



Are persistent organic pollutants and metals in eel muscle predictive for the ecological water quality?



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ABSTRACT

Relationships between the presence of PCBs, OCPs and metals in aquatic ecosystems and the ecological water quality were investigated by combining datasets of long-term monitoring of chemicals in European eel (*Anguilla anguilla*, $N = 1156$) in Flanders (Belgium) and the Ecological Quality Ratio (EQR), based on the assessment of fish assemblages at 185 locations. For most pollutants, EQR scores were lower when pollutant levels were higher. Threshold concentrations for a good quality could be formulated for PCB's, most metals and OCPs. Mixed models suggested that the ecological water quality was significantly correlated with the presence of PCBs. However, the low R^2 indicates that other environmental pressures may significantly influence the biotic integrity of fish communities. Empirical data and their analyses are essential to enable defining threshold values of bioaccumulated levels to allow better protection of the aquatic environment and its biota through associated food webs as demanded by the Water Framework Directive.

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1. Introduction

Biotic indices are widely accepted tools for the assessment of the ecological quality of aquatic environments (Fausch et al., 1984; Karr, 1981; Roset et al., 2007). Well-balanced and adaptive communities can only be maintained by healthy ecosystems, hence community structure will reflect the ecosystem's health. In addition to biotic indices based on the presence and abundance of macro-invertebrates (Bohmer et al., 2004; De Pauw and Vanhooren, 1983; Gabriels et al., 2010; Moya et al., 2011), fish community structure is often used to assess ecological quality. In 1981, Karr developed the Index of Biotic Integrity (IBI), a scoring system to qualify fish community characteristics such as species diversity, trophic position, biomass and condition for Midwestern rivers in the USA (Karr, 1981). Since then the IBI has been accepted and adapted to local conditions in many parts of the world (in Europe (e.g. Schmutz et al., 2007a), America (e.g. Lyons et al., 1995), Australia (e.g. Harris and Silveira, 1999), Asia (e.g. Zhu and Chang, 2008) and in Africa (e.g. Kamdem Toham and Teugels, 1999)). For

water bodies in Flanders (the northern part of Belgium), Belpaire et al. (2000) demonstrated a successful adaptation of the IBI.

The Water Framework Directive (WFD), implemented by the European Union, imposes countries to achieve 'good ecological quality' for all water bodies by 2015 (EU Water Framework Directive, 2000). To monitor and follow up the ecological water status, the European member states needed to create a harmonised tool for measuring the ecological quality in all European countries, i.e. the Ecological Quality Ratio (EQR). The EQR is based on the status of various biological quality elements (phytoplankton, macrophytes and phytobenthos, benthic invertebrates and fish fauna). In this study we focus on fish assemblages as a standard monitoring tool. On a European scale, Schmutz et al. (2007b) described spatially based methods for the ecological integrity of fish communities across European eco-regions. In Belgium, the IBI for Flemish water bodies was further optimized for different water types (Belpaire et al., 2000; Breine et al., 2004, 2007). Subsequently IBI scores were transformed to EQR values to comply with the WFD requirements.

As the EQR reflects the overall quality of an aquatic ecosystem, it may be impacted by a variety of anthropogenic pressures such as fisheries, climate change, spreading of invasive species, habitat deterioration, spatial isolation and pollution. While impact through

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water pollution (e.g. by eutrophication, oxygen depletion, ...) is obvious, reports on the effects of contaminants on fish community indicators are scarce. The analysis of the potential impact of these pollutants on fish based indices is hampered by the complexity of field situations due to the presence of many other anthropogenic and natural stressors. Hence, only few studies were able to show the direct relationship between concentrations of pollutants and the EQR or IBI (Bervoets et al., 2005; Dyer et al., 2000; Hartwell, 1997).

The monitoring of pollutants in the aquatic environment in Europe is still mainly based on measurements in the water column and to a lesser extent in the sediment. However, there is an increasing tendency to also monitor pollutants in aquatic biota. When for a certain pollutant a reliable quantification method in water is unavailable, or when it is not possible to ensure protection against indirect effects and secondary poisoning by quality standards based on surface water alone, it is advisable to monitor concentrations in biota as well (Carere et al., 2012). Currently, the EU is identifying new substances for priority action, setting and updating environmental quality standards (EQS) for them, and setting biota based EQS for some existing and new priority substances.

A potential species that may be used as indicator of the chemical status for the WFD is the European eel (*Anguilla anguilla*) in its yellow stage (Belpaire and Goemans, 2007a). Their longevity, sedentarity in the yellow eel stage, absence of reproduction and associated lipid metabolism in European waters, makes them useful as a monitoring species (Belpaire and Goemans, 2007b; de Boer et al., 2010; Macgregor et al., 2010; Maes et al., 2008). Eel contaminant profiles (pollution fingerprints) appear to give a good reflection of the bioavailable fraction of the contaminants present at a specific site, especially for lipophilic substances (Belpaire et al., 2008). Moreover, several countries are running monitoring efforts for chemicals in eel and a European database compiling the bioaccumulation data across Europe has been initiated by ICES and the Research Institute for Nature and Forest (INBO) (Belpaire et al., 2011a).

The aim of the current study is to investigate possible relationships between the EQR and the presence of pollutants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and metals, in water bodies of Flanders, based on concentrations in European yellow eel. Measuring in tissue has the advantage that only the bioavailable fraction of micro-pollutants is taken into account. To this purpose, databases of long-term monitoring in Flanders of pollution in eels and fish stock assessments are combined and analysed. The main objectives of this study were 1) to investigate possible relationships between ecological water quality (as indicated by EQR) and accumulated levels of individual contaminants in European eel, 2) to determine the contribution of different stressors (bioaccumulated pollutants, water characteristics such as oxygen content, water conductivity, pH...) to the EQR of Flemish water bodies 3) to establish critical tissue concentrations of contaminants for ecological water quality.

2. Material and methods

2.1. Eel contaminant data

The eel contaminant data used in the current study are derived from a long-term monitoring program (1994–2009) which investigated the tissue concentrations of a series of pollutants in yellow eels of Flanders. The trends of these concentrations and implications for the use of the European eel as monitoring tool, were previously reported (Belpaire et al., 2011b; Belpaire and Goemans, 2007b; Bilau et al., 2007; Geeraerts et al., 2008; Maes et al., 2005; Roose et al., 2003). For the current study, pollution data from 1156 individual yellow eels from 185 locations were used. Per location, 3 to 10 eels were sampled. Sampling locations were characterized as rivers, canals, brooks and polders, and were situated in all eleven river basins in Flanders. Eels were collected between 1996 and 2009 using fyke nets or by electrofishing.

Individual eels were measured for total length (cm), weighed (g) and analysed for metals (Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn, metalloid As), OCPs (dieldrin, HCB, α -HCH, γ -HCH, p,p' -DDE, p,p' -DDT, TDE, trans-nonachlor) and PCBs (PCB 28, 31, 52, 101, 105, 118, 138, 153, 156, 180). Measurements below limits of quantification (LOQ) were treated as half the LOQ value of the compound considered. Concentrations of OCPs and PCBs are reported on a lipid weight basis (ng/g lw). Metals are expressed on a wet weight basis (ww).

For the analysis of PCBs and OCPs, eel muscle tissue samples were extracted as described by Bligh and Dyer (1959). After addition of internal standards (tetrachloronaphthalene) and clean-up for lipids, the extract was analysed using a gas chromatograph with an electron capture detector (ECD). For the analysis of Cr, Ni, Cu, Zn, Cd and Pb, muscle tissue was digested with HNO_3 and analysed with ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) (Spectra AA-400 with Zeeman correction, Varian). For detection of arsenic (As) and selenium (Se), fish tissue was heated in a mixture of HNO_3 and H_2O_2 and analysed using GF-AAS (Graphite Furnace Atomic Absorption Spectrometry), while mercury (Hg) was measured in solid phase using AAS (Atomic Absorption Spectrometry) (AMA 254 mercury analyser, Altec). The analyses were performed at two Belgian research institutes (DVZ, the Sea Fisheries Department, Ostend, and CODA, the Veterinary and Agrochemical Research Centre, Tervuren). For a more detailed description of the chemical analyses, we refer to Maes et al. (2008).

2.2. Ecological quality data

The ecological quality data are retrieved from the Fish Monitoring Network of INBO. From the 185 eel sampling locations, data from fish stock assessments were available. These assessments were carried out by electrofishing in the period 1996–2009. Belpaire et al. (2000) give a full description of the methods. Only assessments performed within two days before or after the sampling of the eels for contaminant analyses, were included in the present study. Fish were identified, counted and at each location, maximally 100 individuals from each species were weighed (g) and measured for total length up to 1 mm accuracy. If more individuals are sampled, the rest of the mass was measured in bulk. The EQR, based on the Index of Biotic Integrity (IBI) and established for Flemish water bodies (Belpaire et al., 2000), was calculated for each location. Fish metrics determined for the calculation of the IBI were: total number of species, mean tolerance, mean typical species value, relative presence of type species, total biomass (kg/ha), weight % of non-native species, trophic composition and relative natural recruitment. The overall EQR score for a sampling location ranged from 0 to 1, with score 0 representing locations with no fish present and score 1 representing high ecological conditions. In addition to the EQR, environmental variables (pH, temperature, conductivity and oxygen level) were measured at each sampling location (HQ40D Multimeter, Hach-Lange, Belgium).

2.3. Statistical analyses

First, possible relationships between the EQR and individual accumulated pollutants were explored using scatterplots. To formulate concentration thresholds, above which a good ecological status was never reached, all locations were selected where the EQR score was higher than 0.6. The concentrations threshold was established as the 95th percentile of the concentrations measured in eels from these locations.

To relate ecological water quality to the total pollution load (of the studied compounds) in eel, concentration data were transformed into toxic units (TU) (Bervoets et al., 2005; Meregalli et al., 2000). The TU of a compound was calculated as the concentration in eel divided by the previously formulated threshold of that compound. The TU of the different compounds were summed for each fish and related to the EQR.

For further analyses, pollutant concentrations were standardized by subtracting the mean and dividing by the standard deviation in order to eliminate the effect of each individual pollutant concentration (Eulaers et al., 2011). The assumption of normally distributed data was evaluated using Shapiro–Wilk's tests for normality and QQ plots. Pollutant concentrations and water conductivity were $\log(x + 1)$ -transformed to meet this assumption.

To investigate which pollutants or other factors may influence the EQR, generalized linear mixed effect models were constructed (Zuur et al., 2009). Principle component analysis (PCA) with varimax rotation was performed in order to reduce the underlying complexity in relationships between the pollutants.

In the mixed models, pollutant concentrations and environmental factors were fixed variables while the categorical variables date and sampling location were set as random variables in order to control for pseudo replication. Selection of the most parsimonious model was performed using Akaike's Information Criterion (AIC; Johnson and Omland, 2004). Prior to model selection of the most parsimonious fixed effect structure, the optimal random structure was determined using restricted maximum likelihood. After model selection, the most parsimonious model was refitted using the full dataset. An R^2 was calculated by squaring the correlation coefficient of the correlation between the fitted model values and the actual observations. Statistical analyses were performed using R 2.15.2 (R Development Core Team, 2011) and IBM SPSS Statistics 20.

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