Environmental Pollution 242 (2018) 449–461

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Biomarker responses in fish exposed to polycyclic aromatic hydrocarbons (PAHs): Systematic review and meta-analysis☆

Manuela S. Santana [a, *], Leonardo Sandrini-Neto [b], Francisco Filipak Neto [a], Ciro A. Oliveira Ribeiro [a], Maikon Di Domenico [b], Maritana M. Prodocimo [a]

[a] Departamento de Biologia Celular, Setor de Ciências Biológicas, Universidade Federal do Paraná, CEP 81.531-980, Curitiba, Paraná, Brazil
[b] Centro de Estudos do Mar, Universidade Federal do Paraná, CEP 83255-976, Pontal do Paraná, Paraná, Brazil

ARTICLE INFO

Article history:
Received 8 February 2018
Received in revised form
14 June 2018
Accepted 2 July 2018
Available online 4 July 2018

Keywords:
EROD
Glutathione
Laboratory
Marine
Freshwater

ABSTRACT

Biomarkers of antioxidant and biotransformation systems are commonly used to assess polycyclic aromatic hydrocarbons (PAHs) pollution in fish. Despite their extensive application of biomarkers, contradictory results are vastly reported in the literature, even for the same species in similar contamination scenarios. This study aims to verify response patterns of biomarkers in fish exposed to PAHs. Through systematic reviews and meta-analyses, we were able to evaluate: *(i)* overall magnitude of PAHs effects on biotransformation and oxidative stress biomarkers; *(ii)* patterns of response among experimental approaches (laboratory, field and active biomonitoring), environment (marine and freshwater) and fish habitat (pelagic, demersal, etc.); *(iii)* effects of exposure route, time and concentration of PAHs; and (*iv*) which biomarkers respond best to PAHs exposure. Overall, biomarker responses were significantly affected by PAHs exposure. The activities of ethoxyresorufin-O-deethylase (EROD), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx) and levels of oxidized glutathione (GSSG) and lipid peroxide (LPO) significantly increased in fish exposed to PAHs, whereas catalase (CAT) and glutathione reductase (GR) activities and levels of reduced glutathione (GSH) were not affected. Amongst responsive biomarkers, EROD and GST activities significantly differed among approaches and between marine and freshwater environments, but were not affected by fish habitat. GSSG levels were higher in fish from laboratory bioassays compared to the field, but did not differ between environments nor habitats. Exposure route played a major role only for GST and GPx responses. Finally, increasing PAHs concentration and exposure time had a significant effect on all assessed biomarkers, except for CAT. We conclude that EROD and GST are robust biomarkers to assess PAHs effects in fish. Contrarily, CAT is an inadequate biomarker of PAHs exposure since no significant response was observed. Our study also highlighted some research gaps in PAHs contamination studies, such as a clear lack of active biomonitoring experiments.

1. Introduction

1.1. Polycyclic aromatic hydrocarbons in the aquatic environment

Polycyclic aromatic hydrocarbons (PAHs) represent a class of hydrophobic organic compounds ubiquitously found in freshwater and marine environments (Neff et al., 2005). Even though natural processes such as forest fires or oil seeps (Billiard et al., 2008) contribute to environmental input of PAHs, their major source is anthropogenic, mainly from the incomplete combustion of fossil fuels, wood, oil spills, and discharge from ships and sewage sludge (Suess, 1976; Douben, 2003; Abdel-Shafy and Mansour, 2016; Lawal, 2017). Because a variety of human activities are associated with the widespread contamination of PAHs, their potential toxic effects are continually and increasingly under investigation (Billiard et al., 2008; Rengarajan et al., 2015). Since 1991, PAHs have been ranked on the U.S. Agency for Toxic Substances and Disease Registry priority list (ATSDR, 1991, 2017), which suggests the risk PAHs pose to human health and environmental integrity.

PAHs may be taken up from sediments or directly from the water column into tissues of invertebrates and vertebrates,

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author.
E-mail address: manuelasantana.oc@gmail.com (M.S. Santana).

https://doi.org/10.1016/j.envpol.2018.07.004
0269-7491/

subsequently exerting toxicity through various mechanisms (Livingstone, 2001). Aquatic vertebrates, such as fish, have well-developed biotransformation systems capable of converting PAHs into water-soluble derivatives in order to facilitate excretion (Boelsterli, 2007). During this process, however, electrophilic reactive toxic metabolites may be generated, leading to mutagenesis, carcinogenesis, immunosuppression, as well as adverse developmental and reproductive effects (Tuvikene, 1995; Payne et al., 2003; Rengarajan et al., 2015). In addition, biotransformation of PAHs may lead to an imbalance between the production of pro-oxidant compounds and cellular antioxidants, favouring the former, and potentially leading to oxidative stress (Altenburger et al., 2003; Boelsterli, 2007).

Reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) are the most important pro-oxidants responsible for oxidative stress (Boelsterli, 2007), but potential damage to cells is normally limited by the presence of protective antioxidant defence mechanisms (Davies, 2000). Hence, several studies and monitoring programs have measured fish biotransformation and oxidative stress parameters in order to assess the quality of aquatic environments (Codi et al., 2004; Abrahamson et al., 2008; Lu et al., 2010; Barhoumi et al., 2014). These biochemical responses provide an early-warning signal of adverse effects of contamination and are commonly referred to as biomarkers (van der Oost et al., 2003).

1.2. Biotransformation and oxidative stress biomarkers

Biomarkers are early-warning signals of contamination measured in cells, body fluids, tissues and organs, and may indicate environmental impact before it can be observed at higher biological levels, such as populations and ecosystems (Vidal-Liñan et al., 2010). For the purpose of this review, we will focus on the response patterns of biochemical biomarkers commonly measured in fish livers. The liver contains a variety of PAHs biotransformation enzymes, robust antioxidant defences and is involved in the excretion of PAHs through bile (Oliveira-Ribeiro and Narciso, 2014; Boelsterli, 2007), and therefore, the majority of data on biochemical biomarkers is from fish liver.

Contaminants such as PAHs can be converted to more hydrophilic (water-soluble) compounds to facilitate excretion (Altenburger et al., 2003; Boelsterli, 2007). This biotransformation process is catalysed by phase I enzymes which oxidise, reduce or hydrolyse compounds; and phase II enzymes which catalyse the conjugation of chemical groups (cofactors) such as glutathione (GSH), sulphate and glucuronic acid. Cytochrome P450 isoforms (CYPs) are a large family of membrane-bound heme proteins involved in phase I reactions. The activity and amount of CYPs are important tools to assess environmental disturbances triggered by chemical contamination. For example, the activity of ethoxyresorufin-O-deethylase (EROD) of CYP1A subfamily members is a common and widely used biomarker of planar halogenated and polycyclic aromatic hydrocarbon contamination (Goksøyr and Förlin, 1992; van der Oost et al., 2003), although other CYPs such as CYP1B, CYP1C, and CYP3A can be involved with PAHs biotransformation (Shimada, 2006; Schlenk et al., 2008; Uno et al., 2012).

Glutathione *S*-transferase (GST) belongs to a superfamily of dimeric water-soluble enzymes responsible for a variety of functions, such as intracellular transport, biosynthesis of eicosanoids and more critically, the conjugation of glutathione (GSH) to xenobiotics in phase II reactions (George and Buchanan, 1990; Hayes et al., 2005). Even though GST activity is described as unpredictable (Altenburger et al., 2003; Hellou et al., 2012), it has been used as a biomarker in many ecotoxicological studies because of the significant role GST plays in detoxification of PAHs, DNA-reactive metabolites and oxidative stress products derived from lipid oxidation, nucleic acids and proteins (Schlenk et al., 2008; van der Oost et al., 2003). Like for CYPs, there are several isoforms of GST, with GSTT1 and GSTM1 homologs being involved in PAHs biotransformation (Shimada, 2006; Schlenk et al., 2008). However, ecotoxicological studies usually report the general activity of GSTs, assessed through enzymatic assays using a common substrate for all GST isoforms, the CDNB (2,4-dinitrochlorobenzene), rather than the activity of PAH-specific GSTs. Although there are other families of phase I and phase II enzymes involved in xenobiotic biotransformation, such as peroxidase, epoxide hydrolase, sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT), NAD(P)H quinone oxidoreductase 1, and aldo-keto reductase (Shimada, 2006; Boelsterli, 2007), the majority of studies focus on the EROD and GST activity to understand PAH contamination.

Formation of DNA and protein adducts by electrophilic PAH metabolites and induction of oxidative stress are recognised mechanisms of PAHs toxicity (Payne et al., 2003; Baird et al., 2005). In particular, CYP activity can generate large amounts of ROS that are usually neutralised or degraded by enzymatic and non-enzymatic antioxidant defence mechanisms (Altenburger et al., 2003; Boelsterli, 2007).

GSH is the most important non-enzymatic defence against ROS (Boelsterli, 2007). As a scavenger, GSH reacts with some ROS and radicals, producing GSSG (glutathione disulphide). As a cofactor, GSH is used by GST in detoxification and by glutathione peroxidase (GPx) in H_2O_2 degradation (Winston and Di Giulio, 1991; Davies, 2000). High levels of GSH are maintained by de novo synthesis and by glutathione disulphide reductase (GR)-dependent recycling of GSSG back to GSH (Zhu et al., 2013). Given this paramount role, GSH levels and related enzymes are vastly used as biochemical responses to contaminants (Hellou et al., 2012).

Superoxide dismutase (SOD) and catalase (CAT) are the first enzymatic line of antioxidant defence. SOD is responsible for converting superoxide anions ($O_2^{\bullet -}$) to H_2O_2, which in turn is converted to water by CAT (Abele et al., 2017). By decreasing the levels of $O_2^{\bullet -}$ and H_2O_2, SOD and CAT have an important role in avoiding the production of hydroxyl radicals ($HO^{\bullet}$) through Fenton/Harber-Weiss reactions (Boelsterli, 2007). Hydroxyl radicals are very unstable and reactive radicals are those that can oxidise many biomolecules, such as RNA, DNA, proteins and lipids, leading to toxicity (Farber, 1994; Di Giulio and Meyer, 2008). Thus, the activities of SOD and CAT have been measured in several studies as biomarkers for a variety of contaminants, though there is no consistent pattern of response to establish valid links between SOD-CAT activities and specific contaminants (van der Oost et al., 2003).

Although livers have robust antioxidant defences, excessive production of ROS and impairment of these defences (e.g., by inhibition of enzymatic activities) can cause oxidative damage to biomolecules (Di Guilio and Meyer, 2008). Lipid peroxidation (LPO) is a set of chain reactions triggered by radicals such as hydroxyl radical that can lead to loss of membrane integrity and secondary damage to DNA and proteins through adduct formation with toxic aldehydes produced from lipid peroxides (Davies, 2000). For these reasons, levels of LPO are also recognized as important oxidative stress biomarkers that can be evaluated by assays such as FOX (ferrous oxidation-xylenol orange; Jiang et al., 1992) for lipid hydroperoxides and TBARS (thiobarbituric acid reactive substances) for malondialdehyde (Davies, 2000; Valavanidis et al., 2006).

Even though these biomarkers are well established in the literature, the responses reported by many studies differ due to several sources of variation. Among them, experimental approach, fish species, habitat and environment (i.e. freshwater or marine) have been described as important moderators of contamination effects on fish (Whyte et al., 2000; Altenburger et al., 2003; Payne

et al., 2003; van der Oost et al., 2003; Hook et al., 2014). Therefore, it is paramount to understand how and to what degree each of these sources affect fish responses to PAHs exposure. Therefore, the aim of this review is to explore the scientific literature, in order to understand patterns of response of widely employed biotransformation and oxidative stress biomarkers from fish exposed to PAHs.

1.3. Motivation for meta-analysis

Since the mid-1980s, several studies have developed, described and recommended a broad number of biomarkers for environmental monitoring. A substantial amount of information has accumulated on fish biomarkers, including differences among experimental approaches, environments and species (Beyer et al., 1996; Kirby et al., 1999; Aas et al., 2000; Almroth et al., 2005; Jee et al., 2005; Kopecka-Pilarczyk and Correia, 2009; Lu et al., 2011; Huang et al., 2016). However, results are inconsistent with reports from similar contamination scenarios showing increased (Nogueira et al., 2011; Dussauze et al., 2015; Sadauskas Henrique et al., 2016), decreased (Deer et al., 2010; Theron et al., 2014) or even no alteration (Milinkovitch et al., 2011; Bettim et al., 2016) in biomarker responses.

There is only one quantitative review that has specifically explored EROD activity responses to PAHs (Oris and Roberts, 2007) and many narrative reviews (van der Oost et al., 2003; Whyte et al., 2000; Altenburger et al., 2003; Billiard et al., 2008) examining most of the biomarkers addressed herein. The lack of comprehensive reviews coupled with powerful and integrative analyses may be preventing the examination of general response patterns.

Thus, a thorough, systematic literature review and meta-analysis might be able to shed light on basic and recurring problems in ecotoxicological studies, quantify biomarkers response patterns and identify important sources of variation, such as experimental approaches, fish environment and habitats, route and time of exposure and PAH concentrations used in experimental assays. Additionally, these results may provide support for future experimental design protocols, requiring less sample processing and more power to detect the relationship between xenobiotic and oxidative stress.

1.4. Main questions and hypotheses

Several predictions were made about the strength and direction of PAHs effects on biomarker responses. First, we expect that the magnitude of PAHs effects will be stronger on fish exposed in laboratory compared to field-based studies due to naturally occurring and uncontrollable variables in the field, which may affect uptake and metabolism and are often not accounted for in laboratory settings, leading to an overestimation of response (Goodsell et al., 2009). For example, the bioavailable concentration of PAHs may be maintained throughout the experiment in the laboratory whereas rain, sunlight, microorganisms and sediment may decrease PAHs concentration through dilution, degradation and adsorption in the field (Burgess et al., 2003; Gong et al., 2014). It is also expected that marine and freshwater fish will respond differently to PAHs exposure due to significant physiological differences related to ionic and osmotic regulation, hepatic lipid storage and renal excretion mechanisms (Evans, 1987; Evans et al., 2005; Hinton et al., 2001; Kleinow et al., 2008). Additionally, river discharge, tidal currents and salinity are known to affect the retention, accumulation and transportation of PAHs (Liu et al., 2014), leading to differences in concentration and bioavailability of PAHs between marine and freshwater environments.

Moreover, we presume that fish habitats will influence biomarker responses, specifically, demersal species will have a stronger response to PAHs than pelagic species. Considering that sediment is regarded as an ultimate sink where several classes of contaminants deposit and persist for long periods of time (Cardoso et al., 2016), bottom-associated fish may be more vulnerable due to ingestion of contaminated invertebrates or sediment (Hugla et al., 1995; Gonçalves et al., 2014; Duarte et al., 2017). Additionally, we expect that in laboratory experiments, PAHs delivered through injection techniques (e.g., intraperitoneal injection) will elicit stronger biomarker responses compared to other routes of exposure (i.e. water, diet or sediment) as described by Whyte et al. (2000). Finally, the magnitude of PAHs effects will be stronger in fish exposed for longer periods of time, both in the field and laboratory, due to higher doses/concentrations of PAHs, as described in many primary studies included in our systematic review.

Based on these predictions, we focused on the following questions: *(i)* what is the global net magnitude of the effect of PAHs on fish biotransformation and oxidative stress biomarker responses? *(ii)* does the strength and direction of PAHs effects vary among experimental approaches, environments, habitats and exposure routes? *(iii)* does time and dose dictate the direction of PAHs effects? *(iv)* which are the most responsive biomarkers to PAHs exposure?

2. Methods

2.1. Search strategy and database

Our literature search strategy involved two approaches. First, we extensively searched the literature reporting effects of PAHs on fish biotransformation and oxidative stress biomarkers using the extended database (timespan: “all years”) from *PubMed, Scopus, ScienceDirect* and *Web of Science* until September 2016. The following search strategies were applied in all databases and then cross-referenced against one another providing a final set of studies (3895 publications):

Search 1: (fish* OR teleost*) AND (contamina* OR pollut*)

Search 2: oxidative stress OR antioxidant* OR oxidant* OR oxidative damage OR biomarker* OR biotransformation OR superoxide dismutase OR catalase OR glutathione OR glutathione peroxidase OR lipid peroxidation OR glutathione S-transferase OR ethoxyresorufin-O-deethylase OR EROD OR SOD OR CAT OR GPx OR GST OR GSH OR LPO OR OR MDA OR TBARS.

Search 3: PAHs OR hydrocarbon* OR oil* OR petroleum OR polycyclic organic matter OR polynuclear aromatic hydrocarbon* OR polynuclear aromatics OR polynuclear hydrocarbon*

Second, during our scoping stage of literature search, a seminal paper widely referenced in the field of ecotoxicology was identified: van der Oost et al., 2003. We examined its reference list in order to identify studies that were not published in journals indexed in the databases we had searched. A total of 51 papers were located using this approach. Searches from databases and reference list strongly overlapped, therefore, all duplicates were removed before screening. Next, based on title and abstract, irrelevant papers were excluded and potentially suitable studies were obtained for further examination. All steps of our literature search are summarized in Fig. 1 (PRISMA flowchart – the Preferred Reporting Items in Systematic Reviews and Meta-Analysis, Moher et al., 2009).

We have not requested unpublished data from colleagues or added papers to our dataset after September 2016 since that might lead to sampling bias. Additionally, we have not searched the grey literature, seeing that unpublished studies may have quality and data reliability issues, and ultimately compromise our analysis (Kelly and Jennions, 2011).

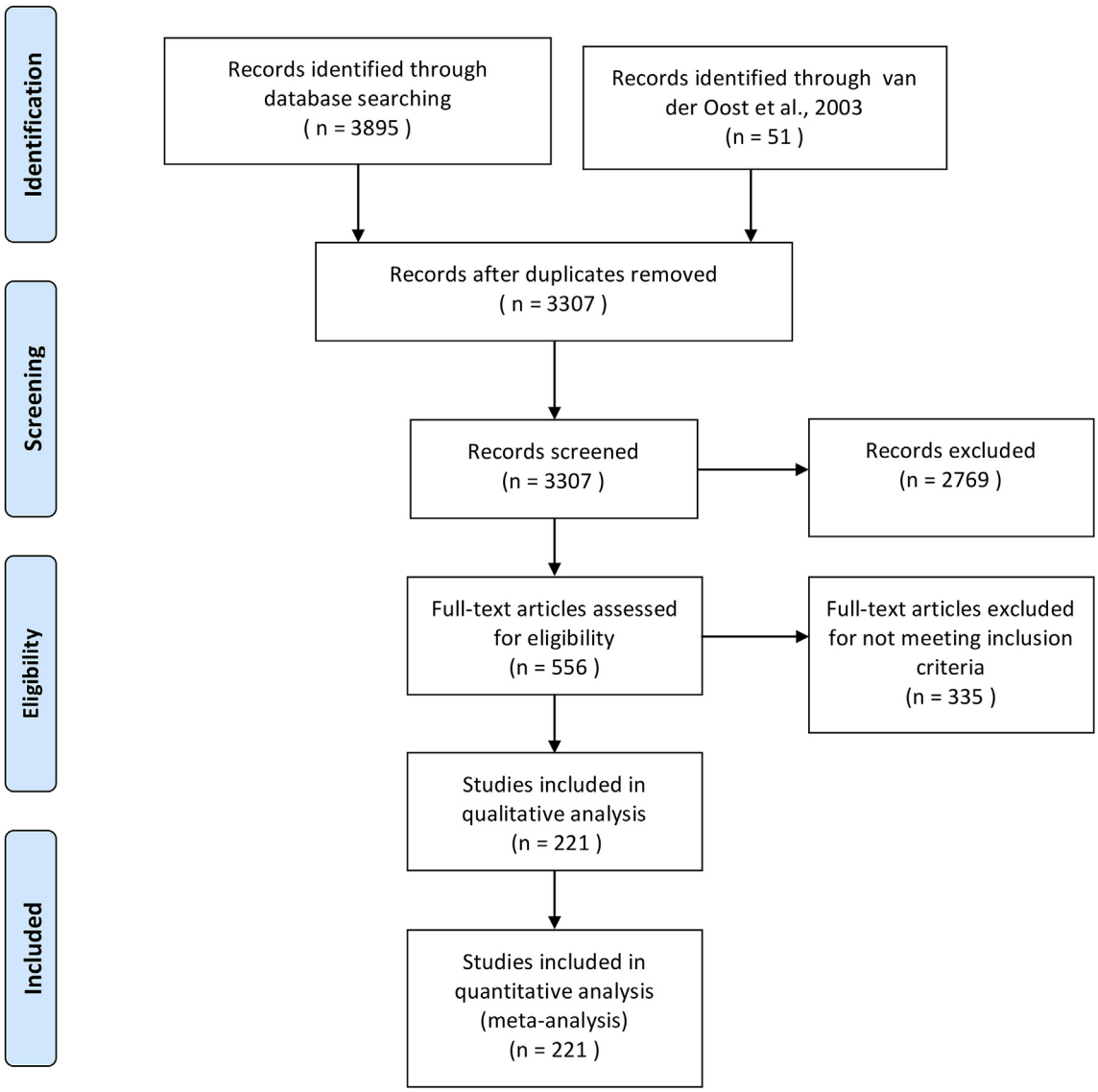

Fig. 1. The screening process used to exclude or retain articles for our meta-analysis; summarized and formatted as a PRISMA flow diagram (Moher et al., 2009).

2.2. Inclusion criteria and data extraction

All peer-reviewed articles had to meet the following inclusion criteria: *(i)* studies had to address our main research question (i.e. PAHs effects on fish biotransformation and oxidative stress biomarkers) *(ii)* studies had to contain extractable data (e.g., mean, standard deviation, sample size); as well as unambiguous details on study design; and *(iii)* both field and laboratory studies had to provide a clear comparison of biomarker responses after exposure to PAHs (impact/treatment setting), and under conditions where PAHs were absent (reference/control setting); *(iv)* only *in vivo* studies that used fish liver to assess PAHs effects were considered.

Field experiments involving the transplant and caging of fish obtained from clean sites to contaminated conditions, both in marine and freshwater environments, were referred as active biomonitoring (ABM) experiments. This practice differs from field approach because fish, usually with known histories of breeding and maintenance, are exposed for a specific amount of time to natural settings.

Since both field and laboratory experiments were included, there were specific criteria for each. For laboratory experiments, only exposure times higher than 12 h were considered, and when studies had two controls; *(i)* fish exposed to the vehicle used to solubilise PAHs, and *(ii)* fish that did not undergo any treatment, only the latter was considered. As for field studies, whenever there were two reference sites, data from both were pooled together as described by Borenstein et al. (2009).

Moreover, fish were categorized as marine or freshwater depending on how authors described the study area or the exposure medium. For example, if fish were acclimated and exposed using seawater, then the species was considered marine, even if they can be found in freshwater or estuaries. When the description was not clear, we searched for information on fish distribution in order to make a decision.

We extracted means, standard deviation and sample size from each study from text, figures and tables. If authors reported another measure of variability (e.g., confidence intervals, standard error or coefficient of variation), we converted them to standard deviations when possible. We used GraphClick (version 3.0.3, Arizona Software, 2012) to obtain data from graphs when needed. Also, we

contacted authors and requested for data whenever it was not reported for biomarkers responses.

Most studies reported response outcomes for more than one period of time, dose, impacted sites, fish species or type of PAH. Such data was extracted and treated as independent studies, which guarantees all information from included studies are accounted for, therefore increasing statistical power (Côté et al., 2013). However, this approach leads to nonindependence among estimates, a common problem in meta-analyses that can affect study conclusions by inflating significance levels for statistical tests and underestimating the standard error of the mean effect (Noble et al., 2017). We dealt with nonindependence by *(i)* treating experiments from the same paper that were conducted in different years or seasons, or in any other setting with independent controls, as independent comparisons; and by *(ii)* modelling sources of nonindependence among estimates (study ID, species and country) and fitting a variance-covariance matrix (described in Meta-analyses section).

2.3. Meta-analysis

The magnitude of PAHs effects on fish biomarker responses was evaluated by converting the data from primary studies to standardized mean difference (SMD) or Hedge's *g*. We selected this effect size because it is designed to compare two means and it includes a correction for small sample sizes (Nakagawa and Santos, 2012; Rosenberg et al., 2013). Standardized differences are >0 when PAHs exposure triggers biomarker responses in fish and their levels are higher than in fish from reference/control settings, and <0 when the opposite occurs.

We performed meta-analyses separately for each of the nine biomarkers, i.e. there is a unique data set for each biomarker. We fitted linear mixed effects models (LMM) with the effect size SMD as the response variable along with its relative sampling error V_{SMD}. We also fitted a variance-covariance matrix in all models to account for nonindependence among effect sizes, which originates from the comparison of a single control with multiple treatments.

For each data set, we ran two different models, an intercept-only model (standard meta-analysis) including only study ID as a random effect to estimate the global effect for every biomarker and a meta-regression adding moderators (i.e. fixed-effects) to the intercept-only model. We chose moderators for the meta-regressions based mostly on what fixed effects are relevant to the questions previously stated in the introduction; experimental approach, environment, habitat, route of exposure, and time and concentration.

Meta-analyses were conducted as follows:

1) Intercept-only model to assess global effect for each biomarker (only ID as random-effect);
2) Type of approach laboratory (Lab), field or active biomonitoring (ABM) was added to intercept-only model to evaluate if effects differ among them. Differences among study ID were added as random-effect. Active biomonitoring refers to the practice of transplanting and fish obtained from clean sit to contaminated field conditions.
3) Within each approach, we ran a meta-analysis assessing the environment marine or freshwater as a source of variation. We included ID, species and country as random effects. Country was added as random effects to our models because we believe that some observations were originated from related research teams from the same country, which may be a source of nonindependence among estimates.

In addition, two-sample t-tests were used to verify if there were differences between marine and freshwater fish baseline levels (i.e. the means form control groups) for each biomarker;

4) Then, we ran a meta-analysis within each environment using habitat (demersal, benthopelagic, reef-associated, demersal, pelagic) as fixed effect and ID, species and country as random effects;
5) Within ABM approach, time of exposure (days) to contaminated sites was set as a fixed effect, and again ID, species and country as random effects;
6) Finally, within Lab approach, exposure routes (diet, injection, water or sediment) and concentration × time interaction were separately assessed as sources of variation, and the type of PAHs (chemical) along with ID, species and country was added as random effects.

For the concentration X time model, we created a specific data set. Since there are different exposure routes and several PAHs, it would be unfeasible to assess the effect of each PAH-exposure route combination separately. Thus, the aforementioned data set consists of all studies that added PAHs (isolated compounds or mixtures of them) to the water tanks of experimental setups, using the same or convertible concentration units. All concentrations were standardized to mg/l.

The consistency among effect sizes was assessed by calculating the overall heterogeneity (I^2_{total}), and I^2 for each random effect as described by Nakagawa and Santos (2012). Values of 25%, 50% and 75% are defined as low, moderate and high levels of heterogeneity, respectively (Higgins et al., 2003). Additionally, total heterogeneity (Q_T) was obtained through Wald-type tests and comparisons between moderators were conducted using Cochran's Q-test (Q_m; Hedges and Olkin, 1985).

Effect size calculations and meta-analyses were performed using the metafor package (version 1.9–9; Viechtbauer, 2010) within the R open-source software environment (version 3.3.1; R Core Team, 2016).

2.4. Publication bias

We assessed potential publication bias in the global effect of each biomarker using two methods. First, we calculated Rosenberg's fail-safe number, which is a statistically improved version of Rosenthal's fail-safe number (Jennions et al., 2013). This method estimates how many additional studies with a mean centred on the null value (i.e. non-significant studies), and with the same average sample size as the included studies, are needed to change the results from significant to non-significant ($P > 0.05$).

Additionally, we visually tested all data sets using funnel plots. Effect sizes were plotted against their corresponding precision values (the inverse of the square root of the sampling variance), and the resulting plots were checked for funnel asymmetry. Publication bias analyses were also performed using the metafor package within R.

3. Results

3.1. Database and overall effects

The search protocol identified 3307 papers that had their title and abstract scanned to identify studies suitable for our meta-analyses. After this initial step, 556 papers were fully screened, i.e. their methods and results sections were read and assessed, and 221 papers were deemed eligible for inclusion. Within those, a total of 1290 estimates of effect of PAHs were computed from 471 independent studies covering several fish species, habitats and from

different experimental approaches (see Appendix A). PAHs effects were assessed mostly through laboratory experiments (69% of estimates) conducted in Europe (44%).

Overall, PAHs had a significant effect on most biomarker responses, i.e. biomarker activity and levels from fish exposed to PAHs (treatment) were higher compared to fish from control/reference settings (Figs. 2–4). EROD, GST, SOD, GPx, GSSG and LPO increased in fish exposed to PAHs, while CAT, GR and GSH levels were not altered after exposure. Given the high amount of heterogeneity (Table S1 – Appendix C), further analyses were conducted exploring differences among approaches, environments and habitats.

3.2. PAHs effects through different approaches and at different environments (marine vs freshwater)

For the biotransformation biomarkers, the effects of PAHs exposure significantly differed among approaches (Fig. 2, Table S2). EROD activity was higher in fish from laboratory bioassays than in active biomonitoring species, but there were no differences between field and other approaches ($Q_m = 12.115$, df = 2, $P = 0.0023$). GST activity from laboratory bioassays was lower and differed only from active biomonitoring experiments ($Q_m = 6.125$, df = 2, $P = 0.047$). As for oxidative stress biomarkers, no significant difference was observed between laboratory and field approaches. An exception to this was for GR activity, which was higher in fish collected in contaminated areas in the field ($Q_m = 5.153$, df = 1, $P = 0.0232$), and GSSG levels that were higher in fish from laboratory bioassays ($Q_m = 4.2622$, df = 1, $P = 0.039$).

Due to high heterogeneity, we performed further analyses within each approach to identify potential moderators of variability. First, we verified if there were differences between marine and freshwater fish baseline levels with a two-sample *t*-test. Except for GPx and GR, no differences between freshwater and marine control means were found for most biomarkers (Table S3). In laboratory experiments, significant differences between marine and freshwater fish were observed only in EROD and GST activities (Fig. 2, Table S2). EROD activity measured in fish transferred from a clean site to contaminated environments (ABM approach) did not differ between marine and freshwater species, whereas despite the absence of effect, GST activity was different between these environments (Table S5). Finally, biomarkers from fish sampled from contaminated sites (Field approach) were similar at both marine and freshwater environments, except for EROD (Fig. 2a) and GR (Fig. 3d) activities, which were higher in freshwater fish (Table S2, S4-S5). Total heterogeneity remained high, and for most biomarkers, a large proportion of it is due to variability among effect sizes (I^2_{ID}).

3.3. PAHs effects at different habitats

Within each environment, biotransformation and most of oxidative stress biomarkers showed no significant difference among habitats, except for GSH levels that were higher on benthopelagic fish compared to demersal fish sampled in contaminated freshwater environments (Figs. S1–S3 – Appendix D). We have also quantified I^2 values for the random effects added to the model and these along with other details are given in Tables S3–S5.

3.4. Exploring further sources of variation within ABM approach

Time had no significant effect on EROD ($Q_m = 0.3589$, $P = 0.5491$) and GST ($Q_m = 0.2641$, $P = 0.607$) activities from fish transferred to contaminated sites (Fig. 5). We have also estimated ID, species and country as random effects. For EROD, the residuals accounted for all variance ($I^2_{ID} = I^2_{total} = 78.35\%$), indicating high variability at the level of effect sizes. As for GST, the largest proportion of total variance was due to differences among species

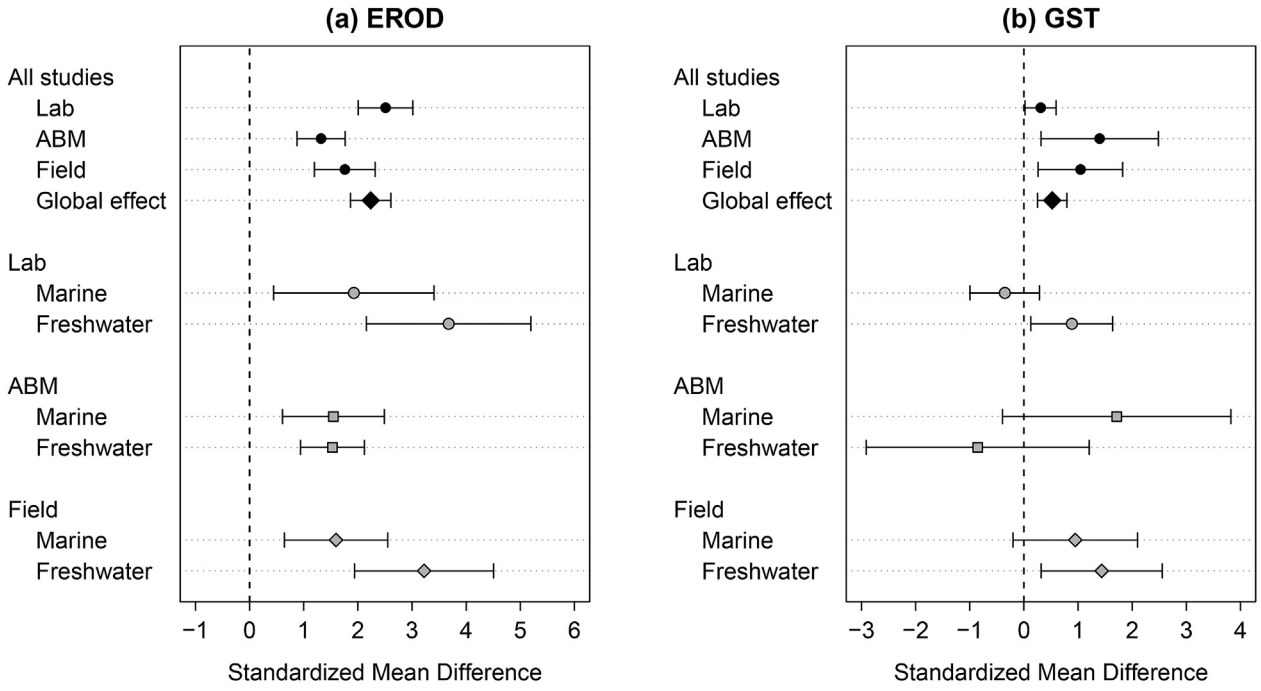

Fig. 2. Effects of polycyclic aromatic hydrocarbons (PAHs) exposure on fish biotransformation biomarkers. (a) ethoxyresorufin-O-deethylase (EROD) and (b) glutathione S-transferase (GST) from laboratory (Lab), active biomonitoring (ABM) and field experiments. Within each experimental approach, the effects on marine and freshwater fish are shown. Dots represent effect size estimates (standardized mean difference – SMD) and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

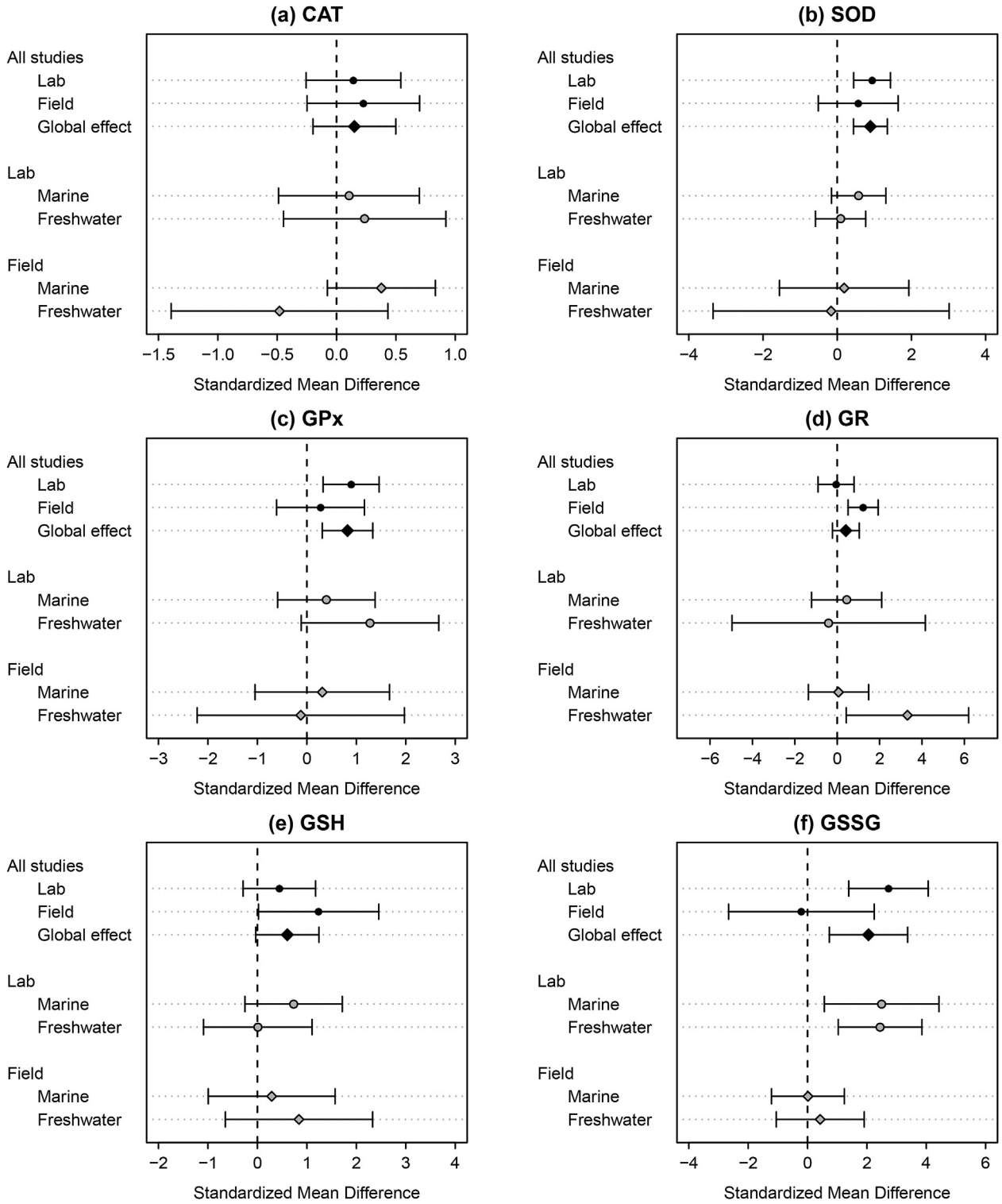

Fig. 3. Effects of polycyclic aromatic hydrocarbons (PAHs) exposure on fish oxidative stress biomarkers. (a) catalase (CAT), (b) superoxide dismutase (SOD), (c) glutathione peroxidase (GPx), (d) glutathione disulphide reductase (GR), (e) glutathione (GSH) and (f) glutathione disulphide (GSSG) from laboratory (Lab) and field experiments. Within each experimental approach, the effect on marine and freshwater fish are shown. Dots represent effect size estimates (standardized mean difference – SMD) and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

($I^2_{species} = 41.39\%$, $I^2_{ID} = 22.94\%$, $I^2_{country} = 27.3\% \rightarrow I^2_{total} = 91.63\%$).

3.5. Exploring further sources of variation within lab approach

First, we assessed the effect of exposure route on biomarker responses, adding the types of PAHs (chemicals) as random effects along with ID, species and country. For most of them, there was no effect, except for GST and GPx activities (Table S6). GST activity was higher in fish exposed to PAHs added to sediment than fish exposed via diet, injection or water (Sediment > Diet = Injection = Water).

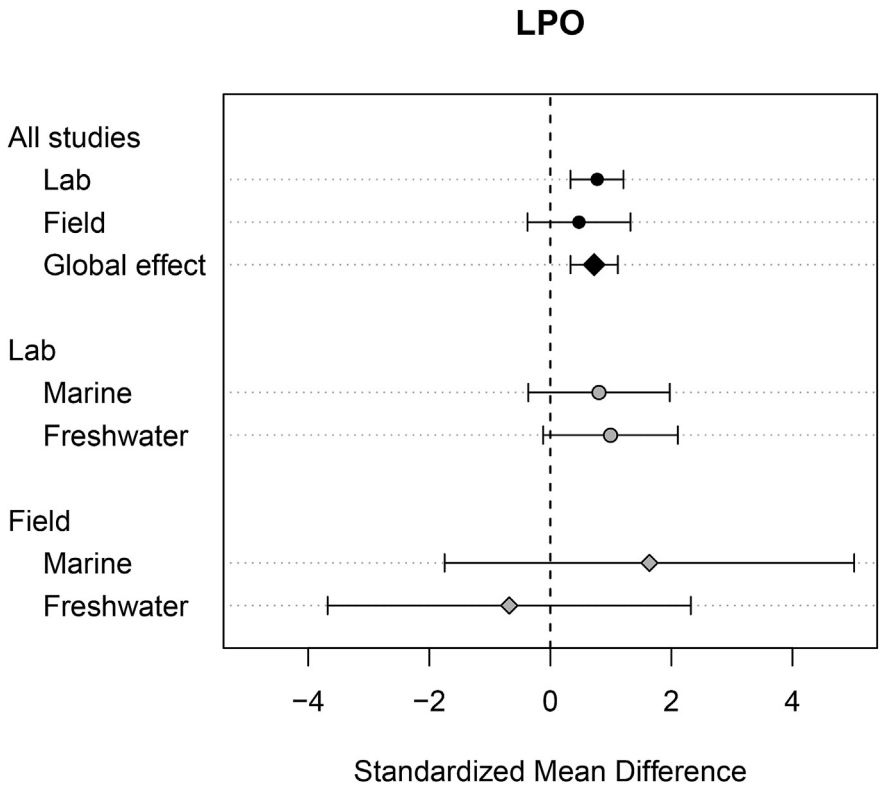

Fig. 4. Effects of polycyclic aromatic hydrocarbons (PAHs) exposure on fish lipid peroxidation (LPO) from laboratory (Lab) and field experiments. Within each experimental approach, the effect on marine and freshwater fish are shown. Dots represent effect size estimates (standardized mean difference – SMD) and error bars represent 95% confidence intervals. Effects are considered different from 0 if 95% confidence intervals do not cross 0 (dashed line).

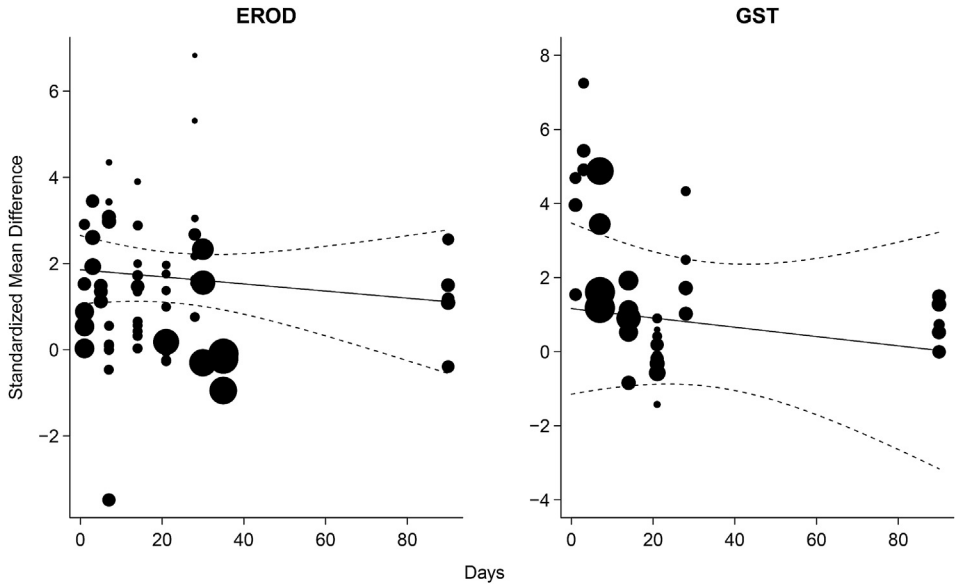

Fig. 5. Effects of polycyclic aromatic hydrocarbons (PAHs) through time in fish transferred from a clean site to a contaminated site (active biomonitoring approach). Time ranged from 12 h to 90 days. Circles represent effect size estimates (standardized mean difference – SMD) and their relative weight (circle size). Dashed lines indicate 95% confidence intervals.

Fish exposed to PAHs added to sediments also showed higher and significantly different GPx activity than fish exposed via water or injection (Sediment > Injection = Water).

The largest proportion of variance was mostly associated with the residuals for several biomarkers, as the chemicals did not explain much of the variance in most cases except for GSH and GSSG levels. For GR activity and LPO levels, the variability was mostly due to differences among countries (Table S6).

We have also assessed the effect of different concentrations of PAHs in water through time (interaction Concentration X Time) on biomarker responses, adding to the model ID, species, country and chemicals as random effects. Except for CAT, GSH and GSSG,

Concentration X Time had a significant effect on most biomarker responses (Table S7). Neither concentration, time nor interaction had a significant effect on CAT activity, and most of the variance was associated with the residuals ($Q_m = 5.982$, $P = 0.1125$). GSH levels were only affected by time, meaning that for each increase in unit of time, the difference between control and treatment means also increases ($Q_m = 110.827$, $P < 0.0001$). As for GSSG, only concentration had a significant effect on its levels, which also means that a unit increase in concentration leads to an increase in mean effect ($Q_m = 20.795$, $P = 0.0001$).

The meta-regressions also found other notable effects. First, there is a significantly negative effect of the interaction between concentration and time on SOD ($Qm = 19.558$, $P = 0.0002$) and LPO ($Q_m = 643.995$, $P < 0.0001$), i.e. the effect of PAHs concentration on these biomarkers decreases by 0.0328 and 0.288, respectively, for every unit increase in time. Additionally, the type of PAHs accounted for very little of the heterogeneity observed in most biomarkers data sets, aside from GSH, which had 36.77% of total variance associated with differences among PAHs ($I^2_{total} = 89.66\%$). Conversely, country accounted for half or more of the variance found for GST, GR, GSSG and LPO. Details on model results and the amount of heterogeneity explained by each random effect are shown in Table S7.

3.6. Publication bias

Funnel plots of all biomarkers were visually inspected (Fig. S4 – Appendix D) and a slight asymmetry was detected in EROD and GPx plots, however, only very few effect sizes (ca. 10) were responsible for this asymmetry. Rosenberg's fail-safe number for each biomarker was as follows: EROD – 930118; GST – 16976; CAT – 0; SOD – 8679; GPx – 9012; GR – 558; GSH – 0; GSSG – 1193; and LPO – 7401. For both CAT and GSH, the number of studies needed to be added was 0 because the main effect size was not statistically significant.

Although unrelated, both methods found little evidence for publication bias. Therefore, our results might be unbiased and robust.

4. Discussion

The overall effect of PAHs on biomarker responses (Table 1) was meta-analytically quantified to test their reliability as research tools since these biomarkers are commonly and widely employed to detect and assess contamination by oil-related compounds. Our meta-analyses have revealed that the overall effect of PAHs on fish is deleterious since the activity and levels of most biomarkers were altered after exposure, with the exception of CAT, GR and GSH for which PAHs showed no significant effect. These findings are compelling by themselves, but the source of variation investigated herein gives us a broader sense of how PAHs exposure is affecting fish worldwide. Below we discuss the importance of our findings for every source of variation accounted in the analyses.

4.1. The role of experimental approach, environment and habitat

Due to the established link between PAH exposure and CYP1A induction, described in several scenarios, EROD activity is expected to increase in fish captured at contaminated sites and exposed in laboratory settings. An extensive examination on EROD activity, covering mechanisms of induction by several classes of contaminants and the main factors influencing the intensity and direction of responses, has been conducted (Whyte et al., 2000; Billiard et al., 2008). Based on these narrative reviews, PAHs are regarded as a weak/moderate inducer of EROD activity both in marine and freshwater species captured in the field or experimentally exposed in laboratory (Whyte et al., 2000; Billiard et al., 2008). Similarly, we expected alterations in all biomarkers even though they are not specific to PAHs contamination. Our meta-analyses were able to corroborate several findings from primary studies and narrative reviews, such as the induction of EROD activity and overall changes in the response patterns of oxidative stress biomarkers after PAHs exposure.

Differences found between experimental approaches were expected, specifically the large magnitude of effects observed in fish from laboratory exposure. Laboratory assays and caging experiments (i.e. active biomonitoring) are designed to understand particular mechanisms of action and determine causality (Goodsell et al., 2009). Because presumed effects of contamination are isolated and hardly simulate the complexity of the natural

Table 1
Summary of biomarker responses to PAHs exposure.

Biomarker	Overall	Approach	Environment	Habitat	Exposure Route	Concentration X Time (waterborne PAHs)	Summary
EROD	✓	✓	✓	×	×	✓	↑ Lab ↑ freshwater fish ↑ concentration X time
GST	✓	✓	✓	×	✓	✓	↑ Field and ABM, ↑ freshwater fish ↑ sediment exposures ↑ concentration X time
CAT	×	×	×	×	×	×	Non-responsive
SOD	✓	×	×	×	×	✓	↓ concentration X time
GPx	✓	×	×	×	✓	✓	↑ sediment exposures ↑ concentration X time
GR	×	✓	✓	×	×	✓	↑ Field ↑ freshwater fish ↑ concentration X time
GSH	×	×	×	✓	×	✓	↑ Freshwater benthopelagic fish ↑ time
GSSG	✓	✓	×	×	×	✓	↑ Lab ↑ concentration
LPO	✓	×	×	×	×	✓	↓ concentration X time

'✓' indicates that the source of variation influences the biomarkers response. '×' indicates that the biomarkers response is not influenced by the predicted source of variation. "↑" indicates increased activity/level and "↓" indicates decreased activity/level.

environment, these approaches often overestimate the responses by fish to any contamination scenario (Forbes et al., 2006), as seen in the increased EROD activity and GSSG levels from fish exposed in the laboratory. Biotic and abiotic variables in the environment, such as rain, sunlight, microorganisms and sediment texture may determine the fate PAHs and consequently the response of organisms (Burgess et al., 2003; Gong et al., 2014). In addition, it should be mentioned that biomarkers can be extremely variable in field conditions and this have a strong impact in the required sample size to detect significant differences between sites (Gagnon and Hodson, 2012). Nonetheless, GST and GR activities were higher in the field than in laboratory exposure. Since they are both less PAH-specific than EROD (Hellou et al., 2012; van der Oost et al., 2003), their induction might be a response to other pollutants present at contaminated sites.

Freshwater fish showed increased EROD activity both in field and laboratory settings compared to marine fish. These different responses were also expected due to inherent physiological differences between marine and freshwater fish, such as osmotic regulation and renal excretion mechanisms (Evans et al., 2005; Kleinow et al., 2008). However, previous studies have recommended caution in the interpretation of these findings since EROD activity baseline levels may significantly vary among species, rendering any degree of induction to be pronounced (Flammarion and Garric, 1997; Balk et al., 1996; Whyte et al., 2000). Nevertheless, by directly comparing reference means from freshwater and marine fish using a two-sample *t*-test, no differences were observed. Therefore, our findings suggest that rather than a variation in baseline levels of EROD, the increased activity observed in freshwater fish might reflect greater susceptibility to PAHs exposure from these species compared to their marine counterparts. The same logic might be applied to GST since there is no difference between control means from freshwater and marine species. GR activity was higher in freshwater fish in the field, but the baseline levels from marine species were significantly higher than freshwater species, suggesting that the difference detected here are more related to intrinsic differences among species. It is important to note that it is difficult to generalize GR differences between environments since there was only one freshwater species studied (*Leuciscus cephalus*).

Additionally, we presumed fish habitat would play an important role in determining the magnitude of PAHs effects since benthic species are more exposed to PAHs and other persistent organic pollutants that are known to associate with aquatic sediments and accumulate in invertebrates (Logan, 2007; Gonçalves et al., 2014; Duarte et al., 2017). However, this was only true for GSH levels in freshwater fish captured at contaminated sites (Benthopelagic > Demersal, Fig. S3). Specifically, for this particular biomarker there were only two freshwater species to compare, the chub (*Leuciscus cephalus*) and the European eel (*Anguilla anguilla*). Clearly there is a lack of field experiments conducted in PAHs contaminated freshwater environments to make any assumption on species sensitivity or habitat vulnerability.

4.2. The role of PAHs exposure routes

We presumed the route of exposure would influence the strength and direction of PAHs effects on biomarkers. More specifically, fish exposed through injection techniques (i.e. intraperitoneal or intramuscular injection) were expected to show increased biomarker responses compared to fish from dietary, waterborne or sediment exposure. Although it does not represent a natural uptake mechanism, previous studies have described a significant increase of EROD activity in fish injected with several PAHs compounds (Pacheco and Santos, 1998; Reynaud et al., 2002; Banni et al., 2009). However, there were no differences among exposure routes for EROD, potentially indicating that PAHs are strong inducers of CYP1A regardless of the delivery method employed in laboratory-based experiments.

Moreover, the response of most biomarkers to PAHs administered via sediment, injection, diet or water are similar, suggesting that even though there was an overall response to PAHs exposure, the exposure route is not an important source of variation. Nevertheless, GST and GPx activities significantly varied among exposure routes. PAHs in the sediment led to a stronger induction of these enzymes compared to water, injection and dietary exposures. Differences in the composition and concentrations of PAHs present in the sediments in comparison to the other routes may explain this finding, since not all PAHs induce GST or are able to promote a redox unbalance that can induce GPx (Altenburger et al., 2003).

4.3. The effect of time and concentration

Contrary to what was expected, the period of exposure in controlled field settings (i.e. ABM experiments) did not affect EROD and GST activities. The relationship between time and these enzyme activities are not always linear and predictive, especially in the natural environment where both inducers, repressors and inhibitors may be present as a complex mixture of contaminants that influences biomarker responses (Sarkar et al., 2006). Induction of EROD activity has been described as high immediately after exposure followed by a steady decline (Whyte et al., 2000), whereas GST activity has been described as quite variable (van der Oost et al., 2003; Hellou et al., 2012). However, a visual analysis of the scatterplots (Fig. 5) indicates no clear pattern between time and biomarker activity. The lack of ABM experiments, for periods of time ranging between 40 and 90 days, is a potential research gap that needs to be fulfilled in order to properly investigate if there is a response-time relationship.

Even though there was no relationship between time of exposure and biomarker response from caging experiments in the field, in laboratory-based experiments, the combination of concentration and time significantly affected the strength of waterborne PAHs effects, as we predicted. Biomarker behaviour towards concentration and time are quite unpredictable, with some biomarkers responding only to specific compounds immediately after exposure while others responding to lower doses and longer time periods (Forbes et al., 2006). However, our analyses revealed that most biomarkers positively related to increasing exposure time and concentrations, except for SOD activity and LPO levels, which decreased with time at a given concentration.

Superoxide dismutase is essential for the dismutation of superoxide in hydrogen peroxide, which is further degraded by GPx, CAT and peroxiredoxins. There are different pools of SOD; a mitochondrial matrix pool (Mn-SOD), and other pools (Cu,Zn-SOD) that are strictly regulated to maintain appropriate levels of hydrogen peroxide and superoxide inside the cells (Fridovich, 1995; Scandalios, 2002). For these reasons, induction of SOD expression may occur immediately after exposure to neutralize the excess of superoxide anion, but once the initial insult is neutralized, SOD activity may reduce steadily, resulting in an overall decrease over time. Varying reports of SOD activity were extracted from the studies reviewed herein, with induction or inhibition occurring after 24 h or 48 h (Lemaire et al., 1996; Dussauze et al., 2015; Nogueira et al., 2013), or no significant changes occurring over time and concentrations. In many cases, a combined effect of time and concentration on biomarker response does exist, however, studies often assess concentration and time independently through one-way ANOVA or multiple t-tests, even when the experimental design requires more complex analyses (Ahmad et al., 2003; Jifa

et al., 2006; Gunawickrama et al., 2008; Nogueira et al., 2013; Frantzen et al., 2015), which may prevent the detection of time X concentration effects as identified here. Additionally, lipid peroxidation levels usually increase with time and concentration, but in our analyses, we were able to verify a decrease in LPO with time at a given concentration. Given all biomarker responses, which mostly increased, it is possible that cellular defences are constantly working to protect cells from eventual burdens.

Another noteworthy finding is that the combined effect of exposure time and concentration of waterborne PAHs did not significantly affect GSH and GSSG levels. Instead, GSH content increased over time regardless PAHs concentration and GSSG increased across concentrations regardless of the exposure time. GSH provides protection against ROS in various disease models and the ratio of GSH/GSSG is kept high in order to maintain normal cellular function and prevent oxidative stress injury (Zhu et al., 2013). Increased GSH levels and GR activity reflect the robustness of the antioxidant system, which dynamically functions to avoid damage to important biomolecules as demonstrated by the reduced levels of LPO.

The combined results of all biomarkers analysed in this dataset provide new insights on how biomarkers behave to PAHs exposure in water, and how cellular redox homeostasis may be maintained.

5. Conclusions

(1) We initially proposed to answer the question, what are the most responsive biotransformation and oxidative stress biomarkers to assess PAHs effects on fish? By covering environment, habitat, exposure route, concentration and time as sources of variation within experimental approaches, we were able to set forth a relevant suite of biomarkers to comprehend the effects induced by PAHs exposure, which may help future ecotoxicological studies.

(2) Among the biomarkers analysed herein, EROD is the most specific to PAHs exposure. Increased and strong responses occur in exposed fish regardless of habitat or route of exposure, indicating that EROD activity is a robust biomarker suitable for a variety of fish species and experimental scenarios. Conversely, CAT is an inadequate biomarker to assess and understand PAHs exposure since no significant response was observed, neither overall, nor relative, to experimental approaches. Therefore, it would not be relevant to measure this enzyme activity in order to establish a causal relationship to PAHs contamination.

(3) GST is an important, albeit, non-specific biomarker. Increased induction at contaminated sites suggests that GST strongly responds to environmental contamination, but the response is not specific to a class of contaminants. In addition, GST along with GPx were the only biomarkers that significantly differed among exposure routes, with high activities observed in experiments using contaminated sediments, suggesting that the exposure route is important to elicit a response from these enzymes.

(4) Even though GR had no overall response, freshwater fish captured at contaminated sites showed significantly increased activities. This difference between freshwater and marine fish might be due to the lack of experiments measuring this biomarker in freshwater environments contaminated by PAHs. Therefore, more research is needed to determine whether GR is indeed a reliable biomarker for PAHs contamination. Likewise, there were only a few experiments conducted in the field for GSH, hindering conclusions.

(5) Lipid peroxidation had an overall increase to PAHs exposure. Nevertheless, no difference was found among moderators, indicating that LPO is an important biomarker for PAHs effects despite approach, environment or habitat, so that LPO should be considered for environmental contamination assessments even if it is not specific to any class of contaminants.

(6) We were able to identify some research gaps to be fulfilled by future studies. First, there is a lack of active biomonitoring studies using oxidative stress biomarkers to assess PAHs contamination and exposing fish for periods of time between 40 and 80 days. This approach is important to establish links between responses found in the laboratory and field, and therefore further investigation would help us understand how fish are affected by PAHs. Additionally, biomarkers such as GSH, GR and GSSG are crucial to understand both pollutant biotransformation and oxidative stress. However, few studies measured these biomarkers on freshwater and marine species, hindering a clear conclusion.

Acknowledgements

Manuela S. Santana acknowledges a scholarship from CAPES (grant number 1620747) (Coordination for Improvement of Higher Education Staff).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.07.004.

References

Aas, E., Baussant, T., Balk, L., Liewenborg, B., Andersen, O.K., 2000. PAH metabolites in bile, cytochrome P4501A and DNA adducts as environmental risk parameters for chronic oil exposure: a laboratory experiment with Atlantic cod. Aquat. Toxicol. 51, 241–258.

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian J. Petroleum 25, 107–123.

Abele, D., Vázquez-Medina, J.P., Zenteno-Savín, T., 2017. Introduction to oxidative stress in aquatic ecosystems. In: Abele, D., Vázquez-Medina, J.P., Zenteno-Savín, T. (Eds.), Oxidative Stress in Aquatic Ecosystems. John Wiley & Sons, Ltd, Chichester, UK, pp. 1–5.

Abrahamson, A., Brandt, I., Brunstrom, B., Sundt, R.C., Jorgensen, E.H., 2008. Monitoring contaminants from oil production at sea by measuring gill EROD activity in Atlantic cod (Gadus morhua). Environ. Pollut 153, 169–175.

Ahmad, I., Pacheco, M., Santos, M.A., 2003. Naphthalene-induced differential tissue damage association with circulating fish phagocyte induction. Ecotoxicol. Environ. Saf. 54, 7–15.

Almroth, B.C., Sturve, J., Berglund, A., Forlin, L., 2005. Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. Aquat. Toxicol. 73, 171–180.

Altenburger, R., Segner, H., Oost, R., 2003. Biomarkers and PAHs – prospects for the assessment of exposure and effects in aquatic ecosystems. In: Douben, P.E.T. (Ed.), PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Ltd, Chichester, UK, pp. 297–328.

ATSDR, 1991. Support document to the 1991 substance priority list. In: Substance and Non-substance Addiction, vol. 1010. Springer Singapore, Singapore, pp. 1–12.

ATSDR, 2017. Support document to the 2017 substance priority list. In: Substance and Non-substance Addiction, vol. 1010. Springer Singapore, Singapore, pp. 1–12.

Baird, W.M., Hooven, L.A., Mahadevan, B., 2005. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ. Mol. Mutagen 45, 106–114.

Balk, L., Larsson, Å., Förlin, L.R., 1996. Baseline studies of biomarkers in the feral female perch (*Perca fluviatilis*) as tools in biological monitoring of anthropogenic substances. Mar. Environ. Res. 42, 203–208.

Banni, M., Bouraoui, Z., Ghedira, J., Clerandeau, C., Guerbej, H., Narbonne, J.F., Boussetta, H., 2009. Acute effects of benzo[a]pyrene on liver phase I and II enzymes, and DNA damage on sea bream Sparus aurata. Fish Physiol. Biochem. 35, 293–299.

Barhoumi, B., Clerandeau, C., Gourves, P.Y., Le Menach, K., El Megdiche, Y., Peluhet, L., Budzinski, H., Baudrimont, M., Driss, M.R., Cachot, J., 2014. Pollution

biomonitoring in the Bizerte lagoon (Tunisia), using combined chemical and biomarker analyses in grass goby, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae). Mar. Environ. Res. 101, 184–195.
Bettim, F.L., Galvan, G.L., Cestari, M.M., Yamamoto, C.I., De Assis, H.C., 2016. Biochemical responses in freshwater fish after exposure to water-soluble fraction of gasoline. Chemosphere 144, 1467–1474.
Beyer, J., Sandvik, M., Hylland, K., Fjeld, E., Egaas, E., Aas, E., Skare, J.U., Goksoyr, A., 1996. Contaminant accumulation and biomarker responses in flounder (Platichthys flesus) and Atlantic cod (*Gadus morhua*) exposed by caging to polluted sediments in Sorfjorden, Norway. Aquat. Toxicol 36, 75–98.
Billiard, S.M., Meyer, J.N., Wassenberg, D.M., Hodson, P.V., Di Giulio, R.T., 2008. Nonadditive effects of PAHs on early vertebrate development: mechanisms and implications for risk assessment. Toxicol. Sci. 105, 5–23.
Boelsterli, U.A., 2007. Mechanistic Toxicology: the Molecular Basis of How Chemicals Disrupt Biological Targets, 2 ed. Taylor and Francis, London.
Borenstein, M., Hedges, L.V., Higgins, J.P.T., Rothstein, H.R., 2009. Introduction to Meta-analysis. John Wiley & Sons, Ltd.
Burgess, R.M., Ahrens, M.J., Hickey, C.W., 2003. Geochemistry of PAHs in aquatic environments: source, persistence and distribution. In: PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Chichester, UK, pp. 35–45.
Cardoso, F.D., Dauner, A.L., Martins, C.C., 2016. A critical and comparative appraisal of polycyclic aromatic hydrocarbons in sediments and suspended particulate material from a large South American subtropical estuary. Environ. Pollut. 214, 219–229.
Codi, S., Humphrey, C., Klumpp, D., Delean, S., 2004. Barramundi as an indicator species for environmental monitoring in North Queensland, Australia: laboratory versus field studies. Environ. Toxicol. Chem. 23, 2737–2744.
Côté, I.M., Jennions, M.D., 2013. The procedure of meta-analysis in a nutshell. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution. Princenton University Press, UK, pp. 14–24.
Davies, K.J.A., 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50, 279–289.
Deer, A.K., Henczova, M., Banka, L., Varanka, Z., Nemcsok, J., 2010. Effects of crude oil and oil fractions on the liver P450-dependent monooxygenase activities and antioxidant defence system of different freshwater fish species. Acta Biol. Hung 61, 262–273.
Di Giulio, R.T., Meyer, J.N., 2008. Reactive oxygen species and oxidative stress. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, Florida, pp. 273–324.
Douben, P.E.T., 2003. Introduction. In: Douben, P.E.T. (Ed.), PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Ltd, Chichester, UK, pp. 1–6.
Duarte, R.M., Sadauskas-Henrique, H., De Almeida-Val, V.M.F., Val, A.L., Nice, H.E., Gagnon, M.M., 2017. Biomarker responses and PAH ratios in fish inhabiting an estuarine urban waterway. Environ. Toxicol 32, 2305–2315.
Dussauze, M., Danion, M., Le Floch, S., Lemaire, P., Pichavant-Rafini, K., Theron, M., 2015. Innate immunity and antioxidant systems in different tissues of sea bass (*Dicentrarchus labrax*) exposed to crude oil dispersed mechanically or chemically with Corexit 9500. Ecotoxicol. Environ. Saf 120, 270–278.
Evans, D.H., 1987. The fish gill: site of action and model for toxic effects of environmental pollutants. Environ. Health Perspect. 71, 47–58.
Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.
Farber, J.L., 1994. Mechanisms of cell injury by activated oxygen species. Environ. Health Perspect. 102, 17–24.
Flammarion, P., Garric, J., 1997. Cyprinids EROD activities in low contaminated rivers: a relevant statistical approach to estimate reference levels for EROD biomarker? Chemosphere 35, 2375–2388.
Forbes, V.E., Palmqvist, A., Bach, L., 2006. The use and misuse of biomarkers in ecotoxicology. Environ. Toxicol. Chem. 25, 272–280.
Frantzen, M., Hansen, B.H., Geraudie, P., Palerud, J., Falk-Petersen, I.B., Olsen, G.H., Camus, L., 2015. Acute and long-term biological effects of mechanically and chemically dispersed oil on lumpsucker (*Cyclopterus lumpus*). Mar. Environ. Res. 105, 8–19.
Fridovich, I., 1995. Superoxide radical and superoxide dismutases. Annu. Rev. Biochem. 64, 97–112.
Gagnon, M.M., Hodson, P.V., 2012. Field studies using fish biomarkers – how many fish are enough? Mar. Pollut. Bull. 64, 2871–2876.
George, S.G., Buchanan, G., 1990. Isolation, properties and induction of plaice liver cytosolic glutathione-S-transferases. Fish Physiol. Biochem 8, 437–449.
Goksøyr, A., Forlin, L., 1992. The cytochrome P450 system in fish, aquatic toxicology, and environmental monitoring. Aquatic Toxicol. 22, 287–312.
Gonçalves, C., Martins, M., Diniz, M.S., Costa, M.H., Caeiro, S., Costa, P.M., 2014. May sediment contamination be xenoestrogenic to benthic fish? A case study with Solea senegalensis. Marine Environ. Res. 99, 170–178.
Gong, Y., Zhao, X., Cai, Z., O'reilly, S.E., Hao, X., Zhao, D., 2014. A review of oil, dispersed oil and sediment interactions in the aquatic environment: influence on the fate, transport and remediation of oil spills. Mar. Pollut. Bull. 79, 16–33.
Goodsell, P.J., Underwood, A.J., Chapman, M.G., 2009. Evidence necessary for taxa to be reliable indicators of environmental conditions or impacts. Mar. Pollut. Bull. 58, 323–331. Elsevier Ltd.
Gunawickrama, S.H., Aarsaether, N., Orbea, A., Cajaraville, M.P., Goksoyr, A., 2008. PCB77 (3,3',4,4'-tetrachlorobiphenyl) co-exposure prolongs CYP1A induction, and sustains oxidative stress in B(a)P-exposed turbot, *Scophthalmus maximus*, in a long-term study. Aquat. Toxicol 89, 65–74.
Hayes, J.D., Flanagan, J.U., Jowsey, I.R., 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45, 51–88.
Hedges, L.V., Olkin, I., 1985. Statistical Methods for Meta-analysis. Academic Press, San Diego.
Hellou, J., Ross, N.W., Moon, T.W., 2012. Glutathione, glutathione S-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. Environ. Sci. Pollut. Control Ser. 19, 2007–2023.
Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. Br. Med. J. 327, 557–560.
Hinton, D.E., Segner, H., Au, D.W.T., Kullman, S.W., Hardman, R.C., 2001. Toxic responses of the liver. In: Schlenk, D., Benson, W.H. (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts. Taylor & Francis, London, pp. 248–298.
Hook, S.E., Gallagher, E.P., Batley, G.E., 2014. The role of biomarkers in the assessment of aquatic ecosystem health. Integrated Environ. Assess. Manag 10, 327–341.
Huang, G.Y., Liu, Y.S., Liang, Y.Q., Shi, W.J., Hu, L.X., Tian, F., Chen, J., Ying, G.G., 2016. Multi-biomarker responses as indication of contaminant effects in *Gambusia affinis* from impacted rivers by municipal effluents. Sci. Total Environ. 563–564, 273–281.
Hugla, J.L., Philippart, J.C., Kermess, P., Goffinet, G., Thome, J.P., 1995. PCB contamination of the common barbel, *Barbus barbus* (Pisces, Cyprinidae), in the river Meuse in relation to hepatic monooxygenase activity and ultrastructural liver change. Neth. J. Aquatic Ecol. 29, 135–145.
Jee, J.H., Kang, J.C., 2005. Biochemical changes of enzymatic defense system after phenanthrene exposure in olive flounder, *Paralichthys olivaceus*. Physiol. Res. 54, 585–591.
Jennions, M.D., Lortie, C.J., Rosenberg, M.S., Rothstein, H.R., 2013. Publication and related biases. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution. Princenton University Press, United Kingdom, pp. 207–236.
Jiang, Z.Y., Hunt, J.V., Wolff, S.P., 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxidein low density lipoprotein. Anal. Biochem. 202, 384–389.
Jifa, W., Yu, Z., Xiuxian, S., You, W., 2006. Response of integrated biomarkers of fish (*Lateolabrax japonicus*) exposed to benzo[a]pyrene and sodium dodecylbenzene sulfonate. Ecotoxicol. Environ. Safety 65, 230–236.
Kelly, C.D., Jennions, M.D., 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. Biol. Rev. 86, 863–884.
Kirby, M.F., Matthiessen, P., Neall, P., Tylor, T., Allchin, C.R., Kelly, C.A., Maxwell, D.L., Thain, J.E., 1999. Hepatic EROD activity in flounder (*Platichthys flesus*) as an indicator of contaminant exposure in English estuaries. Mar. Pollut. Bull. 38, 676–686.
Kleinow, K.M., Nichols, J.W., Hayton, W.L., Mckim, J.M., Barron, M.G., 2008. Toxicokinetics in fish. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, Florida, pp. 55–152.
Kopecka-Pilarczyk, J., Correia, A.D., 2009. Biochemical response in gilthead seabream (Sparus aurata) to in vivo exposure to a mix of selected PAHs. Ecotoxicol. Environ. Saf 72, 1296–1302.
Lawal, A.T., 2017. Polycyclic aromatic hydrocarbons. A review. Cogent Environ. Sci. 71, 1–89.
Lemaire, P., Förlin, L., Livingstone, D.R., 1996. Responses of hepatic biotransformation and antioxidant enzymes to CYP1A-inducers (3-methylcholanthrene, β-naphthoflavone) in sea bass (*Dicentrarchus labrax*), dab (*Limanda limanda*) and rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 36, 141–160.
Liu, F., Yang, Q., Hu, Y., Du, H., Yuan, F., 2014. Distribution and transportation of polycyclic aromatic hydrocarbons (PAHs) at the Humen river mouth in the Pearl River delta and their influencing factors. Mar. Pollut. Bull. 84, 401–410.
Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656–666.
Logan, D.T., 2007. Perspective on ecotoxicology of PAHs to fish. Hum. Ecol. Risk Assess. 13, 302–316.
Lu, G.H., JI, Y., Zhang, H.Z., Wu, H., Qin, J., Wang, C., 2010. Active biomonitoring of complex pollution in Taihu Lake with Carassius auratus. Chemosphere 79, 588–594.
Lu, G.H., Qi, P.D., Qin, J., Wang, C., 2011. Biomarker responses in fish exposed to sediments from northern Taihu Lake. Bull. Environ. Contam. Toxicol 87, 499–505.
Milinkovitch, T., Ndiaye, A., Sanchez, W., Le Floch, S., Thomas-Guyon, H., 2011. Liver antioxidant and plasma immune responses in juvenile golden grey mullet (*Liza aurata*) exposed to dispersed crude oil. Aquat. Toxicol 101, 155–164.
Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., Prisma Group, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 6, e1000097.
Nakagawa, S., Santos, E.A., 2012. Methodological issues and advances in biological meta-analysis. Evol. Ecol. 26, 1253–1274.
Neff, J.M., Stout, S.A., Gunstert, D.G., 2005. Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: identifying sources and ecological hazard. Integrated Environ. Assess. Manag. 1, 22–33.
Noble, D.W.A., Lagisz, M., O'dea, R.E., Nakagawa, S., 2017. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. Mol. Ecol 26, 2410–2425.
Nogueira, L., Da Silva, D.G., Oliveira, T.Y., Da Rosa, J.M., Felicio, A.A., De Almeida, E.A., 2013. Biochemical responses in armored catfish (*Pterygoplichthys anisitsi*) after short-term exposure to diesel oil, pure biodiesel and biodiesel blends. Chemosphere 93, 311–319.

Nogueira, L., Rodrigues, A.C., Tridico, C.P., Fossa, C.E., De Almeida, E.A., 2011. Oxidative stress in Nile tilapia (*Oreochromis niloticus*) and armored catfish (*Pterygoplichthys anisitsi*) exposed to diesel oil. Environ. Monit. Assess. 180, 243–255.

Oliveira-Ribeiro, C.A., Narciso, M.F., 2014. Histopathological markers in fish health assessment. In: Almeida, E.A., Oliveira Ribeiro, C.A. (Eds.), Pollution and Fish Health in Tropical Ecosystems. CRC Press Boca Raton, pp. 206–242.

Oris, J.T., Roberts, A.P., 2007. Statistical analysis of cytochrome P4501A biomarker measurements in fish. Environ. Toxicol. Chem. 26, 1742–1750.

Pacheco, M., Santos, M.A., 1998. Induction of liver EROD and erythrocytic nuclear abnormalities by cyclophosphamide and PAHs in *Anguilla anguilla L.* Ecotoxicol. Environ. Saf 40, 71–76.

Payne, J.F., Mathieu, A., Collier, T.K., 2003. Ecotoxicological studies focusing on marine and freshwater fish. In: PAHs: an Ecotoxicological Perspective. John Wiley & Sons, Ltd, Chichester, UK, pp. 191–224.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., Nishigaki, I., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific J. Tropical Biomedicine 5, 182–189.

Reynaud, S., Marionnet, D., Taysse, L., Duchiron, C., Deschaux, P., 2002. The effects of 3-methylcholanthrene on macrophage respiratory burst and biotransformation activities in the common carp (*Cyprinus carpio L.*). Fish Shellfish Immunol 12, 17–34.

Rosenberg, M.S., Rothstein, H., Gurevitch, J., 2013. Effect sizes: conventional choices and calculations. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution. Princeton University Press, Princeton, pp. 61–71.

Sadauskas-Henrique, H., Braz-Mota, S., Duarte, R.M., De Almeida-Val, V.M.F., 2016. Influence of the natural Rio Negro water on the toxicological effects of a crude oil and its chemical dispersion to the Amazonian fish Colossoma macropomum. Environ. Sci. Pollution Res. 23, 19764–19775.

Sarkar, A., Ray, D., Shrivastava, A.N., Sarker, S., 2006. Molecular biomarkers: their significance and application in marine pollution monitoring. Ecotoxicology 15, 333–340.

Scandalios, J.G., 2002. Oxygen stress and superoxide dismutases. Plant Physiol. 101, 1–6.

Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., van den Hurk, P., Willett, K., 2008. Biotransformation in fishes. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, Florida, pp. 153–234.

Shimada, T., 2006. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. Drug Metabol. Pharmacokinet. 21, 257–276.

Suess, M.J., 1976. The environmental load and cycle of polycyclic aromatic hydrocarbons. Sci. Total Environ. 6, 239–250.

Theron, M., Bado-Nilles, A., Beuvard, C., Danion, M., Dussauze, M., Ollivier, H., Pichavant-Rafini, K., Quentel, C., Le Floch, S., 2014. Chemical dispersion of crude oil: assessment of physiological, immune and antioxidant systems in juvenile turbot (*Scophthalmus maximus*). Water Air Soil Pollut. 225, 1–17.

Tuvikene, A., 1995. Responses of fish to polycyclic aromatic hydrocarbons (PAHs). Ann. Zool. Fenn. 32, 295–309.

Uno, T., Ishizuka, M., Itakura, T., 2012. Cytochrome P450 (CYP) in fish. Environ. Toxicol. Pharmacol 34, 1–13.

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Safety 64, 178–189.

van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol 13, 57–149.

Vidal-Liñan, L., Bellas, J., Campillo, J.A., Beiras, R., 2010. Integrated use of antioxidant enzymes in mussels, Mytillus galloprovincialis, for monitoring pollution in highly productive coastal areas of Galicia (Spain. Chemosphere 78, 265–272.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. J. Stat. Software 36, 1–48.

Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol 30, 347–570.

Winston, G.W., Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19, 137–161.

Zhu, H., Wang, J., Santo, A., Li, Y., 2013. Downregulation of antioxidants and phase 2 proteins. In: Villamena, F.A. (Ed.), Molecular Basis of Oxidative Stress: Chemistry, Mechanisms, and Disease Pathogenesis. John Wiley & Sons, New Jersey, pp. 113–121.