DIOXIN CONCENTRATIONS IN AMERICAN EEL (*ANGUILLA ROSTRATA)* CAPTURED IN EASTERN CANADA

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Abstract

This research was conducted as part of a project to assess whether chemical contaminants may be responsible for the precipitous decline in American eel (*Anguilla rostrata*) recruitment to Lake Ontario and eastern Canada. Current concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and dioxin-like non-ortho polychlorinated biphenyls (dlPCBs), were investigated in whole fish homogenates, due to their persistence and high toxicity. Samples were analyzed by high resolution gas chromatography-mass spectrometry to determine the concentrations of 17 PCDD/F and four dlPCB congeners. Reference eels captured from rivers in eastern Canada that were tributaries to the Gulf of St. Lawrence, ranged from 61 to 80 cm in length, and from 439 to 1260 g in weight. Eels collected from suspected contaminated areas in the St. Lawrence and Lake Ontario ranged from 68 to 127 cm in length, and 518 to 3474 g in weight. Results were expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TEQs), calculated using fish-specific toxic equivalency factors, and were compared to reported toxicity thresholds for European eel (*Anguilla anguilla*). Total TEQ values in eels ranged from 0.07-1.6 pg g^{-1} ww and 0.64-4.0 pg g^{-1} ww for reference and contaminated sites, respectively. These TEQs were lower than toxicity thresholds established for European eels, suggesting little risk of toxicity.

Introduction

American eel (*Anguilla rostrata*) are a catadromous, semelparous, freshwater fish species that spawns in the Sargasso Sea, and drifts as larvae in ocean currents to the coastal waters of North America[1]. Eels may ascend freshwater rivers and streams, and in the St. Lawrence basin can migrate as far as Lake Ontario[2]. The mean age of Lake Ontario eels has been evaluated at 18 years, and they can vary in size up to 150 cm in length[1].

Over the past two and a half decades, the population of eels has declined significantly in Lake Ontario and eastern Canada, highlighted by an 81-fold decrease in abundance from 1985 to 1992[3, 4], and an overall decline of 99% in recruitment of juvenile eels to Lake Ontario since the 1970s[1].

American eel were important historically in eastern Canada. Aboriginal people near the St. Lawrence relied heavily on eels for generations as a source of wintering food. European settlers, as early as the 16th century, reported on the value of the caloric-dense eel for survival. In the modern era, commercial eel fisheries in eastern Canada represented a multi-million dollar industry prior to a population collapse and the closure of the Lake Ontario commercial yellow-eel fishery in 2004[5]. In April 2006, the Committee on the Status of Endangered Wildlife in Canada declared American eel a species of 'special concern'[1] and, in July 2008, it was classified as 'endangered' under the Species at Risk in Ontario List (SARO). This downward trend has not been limited to North American eels, as populations worldwide have declined, illustrated by the 99% reduction in populations of European (*Anguilla anguilla*) and Japanese eel (*Anguilla japonica*) over the past few decades[6].

Several hypotheses exist in the literature as to the reason(s) for the severe decline in American eel[6], ranging from hydroelectric turbine mortality to climate change. While these factors can impact recruitment, there is insufficient information to establish decisive links to the drastic decline in eel abundance. One recent hypothesis for the decline is the accumulation of persistent organic pollutants (POPs), specifically dioxin-like compounds, which have been reported to cause toxicity to salmonid embryos in Lake Ontario when transferred maternally to eggs[7-9]. This hypothesis proposes that Lake Ontario eels, like lake trout, will accumulate high concentrations of POPs from their diet as they grow to sexual maturity. These maternally-derived POPs should then be transferred to eggs, causing embryo-toxicity and impaired recruitment to Lake Ontario.

The main goals of our research were 1) to determine if American eel accumulate sufficient chemical contaminants during their growth and maturation to cause embryo-toxicity and recruitment failure, and 2) to advise Federal and Provincial fisheries agencies on appropriate mitigation options. This paper reports the concentrations of PCDD/Fs and dlPCBs in eels sampled from three (largely uncontaminated) sites in Canada.

Materials and Methods

Figure 1: Eel sampling locations across eastern Canada. Only results for R. Ouelle and the Miramichi and Margaree Rivers are presented (contact jonathan.byer@ec.gc.ca for the complete data set).

Sample Preparation

Eels were collected in 2007 and 2008 throughout eastern Canada (Fig 1), and stored frozen at -20°C at the Fish Contaminants Laboratory of Environment Canada (EC) in Burlington, ON. Before homogenization, several tissues were dissected from each carcass for other analyses, including the liver, small sections of muscle (approx. 10% by weight) and gonad, and otoliths were removed for age determination. Whole fish homogenates were prepared in accordance with standard lab practices[10], sub-divided into 50 and 100 g solvent rinsed jars and stored in EC's National Biological Tissue Archive at -80°C.

Chemical Characterization

Eel tissue extracts were prepared for chemical analysis from approximately 20 g of homogenate. Each sample was dried chemically with anhydrous Na_2SO_4, spiked with $^{13}C_{12}$ CB-170 and 2,3,7,8-[$^{37}Cl_4$]-TCDD, and extracted with dichloromethane (DCM). The extract was split by weight into four portions: 1) 10% for gravimetric lipid determination, 2) 40% as backup, 3) 25% for PCB and polybrominated diphenyl ether (PBDE) analysis (not reported here), and 4) 25% for PCDD/F and dlPCB analysis. The fourth fraction was spiked with a solution of fifteen $^{13}C_{12}$-labelled PCDD/F surrogates and four $^{13}C_{12}$-labelled coplanar PCB surrogates. Sample clean-up consisted of lipid removal using gel permeation chromatography with Biobeads SX-3, and a 2-layered packed 5% deactivated silica-alumina column. The dlPCBs and PCDD/Fs were separated on a Cosmosil 5PYE column by high performance liquid chromatography. The two fractions were reduced in volume and spiked with additional $^{13}C_{12}$-labelled PCDD and PCB surrogates used as instrument standards.

Instrumental Analysis

High resolution gas chromatography-mass spectrometry analyses of dlPCBs and PCDD/Fs was carried out on a Micromass AutoSpec mass spectrometer (Micromass, Manchester, UK) connected to a Hewlett-Packard 6890 GC (Hewlett Packard, Palo Alta, CF, USA) that was equipped with a CTC A200S autosampler (Leap Technologies, Chapel Hill, NC, USA). Chromatographic separation was achieved using a Restek Dioxin-2 column under the following conditions: He carrier gas: 1.5 mL/min, Source temp: 280°C, Front Inlet temp: 280°C, Transfer line temp: 280°C, Splitless injection: 1.5 min @ 30 ml/min (Table 1). GC-HRMS tuning was done using perfluorokerosene as a reference compound (10,000 resolution at 5% peak height definition) over the mass range of PCDD/F and dlPCB congeners.

Table 1: GC temperature program for the analysis of PCDD/Fs and dlPCBs using a Restek Dioxin-2 column.

Oven Temperature Program	PCDD/F Analysis	dlPCB Analysis
Initial Conditions	120°C hold 1.5 minutes	150°C hold 1 minute
1st Ramp Rate	40°C/minute to 200°C	5°C/minute to 200°C
2nd Ramp Rate	3°C/minute to 235°C	3°C/minute to 235°C
Hold	235°C for 10 minutes	235°C for 10 minutes
3rd Ramp Rate	6°C/minute to 300°C	12°C/minute to 300°C
Hold	Hold for 24 minutes	Hold 12 minutes

Quality Control

The result of six replicate samples of certified reference material (CARP-2) purchased from the National Research Council of Canada, Ottawa, ON, show that we were able to meet dioxin analysis performance standards (Fig 2). In the future we plan to participate in a blind round-robin study to further validate our method.

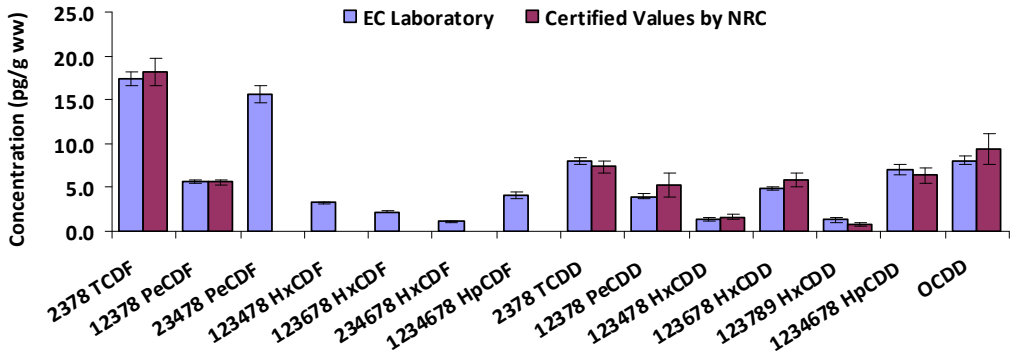

Figure 2: Validation of the analytical method (n =6) using CARP-2, a certified reference material from the National Research Council of Canada (NRC). The measured concentrations for 14 congeners are compared to the concentrations established by the NRC for nine congeners. Error bars represent the measured standard deviations and those reported by NRC.

Typically, samples were analysed in batches of 14 samples; 10 eel samples, one silica blank, one spike solution, one in-house reference material (L. Trout), and one CRM sample. Spike recoveries ranged from 83-110% for the 17 PCDD/Fs, and from 93-143% for the four dlPCBs. Blank samples had 92% non detectable concentrations for all PCDD/F congeners, with the remaining having concentrations below the quantification limit (<0.2 pg g^{-1}); concentrations of all dlPCBs in blanks were below the quantification limit (0.05-1.25 pg g^{-1}).

The experimental data were used to determine the contaminant concentrations in eels on a wet weight basis. The data were converted to 2,3,7,8-TCDD toxic equivalents (TEQs) using the World Health Organization (WHO) 1998 fish specific toxic equivalency factors (TEFs)[11].

$$TEQ = \Sigma (PCDD_i \times TEF_i) + \Sigma (PCDF_i \times TEF_i) + \Sigma (dlPCB_i \times TEF_i) \quad [1]$$

To assess the potential risk to eel reproduction, calculated TEQs were compared to the European maximum residue levels (MRLs) set in 2006[12]; similar guidelines do not exist in Canada.

Results and Discussion

A total of 59 eels from seven different geographic locations (two reference and five 'contaminated' sites) were collected and characterized chemically on a wet weight basis. Silver eels were targeted, although large yellow eels were collected when silver eels were not available (Table 2). This accounts for some of the variation

in length and weight measurements among the sample sites, and some can also be attributed to different ecological conditions (e.g., temperature regimes, productivity) in the different river systems.

Table 2: Eel collection and biological data for spatial samples.

Site	Condition	N	Year	Length (cm)	Weight (g)	Lipid (%)	Dioxin TEQs [b]
Margaree R., NS	Reference	10	2007	67 ± 5	567 ± 151	18 ± 2	0.5 ± 0.4
Miramichi R., NB	Reference	10	2007	74 ± 4	775 ± 227	17 ± 2	0.2 ± 0.2
R. Ouelle, QC	Contaminated	10	2007	112 ± 6	2876 ± 362	21 ± 2	1.7 ± 0.3
Kamouraska, QC	Contaminated	4	2008	92 ± 7	1794 ± 723	22 ± 4	N/A
R. Sud-Ouest, QC	Contaminated	5	2008	86 ± 16	1366 ± 1048	19 ± 5	N/A
Mallorytown, ON	Contaminated	10	2008	97 ± 12	1837 ± 719	N/A[a]	N/A
Lake Ontario, ON	Contaminated	10	2008	106 ± 15	2211 ± 780	N/A	N/A

[a] N/A = data not currently available. [b] Geometric mean.

Chemical profile

The most frequently detected PCDD/F congeners were 2,3,7,8-TCDF and OCDD. High variability in 1,2,3,4,7,8,9-HpCDF and OCDF concentrations was likely due to the low detection frequency; these congeners have been reported to occur less frequently in biota because of their low solubility[13]. Figure 3 illustrates that 2,3,7,8-TCDF concentrations are higher for samples from the Miramichi River, which is consistent with pulp bleaching at a recently-closed (2008) bleached kraft paper mill. Higher OCDD concentrations may also be explained by this activity[14]. High concentrations of 2,3,4,7,8-PeCDF in R. Ouelle may be related to incineration or burning of wood, and elevated concentrations of CB-126 may be due to power generating facilities on tributaries of the St. Lawrence[14]. PCDD/F TEQ concentrations were between 0.06-1.6 pg TEQ g^{-1} ww and total TEQ values ranged from 0.07-1.6 pg g^{-1} ww for the Margaree and Miramichi Rivers. R. Ouelle samples had PCDD/F TEQs between 0.62-3.6 pg TEQ g^{-1} ww and total TEQ values ranged from 0.64-4.0 pg g^{-1} ww.

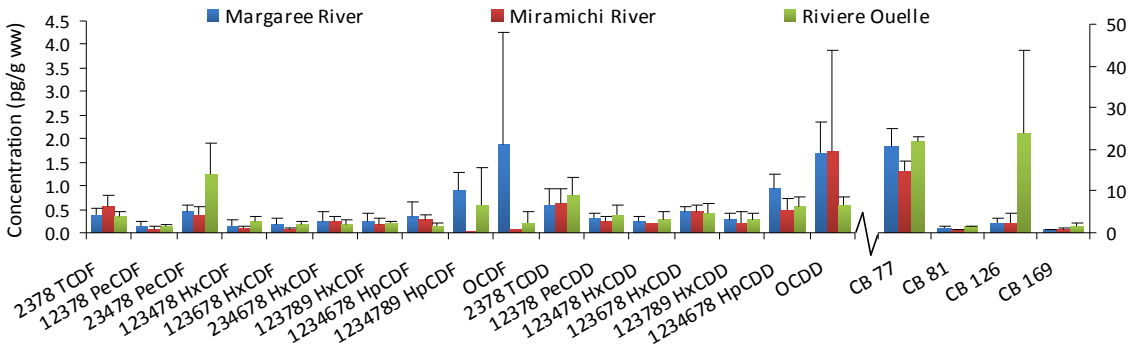

Figure 3: Congener profile for eels captured in the Margaree R., Miramichi R., and R. Ouelle. PCDD/F concentrations are with respect to the left-hand y-axis, and dlPCB concentrations use the right-hand y-axis.

The dlPCBs accounted for 83%, 84%, and 90% of the summed absolute concentrations of analyzed compounds for the Margaree, Miramichi, and R. Ouelle samples, respectively, and contributed 3%, 8%, and 6% to the total TEQs, respectively. The trend of increasing dlPCB concentration from east to west may be linked to population density and industrial activity; however, the data show that the major contributors to total TEQs in eels were the PCDD/Fs due to their orders-of-magnitude higher TEF values. We expected higher concentrations of dioxins in the Miramichi R. samples because of the paper mill; however, the data showed that current contamination from this particular mill may not be environmentally significant any longer due to its closure.

Concentrations of PCDD/Fs were all below the MRL for European eel of 12 pg g^{-1} ww set in 2006, and except for one fish from R. Ouelle (outlier in Fig 4), below the MRL for other fish species of 4 pg TEQ g^{-1} ww

for PCDD/Fs and 8 pg g^{-1} ww for total TEQs. The PCDD/F TEQs in this study are within the range of concentration for European eels reported in a number of studies by van Leeuwen *et al.* (2007) with concentration ranges from 0.2-7.9 pg TEQ g^{-1} ww, and 0.4-2.7 pg TEQ g^{-1} ww[15]. More importantly, our study compares well with Hodson et al. (1994) who reported PCDD/F TEQs ranging from 0.1-1.1 pg g^{-1} ww for American eel captured in the St. Lawrence near Kamouraska[2]. The total TEQs in this study may be an underestimate because only the four coplanar PCBs are reported and not all 12 PCBs with non-zero TEF values.

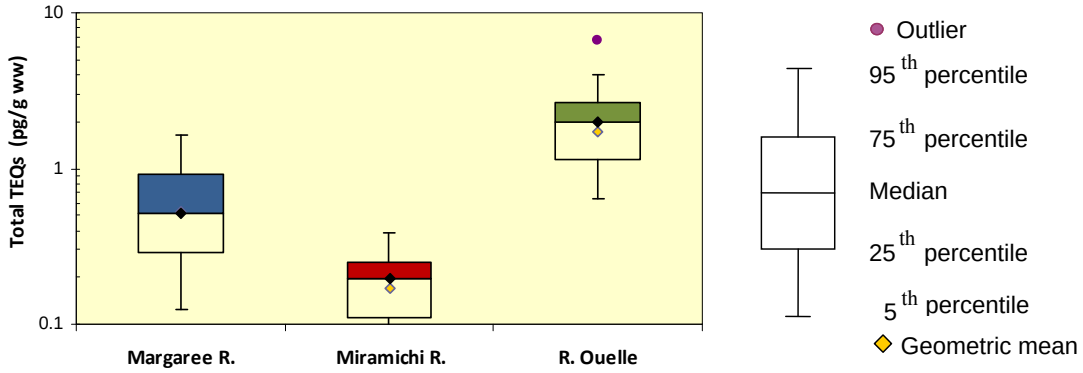

Figure 4: Box and whisker plots of total TEQs for three collection sites (n =10). One fish from R. Ouelle was an outlier at a 95% confidence interval and was not included in the site comparison and calculations.

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