DIOXIN RELATED CONTAMINANTS IN LAKE ONTARIO AMERICAN EEL: A LIKELY CAUSE FOR THEIR DECLINE?

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Introduction

Global eel (*Anguilla sp.*) populations have experienced drastic declines over the past three decades. Since the 1960s, recruitment of Atlantic eels (American and European eel) has declined by as much as 99 percent [1]. American eels have the largest distribution range at over 6000 km of any freshwater fish in North America, and Lake Ontario represents the single largest growth habitat in their distribution. Eels once represented a significant portion of the commercial fishery in Lake Ontario, and provided more than 30% of the fecundity of the species prior to their collapse and the 2004 fishery closure[2, 3]. They were classified as an 'endangered species' under the Species at Risk in Ontario List in 2008[4]. There has been a lot of interest recently in spawner quality as the central link in population decline, specifically the ability of female silver eels to successfully migrate to the Sargasso Sea and spawn, and the viability of their offspring as they attempt to return to continental waters. The former has received a great deal of attention in Europe[5, 6]; however, the latter is relatively unexplored, as Atlantic eel spawning remains a mystery. Our research group is interested in the effect that maternally-derived lipophilic persistent organic pollutants (POPs) may have on the early life stages of developing embryos and how they affect eel recruitment both temporally and spatially[7]. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are classes of aryl hydrocarbon receptor (AhR) agonists that cause early life stage mortality in fish[8]. The objective of this research was to assess past risk of American eel recruitment failure to Lake Ontario due to dioxin related compounds.

Materials and Methods

Whole carcasses of eels captured by electrofishing in eastern Lake Ontario (Fig 1) in 1988 and 1998, purposely archived for this type of study, and whole eels collected in 2008 were chemically characterized for 17 PCDD/Fs, four dl-PCBs, and eight mono-ortho PCBs (mo-PCBs) according to procedures previously described[7]. Briefly, whole fish homogenates were prepared and 20 g of tissue spiked with $^{13}C_{12}$-labeled CB-170 and 2,3,7,8-[$^{37}Cl_4$]-TCDD were extracted with dichloromethane (Table 1). Lipid content was determined gravimetrically (2 g ww). Two fractions, each about 25% by weight (5 g ww), were separated for PCB and PCDD/F analysis. The PCB fraction was spiked with $^{13}C_{12}$-labeled CB-31, CB-52, CB-101, CB-118, CB-153, CB-170, CB-180, and CB-194. The dioxin fraction was spiked with 15 $^{13}C_{12}$-labeled PCDD/Fs and four dl-PCBs. All analytical standards were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

Mo-PCBs 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. PCDD/F and dl-PCB 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).

Table 1: Collection data for eels captured in Lake Ontario in 1988, 1998, and 2008.

Year	No.	Length (cm)	Weight (g)	Age	Ecotype
1988	12	95 ± 7	2075 ± 418	22 ± 3	N/A[a]
1998	12	87 ± 5	1582 ± 440	20 ± 3	N/A
2008	10	106 ± 15	2211 ± 780	N/A	Yellow[b]

[a] N/A = data not currently available. [b]Except for one silver.

Results and Discussion

Current levels of dioxins

The mean concentration of PCDF and PCDD in eels collected in 2008 was 3.6 ± 1.9 and 3.8 ± 2.8 pg/g wet weight (ww), respectively. Dl-PCBs were about 20 fold more concentrated (147 ± 89 pg/g ww), and mo-PCB concentrations were three orders-of-magnitude higher (44 ± 23 ng/g ww). 2,3,7,8-TCDD toxic equivalences (TEQs) were calculated using fish specific toxic equivalency factors (TEFs) established by Van den Berg et al. (1998)[9]. Total TEQs ranged from 1.54-6.50 pg TEQs /g ww (mean = 3.50 pg TEQs/g ww) (Fig 2). PCDD/Fs contributed about 80% to the total TEQs despite being several orders-of-magnitude lower in concentration. 2,3,7,8-TCDD and 2,3,4,7,8-PCDF had the highest concentrations of any PCDD and PCDF congener measured, respectively. They also have the highest TEF values of each group, which resulted in them contributing on average to more than 50% of the total TEQs. PCDD/F and PCB data for 1988 and 1998 eels are currently under analyses.

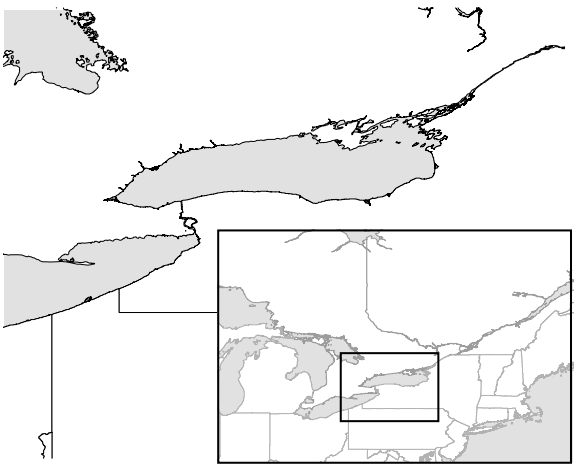

Figure 1: Map of Lake Ontario.

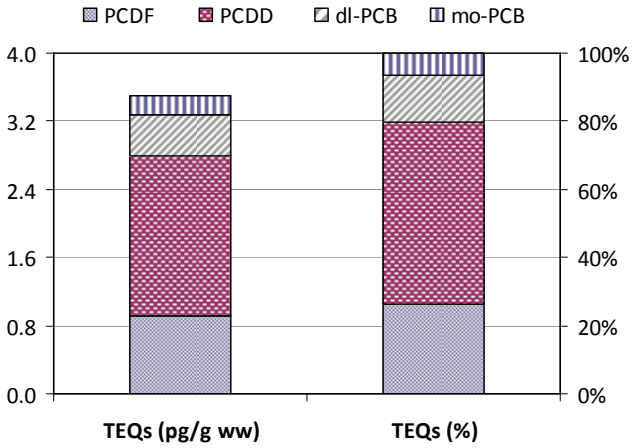

Figure 2: Mean total TEQs and TEQ composition of American eel captured in Lake Ontario in 2008.

The TEQ data from 2008 was compared to the available literature for American eel captured in Lake Ontario and eastern Canada. However, data for historic dioxin related contaminant levels in American eel are extremely limited and in many cases incomplete (Table 2). This highlights the importance of the archived samples from 1988 and 1998 to be analysed in accordance with currently accepted analytical methods and used to fill in the missing data gaps.

Table 2: Examples of American eel TEQs (pg/g ww) for fish from 1980-2008.

Tissues	Compounds	TEFs	% Lipid	TEQs	Location (Year)	Reference
Muscle	2,3,7,8-TCDD dl-PCBs	FISH-98	36.60 ± 4.54	6.4-38.5[a] 3.65-13.2[a,b]	Lake Ontario, Canada (1980)	10
Whole fish	dl-PCBs	FISH-98	Not reported	6.53-9.37[a]	Kamouraska, Canada (1982)	11
Whole fish	6 PCDD/Fs dl-PCBs	FISH-98	18-25	0.03-1.45[a] 1.65-3.69[a,b]	Kamouraska, Canada (1990)	11, 12
Whole fish	17 PCDD/Fs 12 dl & mo-PCBs	FISH-98	23.19 ± 2.69	1.17-5.23 0.36-1.27	Lake Ontario, Canada (2008)	This study

[a]Retrospective TEQs from FISH-98 TEF values[9]. [b]Estimate of dl-PCBs from total PCBs[13].

Past risk of recruitment failure

During spawning migration, eels consume their stored fat for energy and lipophilic contaminants effectively bioconcentrate, increasing in concentration by at least 40%[14]. The effects of these contaminants are typically minimal as long as they are stored in the fat reserves. However, in tandem this fat is mobilized and redistributed from the muscle into the developing gonads, along with POPs such as dioxin-like compounds that have been accumulating in the fat throughout the eel's life. The developmental stages of fish have been shown to be the period most sensitive to dioxin related contaminant toxicity[15]. Contaminants maternally transferred to developing eel embryos may lead to early life stage embryo-toxicity similar to what has been demonstrated in other fish species[8, 15]. This was most notably highlighted by Cook et al. (2003) who concluded that the complete extirpation of lake trout from Lake Ontario in the mid-20th century could be explained by AhR-mediated early life stage toxicity.

The toxic equivalence of an eel egg (TEQ_{egg}) was estimated for the data in Table 2 based on total TEQs and an estimated 40% increase in concentration due to lipid loss during migration[14]. These values were compared to the mortality thresholds suggested by Palstra et al. (2006) for European eel (<4 pg TEQ/g ww) and by Cook et al. (2003) for lake trout (30-100 pg TEQ/g ww), which have similar sensitivity to AhR agonists than American eel[16] (Fig 3). The data show that prior to the early 1990s the concentrations of dioxins in female eels were high enough to potentially cause early life stage mortality in their offspring, while current values suggest low mortality. Equation 1, adapted from Cook et al. (2003), gives a linear relationship from 0-100% mortality based on TEQ_{egg} ranging from 4-100 pg TEQ/g ww. Above 100 pg TEQ/g ww, there is assumed to be 100% mortality.

$$\% \text{ Mortality} = (TEQ_{egg} - 4)/(100/96) \quad (1)$$

Figure 3 compares the percent mortality with time to an index of juvenile young yellow eels (3-to-9-year-old) ascending the upper St. Lawrence River to Lake Ontario[3]. The data indicate that before the early 1980s when percent mortality was at its highest there was a subsequent decline in eel recruitment. There is a 4-7 year delay from embryo hatch in the Sargasso Sea to eel recruitment in Lake Ontario. If one were to apply this offset to the maximum mortality estimated in 1980, it corresponds to the period of steepest recruitment decline. This suggested a significant past risk of recruitment failure related to AhR agonists, and perhaps one of the reasons for the disappearance of American eels in Lake Ontario was the historic loadings of PCDD/Fs and PCBs.

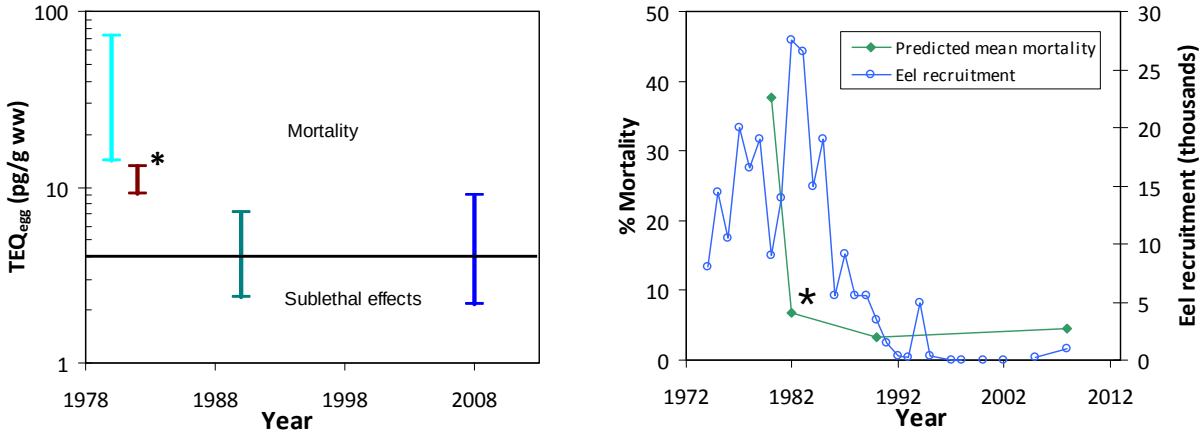

Figure 3: *Left:* Estimated fish specific TEQ_{egg}s for data in Table 2, assuming a 40% increase in concentration due to migration compared to mortality and sublethal effects thresholds. *Underestimate of TEQs and mortality because it only considers dl-PCBs. *Right*: Mean predicted eel embryo mortality against the timeline for eel recruitment to Lake Ontario.

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