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Accumulation of neurotoxic organochlorines and trace elements in brain of female European eel (*Anguilla anguilla*)

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ABSTRACT

Xenobiotics such as organochlorine compounds (OCs) and metals have been suggested to play a significant role in the collapse of European eel stocks in the last decades. Several of these pollutants could affect functioning of the nervous system. Still, no information is so far available on levels of potentially neurotoxic pollutants in eel brain. In present study, carried out on female eels caught in Belgian rivers and canals, we analyzed brain levels of potentially-neurotoxic trace elements (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, MeHg, Mn, Ni, Pb, Sn, Sb, Zn) and OCs (Polychlorinated biphenyls, PCBs; Hexachlorocyclohexanes, HCHs; Dichlorodiphenyltrichloroethane and its metabolites, DDTs). Data were compared to levels in liver and muscle tissues. Eel brain contained very high amounts of OCs, superior to those found in the two other tissues. Interestingly, the relative abundance of PCB congeners markedly differed between tissues. In brain, a predominance of low chlorinated PCBs was noted, whereas highly chlorinated congeners prevailed in muscle and liver. HCHs were particularly abundant in brain, which contains the highest amounts of β-HCH and ϒ-HCH. *p,p*'-DDTs concentration was similar between brain and muscle (i.e., about twice that of liver). A higher proportion of *p,p*'-DDT was noticed in brain. Except for Cr and inorganic Hg, all potentially neurotoxic metals accumulated in brain to levels equal to or lower than hepatic levels. Altogether, results indicate that eel brain is an important target for organic and, to a lesser extent, for inorganic neurotoxic pollutants.

1. Introduction

European eel (*Anguilla anguilla* L.) population has dramatically declined since the 1980s (Dekker, 2003). Changes in ocean currents, climate shifts, habitat loss, overfishing, barriers to migration, increased predation, introduction of parasites and exposure to chemicals have all been postulated as plausible causative factors that could act in a synergistic manner (Miller et al., 2016). Regarding chemical exposure, several authors have pointed to the abundance of organochlorine compounds (OCs) (i.e., polychlorinated biphenyls or PCBs, and organochlorine pesticides or OCPs) in eel tissues, and to the possible impact that this intoxication could have on energy stores and reproduction (Belpaire and Goemans, 2007; Geeraerts and Belpaire, 2010; Van Ginneken et al., 2009). As in most fish species, pollutant analyses in eel usually focus on liver and muscle. This is because of the relative importance of these two organs for detoxification processes and human health, respectively (Harrad, 1999; Hoff et al., 2005). Despite the importance of the central nervous system for animal fitness, and the clear neurotoxic properties of many environmental contaminants, chemical analyses in fish rarely focus on the brain. Neurotoxicity has however been associated with exposure to both organic and inorganic contaminants in this fauna. PCBs and pesticides such as lindane (γ-Hexachlorocyclohexane, γ-HCH) and DDT (Dichlorodiphenyltrichloroethane) are abundant in eel liver (e.g. Maes et al., 2008). These persistent chemicals also are proven neurotoxicants (Slotkin et al., 2006). High levels of neurotoxic trace elements, such as Pb, Cd, Hg, and As have been detected in eel liver and muscle as well (Nunes et al., 2014; Tabouret et al., 2011). If present also in brain, all these contaminants may induce injuries and behavioral deficits

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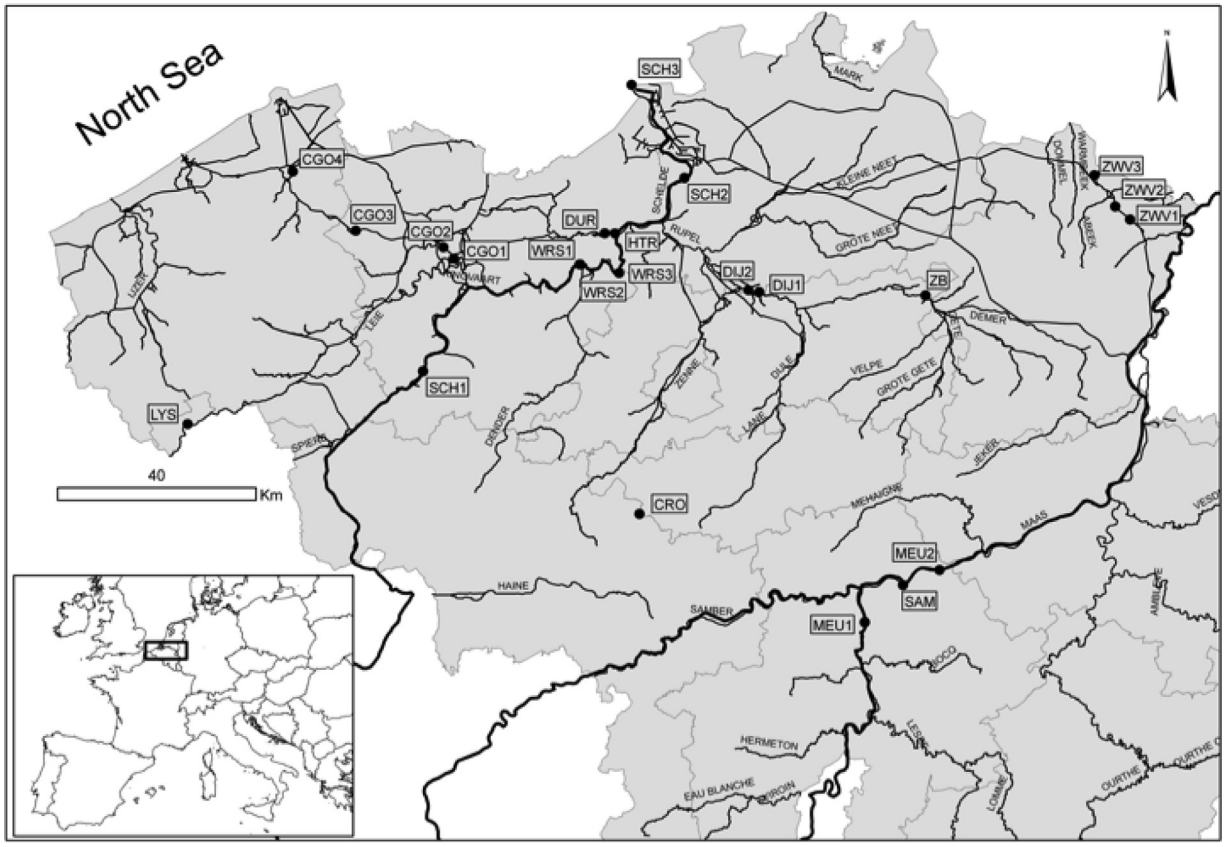

Fig. 1. Map of Belgium with the 23 sampling sites. Black dots indicate yellow eel sampling locations.

affecting individual fitness. Recent studies suggest that cognitive performances are crucial to the successful migration of young eels, especially when facing obstacles such as dams (Podgorniak et al., 2016; Podgorniak et al., 2015a,b). It appears that reduced cognitive performances, together with pollutant-mediated depletion of energy stores (Van Ginneken et al., 2009), could well detrimentally affect silver eels carrying out their 6000-km migration towards their reproduction sites.

To determine whether or not eel brain accumulates neurotoxic organic and inorganic pollutants to levels comparable to those of liver and muscle, we investigated the contamination load in the three organs of female European eels sampled from Belgian rivers and canals.

2. Materials and methods

2.1. Sample collection

Sixty-five female European eels of the yellow stage, with an average length of 70.3 ± 10.8 cm and weight of 714.4 ± 47.1 g (mean ± S.E.M.), were collected from 23 locations in Belgian rivers and canals between May and October 2010 (Fig. 1). Most of them were captured by fyke fishing and a minority by electro-fishing. Eels were transported back to the laboratory in oxygenated freshwater tanks where they were housed for up to 3 days in tanks equipped with a freshwater recirculation system. Eels were euthanized with MS 222 or clove oil, and their total length and weight recorded prior to dissection. Brain (0.22 ± 0.01 g), liver (9.85 ± 0.58 g) and dorsal muscle samples (151.0 ± 10.01 g) were obtained. The brain of each individual was divided into two symmetric equivalent parts, for OC and metal analyses. All tissues were stored at −24 °C prior to lyophilisation and grinding. Samples were weighed before and after lyophilisation as to determine their water content.

2.2. Chemical analyses

The general structures of the OCs quantified in eel tissues are presented in Fig. 2.

2.2.1. PCBs and OCPs

PCB congeners (IUPAC numbers: CB-28, -52, -101, -105, -118, -138, -153 and -180), HCHs (α-HCH, β-HCH + γ-HCH) and DDTs (*p,p*'-DDD, *p,p*'-DDE and *p,p*'-DDT) were analyzed. The method of extraction, modified from Debier et al. (2003) and Schnitzler et al. (2008), was similar for OCPs and PCBs. Briefly, samples were crushed with a Grindomix GM20 (Retsh, Haan, Germany) then freeze-dried with a Benchtop 3L Sentry Lyophilisator (VirTis, New-York, USA). Lipid extraction was performed with *n*-hexane using an Accelerated Solvent Extractor (Dionex 200, Sunnyvale, USA). Cells were heated during 6 min before performing two 5-min cycles of extraction at 1500 psi pressure and 125 °C. All extracts were used for lipid content determination: solvent was evaporated using a Turbovap LV (Zymarck, Hopkinton, Mass., USA). Samples were then diluted in 3 ml of *n*-hexane and an internal standard (CB 112 with a final concentration of 50 pg μl^{-1}) was added in order to quantify possible loss of PCBs during the procedure of sample preparation described hereafter.

To remove organic matter, extracts were subjected to clean-up with sulphuric acid. For this, 2 ml of a mixture of concentrate (95%) and fuming (30%) sulphuric acid (3:1; v:v) were added to each extract. The mixture was shaken and centrifuged for 3 min at 1750*g* (10 °C). The supernatant was removed and 3 ml of *n*-hexane were added to the decanted acid. The shaking-centrifugation-