



Assessment of the quality of European silver eels and tentative approach to trace the origin of contaminants – A European overview

Bastien Bourillon^{a,*}, Anthony Acou^{b,c}, Thomas Trancart^a, Claude Belpaire^d, Adrian Covaci^e, Paco Bustamante^{f,g}, Elisabeth Faliex^h, Elsa Amilhat^h, Govindan Malarvannan^e, Laure Virag^a, Kim Aarestrupⁱ, Lieven Bervoets^j, Catherine Boisneau^k, Clarisse Boulenger^{c,l}, Paddy Gargan^m, Gustavo Becerra-Jurado^{m,n}, Javier Lobón-Cervía^o, Gregory E. Maes^{p,q,r}, Michael Ingemann Pedersenⁱ, Russell Poole^s, Niklas Sjöberg^t, Håkan Wickström^t, Alan Walker^u, David Righton^{u,1}, Éric Feunteun^{a,1}

^a Laboratoire Biologie des Organismes et Écosystèmes Aquatiques (BOREA), Muséum National d'Histoire Naturelle, CNRS FRE 2030, Sorbonne Université, IRD 207, Université de Caen Normandie, Université des Antilles, Centre de Recherche et d'Enseignement sur les Systèmes Côtiers, station de biologie marine de Dinard, 38 rue du Port Blanc, 35800 Dinard, France

^b UMS 2006 Patrimoine Naturel (PatriNat, OFB/CNRS/MNH), Centre de Recherche et d'Enseignement sur les Systèmes Côtiers, station de biologie marine de Dinard, 38 rue du Port Blanc, 35800 Dinard, France

^c OFB, Management of Diadromous Fish in their Environment OFB-INRAE-Agrocampus Ouest-UPPA, 65 rue de Saint Brieuc, 35042 Rennes Cedex, France

^d Institute for Nature and Forest Research (INBO), Dwersbos 28, 1630 Linkebeek, Belgium

^e Toxicological Center, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

^f Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS - Université de la Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France

^g Institut Universitaire de France (IUF), 1 rue Descartes, 75005 Paris, France

^h Centre de Formation et de Recherche sur les Environnements Méditerranéens (Cefrem), UMR 5110 CNRS-Université de Perpignan Via Domitia, 52 Avenue Paul Alduy, F-66860 Perpignan Cedex, France

ⁱ DTU AQUA, National Institute of Aquatic Resources, Section for Freshwater Fisheries Ecology, Technical University of Denmark, Vejløvej 39, 8600 Silkeborg, Denmark

^j University of Antwerp, Systemic Physiological and Ecotoxicological Research group (SPHERE), Groenenborgerlaan 171, 2020 Antwerp, Belgium

^k UMR 7324 CItés, TERitoires, Environnement et Sociétés (CITERES, CNRS, Université de Tours), 33 Allée Ferdinand de Lesseps, 37200 Tours, France

^l INRAE, UMR 985, INRA-Agrocampus, Ecologie et Santé des Écosystèmes, Rennes Cedex, France

^m Inland Fisheries Ireland, 3044 Lake Drive, Citywest Business Campus, Dublin 24, Ireland

ⁿ Institute for European Environmental Policy, Department of Biodiversity and Ecosystem Services, Rue Joseph II 36-38, 1000 Brussels, Belgium

^o Department of evolutionary Ecology, National Museum of Natural Science (CSIC), C/. Jose Gutiérrez Abascal 2, Madrid 28006, Spain

^p Aquaculture, Department of Biosystems, KU Leuven, 3001 Leuven, Belgium

^q Center for Human Genetics, UZ Leuven - Genomics Core, KU Leuven, Leuven 3000, Belgium

^r Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science and Engineering, James Cook University, Townsville, Qld 4811, Australia

^s Marine Institute, Fisheries Ecosystems Advisory Services, Newport, Co. Mayo, Ireland

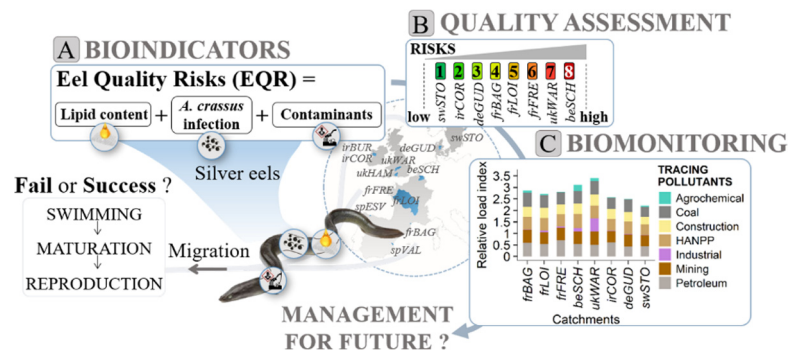
^t Swedish University of Agricultural Sciences, Department of Aquatic Resources, Institute of Freshwater Research, Stångholmavägen 2, SE-178 93 Drottningholm, Sweden

^u Centre for Environment Fisheries and Aquaculture Science, Pakefield Road, Lowestoft, Suffolk NR33 0HT, England, United Kingdom

HIGHLIGHTS

- Among 482 eels, 66% were impacted by *A. crassus* and 27% showed evidence of past infection.
- Among 169 eels, organic and inorganic contaminants showed high prevalence.
- Variability in eel quality at the European scale was mainly driven by organic pollutants.
- According a risk index, eels from Scheldt, Warwickshire and Frémur ranked lowest risk.
- Eel quality risks were positively correlated with a remote sensing anthropization index.

GRAPHICAL ABSTRACT



* Corresponding author.

E-mail address: bastien.bourillon@mnhn.fr (B. Bourillon).

¹David Righton and Eric Feunteun were the two senior researchers in charge of the design, implementation and reporting of this study, which was a work-package within the EU eeliad project.

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ABSTRACT

The European eel is critically endangered. Although the quality of silver eels is essential for their reproduction, little is known about the effects of multiple contaminants on the spawning migration and the European eel management plan does not take this into account. To address this knowledge gap, we sampled 482 silver eels from 12 catchments across Europe and developed methods to assess three aspects of eel quality: muscular lipid content ($N = 169$ eels), infection with *Anguillicola crassus* ($N = 482$), and contamination by persistent organic pollutants (POPs, $N = 169$) and trace elements (TEs, $N = 75$). We developed a standardized eel quality risks index (EQR) using these aspects for the subsample of 75 female eels. Among 169 eels, 33% seem to have enough muscular lipids content to reach the Sargasso Sea to reproduce. Among 482 silver eels, 93% were infected by *A. crassus* at least once during their lifetime. All contaminants were above the limit of quantification, except the 1,2-bis (2,4,6-tribromophenoxy)ethane (BTBPE), Ag and V. The contamination by POPs was heterogeneous between catchments while TEs were relatively homogeneous, suggesting a multi-scale adaptation of management plans. The EQR revealed that eels from Warwickshire were most impacted by brominated flame-retardants and agricultural contaminants, those from Scheldt were most impacted by agricultural and construction activities, PCBs, coal burning, and land use, while Frémur eels were best characterized by lower lipid contents and high parasitic and BTBPE levels.

There was a positive correlation between EQR and a human footprint index highlighting the capacity of silver eels for biomonitoring human activities and the potential impact on the suitability of the aquatic environment for eel population health. EQR therefore represents a step forward in the standardization and mapping of eel quality risks, which will help identify priorities and strategies for restocking freshwater ecosystems.

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1. Introduction

The temperate European eel *Anguilla anguilla* (L., 1758) is a facultative catadromous species characterized by a complex semelparous life cycle. Spawning takes place in the tropical North Atlantic convergence zone (Miller et al., 2015; Righton et al., 2016), and the fertilised eggs hatch into meso-epipelagic leptocephali that are transported for more than a year towards the European and North African coasts (Miller et al., 2015). On the continental shelves, they start metamorphosing, reach estuaries as glass eels and colonize the transitional waters and inland aquatic habitat (Feunteun et al., 2003; Tesch, 2003). After metamorphosis from the juvenile glass and elver stages, eels undergo a growth stage of about 3–30 years as yellow eel in European and North African transitional and inland waters (Tesch, 2003). When yellow eels reach a certain size (35–46 cm for males and 50–100 cm for females, Tesch, 2003), they undergo the silverying metamorphosis to develop adaptations to oceanic-migration conditions, cease their feeding (e.g. degeneration of the digestive tract, Pankhurst and Sorensen, 1984) and begin a seaward migration (Tesch, 2003). Once at sea, the still reproductively immature and fasting silver eels migrate towards the spawning ground (> 5000 km), whose duration remains uncertain (several months to more than a year, Righton et al., 2016) and during which the oocytes and spermatoocytes become mature (Palstra et al., 2005; Tesch, 2003; van den Thillart et al., 2007). Successful migration and breeding is likely to depend on both the quality and quantity of fat stored in the eel during the growth stage. The long-distance migration of the eel, coupled with semelparity, is therefore a risky life-history strategy that risks complete reproductive failure (Robinet and Feunteun, 2002).

The European eel population suffers from several anthropogenic pressures such as habitat modification (Kettle et al., 2011), barriers (Trancart et al., 2020), overfishing (Dekker, 2004), non-native pathogens like the introduced sanguivorous nematode *Anguillicola crassus* (Kirk, 2003), and pollutions (Geeraerts and Belpaire, 2009; Robinet and Feunteun, 2002). As a result, population has declined significantly in the last 30 years, and the European eel is currently listed as critically endangered (Jacoby and Gollock, 2014). To date, eel management targets set by EU Member States in response to the European Commission Regulation EC 1100/2007 (Council of the

European Union, 2007) have mainly focused on reducing fishing, promoting the freshwater migration, and restocking of glass eels to depleted catchments (Belpaire et al., 2019) so as to increase the quantity of eels escaping. However, little attention has been paid to improving the quality of silver eels, which could be used as a proxy for how eels will perform in the future in terms of survival, migratory and reproductive success, since the immature and mature oceanic key stages are not accessible. Three aspects of eel quality are particularly important:

- i) Body fat content. Although little is known about the swimming behaviour towards the spawning area, several laboratory experiments and theoretical assumptions have shown that a fat content representing 12–13% of the body mass is required to fuel the trans-Atlantic migration, while a fat content estimated at 20% is thought to be necessary to ensure successful reproduction (Boëtius and Boëtius, 1980; Palstra and van den Thillart, 2010; van den Thillart et al., 2007).
- ii) The load of *A. crassus* (Kuwahara et al., 1974). This invasive nematode damages the swimbladder in European and American eels, therefore potentially affecting the oceanic swimming performance and the resistance to environmental stress (Kennedy, 2007; Palstra et al., 2007).
- iii) The contaminant load in body tissues. Contaminants such as lipophilic persistent organic pollutants (POPs) and non-lipophilic trace elements (TEs) bioaccumulate in the lipid, muscles and organs (for example: Brinkmann et al., 2015; ICES, 2016; Malervannan et al., 2014; Zacs et al., 2018). During migration, the contaminants are remobilised and directed towards developing organs like gonads and eggs (Freese et al., 2019; Sührling et al., 2015, 2016b). As a result, this remobilisation is thought to affect physiological functions with histological responses, endocrine and osmoregulation disruptions, reduction of the stress resistance, genotoxicity, reprotoxicity and behavioural alterations (Geeraerts and Belpaire, 2009; Robinet and Feunteun, 2002). It could also have an embryotoxicity in future eggs (Palstra et al., 2006) that further compromise the sustainability of the eel population.

Due to the inaccessibility of specific key stages of its life cycle, the impacts on the eel quality is limited by insufficient knowledge in relation

to the thresholds of *A. crassus* and contaminant loads for reducing or compromising spawning migration success (Belpaire et al., 2019). However, assessing the quality of future spawners has several advantages. Firstly, eels are relevant indicators for aquatic management due to their life traits (longevity, high fat content, top predator and benthic dweller, Tesch, 2003). Therefore, measures of the eel quality can pinpoint a part of human pressures on aquatic environment (Belpaire, 2008; Freese et al., 2016) and provide context for relevant management actions on the eel population. Secondly, one of the current eel management actions consists in restocking young glass eels in catchments where natural recruitment is low to enhance the eel stock. Measuring the eel quality in candidate catchments enables the selection of stocking sites producing silver eels with a relatively good quality, potentially favouring the production of future 'healthy' spawners. Current stocking programs typically consider the presence of obstacles to migration, the existing density of eels, and only to a certain extent the quality of the environment (Beaulaton and Azam, 2019). Developing a large-scale eel quality map and the use of standardized quality indicators of eels would help to improve the choice criteria of existing and potential future stocking sites.

Preliminary attempts to assess the quality of yellow eels using epidemiological thresholds relative to human health (Couderc et al., 2015; Quadroni et al., 2013) and to develop bioindicators of the spawner quality have been made, as reviewed in 2015 by the Working Group on Eel (WGEEL, ICES, 2015) involving the European Inland Fisheries and Aquaculture Advisory Commission (EIFAAC), the International Council for the Exploration of the Sea (ICES) and the General Fisheries Commission for the Mediterranean (GFCM). In Flanders, Belpaire and Goemans (2007) and Maes et al. (2005) used contaminants to, respectively, develop the eel quality classes (defined as the quantile values of mean concentrations) and to define the standardized individual mean multi-metal bioaccumulation index (IMBI). The WGEEL also developed the eel "patho-index" as a tool to monitor eel diseases and parasites (ICES, 2015), using data from the restocking project in North Rhine (Germany). In another example, the reproductive potential of female eels based on the size and lipid reserves has been considered by the WGEEL with the RP_{IND} index. Finally, Amilhat et al. (2014) assessed the silver eel quality throughout Mediterranean lagoons with an integrative eel quality index (EQI_{TOT} , ICES, 2015) that integrates toxic contaminants (PCBs, DDTs, Cd, Cu and Zn) and pathogens (*A. crassus* infection and EVEX virus) in four classes (weakly polluted, slightly polluted, polluted, and strongly polluted). However, none of the reported bioindicators adopted an integrative approach including lipid content, parasite burden and contaminant loads. In addition, current bioindicators have been developed only for yellow eels on small biogeographic scales, despite the need for a European consensus on the quality trends of future spawners.

To improve the use of the eel quality in population and environmental assessments, fourteen scientific institutes from the collaborative European EELIAD project (<https://cordis.europa.eu/project/id/212133>) sampled 482 silver eels from 12 catchments located in northern and mid-Europe to assess their quality. A nested sampling strategy was used to gather effective data across as many parameters relating to quality as possible: diagnosis of the *A. crassus* infection ($N = 482$, 12 catchments), measurements of lipid and POPs contents ($N = 169$, 11 catchments) and analysis of TEs ($N = 75$ for which all quality variables were available, 8 catchments). We used the resulting data to address the following aims:

- produce an overview of each subsample to map the range of different quality variables in male and female silver eels throughout several European catchments.
- develop a new integrative bioindicator (the EQR index: eel quality risks) based on current bioindicators and standardized quality variables that we applied on the 75 female silver eels subsample.

- use the standardized quality variables and the EQR index to assess the management of eel habitats with respect to quality and the impacts of related human activities in European catchments.

2. Materials and methods

2.1. Eel sampling

368 female and 114 male silver eels were sampled by scientific or professional fisheries between 2008 and 2010 in 12 European freshwater and brackish waterbodies that ranged from 60 to 117,000 km² in size (Fig. 1, Table 1). At each site, a length stratified sampling protocol was used to select silver eels captured during their seaward migration. Since the gears used were typically passive, the captured eels would have originated from any upstream part of the catchment. The eels were anesthetized using either clove oil / eugenol (3 mL of 30% solution dissolved in 10 L of water), phenoxyethanol, chlorbutol (1 or 2 g of solid in 4 L of water), benzocaine (150 mg/L) or metomidate (400 mg powder for 10 L of water) depending the required ethical standards of each participating institute. Then, biometry was performed (length, weight, length of pectoral fins, vertical and horizontal eye diameters) to assess the silver stage, according to external measurements (Acou et al., 2005), and maturity stages based on anatomic metrics (Durif et al., 2005).

2.2. Quality variables

After euthanasia (through anaesthetic overdoses), the eels were dissected to remove and collect the swimbladders, and to take a dorso-ventral section of muscles (from 5 cm above to 10 cm below the anus). These were weighed and stored at -20°C . Depending on the parameter of interest, analyses were then carried out on three nested

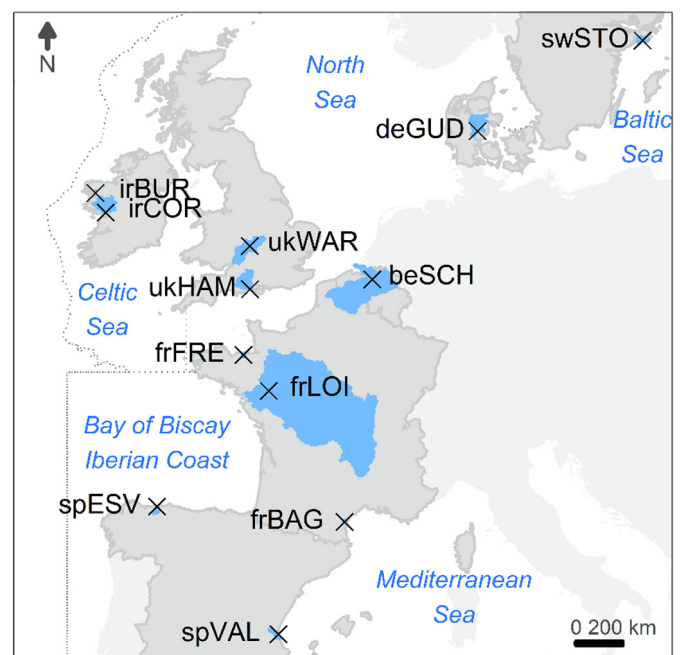


Fig. 1. Sampling locations (crosses) of European silver eels in 12 catchments (blue shading) during the EELIAD program. The involved countries are shaded in dark gray. The eight catchments showing the full quality dataset were frBAG, frLOI, frFRE, beSCH, ukWAR, irCOR, deGUD, and swSTO. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Information about the EELIAD sampling sites. The identifier (ID) is a combination of the country name and site name.

ID	Country	Sampling site	Latitude	Longitude	Area (km ²)	DSS (km)	Waterbody type	Sampling gear	Sampling date (m/d/y)
swSTO	Sweden	Stockholm archipelago	58°57'30.82"	18°02'05.09"	467	8654	S	A	10/12/09
deGUD	Denmark	Gudenå	55°58'01.31"	09°42'16.64"	2684	8053	R	B	11/12 to 12/14/09
irBUR	Ireland	Burrishoole	53°55'13.51"	09°35' 03.30"	89	5682	Cr + L	B	11/19/08
irCOR	Ireland	Corrib	53°16'32.05"	09°03'21.71"	3167	5697	Cr + L	C	11/16/09
ukWAR	U.K.	Warwickshire Avon	52°10'00.46"	01°47'27.39"	4588	6069	R	B	11/05/09
ukHAM	U.K.	Hampshire Avon	50°44'41.56"	01°46'57.18"	2993	5935	R	D	11/16/09
beSCH	Belgium	Scheldt	51°03'58.53"	04°23'19.97"	20,282	6451 ± 55	R + Cp	D + E	10/19 to 10/27/09
frFRE	France	Frémur	48°34'39.80"	02°06'13.1"	60	5949	Cr	B	01/19 to 03/01/10
frLOI	France	Loire	47°22'59.80"	00°50'07.1"	116,962	5539	Cr	F	12/09 to 12/26/09
spESV	Spain	Esva	43°34'09.00"	06°28'11.00"	464	4997	Cr	E	09/13 to 11/22/09
frBAG	France	Bages-Sigean	43°03'39.61"	02°59'38.06"	411	6166	Lg	D	11/08 to 12/16/09
spVAL	Spain	Albufera de Valencia	39°20'59.58"	00°20'44.82"	886	5721	Lg	D	11/06/10

Waterbody type: Cp = canals and ponds, Cr = coastal river, L = lake system, Lg = lagoon, R = river, S = Baltic sea. Sampling gear: A = pound net, B = eel trap, C = Coghill net, D = Fyke net, E = electrofishing, F = stow nets. DSS: distance between sampling site and putative spawning ground.

subsamples among the 482 available individuals (Table 2). The abbreviations and protocols are described in supplementary data (S.1 section).

We investigated the *A. crassus* infection in the swimbladder of all 482 eels, using prevalence (presence or absence of nematode), abundance (number of nematodes per eel), and the swimbladder degenerative index (SDI), that measures three alterations of the swimbladder (transparency, wall thickness, and the presence of pigmentation and exudate), each graded as 0, 1, or 2. The SDI ranges from 0 (not affected) to 6, and a value ≥ 4 indicates a severely damaged swimbladder (Lefebvre et al., 2002). Using the muscle samples of 169 individuals, the lipid content and concentrations of six types of POPs were analysed (BTBPE, DDT and its metabolites, three HBCD isomers, HCB, 13 PBDE congeners, and 40 PCB congeners). For the TEs, we measured Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn in the muscle samples of 75 females (males were excluded because knowledge about lipid requirement for

migration and impact of contaminants on physiology and eggs development are more developed for females). The POPs and TEs mentioned above were chosen due to their more or less known effects on the physiology of eels (or other fishes), and to their proven prevalence in several monitoring networks (Belpaire and Goemans, 2007), excepting the emerging contaminant BTBPE.

The sampling protocol resulted in complete data on the muscular lipid content, swimbladder parasites, POPs and TEs for 75 eels, covering eight catchments: Bages-Sigean (France), Loire (France), Frémur (France), Scheldt (Belgium), Warwickshire Avon (United Kingdom), Corrib (Ireland), Gudenå (Denmark), and Stockholm archipelago (Sweden) (Table 1). These data were then used for the development of the eel quality risks (EQR) index. The data on the muscular lipid content, the swimbladder infestation and muscular POPs concentrations that were not sampled for all characteristics (i.e. outside the subsample $N = 75$) were used to provide knowledge on the overall quality of the studied eel subpopulations.

Table 2

Number (N) and total silvering length [TL in mm, mean \pm SD and range (min–max) in brackets] of sampled female (f) and male (m) silver eels. Three nested subsamples were analysed for the *A. crassus* infection ($N = 368$ f + 114 m), muscular lipids content and POPs (both $N = 166$ f + 3 m), and TEs concentrations ($N = 75$ f).

ID	SUBSAMPLES					
	<i>A. crassus</i>		Lipids & POPs		TEs	
	N	TL	N	TL	N	TL
swSTO	24	851 \pm 43 (754–933)	12	847 \pm 50 (754–929)	9	844 \pm 51 (754–929)
deGUD	37	663 \pm 79 (486–787)	13	637 \pm 88 (486–787)	10	628 \pm 91 (486–787)
irBUR	46	509 \pm 56 (318–635)	13	519 \pm 51 (443–635)	–	–
irCOR	36	674 \pm 137 (356–970)	11	692 \pm 130 (482–970)	10	701 \pm 134 (482–970)
ukWAR	42	656 \pm 73 (538–833)	13	652 \pm 84 (538–833)	9	667 \pm 94 (538–833)
ukHAM	34	568 \pm 101 (333–710)	12	585 \pm 82 (482–710)	–	–
beSCH	45	670 \pm 117 (354–837)	45	670 \pm 117 (354–837)	9	719 \pm 76 (616–837)
frFRE	46	567 \pm 125 (340–796)	13	623 \pm 85 (495–763)	10	615 \pm 88 (495–763)
frLOI	51	669 \pm 190 (373–1005)	13	716 \pm 137 (522–1005)	9	698 \pm 151 (522–1005)
spESV	21	343 \pm 66 (288–610)	–	–	–	–
frBAG	50	464 \pm 152 (354–875)	11	722 \pm 133 (470–875)	9	710 \pm 137 (470–875)
spVAL	50	590 \pm 168 (351–852)	13	726 \pm 76 (583–852)	–	–
TOTAL	482	601 \pm 162 (288–1005)	169	670 \pm 123 (354–1005)	75	696 \pm 122 (470–1005)

2.3. Quality assessment with a standardized bioindicator

2.3.1. Catchments ranking

Our eel quality risks (EQR) index was computed by summing four standardized quality components within the subsample of 75 silver eels described above, as follows: $EQR = RL_{RWLB} + RL_{ACinf} + RL_{POPS} + RL_{TEs}$, with following details:

- The 'RL_i' (relative load) is a function used to standardize the four quality variables between 0 and 1, because of their different orders of magnitude and our consideration without a priori knowledge. The RL_i is similar to the IMBI index (Maes et al., 2005) and reflects the percentage of the maximum value (C_{imax}) detected among all values in the *i* variable: $RL_i = C_i / C_{imax}$, with C_i the individual value of the *i* variable. Thus, EQR index ranged from 0 (high quality or low risks) to 4 (low quality or high risks).
- The RL_{RWLB} component (RWLB: reversed weighted lipid burden) takes into account the muscular lipid content (in %) weighted by the DSS (distance between sampling site and putative spawning ground, in km, Table 1) and penalized by a risk coefficient (λ), as follow: $RL_{RWLB} = RL[\lambda \times (rLipid / DSS)]$, with $rLipid = 1 / lipid$. We assumed the λ values of 0.25, 0.5, and 1 to represent low ($> 20\%$ of the muscular lipid content), medium (12–20%), and high ($< 12\%$) risks of reproduction failure, respectively (Boëtius and Boëtius, 1980; Palstra and van den Thillart, 2010; van den Thillart et al., 2007). In addition, the lipid percentage was reversed to ensure that a high value of RL_{RWLB} represents a risk for an individual with low lipid content and a long distance to travel to reach the spawning ground. The DSS was estimated from QGIS (ver. 2.14.20, QGIS Development Team, 2017), and

trajectories towards the putative Sargasso Sea spawning ground were inspired by [Righton et al. \(2016\)](#) and were calculated to 27°N latitude and 55°W longitude (i.e. the centre of a 22–32°N latitude and 50–60°W longitude square, described by [Friedland et al., 2007](#)).

- iii) The RL_{ACinf} component takes into account the current nematode abundance as well as the swimbladder degenerative index (SDI), where $RL_{ACinf} = RL[(abundance + 0.01) \times (SDI + 0.01)]$.
- iv) The RL_{POPs} and RL_{TEs} are computed as the standardized average of all standardized POPs and TEs respectively, for example $RL_{POPs} = RL[(RL_{BTBPE} + RL_{DDTs} + RL_{HBCDs} + RL_{HCB} + RL_{PBDEs} + RL_{PCBs})/6]$, inferring the same weight to each contaminant, because the effects of contaminants on the eel quality and the sublethal thresholds are currently unknown. Considering this standardized approach adapted for contaminants, we assumed that the maximum concentration of contaminants passed quality controls and were not outliers. For both Ag and V trace elements, RL_i was taken as half of the limit of quantification (LOQ), because all the observed concentrations were below the LOQ. This assumption avoids over-representing the corresponding RL_i values. As recommended by [ICES \(2015\)](#), TEs were expressed as concentration against the dry weight (dw). In order to maintain a same weight between RL_{TEs} and RL_{POPs} components and to promote comparisons, POPs were not expressed by lipid weight (lw) but in wet weight (ww). Furthermore, the EQR index already includes the variation in lipid weight due to the RL_{RWLB} component, and the observed ww and lw concentrations of POPs were positively correlated (Spearman tests; $R^2 = 0.9 \pm 0.04$; $P < 0.001$; Table S1), thereby showing similar standardized values between ww or lw expressions.

Despite the lack of knowledge about the effects of quality variables on the eel spawning migration, we postulate that the probability of spawning migration failure increases with the relative load of penalized and weighted lipid content (RL_{RWLB}), parasite infection (RL_{ACinf}), and muscular contaminants (RL_{POPs} and RL_{TEs}).

We separated the eels into four classes based on the EQR quartile distributions. The eels classed in the Q1 (0–25%) and Q2 (25–50%) quartiles were respectively considered to be representative of habitats that were weakly and slightly impacted by human activities pinpointed by silver eels. The eels found in the Q3 (50–75%) and Q4 (75–100%) quartiles were considered to be either impacted or strongly impacted with the highest probability of failure during spawning migration. To rank catchments according to the EQR index, we computed the rank as the sum of the EQR quartile scores standardized by the number of sampled eels in each catchment.

2.3.2. Identification of quality issues

In order to identify the origin of the eel quality issues within each catchment, the contribution (in %) of each quality component (RL_{RWLB} , RL_{ACinf} , RL_{POPs} and RL_{TEs}) in relation to the EQR value was computed for each individual (contribution = quality component value / EQR value $\times 100$) and then averaged at the catchment scale. Finally, we identified in each quartile and each catchment the six contaminants with the highest cumulative average RL_i values as the priority targets for future conservation measures.

2.3.3. Biomonitoring relevance

We investigated the link between EQR index (EQR with all quality components and EQR without the ACinf component) and a human footprint index (HFP, no unit). Estimated in 2009 by [Venter et al. \(2016a, 2016b\)](#), the HFP integrates several pieces of remote sensing information of human impacts (agriculture, constructions, population density, and navigable waterways). The average HFP values were extracted for each studied catchment (raster package; [Hijmans and van Etten, 2018](#)).

2.4. Variability of quality and approach to trace the origin of contaminants

To assess the intra- and inter-catchment variability of the eel quality, we i) performed a principal component analyse (PCA, FactoMineR package; [Husson et al., 2018](#)), using the RL_i of quality variables measured in 75 females as structuring variables, and ii) computed the coefficients of variation ($CV = (SD/\mu) \times 100$, SD = standard deviation, μ = average) of each quality component (RL_{RWLB} , RL_{ACinf} , RL_{POPs} and RL_{TEs}) and the EQR index.

The European eel is known to reflect human activities in/near the waterbodies that each individual had inhabited ([Belpaire et al., 2008](#); [Freese et al., 2016](#)). This bioindicator property is, therefore, an opportunity to target the human activities responsible for the degradation of the eel quality. The POPs are exclusively produced by anthropogenic activities, such as agrochemistry (DDTs and HCB) and other industries [PCBs and brominated flame retardants (BFRs: BTBPE, HBCDs, and PBDEs)]. For the TEs, it is difficult to distinguish geogenic from anthropogenic sources, and their flows in Europe are poorly known. Therefore, we pooled the TEs according to the anthropogenic flows estimated for USA by [Sen and Peucker-Ehrenbrink \(2012\)](#) as follows: construction (Co, Cr, Fe, Mn, Ni, Pb, Se, V, and Zn), mining (Ag, Cr, Cu, Fe, and Zn), consumption of petroleum (Fe, Hg, Ni, and V) and coal (As, Fe, Mn, Pb, and Zn), and the HANPP (human apportionment of terrestrial net primary productivity; Cd, Co, Fe, Mn, and Zn) activities. For each activity group, we computed the average RL_i of quality variables, and included them as non-structural variables in the PCA as an exploratory approach to target activities responsible of contaminants in the eels.

2.5. Statistical analysis

The statistical analyses were carried out using R ver. 3.6.1 ([R Core Team, 2019](#)). Detailed descriptions of the muscular lipid content ($N = 169$), *A. crassus* infection ($N = 482$), and concentrations of POPs ($N = 169$) and TEs ($N = 75$) are given in supplementary data (S.2 section). All data are described as the mean plus one standard deviation ($\mu \pm SD$). To test the difference of quality variables and indices (EQR and HFP) between catchments, we applied Kruskal-Wallis and Dunn's tests ([Dunn, 1964](#); [Ogle, 2019](#)) because the normality of residues and homoscedasticity were not maintained ($\alpha = 5\%$, with Bonferroni correction for multiple comparisons). The link between EQR and HFP indices was determined by selecting the best-explained deviance from different linear models (polynomial, natural cubic spline, and β -spline; with 1–3 degrees of freedom).

3. Results

3.1. Overview of lipid, parasitic infection, and contaminations

The muscular lipid content for the 169 analysed silver eels ranged between 5.9% (individual from Scheldt) and 31.8% (from Stockholm archipelago). Samples from 3.6% of silver eels contained <12% muscular lipids ($N = 4$ from Scheldt, $N = 1$ from Loire and Burrishoole) and 33.1% of individuals ($N = 56$), essentially from Stockholm archipelago ($N = 12$), Warwickshire ($N = 10$) and Valencia ($N = 7$) catchments, contained >20% muscular lipids (see details in supplementary data, S.2 section, Fig. S1).

Overall, 317 silver eels (66%) hosted nematodes in their swimbladders (Fig. S2a) and were classified as having a damaged swimbladder ($SDI \geq 1$). A total of 131 individuals (27%) were classified as having had a past infection (no nematode but $SDI \geq 1$). The average abundance was 4.5 ± 7.3 nematodes per swimbladder (Fig. S2b). No nematodes were found in silver eels from Burrishoole, while the nematode occurrence reached 93% of all samples in Frémur. In addition to Burrishoole, the abundance was low ($< 1.7 \pm 4.2$ nematodes) in eels from Bages-Sigean, Stockholm archipelago and Esva. The SDI value

exceeded four for 25% ($N = 120$) of individuals, including individuals from Burrishoole ($N = 3$) with no nematode burden (Fig. S2c).

Of the silver eels tested for POPs ($N = 169$, Fig. S3) and TEs ($N = 75$, Fig. S4) of them had concentrations above the limit of quantification (LOQ), except for V, Ag ($< \text{DL}$) and BTBPE, for which 25% of individual ($N = 43$) displayed concentrations above LOQ. Silver eels from Scheldt and Valentia had higher DDTs concentrations ($> 87 \text{ ng} \cdot \text{g}^{-1} \text{ ww}$) compared to other sites (Dunn's tests; $P < 0.05$), except for Bages-Sigean and Warwickshire (64.6 ± 30.1 and $54.2 \pm 26.2 \text{ ng} \cdot \text{g}^{-1} \text{ ww}$). The silver eels from Stockholm, Scheldt and Warwickshire catchments showed highest HCB levels (between 1.3 ± 0.5 and $3.7 \pm 5.3 \text{ ng} \cdot \text{g}^{-1} \text{ ww}$) than others individuals. The Scheldt eels also displayed the highest level of PCBs ($2358 \pm 3284 \text{ ng} \cdot \text{g}^{-1} \text{ ww}$). The HBCDs and PBDEs reached high levels in English (Warwickshire and Hampshire) and Scheldt catchments ($> 21.7 \pm 23.0 \text{ ng} \cdot \text{g}^{-1} \text{ ww}$ for HBCDs, and $> 9.9 \pm 12.2 \text{ ng} \cdot \text{g}^{-1} \text{ ww}$ for PBDEs). The bioaccumulation of Cr, Cu, Fe, and Zn was similar between catchments. The silver eels sampled in Stockholm archipelago were among the least contaminated for Cd, Co, and Se. The Frémur individuals had among the highest concentrations of Co and Hg while Scheldt individuals were highly contaminated by Cd and Pb, and the Bages-Sigean individuals by As and Ni (Fig. S4).

3.2. Quality assessment with a standardized bioindicator ($N = 75$)

3.2.1. Catchments ranking

We computed the four standardized components of quality (RL_{RWLB} , RL_{ACinf} , RL_{POPs} , and RL_{TEs}) for 75 female silver eels with the full set of quality data. The silver eels from Gudenå showed higher RL_{RWLB} values (0.41 ± 0.15) in comparison to eels from Warwickshire Avon (0.16 ± 0.09), Stockholm archipelago (0.17 ± 0.03) and Corrib (0.22 ± 0.1) (Dunn tests; $P < 0.05$; Fig. 2a). In addition, the RL_{RWLB} values of Warwickshire's eels were significantly lower than values of Scheldt (0.34 ± 0.17), Frémur (0.37 ± 0.03) and Bages-Sigean (0.37 ± 0.11)

(Dunn tests; $P < 0.05$). The RL_{ACinf} index identified a higher *A. crassus* infection in silver eels from Frémur (0.24 ± 0.2), Corrib (0.22 ± 0.29) and Gudenå (0.19 ± 0.24) than those from Bages-Sigean (Dunn tests; $P < 0.015$; Fig. 2b). The silver eels from Warwickshire and Scheldt had higher RL_{POPs} values (0.51 ± 0.22 and 0.38 ± 0.30 , respectively) than eels from Corrib (0.04 ± 0.01), Gudenå (0.05 ± 0.02), Stockholm archipelago (0.06 ± 0.02), and Frémur (0.09 ± 0.07 , difference only with ukWAR) (Dunn tests; $P < 0.01$; Fig. 2c). The RL_{TEs} values were relatively homogenous throughout the studied catchments, with lower values for silver eels from Stockholm archipelago (0.37 ± 0.05) in comparison to other catchments: Loire (0.53 ± 0.09), Corrib (0.53 ± 0.12), Frémur (0.55 ± 0.07), Scheldt (0.56 ± 0.14), and Bages-Sigean (0.59 ± 0.13) (Dunn tests; $P < 0.01$; Fig. 2d). In addition, the Gudenå silver eels showed a trace element charge (0.43 ± 0.07) significantly lower than those of Bages-Sigean (Dunn test; $z = -3.17$, $P = 0.04$).

To simplify comparisons between catchments, the four standardized variables discussed above were integrated in the EQR index (Fig. 3a). The eels from Stockholm archipelago displayed a lower EQR index (0.65 ± 0.08) than those from Loire (1.16 ± 0.31), Frémur (1.24 ± 0.22), Warwickshire (1.29 ± 0.31) and Scheldt (1.44 ± 0.49) (Dunn tests; $P < 0.05$). The silver eels from Corrib (1.02 ± 0.40), Gudenå (1.09 ± 0.33) and Bages-Sigean (1.12 ± 0.21) showed intermediary EQR averages. The EQR distribution was split into four quartiles (Fig. 3b) in order of increasing environmental impact: Q1 (weakly impacted) = $0.51\text{--}0.81$, Q2 (slightly impacted) = $0.81\text{--}1.12$, Q3 (impacted) = $1.12\text{--}1.31$, and Q4 (strongly impacted) = $1.31\text{--}2.43$. According to quartiles and the calculated rank, 100% of the tested eels from the Stockholm archipelago showed low impacts ($N = 9$). Eels from Corrib and Gudenå ranked second and third with, respectively, 70 and 50% of individuals classified in Q1 and Q2. Eels from Bages-Sigean were ranked fourth with an intermediary quality status (44% of individuals in Q1 and Q2, 56% in Q3 and Q4). Finally, the Loire, Frémur, Warwickshire and Scheldt displayed a high proportion of impacted and

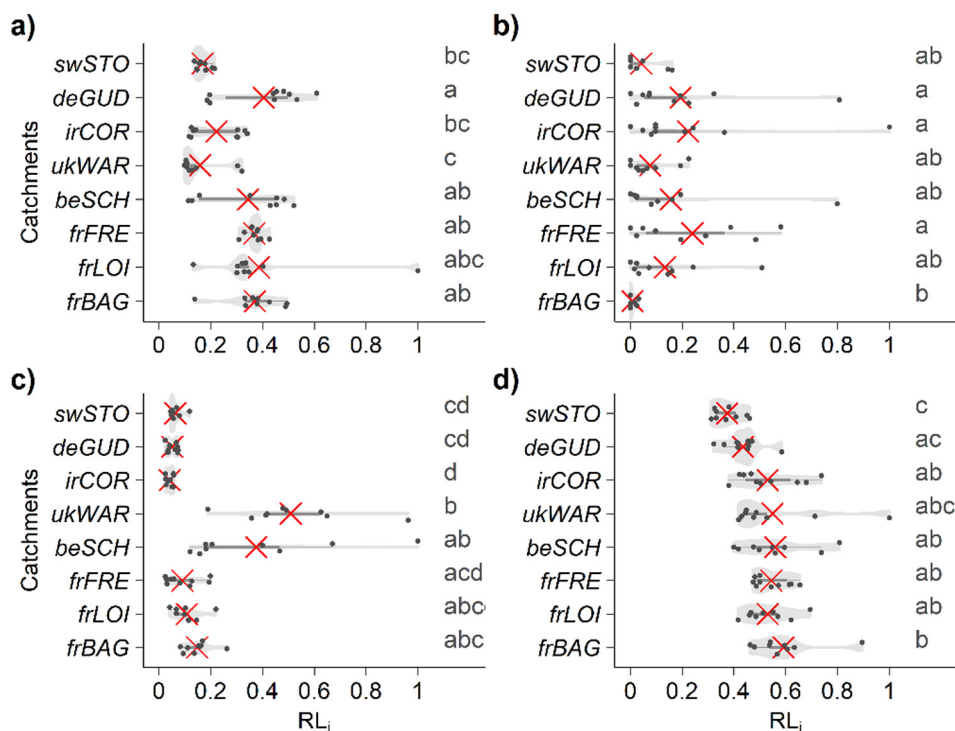


Fig. 2. Violin and box plots of the four quality components (expressed as relative load indices, RL_i) computed for 75 female silver eels in eight catchments from the southern (frBAG) to northern Europe (swSTO): **a)** reverse weighted lipid burden (RWLB), **b)** *A. crassus* infection (ACinf), **c)** persistent organic pollutants (POPs, including BTBPE, DDTs, HBCDs, HCB, PBDEs, and PCBs) and **d)** trace elements (TEs, including Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn). The crosses represent mean values for each catchment. Different letters between catchments (displayed on the right side of each chart) denote means found to be statistically different ($P < 0.05$, Dunn's test comparisons with Bonferroni correction). For the eel number in each catchment refer to Table 2.

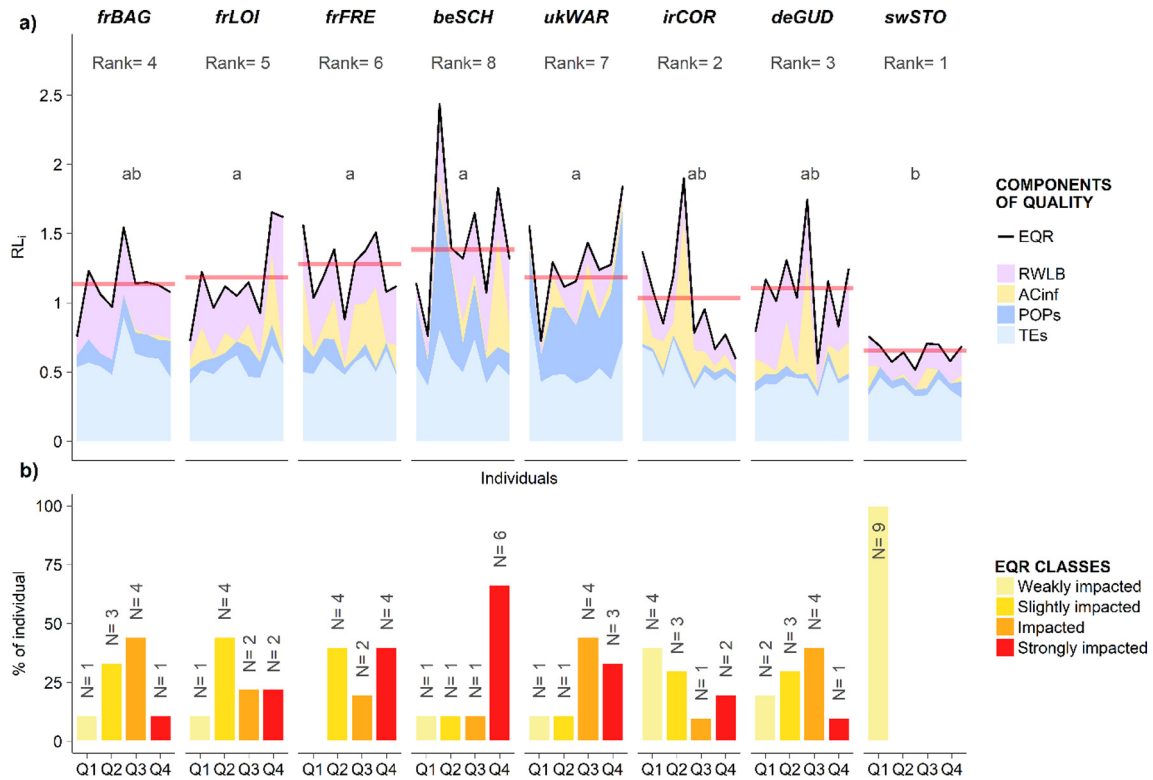


Fig. 3. a) Individual EQR distribution (eel quality risks, black line) of 75 silver eels combined with the cumulative areas of each EQR component (expressed as relative load indices, RL_i): reverse weighted lipid burden (RWLB), *A. crassus* infection (ACinf), and muscular concentrations of persistent organic pollutants (POPs) and trace elements (TEs). The catchments are displayed with the EQR rank (sum of EQR quartile scores divided by the number of eels in each catchment). A high rank means poor eel quality for spawning migration. Horizontal red lines show the average EQR values by catchment. Different letters between catchments (displayed on the center of each catchment chart) denote means found to be statistically different ($P < 0.05$, Dunn's test comparison with Bonferroni correction). For the number of eels sampled in each catchment refer to Table 2. **b)** Percentage of individuals belonging to different EQR quartiles (number of eels is displayed by quartile) for each catchment: Q1 (weakly impacted) to Q4 (strongly impacted). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

strongly impacted eels (44, 60, and both 78% for Warwickshire and Scheldt, respectively). In the Scheldt catchment, 67% of individuals were strongly impacted.

3.2.2. Identification of quality issues

Throughout the catchments, the contribution of RL_{TEs} to the EQR average value (Fig. 4a) was significantly higher ($48.1 \pm 11\%$) than the contribution of RL_{RWLB} ($27.3 \pm 10.9\%$; Dunn test; $z = -5.77$, $P < 0.001$). Both RL_{TEs} and RL_{RWLB} contributed significantly more to the EQR index in comparison to RL_{POPs} ($13.9 \pm 12.8\%$) and RL_{ACinf} ($10.7 \pm 12.5\%$) (Dunn tests; $P < 0.001$). In Warwickshire and Scheldt catchments, the RL_{POPs} ($38.3 \pm 9.8\%$ and $25.4 \pm 14.6\%$, respectively) and RL_{TEs} ($43.0 \pm 11.2\%$ and $55.5 \pm 7.1\%$, respectively) were major sources of poor quality.

Individuals facing the highest risk in spawning migration failure (i.e. high cumulative RL_i) were beSCH19 from Scheldt (EQR = 2.43, high contribution to the EQR index: RL_{POPs} = 44%, RL_{TEs} = 33%), and irCOR026 from Corrib (EQR = 1.9, high contribution: RL_{ACinf} = 52%), despite that Corrib ranked among the highest quality catchments. The only individual displaying a muscular lipid burden below 12% (frLOI138, in Loire catchment) shows the 8th highest EQR value (EQR = 1.62, strongly impacted quartile), for which RL_{RWLB} component contributed 62% to the value of EQR. In Bages-Sigean, the only individual in the Q4 quartile showed the highest sum of RL_i averages (EQR = 1.54) in this catchment, with a significant risk for this individual.

Within each catchment and EQR quartile, major POPs and TEs cocktails (as relative load index) were identified in order to target the main issues linked to pollution by human activities (Fig. 4b). A homogeneous bioaccumulation by Cu, Fe, and Zn appears throughout catchments (see supplementary data, S.2 section), and thus these compounds were

excluded from analyses to better identify the contamination specificities of each catchment. As a result, the six major contaminations cocktails (in descending order of the RL_i averages) were the following for Stockholm archipelago: Hg, Mn, Cd, DDTs, Ni, and BTBPE; for Gudenå: Mn, Hg, Se, Cd, Pb and BTBPE; for Corrib: Hg, Mn, Se, Ni, Cd and As; for Warwickshire Avon: PBDEs, HBCDs, Hg, Pb, Co, and DDTs; for Scheldt: DDTs, Cd, Hg, Se, Pb and Ni; for Frémur: Hg, Ni, Mn, BTBPE, Cd, and Co; for Loire: Hg, Se, Co, Ni, Cd, As; and for Bages-Sigean: Hg, As, DDTs, Ni, Mn, and Co. Regarding these cocktails, we observed a high RL_i average value of Hg in all catchments, especially in Stockholm archipelago (0.23 ± 0.08), Loire (0.35 ± 0.25), Bages-Sigean (0.44 ± 0.31), Corrib (0.53 ± 0.24) and Frémur (0.58 ± 0.21) catchments. We observed specific compounds in several catchments, like Warwickshire Avon with brominated flame retardants (HBCDs and PBDEs), Scheldt with DDTs and Cd, Corrib and Gudenå with Mn and Se, Loire with Se and As, and Bages-Sigean with As and DDTs. Within EQR quartiles, the cocktail showed several specificities, like the bioaccumulation of HCB in Q1 quartile in Scheldt, As in Warwickshire (Q1), and Pb in Loire (Q1) and Frémur (Q4). The brominated flame retardant BTBPE emerged among the six major contaminants mainly in Frémur (Q2 to Q4), but also in Stockholm archipelago (Q1), Gudenå (Q3) and Warwickshire (Q4).

3.2.3. Biomonitoring relevance

In order to validate the relevance of the EQR index for biomonitoring, we used the human footprint index from remote sensing (HFP), that provides clues about the human impacts within each catchment (Fig. 5a). The Corrib catchment and Stockholm archipelago displayed significantly lower HFP index (9.32 ± 6.40 and 10.57 ± 2.69 , respectively) than Bages-Sigean (19.53 ± 9.29), Frémur (22.95 ± 8.19), and Warwickshire

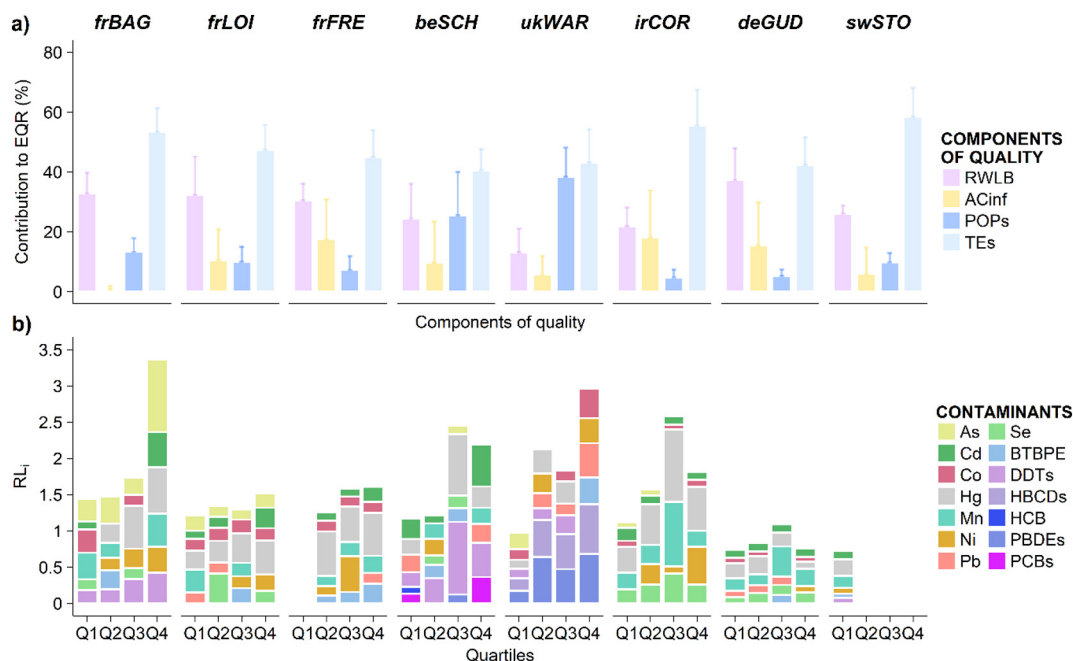


Fig. 4. Identification of major quality risks in each studied catchment (75 silver eels throughout height catchments), with **a)** the average contribution (% \pm standard deviation) of the four standardized components of quality to EQR values [relative load index (RL_i) of RWLB (reverse weighted lipid burden), ACInf (*A. crassus* infection), POPs (persistent organic pollutants) and TEs (trace elements)], and **b)** the sum of average RL_i indices of the six major contaminants that had large contributions to EQR values in each quartile and catchment. Cr, Cu, Fe, and Zn were excluded from the six major contaminants because they were found homogeneously throughout the studied catchments (see the supplementary data section). For the eel number in each quartile and catchment refer to Fig. 3b and Table 2, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(27.60 ± 7.92) (Dunn tests; $P < 0.05$). Similarly, the HFP index of Scheldt (30.36 ± 8.09) was higher than those of Corrib catchment (Dunn test; $z = 6.59$, $P = 1.21 \times 10^{-9}$). The high HFP average values of Scheldt and Warwickshire eels were also significantly different from those of Loire (14.8 ± 8.85 ; Dunn tests; $P < 0.001$). The Scheldt showed a higher HFP index than Gudenå (16.16 ± 8.09 ; Dunn test; $z = 3.77$, $P = 4.49 \times 10^{-3}$). Lastly, our EQR index was significantly and positively correlated with the HFP index ($R^2 = 0.23$, Fig. 5b). When the ACInf component was removed from the EQR index, the correlation remained significant and better explained the variance (EQR without ACInf = $1.6 \times \text{HFP} + 1.0$, Spearman; $N = 75$, $R^2 = 0.34$, $S = 26,891$, $P = 3.62 \times 10^{-9}$).

3.3. Variability of quality and approach to trace the origins of contaminants

According to PCA (Fig. 6a), the variability between individuals originated from POPs, which contribute to 47% in PC1 (DDTs, HCB, PBDEs and PCBs) and 41.6% in PC2 (HBCDs, PBDEs, and PCBs). In addition, the Pb and Cd level also explained a high proportion of the total variance with 14.3% in PC1 and 11.9% in PC2, respectively.

Regarding CV coefficient, the inter-catchment variability of the EQR index was moderate (32.8%) and the intra-catchment variability was ranged as follow: Stockholm (12.1%), Frémur (17.6%), Bages-Sigean (18.8%), Warwickshire (24.0%), Loire (26.5%), Gudenå (30.0%), Scheldt (33.9%) and Corrib (38.6%). The EQR variability between catchments

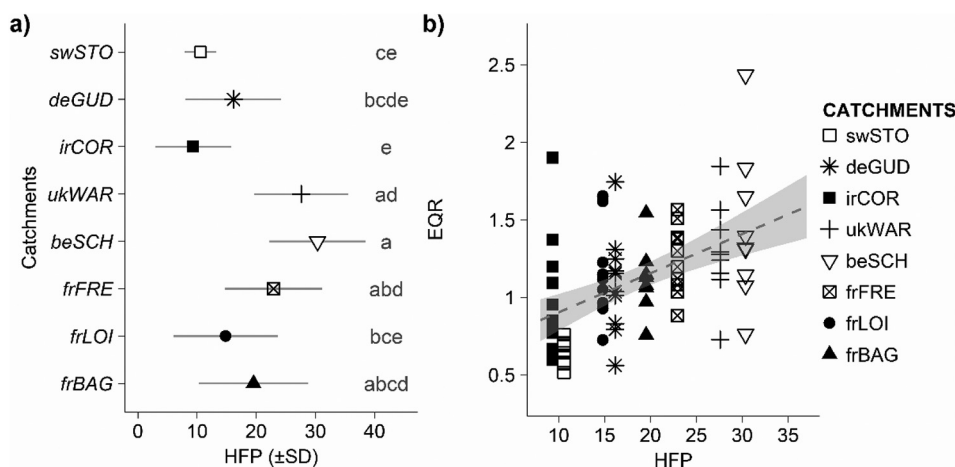


Fig. 5. **a)** Average (\pm SD) of HFP index in the studied catchments. The HFP index (Venter et al., 2016a, 2016b) reflects the cumulative human footprints in 2009. Different letters between catchments (displayed on the right side of the chart) denote means found to be statistically different ($P < 0.05$, Dunn's test comparisons with Bonferroni correction). **b)** Correlation between the mean EQR (eel quality risks) and HFP indices. The displayed linear regression (dotted line, \pm 95% confidence interval): $\text{EQR} = 1.6 \times \text{HFP} + 1.1$ (Spearman; $N = 75$, $R^2 = 0.23$, $S = 35,574$, $P = 6.68 \times 10^{-6}$).

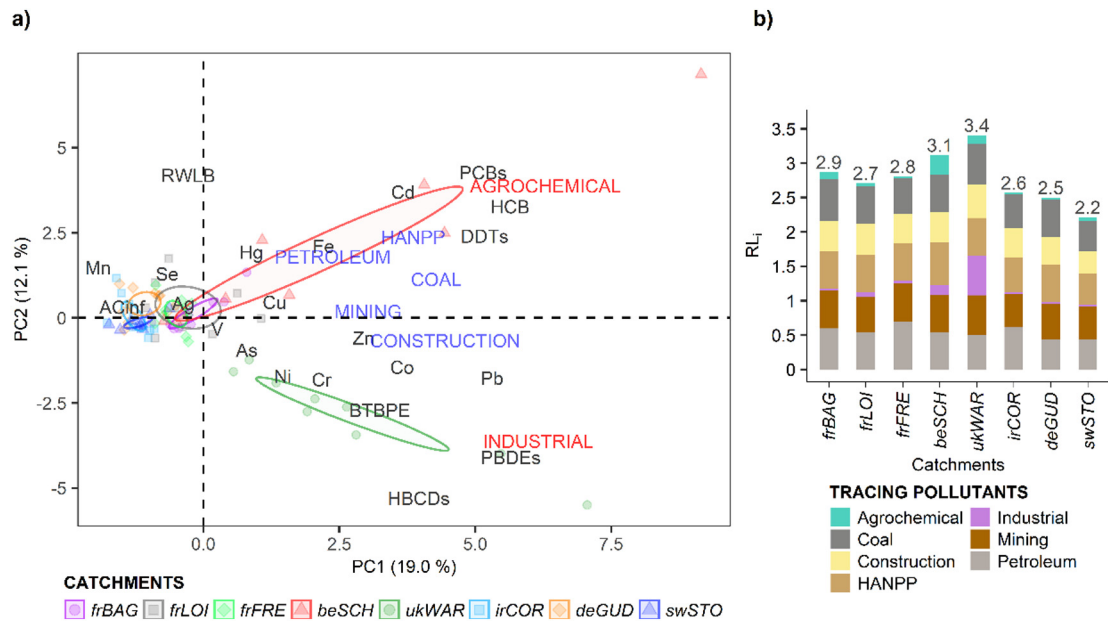


Fig. 6. a) Principal component analysis of the relative load indices (RL_i) of the quality variables (black labels) in 75 female silver eels: RL_{RWLB} (reverse weighted lipid burden), RL_{ACinf} (*A. crassus* infection), and RL_i of each muscular POPs (BTBPE, DDTs, HBCDs, HCB, PBDEs, and PCBs) and TEs (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn). Additional non-structuring variables provide information about the human activities producing POPs (red labels) and TEs (blue labels). b) Cumulative average RL_i values (sums displayed on chart) computed for each catchment and each human activities producing POPs (agrochemical, industrial) and TEs (coal-burning, construction, HANPP: human alteration of photosynthetic productivity on land, mining and petroleum burning). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was primarily related to the level of RL_{POPs} ($CV = 121.1\%$), but also from RL_{ACinf} ($CV = 144.6\%$) and, to a lesser extent, to RL_{TEs} (24.9%) and RL_{RWLB} (51.7%). Within each catchment, the RL_{ACinf} variability was very high, from 84.0% (in Frémur) to 160.2% (in Scheldt), while RL_{POPs} variability was lower, with 31.1% in Corrib, and respectively 72.6% and 78.9% in Frémur and Scheldt catchments.

The sum of average RL_i values computed by anthropogenic contaminant sources (Fig. 6a, b) revealed that silver eels from Warwickshire and Stockholm archipelago were subjected respectively to the strongest and the weakest pressure of human activities according contaminations. The activities producing TEs were relatively similar between catchments, except for the Stockholm archipelago, which also revealed low pressures from industrial and agricultural activities. Silver eels from Scheldt appeared to be at most risk of contamination from agricultural practices (agrochemicals + HANPP activities), activities generating TEs related especially to mining and transportation (coal and petroleum burning), and industrial activities (PCBs). Meanwhile, the Warwickshire catchment showed high industrial pressures (especially brominated flame retardants). The Loire catchments presented intermediary anthropogenic pressures, due to intermediate agrochemical and industrial POPs producing activities and from TEs producing activities that are relatively more important in the other catchments. The Bages-Sigean lagoon and Frémur River suffered from transportation (petroleum burning), agricultural land use (HANPP), mining, and construction activities.

4. Discussion

The quality of eels integrates their entire life history, from the larval stage to the reproductive eel (Jacoby et al., 2015), during which many parameters impact their reproductive potential. When glass eels complete the larval migration and reach transitional and inland waters, they encounter many human activities (dams, fisheries, risk of contaminants bioaccumulation, introduced parasites ...) or natural pressures (predation, indigenous parasites or diseases ...) during their growth stage. Once metamorphosis to the yellow phase occurs, eels can live a

long life as fatty and semelparous top predators that forage on benthos and in sediments (Tesch, 2003), where many contaminants accumulate. However, until eels metamorphose to the silver stage, stop feeding and leave the growth habitat, their lipid reserves and contaminant load are fixed. Silver eels therefore provide the best method to sample the eel quality at a catchment scale than the other life stages. One of the most important challenge for the current eel management plans is to increase considerations and actions to take account of the information that can be derived from measurement of the quality of silver eels. Our study represents a step forward to assess the quality of subadult silver eels by synthesizing information about the muscular lipids content, *A. crassus* infection, and muscular contaminations by POPs and TEs.

4.1. Quality of eels

Among the 482 analysed silver eels, 93% were infected by the sanguivorous *A. crassus* at least once during their lifetime, including 66% with a current infection (presence of nematode and damages) and 27% showing past traces of infection ($SDI \geq 1$), highlighting the invasive nature of this nematode (Kirk, 2003). The observed average burden of 4.6 nematodes per eel is consistent with the finding of Ashworth and Blanc (1997). However, eels from Bages-Sigean, Burrishoole and Stockholm archipelago were less affected by the nematode, possibly as a result of unfavourable temperature and salinity for *A. crassus* (Costa-Dias et al., 2010; Kirk et al., 2000).

To swim towards the spawning ground and reproduce, silver eels need sufficient energy reserves. Because lipids are mainly stored in the muscle tissues (Boëtius and Boëtius, 1985; Dave et al., 1976) which constitute a large part of the body weight (Brinkmann et al., 2015) and since our muscular sampling area reflects the total muscular lipid level (Pohlmann et al., 2019), the measures of lipid content in muscles of our study provide a representative proxy for total energy stores. Several experiments and modelling studies suggest that the migration may consume 60–65% of the total lipid content (or the 12–13% of the body weight that it represents), while maturation requires an additional 40% (8% body weight, Boëtius and Boëtius, 1980; Palstra and van den

Thillart, 2010; van den Thillart et al., 2007). During the downstream migration of 169 silver eels, 63.3% of individuals had muscular lipid ranging from 12% to 20% of the body weight, and 33.1% had >20%. Here, we postulate that only eels with a muscular lipid content >20% could potentially reproduce successfully; and therefore only 33% of the 169 sampled silver eels, mainly from the Stockholm archipelago, Warwickshire and Valencia catchments met this requirement. This result is consistent with the observation of Clevestam et al. (2011) showing that between 45% and 60% of silver eels sampled from the Baltic sea would potentially use up their entire energetic stores during the spawning migration, with no remainder for maturation.

Although some analysed POPs have been banned for decades, almost all the 169 analysed silver eels had a concentration above the limit of quantification for POPs and also TEs (except for BTBPE, Ag, and V). This additional evidence of the widespread and persistent contamination in eels (Belpaire et al., 2019) reinforces the WGEEL suggestion (ICES, 2013, 2015, 2016) that regulations should be quickly introduced or strengthened to improve the quality of waterbodies. Special attention should be given to the BTBPE, an alternate emerging brominated flame retardant (EBFR, currently produced in low quantity to substitute the banned octa-BDE, Oh, 2020), because it was found above the limit of quantification in 25% of silver eels in our study. In addition to legacy contaminants, this result suggests that the European Eel Management Plan should better consider emerging organic and inorganic compounds with potentially persistent, bio-accumulative and toxic properties, and for which occurrence in water or sediments and the bioaccumulation in fish tissues are attested by numerous studies. Sührling et al. (2013, 2014, 2016a), Zacs et al. (2018) and Byer (2013) found new contaminations in juvenile, yellow and subadult eels, by some EBFRs like DBDPE (decabromodiphenyl ethane), HBBs (hexabromobenzene), PBEB (pentabromoethyl), PBT (pentabromotoluene), TBCO (1,2,5,6-tetrabromocyclooctane), TBBPA (tetrabromobisphenol A), TBP – DPBE (2,3-diphenylpropyl-2,4,6-tribromophenyl ether), and TBPH (bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate), and some emerging chlorinated flame retardants as DP (dechlorane plus) and its structural analogues. Some of these previous EBFR compounds have potential effects on fish metabolisms, as oxidative stress or the regulation of gene transcription (Ortega-Olvera et al., 2020). In the same way, some compounds like DPAs (diphenylamine and derivatives) reaching similar or higher contents than those of legacy PCBs are not frequently considered for their effects on the eel quality, despite its transfer capabilities from maternal lipids towards future eggs (Sührling et al., 2016b). Recently, NLs (nonylphenols) and NPEOs (nonylphenols ethoxylates) compounds were found at relatively low levels in muscles and livers of yellow and silver eels (Ruczyńska et al., 2020). Those chemicals have been banned by the EU due to their endocrine disrupting potential, suggesting that the management of aquatic species like the European eel should take into consideration the emerging contamination of banned pollutants (“time bomb” effect). Some studies are starting to study the contamination load and effects of several PPCB compounds (pharmaceuticals and personal care products) that are still scarcely known in *Anguilla* sp. and whose analytical development will enable to take into account these disruptors in the eel quality screening, as food production hormones, human pharmaceutical residues and drugs (paracetamol, non-steroidal anti-inflammatory drugs, cocaine), disinfectants and veterinary drugs (Gay et al., 2013; Nunes et al., 2015; Turnipseed et al., 2019; Zheng et al., 2019). Finally, several heavy industries (aeronautics, naval and hospital industries) release inorganic rare earth elements (REEs: La, Sm, Ce, etc.) in hydrosystems, that have been found in eels, recently (Lortholario et al., 2020). The bioaccumulation properties and physiological effects of these compounds are unknown in fish but which, as a precaution, should be considered by research and management. It would be conceivable to integrate these emerging contaminants generated from textile, agriculture, pharmaceutical, electronic, and plastic industries and bioaccumulated in several organs, or more parasites and viruses in an improved quality indicator.

4.2. Developing an integrated indicator for eel quality

In our study, we developed the EQR index as a tool to quantify the risks incurred by silver eels on their spawning migration, combining muscular lipid reserves (primary condition to ensure the migration), the *A. crassus* pressure (adding cost and structural impairments), and numerous bioaccumulated contaminants. With the decline of European eel population in recent decades, there is insufficient time to study and understand the poorly known complex and potentially cocktail effects of contaminants on eel physiology. In this way, it pragmatic to treat all contaminants with a same weight, even when causes and effects are not well understood, or if effects vary between contaminants. This integrative and without a priori approach is better adapted to target overall quality issues for eels than using separate quality indices. In addition, the EQR index provides information over a given time of the accumulation of numerous contaminants, which represent potential risks for eels (Geeraerts and Belpaire, 2009). Nonetheless, few contaminants are known to be deleterious at different levels for reproduction, nervous, endocrine and immune systems during chronic exposures in fishes (Wood et al., 2011a, 2011b) and eels (Geeraerts and Belpaire, 2009). For example in yellow eels, Cd decreases lipid storage metabolism (Pierron et al., 2007), while Cu impairs the hypothalamo-pituitary-interrenal axis with potential genotoxicity (Oliveira et al., 2008). Hg is considered as the most toxic trace elements in fish (Wood et al., 2011a) and induces DNA damages in the blood of glass eels (Castro et al., 2018). Pb seems to affect the immune system of yellow eels (Santos and Hall, 1990) and has neurotoxic, haematological and developmental effects in fishes (Wood et al., 2011a). PCBs and dioxin-like impair the embryonic development and survival (Palstra et al., 2006). Furthermore, the detoxification process of several organochlorine pesticides seems to be limited (Corsi et al., 2005) and this bioaccumulation could have a metabolic role during the migration (potentially on the lipidogenesis) and be mobilized towards eggs. Thus, while we have treated all contaminants with equal weight in our EQR index so as to provide a standardized assessment, specific contaminants are likely to have different weights on the eel quality in different catchments. Thus, in our study, although subadults from Stockholm archipelago had the lowest contaminant accumulation, individuals may be at special risk in comparison to other catchments with respect to the toxicity of specific contamination found in the archipelago, such as HCB and the emerging BTBPE. Improving knowledge of the impact of contaminants on eel physiology could allow the development of the EQR index so as to include weighting by the toxicity of contaminants and the type of impact (reproduction, immune system, migration, etc.).

4.3. Application of the EQR index

We observed that the EQR index for individuals sampled between 2008 and 2010 was positively correlated with the multi-layer remote sensing-based HFP index estimated in 2009 (Venter et al., 2016a, 2016b), especially due to contaminant and lipid components. This result suggests the relevance of the EQR applied with silver eels as a useful bioindicator for human activities, similar to the finding for yellow eels from several studies (Belpaire et al., 2008; Belpaire and Goemans, 2007). We propose therefore, that the integrated approach based on EQR and HFP could be the foundation of a predictive map of the eel quality through European catchments.

According to the EQR index, human activities producing TEs were relatively similar in all catchments. This homogeneity suggests that the production of TEs by humans has a longer history (and so is more widespread) than the more recent introduction of POPs, and the aquaculture practices, international transport and restocking practices that were responsible for the widespread *A. crassus* infection. The analysis also showed that intra-catchment variability in quality was essentially due to the POP contamination and *A. crassus* infection. A high spatial

variation of contamination was attested by several studies (Belpaire et al., 2008, 2019; Brusle, 1991; Byer et al., 2013), especially for several Belgian catchments (Malarvannan et al., 2014). The Corrib catchment including a lake system and a coastal river displayed the highest intra-catchment variability. The characteristic of lentic water (slow water renewal) and the spatial variability of human activities would promote very different patterns of contaminations or eel productivity at the local scale of a lake, consistent with the different pollution patterns between yellow eels sampled in lake Schullen as described by Belpaire et al. (2001). The second highest intra-catchment variability was observed in Scheldt, which also contains a mixture of lentic (ponds) and lotic (canals, rivers) waterbodies characterized by significant variations in contaminant pressures. The high intra-catchment variability could be explained by the intrinsic characteristics of the tributary catchments (the spatial variability of human activities, salinity, area, etc.) and the different life history of sampled eels (growth rate, habitat selection, diet, individual size, etc.). This result highlights the importance of sampling individuals in the most downstream zone, in order to display a representative diversity of life history traits and to adapt the sample size to the catchment area. This highlights the need to consider quality and management measures at the catchment scale, requiring the balance of European measures that target widespread TEs (Hg, Cr, Cu, Fe, Mn, and Zn in our study) and *A. crassus*, and on the other hand, include local management measures linked to the characteristics of land use and human activities at the scale of the catchment, so as to reduce or limit contamination by POPs and several specific TEs.

By using EQR, we identified the origin of impaired quality in silver eels through the studied catchments. The three worst catchments according to EQR (i.e. Frémur, Warwickshire and Scheldt) showed local characteristics of human activities. Eels from the Frémur River showed a high contribution of RL_{TEs} and RL_{RWLB} components to the EQR index, and seem to have inhabited low-quality habitats, where they were unable to accumulate enough muscular lipid and experienced significant infection levels of *A. crassus*. In addition, specific and potential toxic contaminants of emerging BTBPE and Pb exist in the Frémur River. In Warwickshire, the Avon crosses many conurbations, and the eels sampled here were impaired by industrial BFRs and to a lesser extent agrochemicals (DDTs), in accordance with environmental sampling (Environment Agency, 2014). Human activities also have very high and specific impacts on the Scheldt catchment, which is consistent with the high HFP index. In Scheldt, six silver eels were sampled in canals near at the second largest European cargo port (Antwerp city), and the other eels in ponds about 50 km from Antwerp near farm fields and large conurbations. In that respect, the silver eels were severely affected by agricultural activities (DDTs, HCB), land uses (Cd), industrial PCBs, construction activities and coal burning (Pb). The Scheldt habitats were also affected by HBCDs and PBDEs, but concentrations (162 ± 265 and 66 ± 73 ng·g⁻¹ lw respectively) were lower than earlier results for yellow eels in Flanders between 2000 and 2009 (510 ± 1570 and 110 ± 190 ng·g⁻¹ lw respectively, Malarvannan et al., 2014). Conversely, Malarvannan et al. (2014) measured lower PCBs average concentrations (39 congeners: 6380 ± 2050 ng·g⁻¹ lw) at Flanders scale than in our study in the Scheldt sites ($17,716 \pm 19,952$ ng·g⁻¹ lw). This fact highlights the temporal and spatial heterogeneity of POP contaminations (Freese et al., 2016; Maes et al., 2008; Malarvannan et al., 2014) and the need to establish and manage eel monitoring networks specific for each catchment in a timely manner (Belpaire et al., 2008; Malarvannan et al., 2014).

Tracking the real origin of POP contaminants seems to be difficult due to their long-range atmospheric transport. The low HFP value for the Corrib catchment did not really match with the EQR values of silver eels found there, suggesting that contaminations come from waste incineration plants or leaching from consumer products of the surrounding catchments. Conversely, the high HFP values for Scheldt and Warwickshire catchments and related previous studies (Belpaire et al., 2011b; Malarvannan et al., 2014) indicate that it is highly likely that

POPs come from local sources for these catchments. Because they are long lived, reproduce once, and are migratory species, eels receive and concentrate pollutants that may come from local or remote areas over long periods of time. To that extent they are interesting witnesses of environmental quality over nested scales of space and time. We thought important to state that it would be irrelevant to plan to improve eel quality solely through catchment wide actions because some pollutants were transported by rivers from the surrounding catchments and through atmospheric transport.

It is also important to take into consideration temporal factors and the human activities history. For example, our study shows a high contribution of As in the Bages-Sigean lagoon, probably due to proximity to the former silver mine of Salsigne that was a major site of arsenic contamination in Europe before 2010 (Manlius et al., 2009). Amilhat et al. (2014) observed a higher level of Cd in the silver eels from Bages-Sigean compared to our study. This could be explained by the closure of a factory producing pigments with Cd in the city of Narbonne in 2008. The sampling by Amilhat et al. (2014) was conducted before the factory closure, while our sampling was carried out in November 2009.

Among the studied catchments, the silver eels from Stockholm archipelago were in the most pristine state according to the EQR index. This could be due to low anthropogenic pressures in the sampling area (low HFP index), in combination with eel residence in brackish cold water and geographical properties of the archipelago that could promote fast water turnover and low bioavailability of contaminants. In that respect, the EQR and HFP indices are useful in assessing the suitability of catchments for restocking measures. Restocking operations in catchments displaying both high EQR (or HFP) values and extrinsic stressful factors of eel would likely lead to the development of eel populations with lower quality than could be achieved in catchments with a low EQR index. Optimal restocking emplacements could reflect the following specifications: small catchments (easier to manage and monitor), brackish or low salinity water to minimize *A. crassus* prevalence, an effective water turnover to decrease the residence of potential contaminants, low industrial impacts with agricultural inputs controlled and with less fisheries activities and migration barrier as far as possible. We will consider that the EQR index could be an additional decision-aid criterion to select a catchment for restocking operations. In the future, a more general index than the EQR could be developed by including standardized relative data about the density of eels, obstacles to migration and fisheries that would enable the production of a greater quantity of "healthy" subadults able to contribute to the spawning stocks. However, restocking operations should also consider a diversity of habitats to conserve the large variation of history traits of silver eels. In this way, it would be possible to sample more catchments at European scale than in our study and also improve spatial resolution within a same catchment, such as the 'Flemish eel pollutant monitoring network' (Belpaire, 2008) or the sampling design in Elbe River (Freese et al., 2016), to improve the accuracy of management measures and stocking operations, and to supply the existing European eel quality database (Belpaire et al., 2011a).

4.4. Eel quality and the spawning migration

After leaving the growth catchment, the eel continues its spawning migration towards the tropical North Atlantic convergence zone, whose sea migration corridors and duration are still unknown. Righton et al. (2016) tracked numerous silver eels and observed a migration corridor towards the Azores archipelago. While some individuals migrated quickly, other slower individuals performed the migration over a longer period, perhaps up to eighteen months. In addition to the lack of knowledge on the effect of climate change on oceanic migration of eels, the quality of eels likely has a significant impact on the migration duration and may gradually deteriorate during migration because i) the amount of lipid decreases during swimming and maturation (especially for eels far from the spawning area), and ii) lipophilic

contaminants are remobilised from lipid reserves towards the blood, gonads and eggs during lipids catabolism (Larsson et al., 1990; Sühling et al., 2015, 2016b). Considering only lipid component ($N = 169$ eels), individuals from Scheldt and Gudenå catchments seem to have a higher theoretical migration distance (between about 6400 and 8000 km, Table 1) than eels from Warwickshire (about 6000 km) but have a lower muscular lipid content (16.4 ± 4.1 and $18.3 \pm 2.5\%$, respectively; see supplementary data, S.2 section) compared to those from Warwickshire ($25.2 \pm 4.3\%$), suggesting the importance of taking migration distance into account in the calculation of the RL_{RWLB} . However, individuals from Warwickshire Avon had high muscular lipid content but also high levels of POPs (express as ww or lw), which represent toxicological risks for migrant eels (Geeraerts and Belpaire, 2009; Robinet and Feunteun, 2002) and future eggs (Sühling et al., 2016b). This result highlights that a high muscular lipid content does not warrant reproductive success, and the potential contribution of effective spawners to the next generation could be lower compared to the predicted reproductive success based on measurements of lipid content alone. In addition, Freese et al. (2019) showed the remobilisation capacity of several inorganic TEs, such as Cd, Cu, Hg and Mn relatively homogeneously found in our sampled eels, between somatic tissues and others tissues in which TEs could produce their deleterious effects.

5. Conclusions

According to the WGEEL recommendations (ICES, 2013, 2015, 2016), we believe that developing synthetic quality indices such as the EQR is a new step towards standardizing future European biomonitoring networks to compare and manage the risks of silver eels quality in space (local or regional considerations) and time. A key achievement within the EQR is to link information about contaminant sources and the eel quality, so as to target human activities that are most responsible for the eel quality degradation and therefore require countermeasures.

Upgrading the EQR index with emerging, novel and legacy contaminants, viruses and parasites reflecting several human activities (agriculture, pharmaceutical, heavy industries, aquaculture, etc.) will i) improve the spatial and temporal resolutions for the variability in EQR and ii) lead to the more concrete targeting of specific human activities that impair the eel quality. On the other hand, the development of such indices is limited by several unknown aspects in the ecology of eel (e.g. migration behaviour and physiology), some characteristics of the catchments (e.g. habitat description, sedimentation, and temperature), methods for analysing the emergent contaminants, the costs of analyses, and the requirement for destructive sampling of silver eels. The use of remote sensing (HFP index) as a proxy of silver eel quality risks instead of eel sampling could reduce the requirement to sample large numbers of eels, and help inform the choice of stocking sites. Where sampling of silver eels is necessary, we recommend selecting pollutant categories that depict different kinds of human activities that need prioritized actions to limit the production of contaminants, as well as developing analyses for emerging contaminants that are more persistent and could aggravate the current status of eels. We recommend the use of silver eels for the development of quality metrics rather than yellow eels, because silver eels i) represent the best proxy for the future performance of eels during spawning migration, and ii) silver eels may come from any part of the suitable habitats on their downstream migration and therefore better represent the situation at the scale of areas producing silver eels in catchments. However, we also recommend a finer-scale sampling network with more stations (monitoring network) to dissociate habitats and target risk areas for a hierarchical and spatialized management. It would also be important to analyse links between the quality variables for which we have no a priori knowledge in our study and reproductive success (life history traits of eels like sex, age, length, growth rate...) and it would be more operational to better understand the contaminant fluxes in Europe.

CRediT authorship contribution statement

Bastien Bourillon: Methodology, Formal analysis, Visualization, Writing - original draft. **Anthony Acou:** Methodology, Data curation, Validation, Writing - review & editing. **Thomas Trancart:** Methodology, Validation, Writing - review & editing. **Claude Belpaire:** Resources, Investigation, Visualization, Writing - review & editing. **Adrian Covaci:** Investigation, Writing - review & editing. **Paco Bustamante:** Investigation, Writing - review & editing. **Elisabeth Faliex:** Resources, Investigation, Writing - review & editing. **Elsa Amilhat:** Resources, Investigation, Writing - review & editing. **Govindan Malarvannan:** Investigation, Writing - review & editing. **Laure Virag:** Methodology, Investigation, Data curation. **Kim Aarestrup:** Resources, Conceptualization. **Lieven Bervoets:** Investigation. **Catherine Boisneau:** Resources, Investigation. **Clarisse Boulenger:** Resources, Investigation. **Paddy Gargan:** Resources, Investigation. **Gustavo Becerra-Jurado:** Resources, Investigation. **Javier Lobón-Cervía:** Resources, Investigation. **Gregory E. Maes:** Resources, Investigation. **Michael Ingemann Pedersen:** Resources, Investigation. **Russell Poole:** Resources, Investigation. **Niklas Sjöberg:** Resources, Investigation. **Håkan Wickström:** Resources, Investigation. **Alan Walker:** Resources, Investigation. **David Righton:** Conceptualization, Funding acquisition, Supervision, Project administration, Writing - review & editing. **Éric Feunteun:** Conceptualization, Supervision, Project administration, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.140675>.

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