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The relevance of European Biota Quality Standards on the ecological water quality as determined by the multimetric macro-invertebrate index: A Flemish case study

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ABSTRACT

European Biota Quality Standards (EQS_{biota}), for compounds with low water solubility and high biomagnification, were created to sustain water quality and protect top predators and humans from secondary poisoning. In reality, for multiple compounds, an exceedance of these standards is often reported in literature without a decrease in ecological water quality determined by biotic indices. In the present study, threshold concentrations were defined in biota (from 44 sampling locations throughout Flanders (Belgium)), above which a good ecological water quality, assessed by the Multimetric Macroinvertebrate Index Flanders (MMIF), was never reached. Threshold values were compared to current EQS_{biota}. Accumulated perfluoroctane sulfonate (PFOS), mercury (Hg), hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDEs), dioxins and polychlorinated biphenyls (PCBs) concentrations were measured in muscle tissue of European yellow eel (*Anguilla anguilla*) and perch (*Perca fluviatilis*). Fluoranthene and benzo(a)pyrene were also analyzed in translocated mussels (*Dreissena bugensis, D. polymorpha* and *Corbicula fluminea*). Threshold values could only be calculated using a 90th quantile regression model for PFOS (in perch; 12 μg/kg ww), PCBs (in eel; 328 μg/kg ww) and benzo(a)pyrene (in mussels: 4.35 μg/kg ww). The lack of a significant regression model for the other compounds indicated an effective threshold value higher than the concentrations measured in the present study. Alternatively, the 95th percentile of concentrations measured in locations with a good ecological quality (MMIF≥0.7), was calculated for all compounds as an additional threshold value. Finally, fish concentrations were standardized for 5% lipid content (or 26% dry weight content for PFOS and Hg). Threshold values for PFOS and benzo(a)pyrene and the 95th percentiles for dioxins and fluoranthene were comparable to the existing standards. For all other compounds, the 95th percentile was higher than the current EQS_{biota}, while for HBCD, it was lower. These results strongly advise revising and fine-tuning the current EQS_{biota}, especially for ∑PBDE and HBCD.

1. Introduction

Persistent organic pollutants (POPs) and metals in the aquatic environment, mainly anthropogenically introduced, might lead to chronic and acute toxicity in organisms and biodiversity loss (EC, 2008). Since 2000, the EU implied that a 'good water quality' should be reached and maintained for all water bodies by their member states within the Water Framework Directive (WFD), originally by 2015 (EC, 2000), currently postponed to 2027. Consequently, Environmental Quality Standards were set for a selection of priority substances in order to protect aquatic environments against the adverse effects of chemical pollution (EC, 2008). However, a specific set of hydrophobic/proteonophilic priority compounds needs to be measured in biota because of their low solubility in water (EC, 2013). Due to their

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biomagnification ability, these compounds may reach high bioaccumulated concentrations in higher tropic levels. Therefore, they are to be monitored in fish to avoid the risk of secondary poisoning of top predators (such as fish-eating birds and mammals), including for humans (EC, 2014). An exception was made for polyaromatic hydrocarbons (PAHs), benzo(a)pyrene and fluoranthene, because of their fast metabolization and elimination by fish (EC, 2014; Van der Oost et al., 1994). Instead, these PAHs are to be measured in bivalves or crustaceans.

Besides the chemical and hydromorphological status, the ecological status also determines the water quality. Among other anthropogenic pressures possibly affecting aquatic ecosystems (e.g. fishing, climate change, habitat deterioration), pollution also directly affects the general ecosystem health (Bervoets et al., 2005a; Burdon et al., 2019). Their community structure will reflect healthy ecosystems since they can only be maintained by a well-balanced and adaptive community (Costanza, 1992; Van Ael et al., 2015). To allow for comparison between European member states, the ecological water status assessment is to be presented using a harmonized tool, i.e. the Ecological Quality Ratio (EQR), comparing the local ecological quality to reference locations (EC, 2000). The EQR score ranges between 0 and 1, reflecting a very poor to very good ecological quality. For rivers and lakes, the EQR monitoring should be based on the status of multiple relevant biological quality elements, including phytoplankton, macrophytes and phytobenthos, benthic invertebrates and fish fauna. The ecological quality of aquatic environments is most often assessed using biotic indices.

Macroinvertebrate presence and abundance is considered a longstanding standard monitoring tool for evaluating the general ecosystem health. Biotic indices based on macroinvertebrate communities have been widely used and adapted to local conditions (Moya et al., 2011; Pond et al., 2013; Woodiwiss, 1964). The Multimetric Macroinvertebrate Index for Flanders, the northern part of Belgium (MMIF) was updated by Gabriels et al. (2010) in order to comply with the WFD guidelines and take into account the typology of the sampling site.

The relationship between accumulated concentrations in fish and the ecological water quality might have been used to determine threshold values before (Awrahman et al., 2016; Bashnin et al., 2019; De Jonge et al., 2013; Rainbow et al., 2012; Van Ael et al., 2014, 2015). However, to our knowledge, this has never been done on an elaborate dataset including the priority substances enclosed in the European Biota Quality Standards. The present study aimed to (1) determine threshold values for bioaccumulated concentrations of POPs and mercury above which the ecological water quality was never good and (2) compare these threshold values to existing EQS_{biota} and evaluate their suitability as a protective measure for the aquatic ecosystem quality.

2. Materials and methods

2.1. Sampling locations and species

Biotic samples were collected and analyzed as part of an extensive monitoring study on the European Biota Quality Standards in Flanders (the northern part of Belgium) between 2015 and 2018 (Teunen et al., 2020). Sampling locations (N = 44) were selected from the existing monitoring network used to implement the WFD by the Flanders Environment Agency, with water bodies characterized as canals, rivers and streams. For a detailed view of sampling locations, we refer to Fig. SI-1 and Table SI-1.

2.2. Passive biomonitoring using indigenous fish species

Fish collection was performed by the Research Institute for Nature and Forest (INBO). Two predatory fish species, perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*), were collected using electrofishing (Fishtronics Rudd and Smith Root type VVP 15 C) and/or fyke nets. European eels were targeted in their juvenile yellow eel stage, ranging between 45 and 55 cm total length. Unfortunately, both species could not be caught at all sampling locations. In total, 515 perches and 132 eels were collected. The numbers and species collected per location and sampling years are given in Table SI-1. Fish were sacrificed using MS-222 (Acros Organics, Geel, Belgium), chilled on ice for transport and subsequently frozen at −24 °C.

Using European eels might raise concerns due to their critically endangered status. However, to understand the effects of pollutants on its population decline, it is imperative to continue monitoring and studying this species. Furthermore, the bioaccumulation of lipophilic compounds, as those included in the $EQS_{biota,}$ is incomparable to any other fish species due to the high fat content in eel. In order to minimize the effect on the population, however, accumulated concentrations collected from these samples were used in multiple studies (present, Teunen et al., 2020; Teunen et al., 2021; ICES reports Belgium: e.g. Belpaire et al., 2017). Finally, as previously reported by Belpaire and Goemans (2007), eels used for monitoring purposes are only a negligible portion compared to annual catch and consumption of eel by anglers in Belgium.

2.3. Active biomonitoring using translocated bivalves

In order to have sufficient individuals of the same species to compare among locations, active biomonitoring was performed for measuring PAHs in bivalves. This technique implies the translocation of certain species, preferably collected from a reference location with low background concentrations, to the study sites. The accumulated pollutant concentrations will then reflect the local pollution load after a sufficient exposure time. In the present study, freshwater bivalves of the *Dreissena* genus were used. However, in brackish waters (mean EC20: > 2.4 mS/cm; mean salinity: > 1.2 g/L), Asian clams (*Corbicula fluminea*) were used instead, a species able to cope with higher salinity levels. Mussels were exposed for six weeks at the same locations and in the same period that the fish sampling took place (Table SI-1).

Reference locations were selected based on low background concentrations of organic micropollutants (polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs)) previously measured in indigenous mussels (Bervoets et al., 2005b). Zebra mussels (*Dreissena polymorpha*) were collected from the recreational lake Blaarmeerse in Gent in 2015 and from the drinking water reservoir of the Antwerp Drinking Water Company (Water-link) in Duffel in 2016. From 2017 onward, quagga mussels (*Dreissena bugensis*) collected from the recreational lake the Nekker in Mechelen were exposed due to the declining population of zebra mussels. In 2016, both *D. polymorpha* and *D. bugensis* were exposed in 5 locations simultaneously in order to compare bioaccumulation between the two species. All Asian clams were collected from the Blaarmeerse in Ghent.

At least two weeks prior to exposure, the mussels were acclimated to ambient temperature in a semi-natural pond filled with dechlorinated tap water (mesocosm structure, University of Antwerp, Belgium). A subset of 30–60 individuals was kept separate for analysis of background concentrations. Mussels of comparable size were exposed at each site during six weeks in the water column in two cages made of polyethylene pond baskets (11 × 11 × 22 cm; mesh size 2 × 4 cm) to allow free water circulation (Bashnin et al., 2019; Bervoets et al., 2005b; Smolders et al., 2002). The cages were positioned approximately 1 m below the water surface and were attached to bridges or other solid structures on the river banks using metal chains and locks. Anticipating possible mortality and ensuring sufficient tissue for analysis, 70–75 *Dreissena sp.* or 25–30 *Corbicula fluminea* specimens were exposed per location. To reduce the risk of spreading these alien species to the sampling locations, exposure was performed during autumn and winter since water temperatures below 12 °C reduce mussel reproduction (Wong et al., 2012). Furthermore, a previous Flemish study has shown that *Dreissena* species are already widespread through locations providing the adequate environment for this species to survive and reproduce (i.e. hard substrates to attach to) (Bervoets et al., 2004). After recollection, particle-free water from the respective sampling sites was used to depurate the mussels for at least 15 h at 15–20 °C before dissection.

2.4. Sample preparation and analysis

Before dissection, fish were measured (up to 1 mm) and weighted (Sartorius CP4202S, up to 0.01 g, Göttingen, Germany) (Table SI-1). Fish muscle tissue was collected, while for mussels, the whole soft tissue was removed from the shell and weighted. In the case of *Dreissena sp.,* byssus threads (i.e. the filament bundle used for attachment to substrates) were removed because they complicate digestion and homogenization. Samples were pooled and homogenized (fish: stainless steel kitchen mixer, Bosch, MSM65PER; mussels: Qiagen TissueRuptor, Qiagen, Hilden, Germany) per species per location and frozen at −20 °C until further analysis.

Analytical methods for the bioaccumulated concentrations of the persistent organic compounds and mercury used in the present study have previously been reported (Teunen et al., 2021). In addition to the compounds for which the European Commission (EC, 2013) defined specific EQS_{biota} (i.e. hexachlorbenzene (HCB), hexachlorbutadiene (HCBD), mercury (Hg), perfluoroctane sulfonate (PFOS), hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDEs), dicofol, dioxins, heptachlor and –epoxide, and PAHs fluoranthene and benzo(a)pyrene) also polychlorinated biphenyls (PCBs) were measured in fish muscle tissue, due to their high biomagnification potential.

Total PCB, further referred to as ∑PCB, was calculated as the sum of congeners PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180 (PCB ICES 7). The total of the polybrominated diphenyl ethers (PBDE), further referred to as ∑PBDE, was calculated as the sum of BDE28, BDE47, BDE99, BDE100, BDE153 and BDE154 (PBDE ICES 6). HBCD existed of the sum of α-, β- and γ-HBCD. Dioxins were considered the sum of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDF's) and dioxin-like PCBs (PCB-DL). If the concentration of at least one of the congeners of a specific compound was above the limit of quantification (LOQ), ½ LOQ was used for the congeners with concentrations <LOQ to calculate the sum of that compound (Bervoets et al., 2004; Custer et al., 2000). Of all compounds included in the present study, only those with more than 50% of the measurements above the LOQ were further included in the statistical analysis and determination of threshold values (Table SI-2). This was the case for PFOS (100% > LOQ), Hg (100%), HBCD (perch: 61%, eel: 95%), dioxins (100%), ∑PBDE (perch: 85%, eel: 100%), ∑PCB (100%), fluoranthene (98%) and benzo(a)pyrene (86%) (Table 1; Teunen et al., 2021).

Except for dioxins, all compounds were calculated in µg/kg wet weight (ww). Concentrations of dioxins were expressed in µg WHO_{2005} toxic equivalent (WHO_{2005}-TEQ)/kg ww (Van den Berg et al., 2006).

Table 1
Ranges (and median) of measured concentrations in biota (µg/kg ww), including standardized fish concentrations for a 5% lipid content or 26% dry weight content (for PFOS and Hg).

Parameter	Accumulated concentrations		Standardized concentrations	
	Perch	*Eel*	*Perch*	*Eel*
PFOS	2.4–54 (10)	1.5–65 (8.3)	3–70 (12)	1.1–56 (6.7)
Hg	32–148 (58)	32–332 (132)	41–192 (74)	21–389 (104)
HBCD	<LOQ-1.1 (0.29)	<LOQ-412 (9.0)	0.68–5.7 (1.7)	0.06–290 (4)
Dioxins[a]	0.0003–0.004 (0.001)	0.001–0.04 (0.008)	0.002–0.03 (0.009)	0.002–0.02 (0.004)
∑PBDE	<LOQ-1.4 (0.73)	0.25–106 (7.4)	0.91–9.7 (4.6)	0.13–75 (3.5)
∑PCB	0.75–140 (18)	5.3–1320 (385)	5–769 (117)	7.1–1143 (159)
Fluoranthene[b]	<LOQ-107 (22)	NA	NA	NA
Benzo(a) pyrene[b]	<LOQ-27 (3.0)	NA	NA	NA

LOQs are indicated in Table SI-2. NA: not analysed.
[a] concentrations in µg WHO-TEQ_{2005}/kg ww.
[b] measured in mussels instead of fish.

2.5. Ecological quality assessment

The ecological quality assessment of the aquatic environment was performed using the Multimetric Macroinvertebrate Index of Flanders (MMIF), taking into account water body type. The MMIF has been specifically created for rivers and lakes. However, according to the WFD (EC, 2000), artificial and heavily modified water bodies are assigned the "most similar type". The MMIF is assessed according to that type with a class boundary adjusted according to the ecological potential of that water body. All ecological quality data were available from a long-term monitoring program of the Flanders Environment Agency (http://geoloket.vmm.be/Geoviews/). Only ecological assessments performed no longer than two years before or after sample collection were used. We believe the use of this large time frame to be appropriate since very limited variation in MMIF scores was detected within sites over multiple years (data between 2013 and 2019; Table SI-4).

The MMIF was calculated as described by Gabriels et al. (2010). This index is in line with the WFD definitions for invertebrate assemblages assessment. The metrics included for the calculation of the MMIF were taxa richness, number of Ephemeroptera, Plecoptera and Trichoptera taxa (EPT), number of other (non-EPT) sensitive taxa, the Shannon-Wiener Diversity (SWD; Shannon and Weaver, 1949) index and the mean tolerance score. Gabriels et al. (2010) published reference values for each metric for all lake and river types. Based on threshold values, sampling locations were scored between 0 and 4 for each metric. Here, a value of 4 indicated a value closest to the reference condition. The sum of these scores was divided by 20. This resulted in an overall EQR between 0 and 1, with 0 referring to a very poor ecological quality and 1 representing a location with a very high ecological quality. Macroinvertebrates were collected using a standard handnet (200 × 300 mm frame, 300–500 µm mesh) or deploying artificial substrates for at least three weeks (when the previous method was not possible due to high depth of the water body) as described by De Pauw and Vanhooren (1983) and Van Ael et al. (2015).

2.6. Statistical analysis

Statistical analyses were performed using the software package R (R version 4.0.4; R Core Team, 2021). Relationships between bioaccumulated concentrations and ecological water quality were investigated with two methods (i.e. 95th percentiles, 90th quantile regression model). For both approaches, a MMIF score of 0.7, indicating a good ecological quality, was considered a threshold value (Gabriels et al., 2010). Significant outliers of individual parameters were removed using the Grubbs' test in Graphpad. Adjusted datasets were then used for statistics and figures, while original datasets are presented in Table SI-2. The significance level was set at a p-value < 0.05. All calculations and statistics were performed for the compounds of interest individually.

Two different approaches were used for assessing threshold values for pollutant concentrations in perch and eel (or mussels), above which a good ecological quality with respect to the macroinvertebrate community was never achieved. This was done by calculating the 95th percentile of the accumulated concentrations measured in locations with a MMIF value of at least 0.7 or constructing a 90th quantile regression model of accumulated concentrations against the EQR value (Bervoets et al., 2016; Van Ael et al., 2015).

2.6.1. 90th quantile regression model

A quantile regression model is often used when an ecological response in the field is expected to be caused by multiple environmental factors, besides the stressors of interest (e.g. habitat, species interactions, abiotic conditions) (De Jonge et al., 2013; Iwasaki and Ormerod, 2012; Van Ael et al., 2015). In the present study, also effects and interactions of other pollutants, which are location-specific, might result in a low ecological quality even when the contaminants of interest show low concentrations. A 90th quantile regression model (quantreg package, R) only considers the highest (90th quantile) ecological responses (EQR) as a function of the accumulation of a specific pollutant, compensating for these unmodelled factors. In the case of a significant model, a threshold concentration for a MMIF score of 0.7 could be calculated. This allows to determine the highest concentrations in the sampled biota (fish and/or bivalves) at which a good ecological quality is still achieved. In the case of a significant 90th quantile regression model, a threshold accumulation level for the compound could be derived.

2.6.2. Normalization of fish data based on lipid or dry weight content

Due to the lipophilic characteristics of the priority compounds in the present study, differences in lipid content between species might lead to a variation in accumulation. Eel showed higher and more variable lipid concentrations (12% ± 6.7%) compared to perch (0.84% ± 0.13%) (Teunen et al., 2021), leading to higher concentrations in eel than in perch in general. A standardization based on a lipid content of 5% was proposed in the Guidance document No. 32 of the European Commission on biota monitoring and the implementation of the EQS_{biota} (EC, 2014). An exception was made for Hg and PFOS, which are, because of their high affinity for proteins rather than for lipids (Amlund et al., 2007; Jones et al., 2003; Zhong et al., 2019), normalized based on a dry weight content of 26%. Furthermore, the calculations of the relationships between bioaccumulated concentrations and ecological water quality were repeated for the normalized concentrations to compare results for perch and eel in a more standardized manner.

This normalization was not performed for PAHs in mussels since lipid concentrations were within range (1.2% ± 0.38%) of the 1% that was proposed in the Guidance document (EC, 2014) and multiple species were not exposed at the same locations.

3. Results and discussion

3.1. Accumulated concentrations in biota and ecological quality

Accumulated concentrations of the priority compounds included in the present study have previously been reported, including a comprehensive literature review, in Teunen et al. (2021). Therefore, these were not discussed in the present study. Mean concentrations per location and species are given in Table SI-2.

Zebra mussels collected from the drinking water basin (2016) showed high fluoranthene background concentrations (21 μg/kg ww). However, in some locations the measured accumulated concentrations in mussels after field deployment were lower than the background concentrations (Teunen et al., 2021). This reveals a high elimination rate for fluoranthene (Thorsen et al., 2004). Therefore, we believe that concentrations of this compound measured in mussel tissue after exposure might still reflect the local pollution profile. On the other hand, a possible overestimation of the situation in the field should be taken into account, specifically for fluoranthene. However, no significant difference in accumulated fluoranthene concentrations was detected between sampling years ($H_{(3)} = 2.34$; $p = 0.51$). For benzo(a)pyrene, all reference locations showed background concentrations below the LOQ of 1 μg/kg ww.

In the present study, the EQR based on the MMIF ranged between 0.15 and 0.80 with a mean score of 0.45 (Table SI-2). According to the criteria used, only 20% of the locations showed a good ecological quality (MMIF ≥ 0.7).

3.2. Ecological threshold values

Threshold values for pollutant concentrations in perch and eel (or mussels) above which a good ecological quality was never achieved were calculated using the 95th percentile and 90th quantile regression model. Threshold values, significant regression models and visualization of the results can be found in Tables 2 and 3 and Fig. SI-2, respectively. For all compounds, the eel threshold values on a wet weight basis were much higher than those in perch (Table 2). The threshold values for PAHs were the same when calculated for all mussel species or only including the *Dreissena* spec. (Table SI-5) and were thus reported combining the different mussel species (Fig. 1).

A robust threshold concentration could only be derived when a significant quantile regression model was found. This was the case for PFOS in perch ($p < 0.001$), ∑PCB in eel ($p < 0.001$) and benzo(a)pyrene in mussels ($p < 0.01$), where higher accumulated concentrations resulted in a significantly lower ecological quality. In the other cases where no significant quantile regression could be found, a threshold value was only calculated using the 95th percentile approach. As this value is merely determined by the highest concentrations measured in field situations in the present study, the threshold value may lay even higher in reality. Therefore, it should be stressed that when only the 95th percentile approach could be used, the calculated concentrations were used to compare to the current EQS_{biota} rather than dictate a robust threshold value.

Macro-invertebrate communities can reflect direct effects of contaminant pollution on population size and growth. Exposure to PFOS has been shown to result in decreased fitness and reproduction of *Daphnia magna* (Jeong et al., 2016; Ji et al., 2008). Furthermore, community composition can be altered. Cox and Clements (2013) found that PAH-contaminated sites showed a significantly lower abundance of sensitive amphipods (*Diporeia* spp.) than reference sites. Mercury contamination from mining resulted in a decreased EPT richness and abundance (Costas et al., 2018). In the present study, a negative relationship was found between the accumulated concentrations of PFOS in perch and benzo(a)pyrene in mussels (reflecting the local pollution load) and ecological quality assessed by the MMIF. However, for mercury no such relationship was found.

Multiple studies have investigated the relationship between dissolved or accumulated pollutant concentrations and ecological quality. A lower IBI score (the Index of Biotic Integrity, aka. Fish Index) was found for a pesticide-contaminated river than for a reference site (Mayon et al., 2006). In the Arkansas River in Wichita, Kansas (USA), no relationship was found between the total IBI score and accumulated organochlorine pesticides in fish (Eaton and Lydy, 2000). However, some individual metrics did show a relationship. The same was found along a PCB gradient in Indiana (USA) by Simon et al. (2013). Bashnin et al. (2019), determined threshold values (95th percentile method) of

Table 2
Threshold values (μg/kg ww) for different compounds based on the 95th percentile and 90th quantile regression approaches.

Compound	95th percentile		90th quantile regression	
	perch	*eel*	*perch*	*eel*
PFOS	9.50	48.8	12.0	ns
Hg	133	228	ns	ns
HBCD	0.64	35.2	ns	ns
Dioxins[a]	0.0038	0.011	ns	ns
∑PBDE	1.10	18.5	ns	ns
∑PCB	4.80	312	ns	328
Fluoranthene[b]	45.5		ns	
Benzo(a)pyrene[b]	5.91		4.35	

[a] concentrations in μg $WHO\text{-}TEQ_{2005}$/kg ww.
[b] PAHs were measured in bivalves instead of fish. ns: no threshold value could be calculated because no significant ($p < 0.05$) quantile regression model was found.

Table 3
Results of the 90th quantile regression models. In the case of a significant model ($p < 0.05$) an equation was constructed.

Compound	90th quantile regression model	
	perch	*eel*
PFOS	EQR = −0.012[PFOS] + 0.844 ($p < 0.001$)	ns ($p = 0.85$)
Hg	ns ($p = 0.81$)	ns ($p = 0.65$)
HBCD	ns ($p = 0.49$)	ns ($p = 0.77$)
Dioxins[a]	ns ($p = 0.83$)	ns ($p = 0.30$)
∑PBDE	ns ($p = 0.45$)	ns ($p = 0.33$)
∑PCB	ns ($p = 0.10$)	EQR = −0.0004 [PCB] + 0.831 ($p < 0.001$)
Fluoranthene[b]	ns ($p = 0.25$)	
Benzo(a) pyrene[b]	EQR = −0.026[benzo(a)pyrene] + 0.813 ($p = 0.005$)	

[a] concentrations in µg WHO-TEQ_{2005}/kg ww.
[b] PAHs were measured in bivalves instead of fish. ns: the quantile regression model was not significant ($p > 0.05$).

accumulated pesticides in transplanted *Dreissena polymorpha,* reflecting a decrease in macro-invertebrate community quality, calculated using the MMIF. However, no significant relationships were found using the quantile regression approach.

In contrast to the present study, a significant 90th quantile regression model could be found for total and dissolved Hg concentrations in the water column and MMIF values in a survey of 185 locations in Flanders (Van Ael et al., 2015). However, in that study, critical concentrations determined for Hg were well below the standards for the water column (EC, 2008). In another Flemish study, bioaccumulated concentrations in eel were compared to the IBI based on fish community (Van Ael et al., 2014). That study did not find a significant decrease in the EQR score, even after exceeding the EQS_{biota} of 20 µg/kg ww for Hg. For ∑PCB, however, they found a threshold concentration in eel of 431 µg/kg ww above which a good ecological quality (EQR ≥ 0.6) was never reached, which is even higher than the threshold value suggested by the present study (328 µg/kg ww), although they were all derived without lipid normalization. Besides lipid normalization, another important difference between both studies is the specific biotic index used. The IBI is based on fish community, while MMIF is based on macroinvertebrate community. Since invertebrates are more sensitive for many compounds than fish (Buckler et al., 2005; Xin et al., 2015), this might result in lower EQR values for invertebrate-based indices and thus in lower threshold values. However, community health at different trophic levels is thought to be interconnected since the disappearance of keystone species both at low (e.g. invertebrates) or high trophic levels (e.g. top predators) are known to affect entire ecosystem structures (Collier et al., 2016; Rodríguez-Lozano et al., 2015). On the other hand, it has been shown that, apart from chemical pollutant influences, fish-based indices are strongly affected by physical habitat quality of water bodies (e.g. channel or riffle quality) compared to invertebrate-based indices (Pilière et al., 2014).

As previously mentioned, the dataset of accumulated concentrations in biota used in the present study was already published before (Teunen et al., 2021). In that study, a positive relationship was found between PFOS accumulation in perch and eel and water concentrations and between benzo(a)pyrene concentrations in mussel tissue and water. Furthermore, a positive relationship was also found between fish and sediment for PBDEs and PCBs. In the present study, on the other hand, a significant effect of pollution (measured in fish) on the MMIF was only detected for PFOS (in perch), PCBs (in eel) and benzo(a)pyrene. Although we found some agreements, the absence of relationships might be due to concentrations in the lower trophic levels being sufficiently low not to cause any changes to the macro-invertebrate community. Since the compounds of interest are known to biomagnify, the highest concentrations are to be expected in the higher trophic levels.

The quantile regression approach allows for investigating the relationship between a specific pollutant and the ecological quality and health. However, this does not necessarily reflect a clear causal relationship. In reality, ecosystems are not affected by a single pollutant but rather by a complex mixture of multiple compounds interacting with other environmental stressors (e.g. increasing temperatures, food availability and quality, habitat deterioration). This was reflected in locations with a low MMIF score, despite low accumulated concentrations. Furthermore, to a large extent, ecological quality can be explained by water characteristics (Van Ael et al., 2014, 2015). Nonetheless, the present study, covering different aquatic ecosystems in a temperate climate, allows for the derivation of safe threshold values and evaluation of existing standards for biota, at least for field situations comparable to those in Flanders.

It is important to note that, to a certain degree, the results may depend on the number of selected sampling locations and their characteristics. Apart from pollution load, the macro-invertebrate community might be affected by general environmental characteristics of the location (e.g. structures in and on the sediment, pH, salinity) (Rezende et al., 2014). Furthermore, the calculated threshold values depended on the accumulated concentration in the targeted fish species. Since we merely focussed on lipophilic compounds, lipid content in the fish can strongly affect these concentrations. Eel, for example, is known to accumulate high concentrations due to its high lipid level (Belpaire and Goemans, 2007). This was partly solved by standardization of the

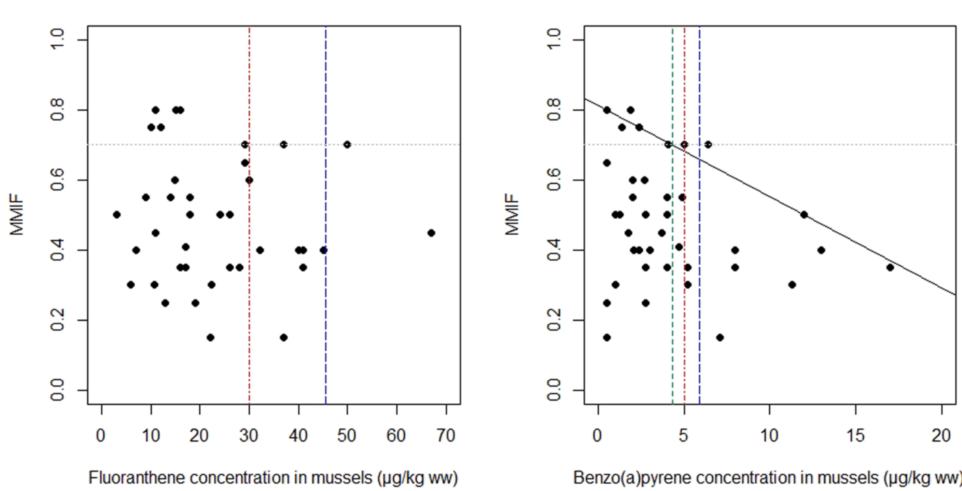

Fig. 1. Scatterplots of the relationship between accumulated concentrations of PAHs in mussels and the ecological quality calculated as the MMIF. The (blue) 'longdashed' line indicates the threshold concentration calculated with the 95th percentile, the green 'dashed' line indicates the threshold concentration based on the 90th quantile regression model and the (red) 'dotdash' line indicates the current EQS_{biota} (Table 2). The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold for good ecological quality. In the case of fluoranthene, a significant 90th quantile regression model could not be constructed (Table 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

concentrations based on lipid content. However, a difference between perch and eel was still visible. This might be caused by differences in exposure routes, lifestyles, age or internal metabolization and elimination pathways. The species used in the present study are generally used monitoring species with a broad distribution throughout Europe (Bashnin et al., 2019; Bervoets et al., 2005b; Fliedner et al., 2018; Foekema et al., 2016; Jürgens et al., 2013; Hendriks et al., 1998; Poma et al., 2014) and therefore our findings could be extrapolated to other European regions. To identify a narrower, more robust threshold value, and counter the above effects, the study should thus be repeated for a broad range of location types and monitoring (fish) species.

3.3. Are the EQS_{biota} protective of the ecological quality of aquatic ecosystems?

For comparison of the derived threshold concentrations with the existing EQS_{biota}, standardized concentrations (on lipid or dry weight basis) as proposed in the Guidance document (EC, 2014) were used for fish (Tables 1, 4, and SI-3). As stated before, all compounds measured in fish, except for PFOS and Hg, were standardized to 5% lipid content. A normalization to 26% dry weight content was performed for PFOS and Hg. After normalization of the fish data, threshold values in general increased for perch and decreased for eel (except for Hg in eel; Tables 4 and SI-6, Figs. 2 and 3), as did the accumulated concentrations (Table 1).

Both PFOS threshold concentrations (95th percentile: 16 µg/kg ww, and quantile regression: 12 µg/kg ww) calculated for perch were comparable to the existing EQS_{biota} for PFOS of 9.1 µg/kg ww (EC, 2013). Contrastingly, for eel, the 95th percentile concentration of 42.6 µg/kg ww was 4.7 times higher than the current EQS_{biota}. The threshold concentrations for Hg calculated with the 95th percentile concentrations were between 8.6 and 12 times higher than the existing EQS_{biota} of 20 µg/kg ww (EC, 2013) for perch (172 µg/kg ww) and eel (241 µg/kg ww), respectively. For HBCD, on the other hand, the 95th percentiles concentrations were between 46 (perch: 3.6 µg/kg ww) and 22 (eel: 7.6 µg/kg ww) times lower than the existing EQS_{biota} (167 µg/kg ww; EC, 2013). The threshold values (95th percentile) for dioxins in perch and eel ranged from 0.003 to 0.021 µg $TEQ_{WHO\text{-}2005}$/kg ww, which included the existing standard of 0.0065 µg $TEQ_{WHO\text{-}2005}$/kg ww (EC, 2013). For ∑PBDE these values were comparable between perch and eel (6.09 and 6.52 µg/kg ww respectively, but were between 716 and 767 times higher than 0.0085 µg/kg ww, the current biota standard (EC, 2013). The estimated threshold values for PAHs in the present study were comparable to EQS_{biota} (Table 4). As discussed before, PAH concentrations were not standardized for lipid content. A 95th percentile concentration of 45.5 µg/kg ww was found for fluoranthene, similar to the EQS_{biota} of 30 µg/kg ww (EC, 2013). Finally, both threshold values for benzo(a)pyrene (95th percentile: 5.91 µg/kg ww, and quantile regression: 4.35 µg/kg ww) were around the existing standard of 5 µg/kg ww (EC, 2013).

Table 4
Threshold values (µg/kg ww) for different compounds based on the 95th percentile and 90th quantile regression approaches for data normalized for lipid content (HBCD, dioxins, ∑PBDE and ∑PCB) or dry weight (PFOS and Hg). The European environmental quality standard for biota (EQS_{biota}) is also given (EC, 2013).

Compound	95th percentile		90th quantile regression		EQS_{biota}
	perch	*eel*	*perch*	*eel*	
PFOS	12.0	42.6	16.0	ns	**9.1**
Hg	172	241	ns	ns	**20**
HBCD	3.6	7.6	ns	ns	**167**
Dioxins[a]	0.021	0.003	ns	ns	**0.0065**
∑PBDE	6.09	6.52	ns	ns	**0.0085**
∑PCB	25.9	98.5	ns	183	NA

[a] concentrations in µg WHO-TEQ_{2005}/kg ww. ns: no threshold value could be calculated because no significant ($p < 0.05$) quantile regression model was found. Significant regression models can be found in Table SI-6. NA: no EQS_{biota} exists up to date.

From the above results, it can be observed that the current EQS_{biota} for HBCD is exceptionally high, even though effects to ecosystem health were detected at much lower concentrations and thus might need to be revised. In the present study, one outlier in eel contained 412 µg/kg ww, exceeding the standard. This is in line with the study published by Eljarrat and Barceló (2018), stating that the EQS_{biota} for HBCD is exceeded rarely on a global scale. In contrast to HBCD, the current EQS_{biota} for ∑PBDE is extremely low, even below LOQ of current analysis methods. Effects on ecosystem health are only detected at concentrations more than 700 times higher than the current standard. Thus the EQS_{biota} might need revising to a higher threshold concentration. This vast exceedance of the standard was also found by Eljarrat and Barceló (2018), who found that 25% of fish samples from studies over the world even showed exceedances up to ten thousand times. The EQS_{biota} for PBDEs has previously been criticized by multiple authors since it was based on the observed effects of one congener (BDE99) on mice and was determined, including very large safety factors (EC, 2011; Eljarrat and Barceló, 2018; Jürgens et al., 2013).

Based on our results, the current EQS_{biota} seems to be sufficiently protective of aquatic ecosystem quality for benzo(a)pyrene and PFOS (measured in perch). Furthermore, we found a strong indication that an EQS_{biota} for ∑PCB should range between 98.5 and 183 µg/kg ww (as was measured for both approaches in eel; Table 4). For PCBs, no EQS_{biota} exists to date. However, the consumption limit for muscle tissue of wild eel or product thereof specifically (EC, 2011) is set at 300 µg/kg ww (although calculated for the sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180). This value is comparable to the threshold derived for eel (without standardization) of 328 µg/kg ww (Table 2). The 95th percentiles for fluoranthene and dioxins were also close to the existing EQS_{biota}, but could not be confirmed with a 90th percentile regression model (as was indicated before).

Besides the general protection of ecosystem health, the EQS_{biota} were set with a specific double purpose in mind (EC, 2014). Firstly, these standards are meant to protect the aquatic ecosystems and prevent secondary poisoning of top predators ($EQS_{biota,\ secpois}$). Secondly, human health risks were taken into account ($EQS_{biota,\ hh}$). However, any statements on the protective value of the current EQS_{biota} on the protection of ecosystem integrity, with the focus on macro-invertebrate community, are relevant. The main focus of the present study was on investigating whether we could define threshold values that guaranteed protection of the ecological quality as assessed by the macro-invertebrate community. As previously discussed, macro-invertebrates, occupying relatively low levels in the food chain, can reflect the general health of the ecosystem and therefore also higher trophic levels. However, to further investigate the relevance of the current EQS_{biota}, we recommend repeating the current study using a fish-based index. On the other hand, since no effects of secondary poisoning or human health risk were investigated in the present study, no conclusive statements can be made on the effectiveness of EQS_{biota} as originally proposed by the EU. Therefore, the results should be merely interpreted as an indication for further investigation.

Between the $EQS_{biota,\ secpois}$ and $EQS_{biota,\ hh}$, the most sensitive was selected as EQS_{biota}. As in the present study, however, the main focus is on the ecological risk of the ecosystem health; secondary poisoning seems like a more relevant endpoint than the human health risk. Therefore, threshold values were further compared to the $EQS_{biota,\ secpois}$ specifically. This latter differed from the used EQS_{biota} for ∑PBDEs (44 µg/kg ww), fluoranthene (11,522 µg/kg ww), hexachlorobenzene (16.7 µg/kg ww), PFOS (33 µg/kg ww), dioxins (0.0012 µg $TEQ_{WHO\text{-}2005}$/kg ww) and heptachlor(-epoxide) (33 µg/kg ww). For benzo(a) pyrene, included in the PAHs, no separate $EQS_{biota,\ secpois}$ was available.

The biota quality standards specified for the risk of secondary poisoning resulted in values much higher than the calculated threshold values in the present study for ∑PBDE (6.09–6.52 µg/kg ww),

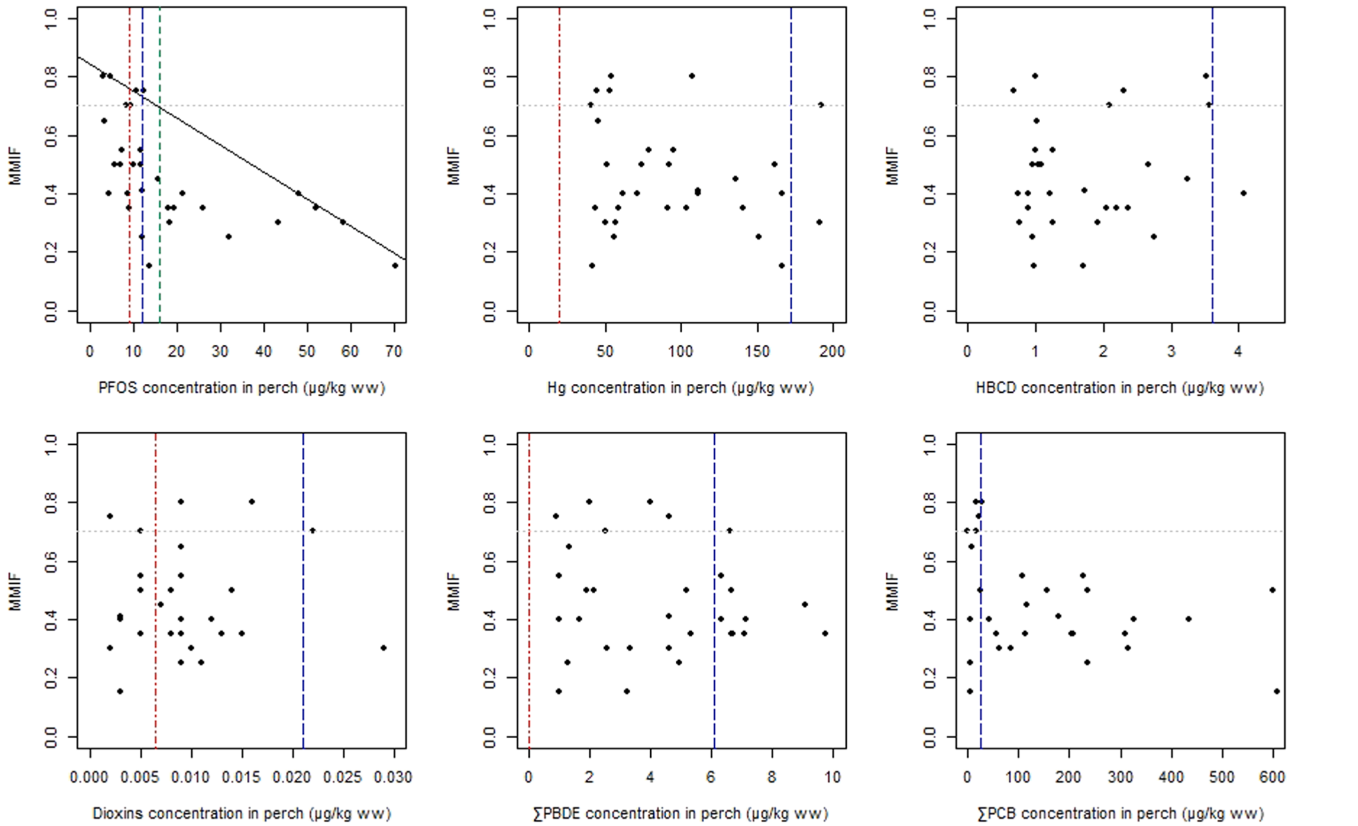

Fig. 2. Scatterplots of the relationship between (standardized) accumulated concentrations of priority compounds in perch and the ecological quality calculated as the MMIF. The (blue) 'longdashed' line indicates the threshold concentration calculated with the 95th percentile, the green 'dashed' line indicates the threshold concentration based on the 90th quantile regression model and the (red) 'dotdash' line indicates the current EQS_{biota} (Table 4). The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold for good ecological quality. Regression lines were only indicated when the quantile regression model was significant (Table SI-6). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fluoranthene (45.5 µg/kg ww). For dioxins, the $EQS_{biota,\ secpois}$ were lower than the calculated threshold values (0.003–0.021 µg $TEQ_{WHO\text{-}2005}$/kg ww) and for PFOS they were comparable to the threshold concentration calculated for eel (42.6 µg/kg ww). However, as stated before, since no significant quantile regression model could be derived for ∑PBDE, PFOS (in eel), dioxins or fluoranthene, it might be possible that, in reality, the threshold values are higher and closer to the current $EQS_{biota,\ secpois}$. To further investigate this, ecosystems with a higher pollution load need to be studied in order to determine the actual threshold concentration resulting in a decrease in ecological quality.

Although the findings of the current study might indicate that some of the current EQS_{biota} might be too strict or too high, this only serves as an indication for revision of the current standards. The alteration of current standards should be investigated with care and more research on this topic is needed. Firstly, the present study should be repeated using higher trophic levels for ecological quality assessment (e.g. fish-based indices), since these show a more direct relationship with the accumulated concentrations in fish. However, the selection of sampling location in the current study did not allow for this approach. Furthermore, these studies should be replicated in other European member states in order to verify and strengthen our findings. Especially, for the lowering of current standards, their effects both at the individual and ecosystem level should be extensively investigated.

4. Conclusions

Threshold concentrations based on the 90th quantile regression model could only be calculated for PFOS (in perch), ∑PCB (in eel) and benzo(a)pyrene. The present study revealed that for PFOS (in perch) and benzo(a)pyrene, the EQS_{biota} is sufficiently protective of the aquatic ecosystem quality. For dioxins and fluoranthene, the calculated 95th percentile thresholds were comparable to the existing standards. However, no significant quantile regression model could be derived for these compounds. Thus, since the threshold values were calculated on the contamination load and ranges of the sampling locations included in the present study, they might be even higher in reality.

As a consequence, the threshold values of the present study should be validated in other aquatic ecosystems. For all other compounds, the current EQS_{biota} was too strict to protect the ecosystem quality with respect to macroinvertebrate community structure and needs re-evaluation. For HBCD, on the other hand, the EQS_{biota} was not sensitive enough. Furthermore, since fish concentrations were standardized based on lipid (or dry weight) content, threshold concentration ranges can be extrapolated to other fish species. Our findings should be taken into account for revision and fine-tuning the current EQS_{biota}.

CRediT authorship contribution statement

Lies Teunen: Conceptualization, Resources, Formal analysis, Writing – original draft. **Maarten De Jonge:** Resources, Writing – review & editing. **Govindan Malarvannan:** Resources, Writing – review & editing. **Adrian Covaci:** Resources, Writing – review & editing. **Claude Belpaire**: Resources, Writing – review & editing. **Jean-François Focant:** Resources, Writing – review & editing. **Ronny Blust:** Writing – review & editing. **Lieven Bervoets:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial

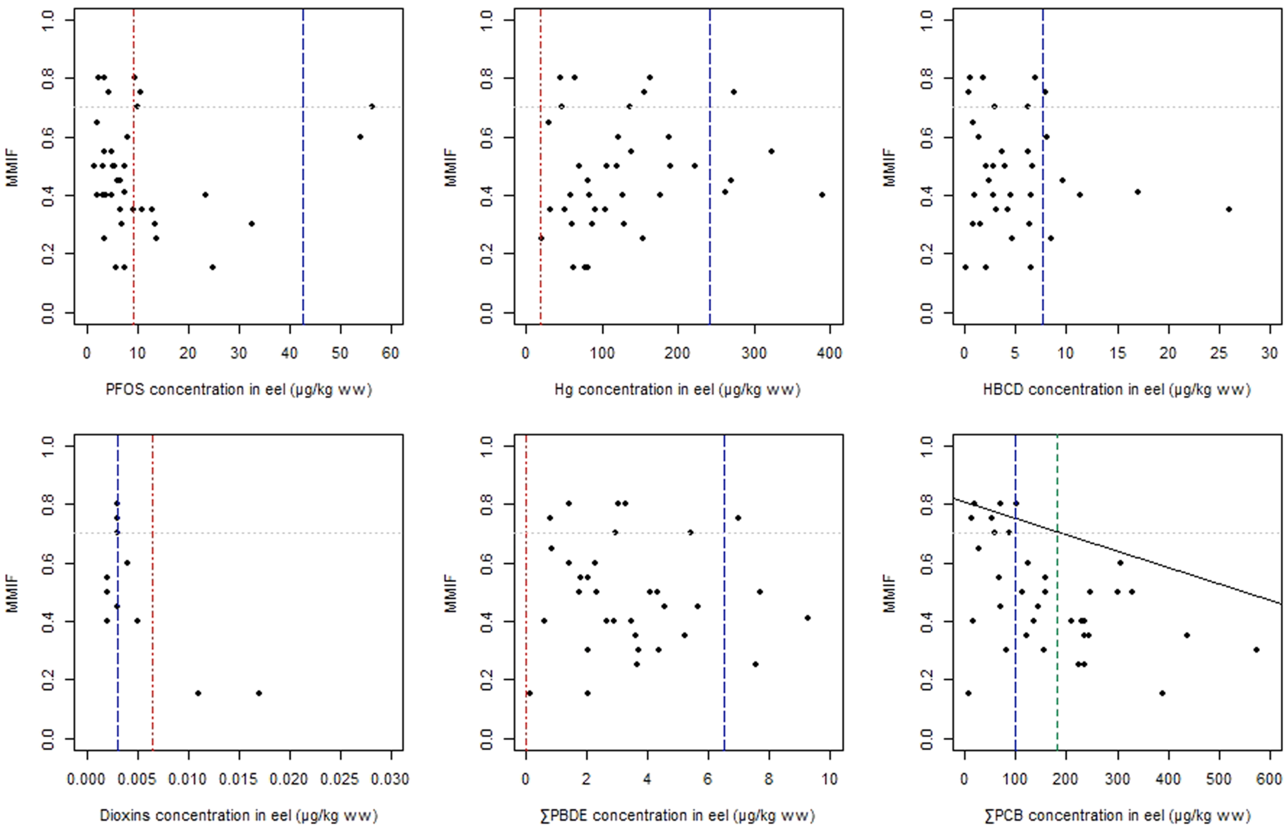

Fig. 3. Scatterplots of the relationship between (standardized) accumulated concentrations of priority compounds in eel and the ecological quality calculated as the MMIF. The (blue) 'longdashed' line indicates the threshold concentration calculated with the 95th percentile, the green 'dashed' line indicates the threshold concentration based on the 90th quantile regression model and the(red) 'dotdash' line indicates the current EQS_{biota} (Table 4). The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold for good ecological quality. Regression lines were only indicated when the quantile regression model was significant (Table SI-5). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113222.

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