLCs from muscle lipids towards ovarian tissues in European eels. Some detected DLC concentrations in eel eggs taken from animals from comparably low contaminated habitats exceeded levels responsible for potentially impairing embryo development and survival. Due to the rather low number of analysed individuals and the high variability of occurring

chemical contamination in eels under natural conditions, results of this study must be regarded with caution. Still, the presented findings can now help to further investigate this topic and eventually help improve the management of these endangered species. With reference to the toxicological role of POPs in the reproductive biology of eels, their potential for high accumulation may result in consequences for the success of stock management measures in the long run. Therefore it is crucial to consider contamination of escaping silver eels when identifying and evaluating the suitability of habitats for restocking measures considered for stock enhancement. Our results may bring important new insights to the question whether escaping silver eels are capable of entering the effective spawning stock biomass in the future. Management strategies could use these findings by combining pollution monitoring with protective measures such as harvest restrictions specifically for silver eels escaping habitats of low contamination levels or with regard to site selection for eel stocking programs.

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762 crassus in inland and coastal waters of northern Germany. J. Coast. Conserv. 18, 121–130.
763

764 Table 1 Biometric parameters (if applicable) including bodylength, bodyweight (before and after
765 treatment) and lipid content of eels used in this study

Life stage	n	Length (cm)	Start weight (g)	End weight (g)	Muscle lipid (%)	Gonad lipid (%)	Egg lipid (%)
Hormone treated	5	73.6±8.8 (63-81)	755.0±294.1 (405-1042)	957.2±336.2 (567-1385)	27.7±6.5 (21.9-35.0)	11.8±4.1 (6.6-15.3)	5.2±0.6 (4.3-5.9)
Untreated	10	69.7±4.1 (62-76)	654.3±117.1 (474-875)	N/A	25.3±3.3 (20.2-30.8)	18.9±5.8 (11.5-26.8)	N/A

766 Data are given in mean values ± standard deviation (minimum-maximum) where applicable.

767
768 Table 2 Overview of amalgamated data obtained for samples of analysed hormonally treated and
769 untreated eels. Units or sample specifications are indicated in brackets

		HORMONE-TREATED (n=5)	UNTREATED (n=10)
dl-PCBs	Σdl-PCB in muscle (pg/g ww)	28500±26500 (10609-73808)	14300±14550 (2780-46861)
	WHO_{2005}-PCB-TEQ (muscle)	28.0±27.9 (8.35-75.56)	12.2±12.5 (1.98-40.35)
	Σdl-PCB in gonads (pg/g ww)	8400±5800 (4904-18701)	8400±10900 (2134-37426)
	WHO_{2005}-PCB-TEQ (gonads)	6.5±5.4 (2.53-15.99)	7.5±9.2 (1.64-25.92)
	Σdl-PCB in eggs (pg/g ww)	4450±4862 (1843-13062)	N/A
	WHO_{2005}-PCB-TEQ (eggs)	3.8±4.4 (1.04-11.46)	N/A
	Transfer Ratio muscle/gonads	3.2±1.4 (1.89-5.16)	2.3±1.9 (0.85-6.68)
	Transfer Ratio muscle/eggs	7.0±1.5 (5.27-8.92)	N/A
		HORMONE-TREATED (n=3)	**UNTREATED (n=4)**
PCDD/ PCDFs	ΣPCDD & PCDF in muscle (pg/g ww)	9±0 (9-9)	13±7 (9-25)
	WHO_{2005}-PCDD/F-TEQ (muscle)	1.9±0.00 (1.91-1.91)	3.4±2.9 (1.91-7.77)
	ΣPCDD & PCDF in gonads (pg/g ww)	9±0 (9-9)	14±9 (9-28)
	WHO_{2005}-PCDD/F-TEQ (gonads)	1.9±0.00 (1.91-1.91)	3.8±3.8 (1.91-9.41)
	ΣPCDD & PCDF in eggs (pg/g ww)	23±16 (9-40)	N/A
	WHO_{2005}-PCDD/F-TEQ (eggs)	2.0±0.1 (1.91-2.15)	N/A

770 Data are given in mean values ± standard deviation (minimum-maximum) where applicable.
771

Figure 1 Tissue burdens (based on wet weight) of dl-PCBs bound in muscle and gonadal tissue in hormone-treated and untreated silver eels. Median values indicated by horizontal lines in boxes, whiskers represent data range.

Figure 2 Mean contributions of dl-PCBs, middle bound LOD and/or detected PCDD & PCDFs to total dioxin TEQ_{2005} concentrations based on wet weight in three matrices (muscle, gonad and eggs) of hormone-treated eels (n=3) and in two matrices (muscle and gonad) of untreated eels (n=4). For TEQ calculations, concentrations below LOD were considered as half the LOD (middle bound).

Figure 3 Percentaged contributions of analysed dl-PCB congeners to total dl-PCB concentration (per wet weight) in different matrices of grouped samples (means) of hormone-treated (n=5) and untreated eels (n=10).

Figure 4 Means of lipid-normalized contributions of analysed dl-PCB congeners to total dl-PCB concentration in different matrices of grouped samples of hormone-treated (n=5) and untreated eels (n=10).

Figure 5 Measured and estimated (black circles and white circles) TEQ values found in eggs from artificially matured eels. Angled symbols represent estimated concentrations, projected from muscle concentrations found in silver eels from different German water bodies (Freese et al, 2016).
Horizontal lines represent different threshold effect concentrations taken from literature. (Thin, dotted line at 4 pg TCDD equivalence/g gonadal mass represents the threshold for occurrence of disrupting effects found in eel embryos presented by Palstra et al in 2006. The thick line at 29pg TEQ/g egg represents beginning of direct egg mortality in lake trout King-Heiden et al. 2012

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