Brominated Flame Retardants in American Eel: a Reason for the Eel's Decline?

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Introduction

Eel populations worldwide have undergone dramatic decline since the early 1980s. For example, American (*Anguilla rostrata*), European (*Anguilla anguilla*), and Japanese eel (*Anguilla japonica*) have seen a reduction in glass eel recruitment by up to 99% over this time frame (van Ginneken & Maes 2005). Thus far, efforts have been unsuccessful at establishing a cause of their declining numbers. Our group recently proposed that persistent organic pollutants (POPs) may be responsible for diminishing American eel stocks, particularly for eels inhabiting highly contaminated areas such as Lake Ontario (Byer et al. 2009). Regulations and environmental improvements have reduced concentrations of organochlorine chemicals, but as yet there is no evidence of eel stock recovery. As an extension of investigations into conventional POPs, this research investigates contamination of American eels by brominated flame retardants (BFRs) due to their reported endocrine disrupting and carcinogenic properties in other fish and wildlife (Goosey 2006). The emergence of BFRs may be linked to the lack of recovery of eels in recent years, since their concentrations have increased since the early 1980s. Several European studies (Roosens et al. 2008; Geeraerts & Belpaire 2009) have measured BFRs in European eels, but few data exist for American eel (Ashley et al. 2007; Law et al. 2003), especially in Canadian waters, and virtually no information is available for emerging BFRs. The objectives of this research were to determine the levels of polybrominated diphenylethers (PBDEs) for American eel captured in eastern Canada, and to identify new and/or emerging organobromine compounds using gas chromatography/mass spectrometry (GC/MS). The future goal is to assess if these 'new' chemicals represent a stress prohibiting the eel's recovery.

Materials and Methods

Sample Collection. A total of 58 eels were collected in 2007 and 2008 from seven locations throughout eastern Canada (Fig 1) for chemical characterization. Several tissues were dissected from each carcass for other analyses, including the liver, otoliths, small sections of muscle (approx. 10% by weight) and gonad. Whole fish homogenates were prepared for the remainder in accordance with standard lab practices (Kiriluk et al. 1998).

Extraction and Clean-up. Whole fish homogenates (10 g) were dried chemically with anhydrous sodium sulfate, spiked with $^{13}C_{12}$-chlorinated biphenyl-170, and extracted with dichloromethane. Lipid content was determined gravimetrically using 2 g wet weight equivalent (ww eq.). Each extract was additionally spiked with $^{13}C_{12}$-BDE-47, -BDE-99, -BDE-100, -BDE-126, and -BDE-153, and underwent gel permeation chromatography for bulk lipid removal. A portion (5 g ww eq.) of extract was further cleaned up and analysed for PBDEs according to the method previously described (Lebeuf et al. 2006). A total of 14 BDE congeners were quantified and reported individually and as the sum of

the tri-tetra (17, 25, 28, 47, 49, 66, 75), penta (99, 100, 118, 119), and hexa (153, 154, 155) substituted congeners. The remaining fraction (3 g ww eq.) was analysed using GC/electron capture negative ion-(ECNI)-MS to probe for other organobromine compounds, including 27 emerging BFRs available from Wellington Laboratories Inc. (Guelph, ON).

Mass Spectrometry. PBDEs were determined using a ThermoQuest TraceGC equipped with a Finnigan PolarisQ ion trap, the ion source was operated in electron ionization (EI) mode and the ion trap in MS/MS mode. Organobromine compounds were determined on an Agilent 5975 MSD coupled to a 7890 GC (Agilent Technologies, Palo Alto, CA, USA) in electron capture negative ionization mode. The MSD functioned as an amplified bromine detector highlighting organobromine compounds based on their $^{79/81}$Br isotope ratios and, in some cases, their molecular ion. Using retention time matching against BFR standards, we identified several peaks of interest, which were subsequently confirmed by GC/high resolution MS (GC/HRMS).

GC/HRMS analyses were carried out on a Micromass AutoSpec MS (Micromass, Manchester, UK) operated in EI mode, connected to a Hewlett-Packard 6890 GC (Hewlett Packard, Palo Alta, CA, USA). We confirmed the presence of allyl 2,4,6-tribromophenyl ether (ATE), brominated biphenyl (BB-153), 2,3-dibromopropyl-2,4,6-tribromophenylether (DPTE), decabromodiphenylethane (DBDPE), hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), pentabromotoluene (PBT), and 2,3,5,6-tetrabromo-p-xylene (p-TBX) in the fish samples, in addition to some other compounds that are not quantifiable by GC including 3,3',5,5'-tetrabromobisphenol-A (TBBP-A), rac-(1R,2R)-1,2-dibromo-(4S)-4-((1R)-1,2-dibromoethyl)cyclohexane (α-TBECH), and rac-(1R,2R)-1,2-dibromo-(4S)-4-((1S)-1,2-dibromoethyl)cyclohexane (β-TBECH).

Results and Discussion

PBDE Profile. The measured PBDE levels (ng/g lipid weight) in eels captured at three different geographic locations (Fig 2) and their corresponding congener profiles (Fig 3) indicate that PBDEs accumulate in American eel. Eels from Rivière Ouelle had the highest concentration of PBDEs measured in this study, with a mean ΣPBDEs equal to 68 ± 29 ng/g lw (n =9); one fish (outlier in Fig 2) measured 331 ng/g lw. The concentration and number of detected PBDE congeners decreased from west to east geographically, which corresponds well with known anthropogenic activities and population density. The concentrations measured were generally less than reported for European eels (Law et al. 2006; Roosens et al. 2008; van Leeuwan & de Boer 2008). More importantly, the concentrations in this study were less than reported in the muscle of American eel collected at Kamouraska in 1999, which had a mean ≈ 420 ng/g lw (Law et al. 2003), and for the Delaware River in 1998, reported to range from 10 to 5652 ng/g lw (Ashley et al. 2007). However, our values are within the lower ranges reported by these studies. The difference in concentration between Rivière Ouelle (2007) and Kamouraska (1999) could be reflective of the origin and/or distance migrated by the eels (i.e. resident of local rivers vs. upstream migrant). PBDEs were measured in every fish sample analysed with BDE-47, BDE-100, BDE-49, and BDE-99 detected most frequently, indicative of past use of the penta-BDE technical mixture. In addition, BDE-154 was often detected and has been reported to be the possible result of the debromination of BDE-209 (Stapelton et al. 2004). The mean ΣPBDEs differed by about 5-fold between the sites that were furthest apart. As expected, PBDE

patterns were less complex in eels from areas of low PBDE contamination, likely due to the limit of detection (LOD) for trace PBDEs. For example, BDE-25, -75, -119, and -153 were quantifiable only in the R. Ouelle eels. In the future we plan to complete the PBDE analysis of eels from the remaining sites.

Emerging BFRs. All 58 eel samples were scanned for organobromine compounds using GC/ECNI-MS and compared against various BFR standards. We were able to identify several organobromine compounds, some of which were subsequently confirmed by GC/HRMS as described previously. However, only five BFRs (ATE, p-TBX, PBEB, HBB, and DBDPE) were quantified using the low resolution method (Table 1). ATE was quantifiable in all but three samples (55 of 58) and p-TBX was measured in about half of the samples (30 of 58); whereas, HBB was quantified in just 14% of the samples (8 of 58), PBEB was determined once in a fish from the Miramichi and Margaree Rivers, and DBDPE was quantified one time in a fish from L. Ontario. Relatively high limits of detection were most likely due to the dilution factor (1 µL injection from a 1 mL final volume), and prevented some compounds from being quantified. Moreover, coelution occurred for some compounds, e.g. PBT co-eluted with BDE-28 and these are indistinguishable by the GC/ECNI-MS method. Since this method was implemented to screen for emerging BFRs and the samples were not cleaned up further after GPC, lipid related effects prevented the further reduction of sample volume to improve the LOD. In the future, we will develop a targeted method for analysis of the emerging BFRs confirmed by GC/HRMS.

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Fig 1: Geographic distribution of eel sampling locations in eastern Canada.

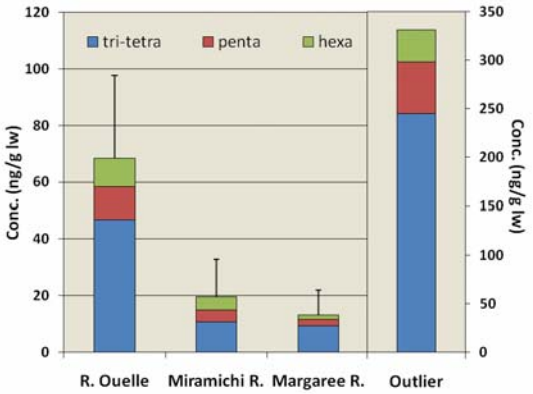

Fig 2: Spatial variation in mean summed PBDE concentrations. Standard deviation of the sums are represented by the error bars (n=10).

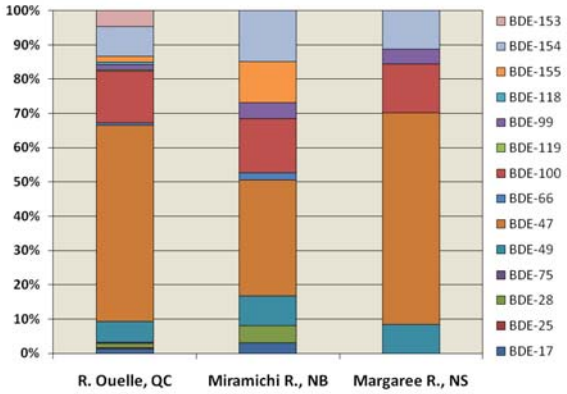

Fig 3: Spatial variation in the averaged PBDE congener profiles of eels from three locations.

Table 1: Biological and emerging BFR data (ng/g lw) for geographical different eel samples.

Site	Location	N	Year	Length (cm)	Weight (g)	Lipid (%)
1	Margaree R., NS	10	2007	67 ± 5	567 ± 151	18 ± 2
2	Miramichi R., NB	10	2007	74 ± 4	775 ± 227	17 ± 2
3	R. Sud-Ouest, QC	4	2008	86 ± 16	1366 ± 1048	19 ± 5
4	Kamouraska, QC	4	2008	92 ± 7	1794 ± 723	22 ± 4
5	R. Ouelle, QC	10	2007	112 ± 6	2876 ± 362	21 ± 2
6	Mallorytown, ON	10	2008	97 ± 12	1837 ± 719	23 ±6
7	Lake Ontario, ON	10	2008	106 ± 15	2211 ± 780	23 ±3

Site	1	2	3	4	5	6	7
ATE	0.68 ± 0.19	1.3 ± 0.4	0.62 ± 0.22	0.60 ± 0.21	0.59 ± 0.23	0.25 ± 0.13	0.26 ± 0.10
p-TBX	0.15 ± 0.01	0.43 ± 0.22	0.32 ± 0.11	0.36 ± 0.10	0.31 ± 0.13	0.12 ± 0.03	0.09 ± 0.04
PBEB	1.81[a]	12.48[a]	—	—	—	—	—
HBB	0.09[a]	5.7 ± 2.3	—	—	0.67 ± 0.31	—	0.40 ± 0.24
DBDPE	—	—	—	—	—	—	0.21[a]

Mean values quantified using GC/ECNI-MS. — Not detected/quantifiable. [a] Quantified in one sample.