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Impact of chemical pollution on Atlantic eels: facts, research needs and implications for management

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Abstract

Many eel species of the genus Anguillidae are under anthropogenic pressure. This review presents strong evidence that chemical pollution is a driving force behind the catastrophic decline in recruitment and abundance of both the European (*Anguilla anguilla*) and the American eel (*A. rostrata*). In response to this crisis, stock and habitat management policies have blindly focused on increasing the areas available for the recruitment and rearing of yellow eels, and increasing the numbers of silver eels escaping to spawn in the Sargasso Sea. No specific policies or regulations have been adopted to foster recruitment of yellow eels to uncontaminated watersheds, to monitor the quality and condition of silver eels, or to protect silver eels from contaminated environments. Research is needed to identify existing and emerging contaminant problems, to understand their potential impacts on eel reproduction, and to develop indicators of spawner quality and management actions that would increase the likelihood of successful eel reproduction and recruitment.

1. Introduction

Many Anguillid eel species are threatened or near-threatened due to continuous and persistent declines in recruitment and abundance over past decades. The most affected is the European eel *Anguilla anguilla*, for which recruitment has decreased to 2.1% of the 1960-1979 average in the North Sea data series [1]. Despite measures taken at national levels there is no clear recovery, and in most (84%) eel management units, stock indicators remain far below management targets [1]. At the same time, there have been alarming declines in stocks of two other temperate eel species of high commercial value, the American eel *A. rostrata* and the Japanese eel *A. japonica*. These dramatic developments prompted global interest in anthropogenic causes, including over-fishing, habitat degradation, barriers to migration, diseases, pollution, and climate change. While the causes may interact synergistically, only pollution and climate change affect every single life stage [2].

Anguillid species are semelparous (once-in-a-lifetime spawners) and panmictic (random mating), reproducing far from their continental habitats (e.g., the Sargasso Sea for *A. anguilla* and *A. rostrata*). The oceanic larvae drift and develop for 0.5 to >2.5 years before they metamorphose into glass eels at the continental slopes and enter estuaries and rivers [3]. After pigmentation, they begin to feed and grow for 6 to >20 years as yellow eels. In their final life stage, they cease feeding, transform to silver eels and mature sexually while migrating back to the Sargasso to spawn and die. Silver eels rely on lipid stores to fuel gonadal maturation and migrations up to 7000 km. This review summarizes the current knowledge and critical research needed to understand how chemical pollution impairs the survival, growth and reproduction of Atlantic eels.

2. Unique sensitivity of anguillid species to chemical contamination

Eels are benthic and opportunistic predators that accumulate extraordinarily high amounts of body fat during their continental lives in coastal and freshwater habitats. Thus, they are particularly prone to accumulating and biomagnifying lipophilic and persistent organic pollutants (POPs) and other chemicals of concern [4-9].

Semelparous eels cannot reduce contaminant burdens by releasing gametes during repeated spawning, so their body burdens of contaminants clearly exceed those of other fish species from the same habitat [10]. Fat stores catabolized during migration release these stored contaminants to the bloodstream, where they can contaminate and affect reproductive organs and gametogenesis. Concentrations of tissue contaminants provide a crucial benchmark for the quality of spawners and their overall reproductive success [11-16].

Pioneering work in analyzing and monitoring eels and developing standard methods for assessing bioaccumulating chemicals was done in The Netherlands and Belgium for *A. anguilla* [4,17] and in Canada for *A. rostrata* [18-19]. Larsson et al. [20] were probably the first to suggest that declining stocks of *A. anguilla* might be explained by chemical contamination. Ground-breaking research in The Netherlands on the toxicity to European eel embryos of maternally-derived dioxin-like compounds (DLCs) [21], and comparisons of the swimming performance of adult eels to their chemical burdens [22] suggested realistic mechanisms linking contamination to impaired reproduction.

3. Bioaccumulation in eels – spatially and physiologically driven

Pollutant concentrations in both Atlantic eel species are characterized by extreme variability [7,23-26], and body burdens reflect atmospheric transport and the proximity of rearing habitats to urban, agricultural and industrial development (Table 1). There are clear correlations between local contamination pressure and the pollution fingerprint of wild yellow eels. Yellow eels are efficient bio-indicators for monitoring the sources and distribution of metals and lipophilic compounds [8,23,27-28]. For example, concentrations of mirex (i.e. organochloride insecticide) in *A. rostrata* provided a clear chemical marker of eels migrating from L. Ontario, which is uniquely contaminated by a single point-source [18,19]. For both species, tissue concentrations of legacy chemicals (e.g., lead; PCBs) that first attracted attention in the 1970s have since declined [12,19,29-31], to be replaced by emerging chemicals (e.g. brominated and fluorinated compounds). Many of those new chemicals are ubiquitous in eel (Table 2), at

concentrations that reflect the extent of habitat degradation. In general, the effects on eels of these newly recognized compounds are poorly understood, yet some are known for their toxic and endocrine-disrupting properties.

In eels, lipophilic contaminants are usually measured in muscle where most lipids are found as stored energy. However, contaminants are not distributed evenly among eel tissues. This makes impact studies more challenging because each contaminant can exert specific damage in the target organ where it accumulates. For example, the eel brain is an important target for DDT, a neurotoxic pollutant [32]. Similarly, mercury is typically measured in muscle due to concerns for human safety, but it accumulates mainly in the liver, kidneys and brain [33].

Physiologically-based toxicokinetic (PBTK) models estimate uptake and distribution of chemicals in distinct body compartments during exposure. Brinkmann et al. [34] developed the first PBTK model for European eels with excellent predictive precision for moderately hydrophobic chemicals. The same model described the metabolic pathways of the pesticide Fipronil and two of its metabolites in muscle and liver of eels from a German river [9]. Further model development may help in future quantification and assessment of potential pollution impacts.

4. Pollution impairs the health of eels: spawner energetics, embryo-larval survival, and endocrine disruption

Research on contaminant effects on eels has focused on traits affecting their fitness to complete their life cycle, including their ability to swim, accumulate energy reserves, develop healthy oocytes, and reproduce. Lipid stores are crucial for eel reproduction. It has been estimated that a minimum of 20% in muscle is needed for normal migration and reproduction [35]. Lipid concentrations in female silver European eels vary considerably over their distribution range, suggesting large differences in their capacity to complete spawning migrations and in reproductive potential (number of eggs produced) [55], particularly because pollutants impair lipid metabolism [36]. Significant declines in lipid levels in European and

American eels from polluted watersheds [30-31,37] suggest that eel stocks might well be governed by pollution-impaired lipid storage, spawning migrations, and/or fecundity [24].

The release of organic contaminants from lipid stores mobilized to support eel migration and gonadal development represents a risk of toxicity to migrating adults, developing oocytes, and early developmental stages of fertilized eggs [7,21,36-37]. Similarly, stored metals can be conveyed to oocytes by vitellogenin [11]. For *A. rostrata*, DLCs extracted from muscle lipids of Lake Ontario yellow eels captured between 1988 and 1998 were toxic to mummichog (*Fundulus heteroclitus*) embryos; extracts from eels captured in 2008 were not [39]. The decline in embryotoxicity corresponded to parallel declines in tissue concentrations of DLCs [31].

The rates of survival and deformities of European eel embryos were correlated to concentrations of DLCs in ovaries of contaminated females induced to spawn in the lab [21]. However, this land-mark study was limited by low sample numbers. More recent studies demonstrate that substituted diphenylamines, flame retardants (FRs), DLCs and metals can be transferred from artificially matured females to eggs [13-16,40].

While most studies focus on chemical effects on female reproduction and embryo development, contaminants also impair the reproductive capacity of males by endocrine disruption, either by feminization or reduced fertility, as occurs for other fish species (reviewed in Matthiessen et al. [41]). Even though metals such as cadmium may disrupt eel endocrine pathways and gonadal maturation [11], this field is understudied.

5. The role of pollution in eel decline: confounding factors and evidence from other species

Although there is substantial evidence of contaminant effects on eel physiology (reviewed in Geeraerts and Belpaire [36]), most is derived from experiments and is limited to specific life stages and endpoints with unrealistic - exposure times and pathways. The effects on eels of life-time exposures to complex mixtures of chemicals are essentially unknown. Some promising *in situ* approaches to produce valid effects data include the measurement of molecular

biomarkers (reviewed in ICES [42]). Although transcriptomic responses demonstrated pollution impacts on Atlantic eels (e.g. [43-46]), changes in gene transcription are not yet reliable indicators of the potential for eels to successfully migrate and reproduce [42]. Moreover, because many environmental factors unrelated to pollution also affect these indices, simple comparisons of individuals between clean and contaminated sites could be misleading. Interpretation of transcriptomics is especially challenging and should consider the interindividual variability and diversity of life history traits of eels [46-47]. Nonetheless, high-throughput sequencing technologies hold promise for further progress. Laporte et al. [48] recently applied restriction site associated DNA sequencing to demonstrate within-generation polygenic selection of wild Atlantic eels exposed to PCB153, *p,p'* DDE and selenium. The evidence suggests non-random mortality of Atlantic eels by human-driven environmental selection with potential long-term impacts on genetic diversity and evolutionary potential.

Compared to other fish species, assessing the comprehensive effects of pollution on the stock of Atlantic eels is extremely challenging due to their eurytopic behavior and specialized biology. Oceanic mating and subsequent distribution of larvae to freshwater rearing habitats are considered totally random (see [49]) so there are no clear links between reduced recruitment to polluted freshwater habitats and embryo-larval toxicity caused by maternally-transferred contaminants [50]. Chemical effects on other species may improve the understanding of the effects of maternally-derived contaminants on larval development, condition, and survival and on subsequent stock recruitment. Well-known examples include the population collapse of several birds of prey due to DDT (e.g. [51]), the total elimination of natural reproduction of Lake Ontario lake trout (Salvelinus namaycush) by DLCs [52], reductions in abundance of Atlantic salmon (Salmo salar) after large-scale forest treatment with an insecticide containing nonylphenol [53], and reproductive disturbances and lower fecundity in populations of brown bullhead Ameiurus nebulosus from agricultural watersheds [54]. Based on toxicity thresholds for PCB effects on reproduction of other fish species, ICES [55] estimated that >60% of European eels from eight countries were at risk of reproductive impairment (e.g. compared to North Sea whiting Merlangius merlangus). Similar conclusions were drawn for American and

European eels when tissue concentrations of DLCs were compared to threshold concentrations affecting lake trout reproduction [15,31].

Declines in fish reproduction and abundance followed the release of a panoply of new chemicals from the 1940s onwards (e.g. [52]). However, the decline in eel stocks occurred later, in the early eighties, corresponding to the longer generation times of eels. PCBs likely attained their highest concentrations in eel by the late seventies, contributing to lower recruitment during the early eighties [24,31]. Finally, the concurrent timing of recruitment decline in *A. anguilla*, *A. rostrata* and *A. japonica* suggests that a common global pressure was involved, including the global distribution of one or more legacy or emerging contaminants of concern, combined with other stressors such as climate change.

6. Research needed to understand the impact of chemicals on eel stocks

Apart from monitoring to assess the status of contaminants and the quality of eels over their range [42], collaborative international research on pollution impacts is urgently required [42,56] (Table 3), taking advantage of new tools and technologies (e.g. artificial reproduction, swimming tunnels, analytical chemistry, biomarkers, genetic work). As detailed in Table 3, research is needed on: the effects of specific contaminants on eel reproduction, lipid metabolism, epigenetics during metamorphosis, and toxicogenomics; contaminant distributions among tissues; and development of methods to support reproduction of eels in the laboratory and to assess the capacity of wild eels to migrate and reproduce.

7. Do eel management policies account for the effects of pollution?

For the European eel, current stock management is focused on regulating fisheries, assisting migration, or translocating and stocking wild-caught recruits to areas with low natural recruitment [57]. These policies will allow more spawners to escape and reproduce in the short term. However, they do not recognize, integrate, or implement measures that would reduce pollution as a factor contributing to stock decline. The regulations target a defined quantity of

silver eels to leave continental catchments, but fail to consider their quality, even though pollution effects on quality have been identified as a crucial cause of recruitment failure.

The situation is little different for the American eel but aggravated by eel habitats that are distributed among numerous watersheds of the Caribbean, the Gulf of Mexico, eastern United States and eastern Canada. Unlike the EU, there are no consistent approaches to habitat or fisheries management. Some jurisdictions such as the Province of Ontario, Canada have detailed and scientifically-sound eel recovery strategies [58], but this is the exception not the rule. And even in Ontario, the impacts on eels of chemical pollution are given only the briefest of nods. Although 'historic' problems are acknowledged (e.g., DLC toxicity to fish embryos), there are no recommendations to mitigate widespread problems of pollution and habitat quality, and none at all for assessing the reproductive quality of silver eels.

As recently suggested by Freese et al. [7] and De Meyer et al. [59], stock management of anguillids must integrate the condition and quality of rearing habitats and of the eels leaving continental waters. Effective eel stock management must be re-defined to include standards for judging the success of eel and watershed management. These standards must ensure that recruits have access to un-polluted watersheds, that productive but contaminated watersheds are rehabilitated as suitable habitat for healthy eels that are safe to eat, and that targets are set for spawner quality (e.g, lipid and contaminant content, parasites and viruses). Given the complex life cycle of anguillids and their wide range of habitats and sensitivity to multiple stressors, effective management requires a multifaceted approach.

Habitat remediation requires a reduction of chemical discharges and removal of contaminated sediments. However, these are long-term solutions. Given the precarious status of eel stocks, relying solely on existing regulations to decrease pollutant pressure (e.g., EU WFD, REACH) is not sufficient. Specific new measures are needed to further document and understand the impact of pollutants on eels, and to recognize current knowledge in management actions. ICES [42,60] initiated work to harmonize monitoring strategies and to understand contaminant

effects on the stock. However, pollution-related monitoring and management of eels is not yet coordinated and there is no clear indication of its effectiveness in improving the spawning stock and recruitment. Possible management measures may include refraining from stocking glass eel in heavily polluted catchments and maximizing protection of less-polluted catchments that produce well conditioned females. These valuable habitats must be identified and used as 'reserves' to foster appropriate stocking. The production of escaping high quality spawners must be also maximized by removing obstacles to migration, restricting development that affects habitat, and banning fisheries.

8. Conclusion

Currently, a clear and quantitative assessment of pollution impacts on eel stocks is not available. While new chemical and bio-analytical tools have enabled significant progress, research, monitoring and management are inadequate to understand and mitigate stock-wide impacts of contaminants. A reliance solely on fisheries measures to restore declining stocks risks losing the species if contaminant issues crucial for eel restoration are over-looked.

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Table 1. Contaminant concentrations (ranges) in eels from different watersheds, sorted by species and country. More data can be found in other reports e.g.[5, 29, 74]

Species	Contaminant and concentration	Matrix	Site	Reference
	range			
A.a.	DDT 4.9-392.3 ng/g	Muscle (all),	5 sites in Poland, 2010-	Szlinder-Richert et al.
	PCBs 1.7-288.5 ng/g	Liver (ww)	2012	2014 [62]
	DLCs(PCDD/F/dl-PCBs) 1.42-14.59	(PBDEs;PCBs;		
	pg TEQ/g	Pesticides)		
	PBDEs 0.07-8.19 ng/g			
	HBCDD 0.16-17.52 ng/g			
A.a.	PAH metabolites	Bile	5 river systems, 10	Kammann et al. 2014
	1-OHPyr 323-3806 ng/mL		sampling sites in	[28]
	1-OHPhen 110-699 ng/mL		Germany, 2011-2012	
A.a.	Σdl-PCBs 2.3-266.0 ng/g	Muscle (ww)	6 river systems, 13	Freese et al. 2016 [7]
			sampling sites in	
			Germany, 2011-2012	
A.a.	Sum 7 PCBs 3.5-12455 ng/g	Muscle (ww)	365 sites in Belgium	Maes et al. 2008 [29]
	Sum DDTs 1.5-3995 ng/g		1994-2005	
	Hg 5-1185 ng/g			
	Cd 1-2474 ng/g			
	Pb 1-3453 ng/g			
A.a.	Sum 6 PCBs 5-2600 ng/g ww	Muscle (ww);	60 sites in Belgium	Malarvannan et al.
	Sum DDTs 110-7000 ng/g lw	muscle (lw)	2000-2009	2014 [61]
	PBDEs 12-1400 ng/g lw			
	HBCD 7-9500 ng/g lw			
A.a.	Hexachlorobenzene 2.1-3.2 ng/g	Muscle (dw)	4 sites in France, 2011-	Laporte et al. 2016
	Lindane 0.47-9.87 ng/g		2012	[48]
	Sum DDTs 4.6-149.1 ng/g			
	Sum 7 PCBs 53-1220 ng/g			
A.a.	Metals	Muscle; Liver,	4 sites in France, 2011-	Pannetier et al. 2016
	Cu 70-125 μg/g muscle	Kidney (dw)	2012	[8]
	Se 22-52 μ/g liver			
	Zn 250-290 μg/g liver			
	Ag 0.65-2.0 μg/g liver			
	As 1.5-15 μg/g muscle			

Cr.1.5-1.2 µg/g liver Hg 0.2 0.9 µg/g liver Ni 0.5-0.8 µg/g kidney Pb 0.2-1.8 µg/g kidney A.r. Hexachforobenzene 0.8-2.3 ng/g Lindane 0.16-0.21 ng/g Sum DDTs 8.1-63.8 ng/g Sum 7 PCBs 21-120 ng/g A.r. Metals Cu 60-270 µg/g muscle Se 22-80 µ/g liver Ag 1.1-2.1 µg/g liver As 0.5-3.5 µg/g muscle Cd 0.5-14 µg/g kidney Cr 1.9-5.8 µg/g liver Hg 0.3-1.8 µg/g liver Ni 0.8-1.1 µg/g kidney Pb 0.1-0.6 µg/g kidney A.r. A.r. Various pesticides 87-1480 ng/g Hg 50-990 ng/g PCBs 142-5391 ng/g A.r. Sum DDTs 11-250 ng/g ww Sum HCH 0.10-0.83 ng/g ww Sum PCBs 12-5-2345 ng/g ww		Cd 0.5-37 μg/g kidney			
Hg 0.2-0.9 µg/g liver Ni 0.5-0.8 µg/g kidney Pb 0.2-1.8 µg/g kidney Pb 0.2-1.8 µg/g kidney Pb 0.2-1.8 µg/g kidney Pb 0.2-1.8 µg/g kidney A.r. Hexachlorobenzene 0.8-2.3 ng/g Lindane 0.16-0.21 ng/g Sum DDTs 8.1-63.8 ng/g Sum 7 PCBs 21-120 ng/g A.r. Metals Cu 60-270 µg/g muscle Se 22-80 µ/g liver Ag 1.1-2.1 µg/g liver As 0.5-3.5 µg/g muscle Cd 0.5-14 µg/g kidney Cr 1.9-5.8 µg/g liver Hg 0.3-1.8 µg/g liver Hg 0.3-1.8 µg/g liver Hg 0.3-1.8 µg/g liver Hg 0.9-90 ng/g PcBs 142-5391 ng/g Mirex 1-474 ng/g head estuary, 1990 (includes PCBs 142-5391 ng/g month of the st. Lawrence R. and tributaries) North America A.r. Sum DDTs 11-250 ng/g ww Sum HCH 0.10-0.83 ng/g ww Sum PCBs 12.1-39.4 ng/g ww Sum PCBs 12.5-2345 ng/g ww Sum PCBs		Cr 1 5-4 2 µg/g liver			
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A.r. Pb 0.2-1.8 µg/g kidney		Hg 0.2-0.9 μg/g liver			
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Ni 0.8-1.1 μg/g kidney Pb 0.1-0.6 μg/g kidney A.r. Various pesticides 87-1480 ng/g Mirex 1-474 ng/g Hg 50-990 ng/g PCBs 142-5391 ng/g A.r. Sum DDTs 11-250 ng/g ww Sum chlordanes 1.1-10.5 ng/g ww Sum Nonachlor 1.89-17.9 ng/g ww Mirex 0.037-19.6 ng/g ww Sum PBDE 2.1-39.4 ng/g ww Sum PCBs 12.5-2345 ng/g ww Sum PCBs 12.5-2345 ng/g ww Sum PCBs 12.5-2345 ng/g ww North America Migrating silver eels in Hodson et al. 1994 Migrating silver eels in Hodson et al. 1994 Lawrence R. and tributaries) North America Estuary, 1990 (includes eels from Lake Ontario, the St. Lawrence R. and tributaries) North America Dontario, the St. Lawrence R. (ON), R. Sud Ouest (Qc), Miramichi R., NB, Margaree R., NS, Hudson otoliths; R. NY; Silver eels – St. Lawrence R. R. (NY; Silver eels – St. Lawrence R. (NY; Silver eels – St. L		Cr 1.9-5.8 μg/g liver			
A.r. Various pesticides 87-1480 ng/g Gutted Migrating silver eels in Hodson et al. 1994 Mirex 1-474 ng/g carcass w/o the St. Lawrence R. [19] Hg 50-990 ng/g head estuary, 1990 (includes eels from Lake Ontario, skeleton, skin) the St. Lawrence R. and tributaries) North America A.r. Sum DDTs 11-250 ng/g ww Sum chlordanes 1.1-10.5 ng/g ww Sum HCH 0.10-0.83 ng/g ww Sum Nonachlor 1.89-17.9 ng/g ww Sum PBDE 2.1-39.4 ng/g ww Sum PCBs 12.5-2345 ng/g ww Sutted Migrating silver eels in Hodson et al. 1994 the St. Lawrence R. and tributaries) North America Byer et al. 2013b, Table SI-2 [26] CQC, Miramichi R., NB, Margaree R., NS, Hudson R. NY; Silver eels – St. Lawrence River estuary.		Hg 0.3-1.8 μg/g liver			
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Mirex 1-474 ng/g Hg 50-990 ng/g PCBs 142-5391 ng/g A.r. Sum DDTs 11-250 ng/g ww Sum chlordanes 1.1-10.5 ng/g ww Sum Nonachlor 1.89-17.9 ng/g ww Sum Nonachlor 1.89-17.9 ng/g ww Sum PBDE 2.1-39.4 ng/g ww Sum PCBs 12.5-2345 ng/g ww Sum PCBs 12.5-2345 ng/g ww Sum PCBs 12.5-2345 ng/g ww North America Whole fish Large yellow eels in L. Ontario, the St. Lawrence R. (QC), Miramichi R., NB, Margaree R., NS, Hudson R. NY; Silver eels – St. Lawrence R. and tributaries) North America Byer et al. 2013b, Table SI-2 [26]		Pb 0.1-0.6 μg/g kidney			
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PCBs 142-5391 ng/g (muscle, skeleton, skin) A.r. Sum DDTs 11-250 ng/g ww Sum chlordanes 1.1-10.5 ng/g ww Sum HCH 0.10-0.83 ng/g ww Sum Nonachlor 1.89-17.9 ng/g ww Mirex 0.037-19.6 ng/g ww Sum PBDE 2.1-39.4 ng/g ww Sum PCBs 12.5-2345 ng/g ww (muscle, skeleton, skin) Whole fish Large yellow eels in L. Homogenates Ontario, the St. Lawrence R. (ON), R. Sud Ouest few grams of (Qc), Miramichi R., NB, Margaree R., NS, Hudson Otoliths; R. NY; Silver eels – St. Lawrence River estuary.		Mirex 1-474 ng/g	carcass w/o	the St. Lawrence R.	[19]
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A.r. Sum DDTs 11-250 ng/g ww Whole fish Large yellow eels in L. Sum chlordanes 1.1-10.5 ng/g ww homogenates Sum HCH 0.10-0.83 ng/g ww minus liver, a Sum Nonachlor 1.89-17.9 ng/g ww few grams of Mirex 0.037-19.6 ng/g ww ovary, and Sum PBDE 2.1-39.4 ng/g ww Sum PCBs 12.5-2345 ng/g ww Sum PCBs 12.5-2345 ng/g ww Lawrence River estuary.		PCBs 142-5391 ng/g	(muscle,	eels from Lake Ontario,	
A.r. Sum DDTs 11-250 ng/g ww Whole fish Large yellow eels in L. Sum chlordanes 1.1-10.5 ng/g ww homogenates Sum HCH 0.10-0.83 ng/g ww minus liver, a Sum Nonachlor 1.89-17.9 ng/g ww few grams of Sum PBDE 2.1-39.4 ng/g ww otoliths; Sum PCBs 12.5-2345 ng/g ww Sum DDTs 11-250 ng/g ww North America Whole fish Large yellow eels in L. Ontario, the St. Lawrence River et al. 2013b, Table SI-2 [26] Table SI-2 [26] R. (ON), R. Sud Ouest (Qc), Miramichi R., NB, Ovary, and Margaree R., NS, Hudson R. NY; Silver eels in L. Sum PCBs 12.5-2345 ng/g ww			skeleton, skin)	the St. Lawrence R. and	
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Sum HCH 0.10-0.83 ng/g ww minus liver, a few grams of (Qc), Miramichi R., NB, Mirex 0.037-19.6 ng/g ww ovary, and Sum PBDE 2.1-39.4 ng/g ww otoliths; R. NY; Silver eels – St. Lawrence River estuary.	A.r.	Sum DDTs 11-250 ng/g ww	Whole fish	Large yellow eels in L.	Byer et al. 2013b,
Sum Nonachlor 1.89-17.9 ng/g ww few grams of Mirex 0.037-19.6 ng/g ww ovary, and Sum PBDE 2.1-39.4 ng/g ww otoliths; Sum PCBs 12.5-2345 ng/g ww Lawrence River estuary.		Sum chlordanes 1.1-10.5 ng/g ww	homogenates	Ontario, the St. Lawrence	Table SI-2 [26]
Mirex 0.037-19.6 ng/g ww ovary, and Margaree R., NS, Hudson Sum PBDE 2.1-39.4 ng/g ww otoliths; R. NY; Silver eels – St. Lawrence River estuary.		Sum HCH 0.10-0.83 ng/g ww	minus liver, a	R. (ON), R. Sud Ouest	
Sum PBDE 2.1-39.4 ng/g ww otoliths; R. NY; Silver eels – St. Sum PCBs 12.5-2345 ng/g ww Lawrence River estuary.		Sum Nonachlor 1.89-17.9 ng/g ww	few grams of	(Qc), Miramichi R., NB,	
Sum PCBs 12.5-2345 ng/g ww Lawrence River estuary.		Mirex 0.037-19.6 ng/g ww	ovary, and	Margaree R., NS, Hudson	
		Sum PBDE 2.1-39.4 ng/g ww	otoliths;	R. NY; Silver eels – St.	
N= 3-17		Sum PCBs 12.5-2345 ng/g ww		Lawrence River estuary.	
				N= 3-17	

A.r.	Various pesticides 0.4-209 ng/g	Whole boo	y Lake Ontario	Byer et al. 2015 [31]
	Mirex 5.2-39 ng/g	minus live	r North America, 2008	
	Hg ND	and sma	П	
	PCBs 163-719 ng/g	samples	of	
	PBDE 5.9-63 ng/g	gonad ar	d	
	PCDD/PCDF 3.8-13 ng/g	muscle		

ww - wet weight; lw- lipid weight; dw – dry weight; A.a. – Anguilla anguilla; A.r. – A. rostrata

Table 2. Examples of new emerging chemicals as reported from eel studies

Anguillid Species	<u>Chemical</u>	Country	<u>Reference</u>
A.a.	brominated flame retardants	Belgium;	Sühring et al., 2013a,b; 2015 [5-
	(PBDEs) and dechloranes	Germany;	6,13]; Malarvannan et al. 2014 [61];
		Poland; France	Szlinder-Richert et al. 2014 [62]:
			Laporte et al. 2016 [48]
A.a.	(per)fluorinated substances	Belgium and	Sühring et al. 2013b [6]; Roland et
		Germany	al. 2014 [62]
A.a.	organophosphorus flame	Belgium	Malarvannan et al. 2015 [64]
	retardants and plasticizers		
A.a.	Fipronil (insecticide)	Germany	Michel et al., 2016 [9]
A.a.	Toxic textile dyes (such as	Belgium	Belpaire et al. 2015 [65]
	malachite green)		
A.a A.r.	Thallium	France; Canada	Rosabal et al. 2015 [66]
A.r.	Brominated flame retardants	USA; Canada	Ashley et al. 2007 [67]; Byer et al.
	(PBDEs) and dechloranes		2013b [26]; Sühring et al. 2013b [6];
			Laporte et al. 2016. [48]

A.a.– Anguilla anguilla; A.r.– A. rostrata

Table 3. Summary of research needed to understand the role of contaminants in eel decline

<u>Objective</u>		<u>Tools</u>	Relevant references
1.	Improving the controlled reproduction of eels is crucial for research on reproductive impacts of contaminants (see 2). One major obstacle to understanding the contaminant effects on eel reproduction, is the lack of tools and understanding needed to experimentally reproduce Atlantic eels in the laboratory. While the production of fertilized eggs and early stage larvae (<20 days) is feasible, rearing of larvae beyond 20 days remains a major bottle-neck.	Aquaculture zootechnical tools. Artificial reproduction in <i>A. anguilla</i> and <i>A. rostrata</i> , benefitting from experiences with <i>A. japonica</i> . Development of early stage food.	Butts et al. 2014 [68]; Masuda et al., 2012 [69]
2.	Assessing the effects of specific contaminants on eel reproduction. Taking advantage of progress under 1, assess the effects of specific legacy and emerging contaminants on eel reproduction, gamete viability and development of larvae and juveniles.	Classical ecotoxicological approaches tailored to eel. Challenge experiments at different doses combined with artificial reproduction.	Palstra et al., 2006 [21]; Pierron et al. 2008 [11]
3.	Assessing partitioning and distribution of contaminants among eel tissues. Contaminant concentrations are usually measured in muscle tissue but may not be evenly distributed among other organs. Tissue distributions will help predict toxic concentrations in target organs of wild eels.	Experimental work and development of physiologically based toxicokinetic (PBTK) models; Identification of critical body burdens for specific contaminants	Brinkmann et al. (2015) [34]; Michel et al. (2016) [9]; Sühring et al. 2015; 2016 [13-14]; Freese et al. 2017 [15]; Nowosad et al 2018 [40]; Freese et al., 2019 [16]
4.	Contaminant effects on lipid metabolism. In other species, contaminants alter lipid physiology, but there are few studies of eel, despite the crucial role of lipids in migration and reproduction.	Chemical exposures combined with swim tunnels and physiological responses and energetical studies	See for an overview Geeraerts and Belpaire, 2010 [36](e.g. Palstra and van den Thillart [38])
5.	Tools to assess the quality of eels over its distribution area in relation to their capacity to migrate and reproduce. Biomarkers are needed to assess survival, migration and reproduction capacity.	Monitoring of chemicals in silver eels over their distribution area. Development of biomarkers	Couillard et al., 2011 [70]; see for an overview ICES, 2015 [42]
6.	Epigenetic mechanisms of pollutant impacts on eels. Role of epigenetic marks and their potential pollutant-induced changes during the critical windows of metamorphoses, the early stages of eels, and their subsequent consequences on the completion of the eel's life cycle.	Use of molecular methods to investigate the impacts of contaminants on epigenetic marks. Correlate changes in DNA methylation, histone marks or miRNAs expression with contaminant burden in critical life phases	Trautner et al. 2017 [71]; Pierron et al. 2014 a,b; 2019 [47,72-73];
7.	Toxicogenomic studies of pollutant effects on eel stocks. Assessments of eels from contaminated environments to establish links between pollution and genetic biomarkers	Toxicogenomics, biomarkers	Maes et al., 2013 [43]; Pujolar et al., 2012; 2013 [44-45]; Baillon et al., 2015 [46]