Assessing the impact of chemical pollution on endangered migratory fish within a catchment using a Potentially Affected Fraction of species (PAF) approach: a case study at main rivers and spawning grounds scales

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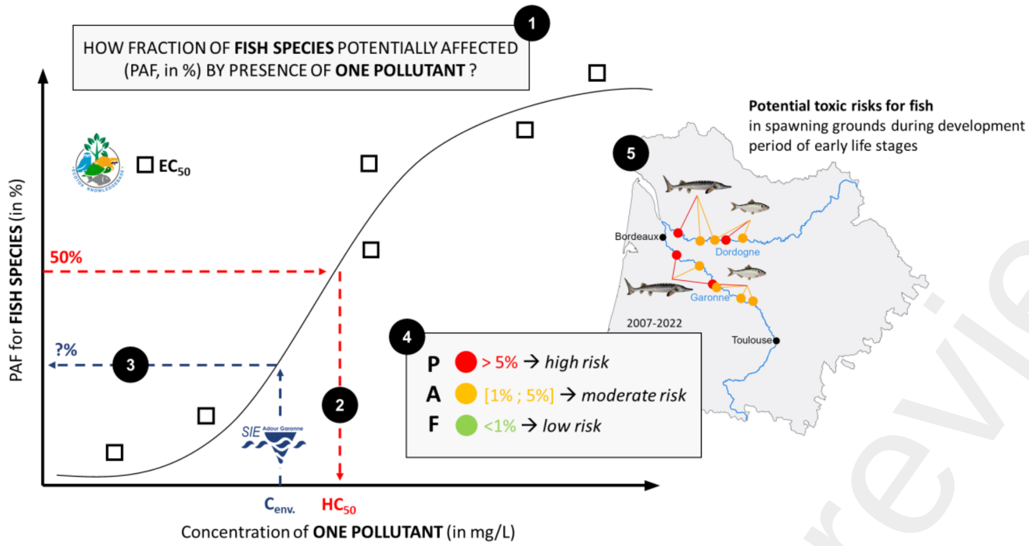
Keywords. Water contamination, risk assessment, mixtures, Garonne, Dordogne

ABSTRACT.

Water pollution is a one of the most contributors to aquatic biodiversity decline. Consequently, ecological risk assessment methods have been developed to investigate the effects of existing stresses on the environment, including the toxic effects of chemicals. One of the existing approaches to quantify toxic risks is called “Potentially Affected Fraction of species” (PAF), which estimates the potential loss of species within a group of species studied. In this study, the PAF method was applied to the Garonne catchment (southwest France) due to the limited information available on the involvement of water pollution in the decline of diadromous fish populations. This approach was used to quantify the potential toxic risk associated with chemical contamination of water for fish species. The objectives were to quantify this risk (1) in the Garonne and Dordogne rivers and (2) in the spawning grounds of two endangered anadromous fish species: the allis shad and the European sturgeon during the development period of their early life stages. Environmental pollution data was provided for 21 sites within the Garonne catchment between 2007 and 2022, and toxicity data was obtained specifically from freshwater toxicity tests on fish species. Then, for each site and each year, the potential toxic risk for a single substance (ssPAF) and for a mixture of substances (msPAF) was calculated and classified as high (>5%), moderate (>1% and <5%) or low (<1%). Potential toxic risks were mostly moderate and mainly associated with: metals > other industrial pollutants and hygiene and care products > agrochemicals. In summary, this study highlights the probable involvement of water contamination on the decline, fate and restoration of diadromous fish populations in the Garonne catchment, focusing notably on the toxic effects on early life stages, a previously understudied topic.

31 **GRAPHICAL ABSTRACT.**



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1. INTRODUCTION

Water is generally considered as the most essential natural resource, but the quality of freshwater ecosystems has deteriorated considerably, mainly due to anthropogenic activities (Carpenter et al., 2011; Meybeck, 2003; Vörösmarty et al., 2010). This situation raises growing concerns about the preservation of our water resources and requires urgent measures to limit the degradation of these environments. One of the main factors in the degradation of these ecosystems is water pollution, caused mainly by population growth and industrialization, which is contributing to the decline in freshwater biodiversity (Dudgeon et al., 2006; Pimentel et al., 1997). The toxic effects of chemicals (metals, agrochemicals, *etc.*) on the fitness of aquatic species are well documented (*e.g.* Kahlon et al., 2018; Schafer et al., 2011) and hypotheses have been discussed concerning the involvement of these substances in the collapse of some aquatic populations (*e.g.* Limburg & Waldman, 2009; Van Dijk et al., 2013), compromising ecosystems and its associated resources. This is especially the case for diadromous fish populations (*i.e.*, migrating between marine and freshwater systems) for which Limburg & Waldman (2009) described a widespread decrease in numbers in the North Atlantic. According to their assumptions, this dramatic decline could be caused partially by water pollution. Indeed, these species have seen their populations maintained at very low levels of abundance in many parts of the world (Verhelst et al., 2021).

In France, the Garonne catchment is no exception to this statement (Boyer et al., 2000), although it was once previously considered an ichthyological reference (Maury-Brachet et al., 1999). Indeed, the numbers of anadromous fish (*i.e.*, growing in marine environments and reproducing in rivers) such as European sturgeon (*Acipenser sturio*), allis shad (*Alosa alosa*), twaite shad (*Alosa fallax*), Atlantic salmon (*Salmo salar*), sea lamprey (*Petromyzon marinus*) and river lamprey (*Lampetra fluviatilis*); and catadromous fish (*i.e.*, growing in rivers and reproducing in marine environments) such as the European eel (*Anguilla anguilla*) are currently at historically low levels (Almeida et al., 2021; Aprahamian et al., 2003; Castelnaud & De Verdilhac, 1981; ICES, 2014; Martin-Vandembulcke, 1999; Prouzet, 1990; Williot & Castelnaud, 2011). Presently, all of these species are classified as more or less endangered (ranging from "near threatened" to "critically endangered") on the IUCN France red list (IUCN, 2019). In this catchment, various factors, including overfishing, migration barriers, degradation of spawning grounds, global changes and water contamination, have been investigated as potential cumulative causes (Legrand et al., 2020). However, there are still numerous questions regarding the effects of water contamination (Pannetier et al., 2016), especially on the early life stages of anadromous fish species, which are particularly sensitive to environmental factors including chemical pollution (McKim, 1977). For example, in the GGD catchment, Delage (2015) demonstrated a deleterious effect of the contaminated sediments on the development of European sturgeon embryos and Blaya et al. (2022) have suggested a potential effect of water contamination on the development of allis shad embryos.

Within the Garonne catchment, the contamination of the water column and sediments by metals, agrochemicals and other industrial substances has been well documented (e.g. Aminot, 2013; Bernard, 2018; Budzinski et al., 1997; Grousset et al., 1999), as well as pollutant accumulation in various fish species (e.g. Acolas et al., 2020; Daverat et al., 2011). Furthermore, the establishment of the Water Framework Directive in France has led to the creation of monitoring networks for water bodies, notably to monitor the presence of chemicals in water. However, although the presence of these substances is well monitored and known, it is rarely associated with toxicity data, and when it is, it focuses only on a single group of substances and/or a small number of substances

(Budzinski et al., 1997; Daverat et al., 2011; Grousset et al., 1999). As a result, the toxic risk of this broad spectrum of pollutants for fish is rarely considered, and even less quantified. It is therefore important to use methods for quantifying this toxic risk, given the availability of historical contamination data, while considering the environmental context of endangered migratory fish species.

However, few tools are currently available to quantify these toxic effects, which represents a major challenge for research in this field. One of the existing methods in ecotoxicological risk assessment is called "Potentially Affected Fraction of species" (PAF) (Beaumelle et al., 2017; Rämö et al., 2018), which estimates the potential loss of species within a group of species studied. It is based on the concept of "Species Sensitivity Distribution" (SSD), which models the differences in a species sensitivity to a pollutant (Posthuma et al., 2002). Thus, by comparing environmental concentrations of one or more pollutants with reference toxicity values, it is possible to predict the percentage of species potentially affected by the presence of this/these substance(s) in the environment (Posthuma & de Zwart, 2006). Although initially developed to estimate toxic risk of pollutant(s) on an ecosystem, PAF approach has also been used to visualize the expected effects on a single taxon, and particularly for the fish species (de Zwart & Posthuma, 2005; Merga et al., 2021; Rämö et al., 2018). However, this type of study often focuses on a single group of substances (e.g. Faggiano et al., 2010; Liu et al., 2020; Silva, 2015) and/or a small number of substances (e.g. He et al., 2014; Posthuma & de Zwart, 2012). In the Garonne catchment, the PAF method has been used specifically to assess the agrochemical pressure on various taxa (Faggiano et al., 2010), and particularly on fish species (Shinn et al., 2009). However, none has considered the risk to fish from substances other than agrochemicals. Consequently, in order to determine the existing overall toxic pressure for fish, it is essential to consider the environmental context (past and current contamination pressures, relevant spatio-temporal scales, use of the habitat by the species considered, *etc.*) and to integrate the widest variety of contaminants present in the environment.

This study proposes to use the PAF method specifically to determine the impact of chemical contamination of water (by metals, agrochemicals and other industrial substances) on fish in the Garonne catchment. The PAF method was applied specifically to fish species at two different spatial scales: rivers and spawning grounds within the Garonne catchment, with the aim of studying the global potential impact of chemical contamination for fish species and, more specifically, during the development period of the early life stages of two anadromous species. The objectives of this study were to quantify the potential toxic risk associated with chemical contamination of water for fish species (1) in the Garonne and Dordogne rivers and (2) in the spawning grounds of two emblematic endangered anadromous fish species: allis shad and European sturgeon during the development period of the early life stages (embryo and larva). The first objective has been named "main rivers scale" and spatially refers to the catchment's main rivers, *i.e.* sites within the Garonne and Dordogne rivers. In this case, environmental data for the whole year and toxicity data for all fish life stages were used. The second objective has been named "spawning grounds scale" and spatially refers to European sturgeon and allis shad spawning grounds in both catchment's rivers, *i.e.* sites within these spawning grounds. In this other case, environmental data considering the reproduction and development period of the early life stages of European sturgeon and allis shad (*i.e.*, April to August), and toxicity data considering specifically the early life stages of fish were used.

2. MATERIALS & METHODS

A. Study area & environmental contamination data

The Garonne catchment (south-west France, Fig. 1) is characterized by the presence of two main rivers (Garonne and Dordogne), as well as two main urban areas (Toulouse and Bordeaux, with nearly 500,000 inhabitants and 250,000 inhabitants, respectively, in 2020 according to the INSEE). In 2023, the number of industrial installations classified for environmental protection (ICPE in French, *i.e.* including activities presenting a risk to the environment) in this catchment was 16,443 (Ministère de la Transition Écologique et de la Cohésion des Territoires, 2023). Furthermore, a high level of agricultural activity is observed within this catchment, with over 50% of the land used for agriculture: ~50% for cereals including corn, wheat and oilseeds, and 50% for vineyards and fruit trees (Bernard, 2018; Faggiano et al., 2010).

Environmental data on chemical contamination were extracted from the "physicochimie.csv" and "phytos.csv" files in the database of the "Système d'Informations sur l'Eau Adour-Garonne" (SIEAG, available at https://adour-garonne.eaufrance.fr/data). The data was obtained for 21 sites (Fig. 1, Table S1) along the two main rivers (12 in the Garonne and 9 in the Dordogne). Data covered the years 2007 through 2022. Known European sturgeon spawning grounds (between ~Beautiran and ~Agen in the Garonne, and between ~Libourne and ~Bergerac in the Dordogne) are situated further downstream than those of allis shad (between ~Aiguillon and ~Golfech in the Garonne, and between ~Sainte-Foy la Grande and ~Mauzac in the Dordogne) (MIGADO, 2022). Given the proximity of Port St-Pardon to European sturgeon spawning grounds and of Trémolat and St-Nic. de la Grave to allis shad spawning grounds (~10-15km), they have been added to these respective spawning grounds.

Figure 1. Study area with location of sites.

Chemical substances were categorized into four classes: metals, agrochemicals, hygiene and care products and other industrial pollutants (Tab. 1). This classification was done using the "family.csv" file in the SIEAG database and the standardized "PubChem" database (available at https://pubchem.ncbi.nlm.nih.gov/). Hygiene and care products only began to be monitored in 2016. All chemical data quantified in water and sediment were retained but the matrix of interest for this study was water, since toxicity data for fish are classically obtained in this matrix. Indeed, ~95% of fish toxicity data in the ECOTOX Knowledgebase are associated with the water matrix in 2023 (available at https://cfpub.epa.gov/ecotox/). However, as some substances are particularly hydrophobic, they can easily be accumulated in sediment and secondary resuspended in the water column through hydrodynamical processes (Geffard, 2001). Given these processes, some substances may or may not be quantified in the water depending on sampling time. Therefore, to estimate the concentration of the pollutants that could be resuspended in water from sediments and avoid neglecting some pollutants, soil-water partitioning coefficients were calculated as in the study by Bockting et al. (1993). Thus, quantified measurements obtained from sediments were transformed into quantified measurements obtained in water.

For each substance quantified in water (directly in water and using soil-water partitioning coefficients), the 95th percentile of concentration value was calculated for each site and year studied, aiming to represent a value near the maximum concentration while mitigating the impact of potential outliers (*e.g.*, human error). On the main rivers scale, data collected throughout the year were used, resulting in 284 quantified pollutants. On the spawning grounds scale, only data collected between April and August were used, *i.e.* during the reproduction and early development period for European sturgeon and allis shad (Table S2), resulting in 198 quantified substances.

Table 1. Classification of the chemical pollutants.

Categories	Details
Agrochemicals	Herbicides, Fungicides, Insecticides, Other biocides and Metabolites
Metals	Alkaline earth metals, Transition metals, Post-transition metals, Metalloids, Actinides and Other nonmetals
Hygiene and Care Products	Pharmaceuticals, Cosmetics and hygiene products, Hormones and Metabolites
Other Industrials Pollutants	Polycyclic Aromatic Hydrocarbons, PolyChlorinated Biphenyls, Per- and PolyFluoroAlkylated Substances, Flame retardants, Plasticizers, Solvents, Degradation products, Others and Metabolites

B. Toxicity data & potential toxic risk estimation

To quantify the potential toxic risk caused by chemicals in the Garonne catchment, a comparison of the environmental concentration of each pollutant with its "Hazardous Concentration for 50% of species" (HC_{50}) value was performed. The HC_{50} for each substance can be calculated as the geometric mean of “median Effect Concentrations” (EC_{50}) specific to each species studied (Payet, 2004). Thus, various EC_{50} and “NO Effect Concentration” (NOEC) from freshwater toxicity tests on fish species ; *i.e.* the most numerous toxicity data for this kind of toxicity tests (Aurisano et al., 2019) ; was extracted from the "ECOTOX Knowledgebase" (available at https://cfpub.epa.gov/ecotox/). NOEC were recovered in addition to EC_{50} to recover as much toxicity data as possible. Among the substances quantified in water, 125 substances had toxicity data on the main rivers scale and 78 on the spawning grounds scale. Then, every recovered toxicity data (EC_{50} and NOEC) were transformed into chronic EC_{50} using extrapolation factors calculated in the study of Aurisano et al. (2019), which allowed to represent a long-term exposure for fish (≥ 7 days):

- For acute EC_{50}: $EC_{50ch} = EC_{50ac} / 1.71$
- For chronic NOEC: $EC_{50ch} = NOEC_{ch} * 3.41$
- For acute NOEC: $EC_{50ch} = NOEC_{ac} * 3.41 / 3.14$

Where EC_{50ch} represented chronic EC_{50}, EC_{50ac} represented acute EC_{50}, $NOEC_{ch}$ represented chronic NOEC and $NOEC_{ac}$ represented acute NOEC.

A categorization of the life stages of fish was performed (Table S3) to use toxicity data representing, all life stages for the main rivers scale and only embryos and larvae for the spawning grounds scale, in order to calculate substance-specific HC_{50} (Table S4).

As described by Pennington et al. (2004), assuming linearity of SSD curve under the HC_{50}, the percentage of fish species potentially affected can be calculated as follows:

- For single substance: $ssPAF = 0.5*(C_S/HC_{50S})*100$
- For a mixture of substances (considering an additive model): $msPAF = 0.5*\sum(C_S/HC_{50S})*100$

Where C_S represented the 95th percentile of the environmental concentration of a substance "S" specific to a year and site, and HC_{50S} represented the HC_{50} specific to a substance "S".

The intensity of the potential toxic risk was determined based on the result (in percent) of the ssPAF and msPAF calculations as in the study by Rämö et al. (2018). Consequently, a result below 1% was considered low potential toxic risk, while a result between 1% and 5% was considered moderate potential toxic risk, and a result above 5% was considered high potential toxic risk.

3. RESULTS

A. Main rivers scale

The potential toxic risk seems to have decreased predominantly throughout the period for most sites (Fig. 2). In the Garonne, among the 13 msPAF values exceeding 10% of potentially affected fish species, 11 were recorded during the period 2007-2012 and 2 after 2012. In the Dordogne, among the 7 msPAF values exceeding 10% of potentially affected fish species, 6 were observed during the period 2007-2012 and 1 after 2012. However, in 2022, solely 2 of the 21 sites studied (1 in both rivers) present a low potential toxic risk (msPAF < 1%).

Sites in the Garonne and Dordogne rivers showed mainly a moderate potential toxic risk (1% < msPAF < 5%) over the entire period. Indeed, 96 of the 188 years studied for the Garonne and 69 of the 141 years studied for the Dordogne presented a moderate potential toxic risk. In addition, a significant proportion of the years studied presented a high potential toxic risk (msPAF > 5%): 54 of the 188 years studied for the Garonne and 36 of the 141 years studied for the Dordogne. Over the period studied, all sites for the Garonne and 2/3 of sites for the Dordogne had at least one msPAF value representing a high potential toxic risk (msPAF > 5%). Furthermore, 38 of the 188 years studied presented a low potential toxic risk (msPAF < 1%) for the Garonne and 36 of the 141 years studied for the Dordogne.

Regarding contributions, metals seem to explain most of the high potential toxic risk values (msPAF max. 48.5% in the Garonne and 44.4% in the Dordogne). However, some contributors generating a high potential toxic risk are also associated with hygiene and care products (msPAF max. 8.41% in the Garonne), and other industrial pollutants (msPAF max. 26.1% in the Garonne and 7.26% in the Dordogne). In contrast, a very small contribution from agrochemicals was highlighted (msPAF max. 3.04% in the Garonne and 2.00% in the Dordogne).

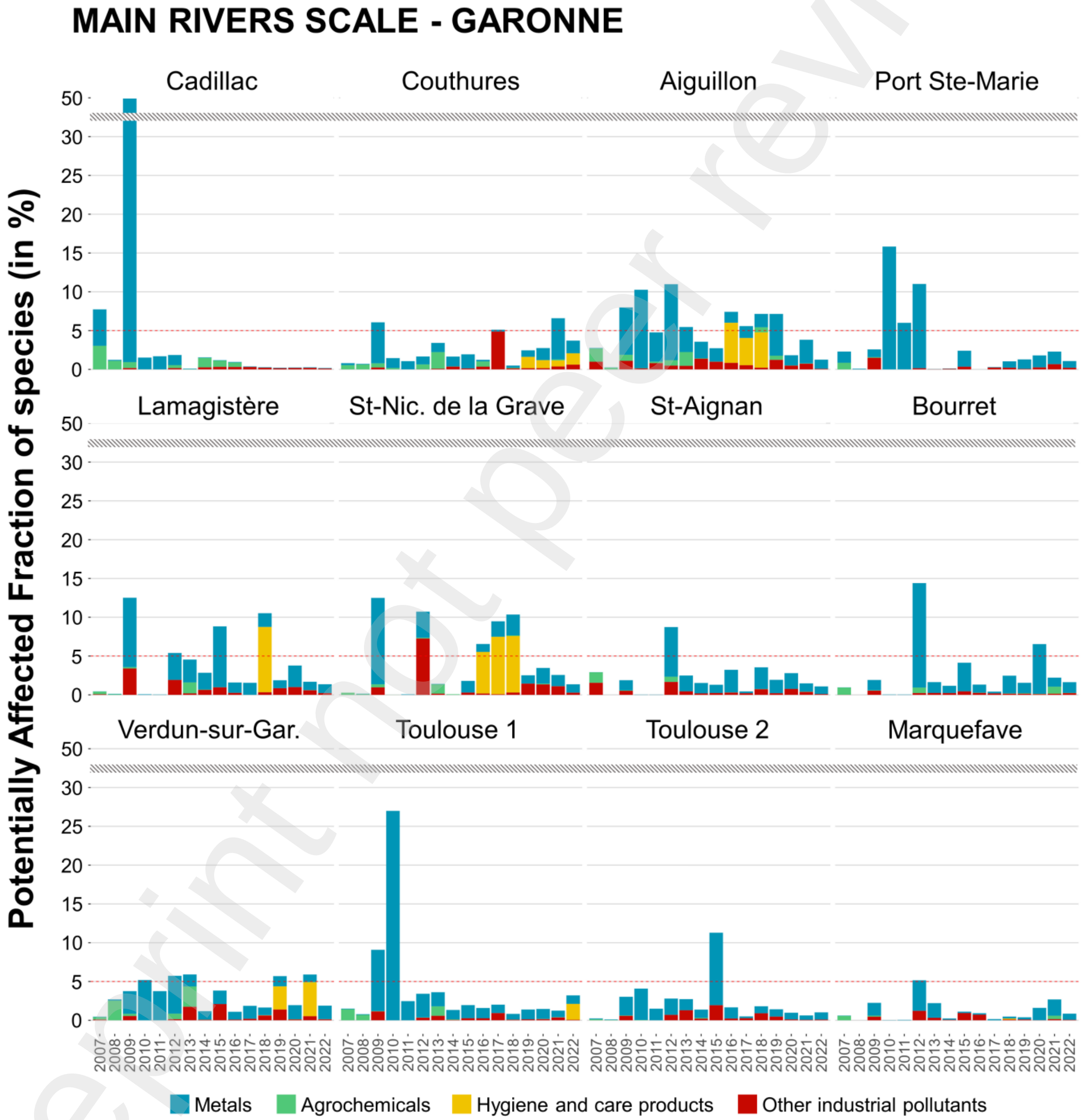

Figure 2. Percentage of fish species potentially affected for each site, year and category in the Garonne and Dordogne rivers at the main rivers scale (*i.e.*, values were calculated using all sites, all months and toxicity data recovered from all life stages). Sites are ordered from downstream to upstream. The dotted red line represents the 5% threshold, exceeding which generates a high potential toxic risk. The gray lines represent jumps in scale on the y-axis.

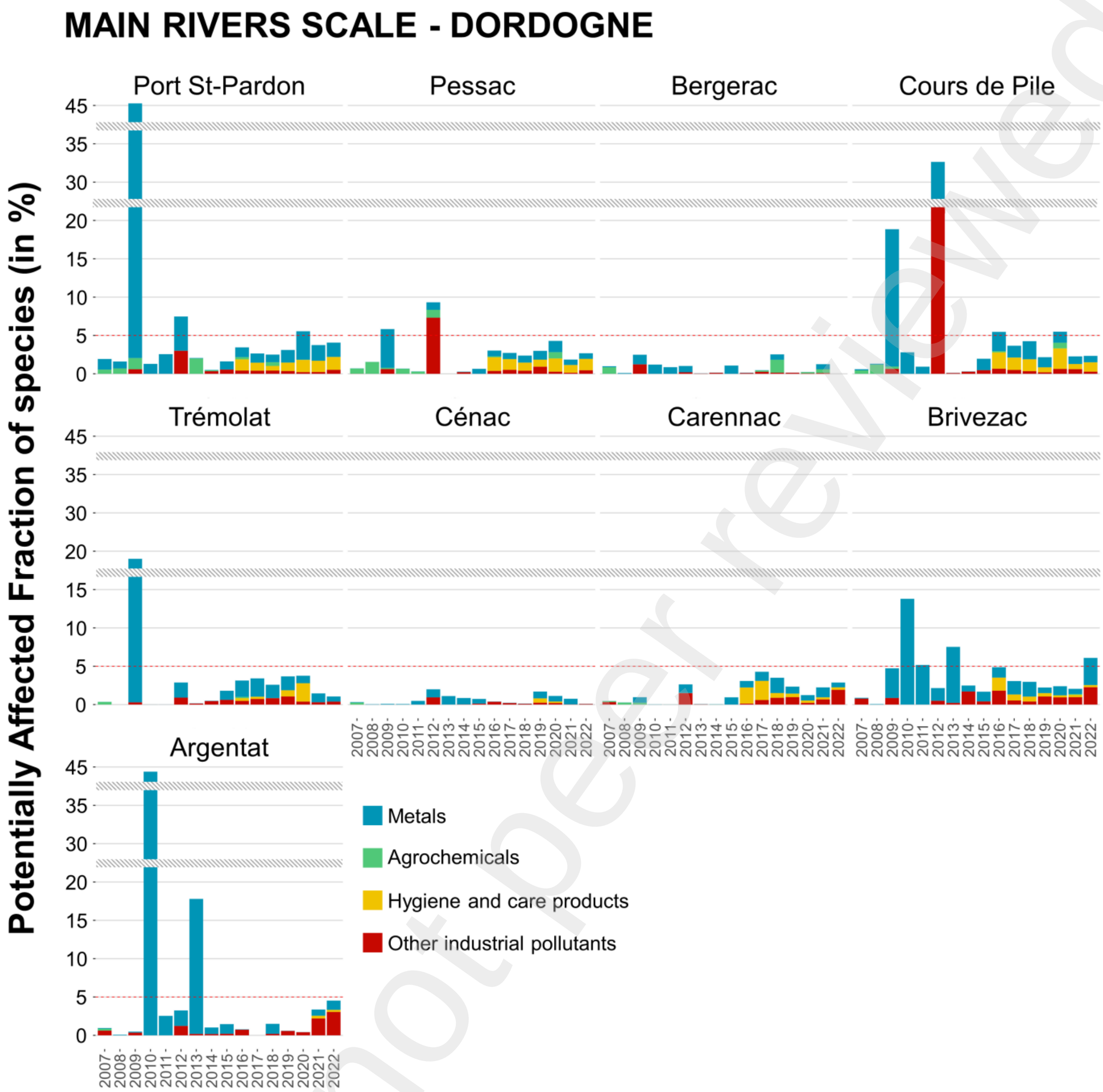

Figure 2. Continued.

All categories of studied pollutants (metals, agrochemicals, hygiene and care products, and other industrial pollutants) generated moderate potential toxic risk (1% < ssPAF < 5%) in the Garonne and Dordogne (Fig. 3). In both rivers, three substances had more than 10 ssPAF values representing at least a moderate potential toxic risk (1% < ssPAF < 5%) throughout the study period: copper with 69 values, zinc with 21 values and lead with 17 values in the Garonne, and copper with 23 values, metformin with 20 values and zinc with 13 values in the Dordogne. Other substances had less than 10 ssPAF values representing at least a moderate potential toxic risk (1% < ssPAF < 5%): phenol-4-nonyl-branched, diuron, dioctyl-phthalate, benzo[a]pyrene, fluoranthene, metformin and estrone in the Garonne; cypermethrin, bisphenol S, lead and bisphenol A in the Dordogne; and oxadiazon, cadmium, ziram, hydroxyterbuthylazine and iron in both rivers.

Only metals and hygiene and care products in the Garonne, and metals and other industrial pollutants in the Dordogne represented a high potential toxic risk (ssPAF > 5%). In both rivers, four substances had ssPAF values representing a high potential toxic risk (ssPAF > 5%): lead with 2 values, copper with 6 values, estrone with 3 values and zinc with 2 values in the Garonne, and copper with 4 values, bisphenol A with 2 values, lead with 3 values and zinc with 4 values in the Dordogne. None agrochemicals had ssPAF values representing a high potential toxic risk (ssPAF > 5%). The highest ssPAF value was obtained by two metals: lead in the Garonne (nearly 25%) and copper in the Dordogne (nearly 40%).

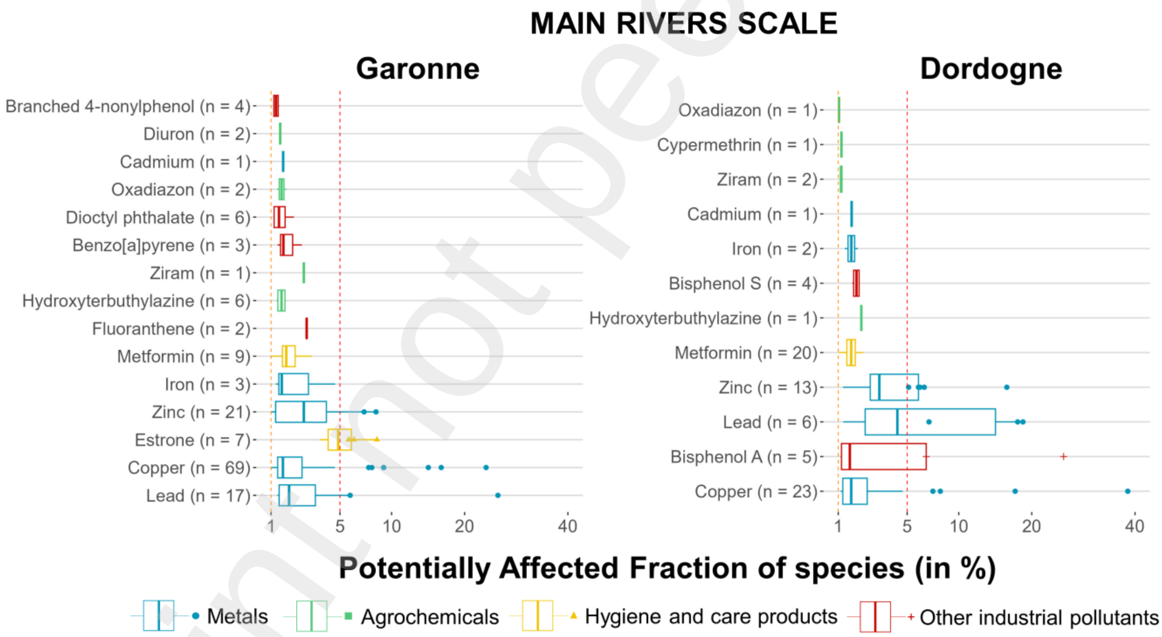

Figure 3. Boxplots of substance values exceeding 1% (moderate potential toxic risk) of potentially affected fish species per river at the main rivers scale (*i.e.*, values were calculated using all sites, all months and toxicity data recovered from all life stages). The dotted red line represents the 5% threshold, exceeding which generates a high potential toxic risk. Points (point, square, triangle and cross) indicate values exceeding 5% (high potential toxic risk) of potentially affected fish species.

B. Spawning grounds scale

The potential toxic risk seems to have decreased predominantly throughout the period for most sites (Fig. 4). In the Garonne, among the 7 msPAF values exceeding 10% of potentially affected fish species, 6 were recorded during the period 2007-2012 and 1 after 2012. In the Dordogne, among the 5 msPAF values exceeding 10% of potentially affected fish species, all were observed during the period 2007-2012. In 2022, no sites present a low potential toxic risk (msPAF < 1%).

Sites in the Garonne and Dordogne rivers showed mainly a moderate potential toxic risk (1% < msPAF < 5%) over the entire period. Indeed, 42 of the 86 years studied for the Garonne and 36 of the 68 years studied for the Dordogne presented a moderate potential toxic risk. In addition, a significant proportion of the years studied presented a high potential toxic risk (msPAF > 5%): 21 of the 86 years studied for the Garonne and 13 of the 68 years studied for the Dordogne. Over the period studied, 100% of sites for both rivers had at least one msPAF value representing a high potential toxic risk (msPAF > 5%). The spawning grounds of both species have msPAF values generating a high potential toxic risk (msPAF > 5%), with the highest value on European sturgeon spawning grounds (msPAF max. 100% for European sturgeon and msPAF max. 55% for allis shad). Furthermore, 23 of the 86 years studied presented a low potential toxic risk (msPAF < 1%) for the Garonne and 19 of the 68 years studied for the Dordogne.

Regarding contributions, metals seem to explain most of the high potential toxic risk values (msPAF max. 100% in the spawning grounds of both rivers). In contrast, a smaller contribution from hygiene and care products (msPAF max. 3.81% in the Garonne and 1.48% in the Dordogne), other industrial pollutants (msPAF max. 3.12% in the Garonne and 3.00% in the Dordogne), and agrochemicals (msPAF max. 1.75% in the Garonne and 1.99% in the Dordogne) is visible.

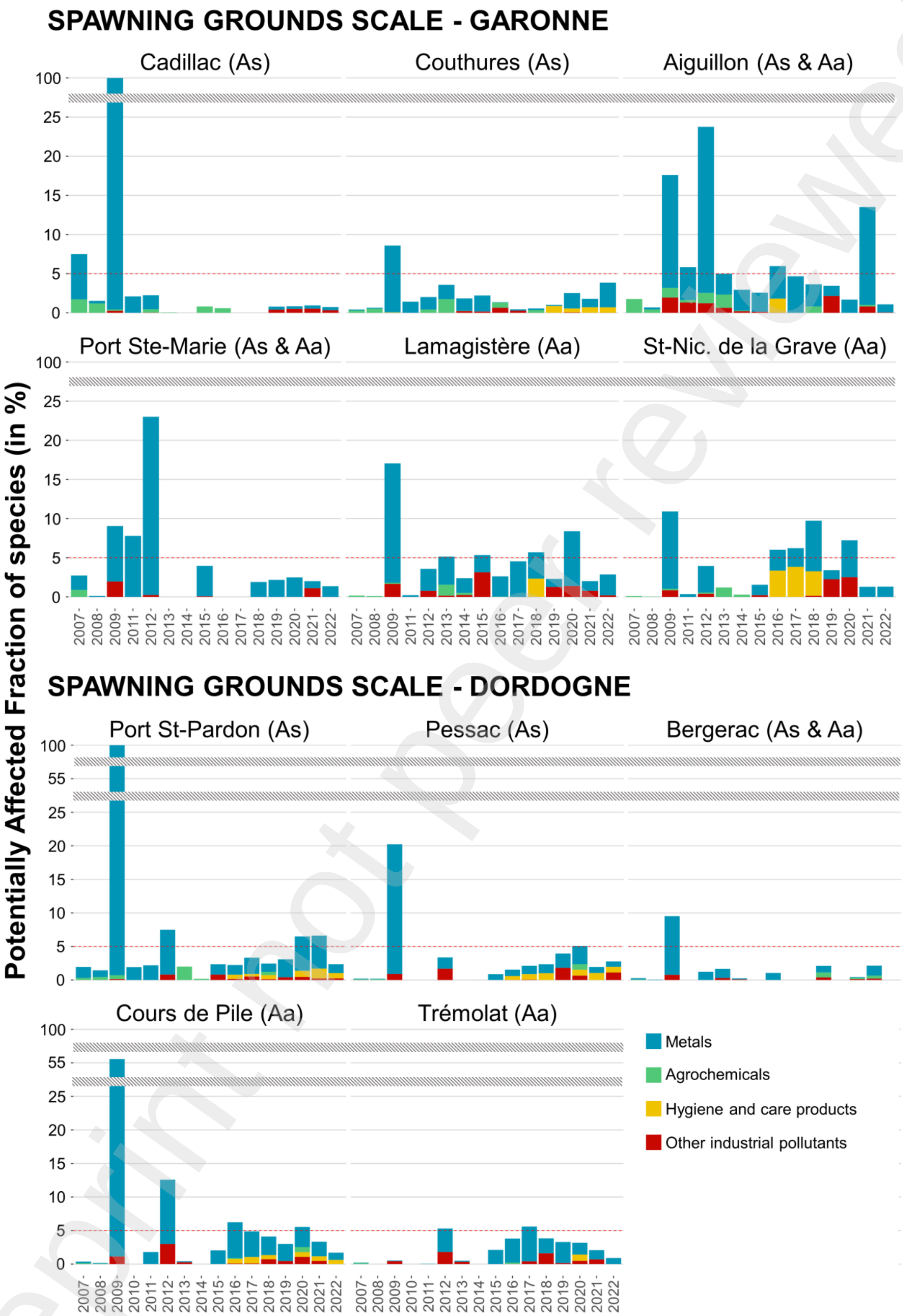

Figure 4. Percentage of fish species potentially affected (at early life stages) for each site, year and category in the Garonne and Dordogne rivers at the spawning grounds scale (*i.e.*, values were calculated using sites in spawning grounds, April-August months and toxicity data recovered from early life stages). Sites are ordered from downstream to upstream. As = *Acipenser sturio* spawning grounds; and Aa = *Alosa alosa* spawning grounds. The dotted red line represents the 5% threshold, exceeding which generates a high potential toxic risk. The gray lines represent jumps in scale on the y-axis.

All categories of selected pollutants (metals, agrochemicals, hygiene and care products, and other industrial pollutants) generated moderate values (1% < ssPAF < 5%) in the Garonne and Dordogne spawning grounds (Fig. 5). In the Garonne spawning grounds, three substances had more than 10 ssPAF values representing at least a moderate potential toxic risk (1% < ssPAF < 5%) throughout the study period: copper with 37 values, lead with 21 values and zinc with 11 values, and two in the Dordogne spawning grounds: copper with 23 values and lead with 11 values. Other substances had less than 10 ssPAF values representing at least a moderate potential toxic risk (1% < ssPAF < 5%): branched 4-nonylphenol, dioctyl phthalate, 4-tert-octylphenol, acetochlor and estrone in the Garonne spawning grounds; bisphenol A, benzo[a]pyrene and zinc in the Dordogne spawning grounds; and metformin, hydroxyterbuthylazine and iron in both rivers. Of the 232 ssPAF values giving rise to a moderate potential toxic risk (1% < ssPAF < 5%), 64 were specific to European sturgeon spawning grounds, 112 were specific to allis shad spawning grounds and 56 were common to the spawning grounds of both species.

Only metals in both rivers represented a high potential toxic risk (ssPAF > 5%) in the Garonne and Dordogne spawning grounds. Four substances had ssPAF value(s) representing a high potential toxic risk (ssPAF > 5%) in the Garonne spawning grounds: lead with 5 values, copper with 1 value, zinc with 3 values and iron with 1 value, and three substances in the Dordogne spawning grounds: lead with 4 values, copper with 2 values and zinc with 2 values. None agrochemicals, hygiene and care products, and other industrial pollutants had ssPAF values representing a high potential toxic risk (ssPAF > 5%). The highest ssPAF value was obtained for lead in the Garonne spawning grounds (100%) and Dordogne spawning grounds (nearly 100%), both in 2009. Of the 36 ssPAF values giving rise to a high potential toxic risk (ssPAF > 5%), 16 were specific to European sturgeon spawning grounds, 9 were specific to allis shad spawning grounds and 11 were common to the spawning grounds of both species.

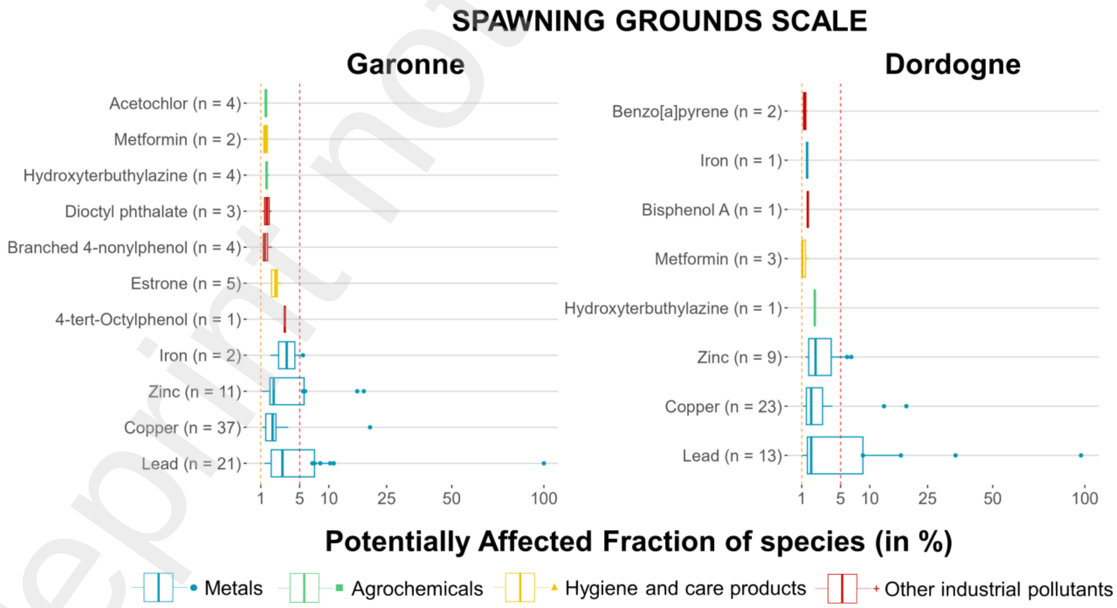

Figure 5. Boxplots of substance values exceeding the 1% (moderate potential toxic risk) threshold of potentially affected fish species (at early life stages) per river at the spawning grounds scale (*i.e.*, values were calculated using sites in the spawning grounds, April-August months and toxicity data recovered from early life stages). The dotted red line represents the 5% threshold, exceeding which generates a high potential toxic risk. Points (point, square, triangle and cross) indicate values exceeding 5% (high potential toxic risk) of potentially affected fish species.

4. DISCUSSION

A. Study limitations

On the Garonne catchment, this work is the first to use the PAF method specifically estimating toxic risk for fish species with a wide range of pollutants (n = 125 on the main rivers scale and n = 78 on the spawning grounds scale) and over a long-term period (16 years) and to propose a specific adaptation of the method to early life stages. To our knowledge no previous work had adapted the PAF method to the specific life stages of a particular taxon. It has enabled to quantify potential toxic risks in space (sites contained in the main rivers scale and the spawning grounds scale) and time (throughout the period) and to identify substances potentially affecting fish in the Garonne catchment. However, this method is subject to various inherent data limitations.

In reality, of all the chemical substances on the European market in 2019 (at least 144,000), *i.e.* potentially present in the environment, only 0.25% (*i.e.*, 350 substances) were monitored in the water framework directive, assessing water quality in Europe (Posthuma et al., 2020). According to the most recent estimations, this percentage would even be lower given that there are at least 225,000 substances on the European market (ECHA, 2023). Based on these available data only, 39% of European waters are insufficiently protected against individual and combined chemical hazards (Rorije et al., 2022), a high percentage despite the low level of monitoring. Furthermore, among this few number of substances monitored, only 10% are estimated to be quantified in water (Rorije et al., 2022). In this study, using the SIEAG database, only 284 substances of the 526 substances in the database (~54%) were quantified at least once over the area and period studied. Furthermore, 96% of the available data was unquantified in water due to low concentrations and/or excessively high quantification thresholds. By the way, no increase in terms of quantification frequency was observed over the period (maximum of quantification in 2010 with ~10%). One of the hypotheses is that no lowering of quantification thresholds occurred during the study period. Thus, the limited environmental data available for some substances has probably affected the quality of the data used to calculate the 95th percentile, particularly for substances of little interest, and therefore little monitored. These findings underline the need to monitor more substances and lower the quantification thresholds in order to process more data and ensure greater robustness of results. In addition, among the substances quantified, ~56% at the main rivers scale and ~61% at the spawning grounds scale had no fish toxicity data available. As in the case of environmental data, the lack of available toxicity data may have resulted in a loss of quality in the toxicity data used. Indeed, when few EC_{50} values were available, the HC_{50} values calculated were hardly representative of fish taxon, and therefore potentially more representative of a fish species or group of fish species with low or high sensitivity to a pollutant. In addition, toxicity data were obtained on a limited range of species, including toxicity-model species, which may reflect a relatively different sensitivity to substances than the species present in the Garonne catchment. Thus, given the limited environmental and toxicity data available, the potential toxic risk calculated in this study is based on an underestimated number of substances (except for metals whose number is limited, and which have long been the focus of ecotoxicological studies) which may have degraded data quality.

While most of the previous biases appear to have underestimated the potential toxic risk, the use of the 95th percentile (close to the maximum estimate) may have overestimated it. The mean could provide more realistic results. Furthermore, the additive model (*i.e.*, addition of concentrations and responses) simplifies calculations, but is less realistic than a model including the antagonistic and synergistic effects generated by interactions between substances (Altenburger et al., 2003), which can modify, by either decreasing or increasing, the calculated potential toxic risk.

B. Origins and effects of potentially impacting substances

Although existing biases, the PAF method allowed to identify trends over the period studied, the sites most at risk and the potentially problematic substances or groups of substances. The potential toxic risk to fish appeared to decrease over the period, with higher msPAF values (msPAF > 10%) from 2007 to 2012, in both scales. In 2022, only 2 of the 21 sites studied presented a low potential toxic risk (msPAF < 1%) at the main rivers scale and none at the spawning grounds scale. Metals were identified as the main contributors to high msPAF values in both scales and rivers studied. The most problematic substances (high ssPAF values and/or significant number of ssPAF values exceeding at least 1% of fish species potentially affected) were lead, copper, zinc, metformin, estrone (only in the Garonne) and bisphenol A (only in the Dordogne) at the main rivers scale, and lead, copper, zinc and iron (only in the Garonne) at the spawning grounds scale. In addition, 18 and 13 substances (with at least 1 substance per category), had ssPAF values exceeding the 1% threshold (*i.e.*, moderate potential toxic risk) at the main rivers scale and the spawning grounds scale, respectively. Of these substances, 11 were common to both scales (iron, zinc, lead, copper, hydroxyterbuthylazine, metformin, estrone, benzo[a]pyrene, dioctyl-phthalate, bisphenol A and branched 4-nonylphenol), 7 were found only at the main rivers scale (cadmium, cypermethrin, diuron, oxadiazon, ziram, bisphenol S and fluoranthene) and 2 were found only at the spawning grounds scale (acetochlor and 4-tert-octylphenol). Some pollutants were found specifically in one or other river at concentrations generating at least a moderate potential toxic risk (1% < ssPAF < 5%). On the main rivers scale, 6 substances were specifically impacting in the Garonne (branched 4-nonylphenol, diuron, dioctyl phthalate, benzo[a]pyrene, fluoranthene and estrone), 3 in the Dordogne (cypermethrin, bisphenol S and bisphenol A) and 9 in both rivers (cadmium, oxadiazon, ziram, hydroxyterbuthylazine, metformin, iron, zinc, copper and lead). On the spawning grounds scale, 5 substances were specifically impacting in the Garonne (acetochlor, dioctyl phthalate, branched 4-nonylphenol, estrone and 4-tert octylphenol), 2 in the Dordogne (benzo[a]pyrene and bisphenol A) and 6 in both rivers (metformin, hydroxyterbuthylazine, iron, zinc, copper, lead). More ssPAF values and a greater variety of pollutants generating at least a moderate potential toxic risk (1% < ssPAF < 5%) and more msPAF values generating a high toxic risk (msPAF > 5%) were observed in the Garonne than in the Dordogne.

The three most impacting metals (copper, lead and zinc) were quantified at concentrations generating a high potential toxic risk for fish (ssPAF > 5%) at both scales and in both rivers. These substances are relatively well documented and are well-known to impact fish at all life stages (Authman, 2015) and particularly during in the early life stages by disrupting embryonic development, which can result in fewer hatchlings and deformed larvae, making them less efficient (Jezierska et al., 2009). Historically, metal pollution in the Garonne catchment has been documented and associated with various industrial activities such as mining, tanneries, *etc*. (Grousset et al., 1999). In this catchment, copper and zinc have been used in agriculture. For example, copper is a compound of the "bouillie bordelaise" particularly used for fungicide treatments in vineyards in France (Baize & Saby, 2006). In addition, ziram (also impacting in this study) is a zinc-based fungicide used and authorized to combat fruit diseases (Cao et al., 2019). In this catchment, vines and fruit trees are particularly exploited by agriculture and could explain their high presence in Garonne and Dordogne rivers (Bernard, 2018; Faggiano et al., 2010; Masson et al., 2006). Regarding cadmium, it had an impact solely in 2009 at Bourret (Garonne) and Brivezac (Dordogne), despite it was a substance particularly problematic in the past (Blanc et al., 2006). Iron toxicity, for its part, is poorly documented. Among hygiene and care products, estrone and metformin have proved particularly impactful, reaching ssPAF values of 8.4% (in 2018 at Lamagistère, Garonne) and 4.3% (in 2021 at Verdun-sur-Gar., Garonne), respectively. Estrone is a natural estrogen which may result from the transformation of estradiol or ethinyl estradiol (compound found in the most widely used contraceptive pills) (Adeel et al., 2017; Stanczyk et al., 2013). In 2018, downstream of the city of Toulouse, estrone was one of the 7 drug residues considered most at risk for the Garonne (Destrieux, 2018). The quantified concentrations of estrone solely in the Garonne are consistent with greater human density (probably associated with the influence of the city of Toulouse). This substance can cause adverse effects in fish, such as reducing the number of fertilized eggs (Imai et al., 2007). As for metformin, is worldwide used oral hypoglycemic agent for the treatment of diabetes (Niemuth & Klaper, 2015). It is known to be an endocrine disruptor with estrogenic activities (*i.e.*, mimicking/antagonizing the activity of natural oestrogens) may induce the development of intersex gonads and a reduction in size in males and a decrease in fertility in pairs in fish (Niemuth & Klaper, 2015). Furthermore, among the other industrial pollutants, bisphenol A have proved particularly impactful, reaching values of 25.5% (in 2012 at Cours de Pile, Dordogne) and 6.6% (in 2012 in Pessac, Dordogne). It is mainly used in the production of polycarbonate plastics (~70%), epoxy resins (~20%) and as antioxidant or inhibitor of polymerization (~10%) (Eladak et al., 2015). Bisphenol A can cause significant toxic effects in fish affecting hatching, embryo development and behavior (spontaneous movements and heartbeat rate) and larval development (yolk sac edema, pericardial edema, spinal deformation) (Gao et al., 2022).

Acetochlor and 4-tert-octylphenol were specifically toxic to embryos and larvae. Acetochlor is a herbicide banned in 2013 in France and formerly used for weed control in corn (INERIS, 2016), a culture particularly present in this catchment (Bernard, 2018; Faggiano et al., 2010). This substance exceeded 1% of potentially affected fish species at Cadillac (Garonne) in 2007 and Aiguillon (Garonne) in 2007, 2009 and 2012, before its ban. Acetochlor is an endocrine disruptor, particularly affecting thyroid hormones, which can lead to bioenergetic, development and behavioural problems in early life stages of fish (Huang et al., 2021). 4-tert-octyl-phenol is a degradation product of non-ionic surfactants (*i.e.*, with a low foaming power) used in the manufacture of resins used to produce plastics, agrochemicals, detergents, hygiene and care products, *etc.* (Madsen et al., 2006). A value for this chemical compound exceeded 1% of potentially affected species at Lamagistère (Garonne) in 2015. It is an endocrine disruptor with estrogenic activity that can affect hatching success and cause developmental problems (blood circulation and swim bladder) at the embryo-larval stages of fish (Gray & Metcalfe, 1999).

We observed other contributors above 1% of potentially affected species, but with values near 1% and never exceeding 5% (some agrochemicals and other industrial pollutants). Among agrochemicals, in addition to acetochlor, cypermethrin, diuron, oxadiazon, ziram and hydroxyterbuthylazine were in this case. In this study, the maximum ssPAF value for agrochemicals was 2.6% (associated with hydroxyterbuthylazine in 2013 at Verdun-sur-Gar., Garonne), close to the maximum value of 3.7% associated with carbofuran obtained in the same catchment in the study by Faggiano et al. (2010). These agrochemicals have toxic effects on fish (Cao et al., 2019; Carriquiriborde et al., 2009; Saglio & Trijasse, 1998; Velisek et al., 2014; Zanjani et al., 2018). For example, cypermethrin can reduce survival and cause growth problems (Carriquiriborde et al., 2009), diuron can disrupt behaviour (Saglio & Trijasse, 1998), oxadiazon can affect hematological and biochemical parameters (Zanjani et al., 2018), ziram can disrupt embryonic development and larval behaviour (Cao et al., 2019), and therbutylazine can affect development, biochemical parameters and histological parameters in fish. Among other industrial pollutants, in addition to 4-tert-octylphenol, six substances belonging to other industrial pollutants were also in this case: branched 4-nonylphenol, dioctyl phthalate, bisphenol A, bisphenol S, benzo[a]pyrene, fluoranthene. The first three substances are considered as endocrine disruptors in fish (Amaninejad et al., 2018; Adeogun et al., 2018; Moreman et al., 2017) and the last two substances can cause larval abnormalities (edema and spinal curvatures) in fish (Le Bihanic et al., 2014).

Per- and polyfluoroalkylated substances and polychlorinated biphenyls contributed negligibly to ssPAF/msPAF values. The highest ssPAF value were ~0.001% for per- and polyfluoroalkylated substances and ~0.002% for polychlorinated biphenyls. These substances (except short-chain per- and polyfluoroalkylated substances) are particularly hydrophobic and can therefore strongly bind to suspended matter and accumulate in sediment (Ranjbar Jafarabadi et al., 2019; Labadie & Chevreuil, 2011) that could explain their low contributions in this study, despite the use of sediment values that can be resuspended in water.

C. Pressure factors and diadromous fish populations

The PAF method has furnished insights on the main potential contributors and, indirectly, on the biological disturbances they may cause to fish. More concretely, this method has been applied to two spatial scales (rivers and spawning grounds), revealing the probability that water contamination can affect all fish species in this catchment, and particularly the early life stages during the development period of anadromous fish species. Moreover, considering the absence of a substantial upstream-downstream gradient in the response to potential toxic risks, migratory species could be impacted across a significant part of their migration.

Hypotheses regarding the impact of pollutants in water on aquatic populations have been previously addressed in the scientific literature (Dethlefsen, 1988). For example, Slooff (1982) noted greater morphological anomalies in fish in "more polluted" areas compared to "less polluted" areas. More recent research by Van Dijk et al. (2013) suggested that the insecticide imidacloprid might lead to a reduction in taxonomic richness and abundance of macroinvertebrates in Netherlands surface waters. However, Vijver & Van Den Brink (2014) criticized this work, concluding that imidacloprid was just one stress factor among agrochemicals, themselves being only one stress factor among many others (climate change, invasive species, *etc.*). Indeed, it is widely acknowledged that pollutant effects can be exacerbated by additional factors such as temperature (e.g. Laetz et al., 2014; Willming et al., 2013). With increasing pressures on ecosystems (climate change, invasive species, acidification, land use change, *etc.*), research to preserve our freshwater ecosystems appears essential. Therefore, while there is potential toxic pressure in the Garonne catchment, the percentages of potentially affected species quantified should be considered in perspective, as other factors like temperature have not been accounted in the method. Moreover, Smetanová et al. (2014) demonstrated that, although the PAF indicator correlated well with field observations, the use of the 5% threshold often underestimated the observed effects. Thus, the quantifications of this study serve as a relative indicator of toxic pressure, challenging to extrapolate but providing an insight into the actual situation. Consequently, further field studies (ecotoxicological and multi-stress) are indispensable to draw conclusions about the precise implication of water contamination on the current state of diadromous fish.

This study serves as a cautionary note by raising questions about the impact of water pollution on the status of migratory fish species, a concern that has been overlooked for too long. Indeed, a deeper understanding of chemical water status in the Garonne catchment and associated toxicity processes could potentially lead to a resurgence in diadromous fish populations if appropriate environmental restoration measures are implemented. For example, in the northeastern United States, resurgent populations of American shad, striped bass, and Atlantic sturgeon have been observed following pollution reductions (Waldman & Quinn, 2022). Le Pichon et al. (2020) have also highlighted alternating declines/resurgences of diadromous fish populations in the Seine estuary, associated with the appearance/disappearance of physical as well as chemical barriers. Thus, the influence of chemical factors on the presence/disappearance of diadromous fish populations appears significant and needs to be investigated further. Indeed, 6 of the 9 planetary limits have been exceeded, including that associated with the introduction of new entities (Richardson et al., 2023). This suggests there will be an ever-increasing number and diversity of chemical cocktails that will be difficult to identify and control. Given the tendency of chemicals to settle in estuaries, diadromous fish are among the species most vulnerable to this type of stress (Waldman & Quinn, 2022). Consequently, the use of risk assessment methods, such as the PAF method, to quantify the ecological risks faced by specific groups of species appears essential.

5. CONCLUSION

To conclude, this study shows the potentially involvement of water quality in the collapse of diadromous fish species in the Garonne catchment, which has so far remained mainly unexplored. The PAF method has proven to be valuable in identifying spatiotemporal trends in potential toxic risk and pinpointing certain pollutants that could potentially affect the species studied. Additionally, it has demonstrated flexibility in its application, ranging from assessing impacts widely on fish species to specific effects on particular life stages. The identification of the biases inherent in the data showed mainly a limited amount of environmental and toxicity data, which may have an impact on the data quality and, *in fine*, on the results obtained. According to our results, contamination of the catchment water could indeed be a problem for the recovery and fate of migratory fish populations, particularly through toxic effects of pollutants on early life stages during the development period of anadromous fish species. Consequently, more ecotoxicological field studies using fish models representative of the Garonne catchment and multi-stress studies should be envisaged to better estimated the chemical risk for endangered fish species, and more broadly for declining fish populations.

ASSOCIATED CONTENT

Supporting information

Table S1. Study sites; Table S2. Life cycle of the European sturgeon (*Acipenser sturio*) and allis shad (*Alosa alosa*); Table S3. Categorization of fish life stages; Table S4. HC_{50} values obtained for each substance quantified in water at the main rivers scale (Garonne and Dordogne rivers) and the spawning grounds scale (European sturgeon and allis shad spawning grounds).

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We thank the anonymous reviewers for improving this manuscript. This work is part of the “ESPECE EX-SITU” research project (n° 00006189), financed by the “Agence de l’Eau Adour-Garonne” (AEAG, 90 rue Férétra, 31078, Toulouse Cedex 4, Occitanie, France) and by two research units of the “Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement” (INRAE): the UR EABX (50 Avenue de Verdun, 33612, Cestas Cedex, Nouvelle-Aquitaine, France) and the UR RiverLy (5 Rue de la Doua, 69100, Villeurbanne Cedex, Auvergne-Rhône-Alpes, France).

ABBREVIATIONS

SIEAG: “Système d’Informations sur l’Eau Adour-Garonne”;

ICPE: “Installations Classées Protection de l'Environnement”;

C_S: environmental concentration of a substance "S";

NOEC: NO Effect Concentration;

$NOEC_{ac}$: acute NO Effect Concentration;

$NOEC_{ch}$: chronic NO Effect Concentration;

EC_{50}: median Effect Concentration;

EC_{50ac}: acute median Effect Concentration;

EC_{50ch}: chronic median Effect Concentration;

HC_{50}: median Hazardous Concentration;

HC_{50S}: median Hazardous Concentration specific to a substance "S";

PAF: Potentially Affected Fraction of species;

ssPAF: Potentially Affected Fraction of species by a single substance;

msPAF: Potentially Affected Fraction of species by mixture of substances.

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