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Review article

Use of wild trout for PBDE assessment in freshwater environments: Review and summary of critical factors

Juan M. Ríos [a], Nerina B. Lana [a, b], Paula Berton [a, b], Néstor F. Ciocco [b, c], Jorgelina C. Altamirano [a, b, *]

[a] Laboratorio de Química Ambiental, Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales (IANIGLA)-CONICET, Mendoza, P.O Box 131, 5500 Mendoza, Argentina
[b] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, 5500 Mendoza, Argentina
[c] Instituto Argentino de Investigación de Zonas Áridas (IADIZA)-CONICET, Mendoza, P.O Box 507, 5500 Mendoza, Argentina

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ABSTRACT

Certain wild animals represent sentinels to address issues related to environmental pollution, since they can provide integrative data on both pollutant exposure and biological effects. Despite their technological benefits, PBDEs are considered a threat to environmental health due to their persistence, toxicity, and capacity to be accumulated. These pollutants have been found geographically widespread in fish, particularly in predator species such as trout. The aim of this work is to critically review the applicability and usefulness of wild trout for assessing PBDEs in freshwater environments. Reviewed reports include data from highly industrialized areas as well as areas from remote regions with relatively low human activity, including European and North American great lakes and freshwater environments in Europe, Greenland, subarctic areas and Patagonia, respectively. A summary of relevant factors were grouped into organism-specific factors (food habits, age, size, lipid content, sex and reproduction, tissue type, mechanism of contaminant uptake and metabolism), and PBDE levels in the surrounding environment (sediment). Five wild trout species [rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), arctic char (*Salvelinus alpinus*), and brook trout (*Salvelinus fontinalis*)], collected worldwide within the 1994 to present time frame, were considered. Multivariate techniques (principal component analysis-PCA) and mapping approach, showed clear differences in geographic distribution patterns of PBDE levels in trout depending on the region studied: wild trout from European and North American great lakes have the highest PBDE loads. This pattern could be due to high industrial activity at these locations. A correlational approach used to explore intraspecific relationships between PBDE levels and morphometry, showed positive relationships only for brown trout. Further, brown trout showed the highest trout-to-sediment ratios, which is suggestive of a relatively greater capacity of this species to accumulate PBDEs in relation to sediment levels. Overall, results suggest that adult wild trout could be useful as a PBDE bioindicator.

* Corresponding author. Laboratorio de Química Ambiental, Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales (IANIGLA)-CONICET, Mendoza, P.O Box 131, 5500 Mendoza, Argentina.
E-mail addresses: jrios@mendoza-conicet.gob.ar (J.M. Ríos), blana@mendoza-conicet.gob.ar (N.B. Lana), pberton@mendoza-conicet.gob.ar (P. Berton), nciocco@mendoza-conicet.gob.ar (N.F. Ciocco), jaltamirano@mendoza-conicet.gob.ar (J.C. Altamirano).

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Contents

1. Introduction

A challenge task in environmental assessments has been to measure pollutant concentrations in key ecological compartments (e.g. air, soil, sediment and biota) and then make toxicological judgements based on their known or suspected health effects [1,2]. Certain wild animals represent models, or sentinels, to address issues related to environmental pollution, since they can provide integrative data on both exposure (i.e., information on type, amount, distribution of contaminants) and effects (i.e., information on biological responses). Several freshwater invertebrates and vertebrates were claimed as valuable models for monitoring PBDEs: amphipod crustaceans (*Echinogammarus stammeri*) [3], decapod crustaceans (*Macrobrachium nipponense* and *Eriocheir sinensis*) [4], birds (*Larus argentatus*, *Uria aalge* and *Sturnus* spp) [2,5], and fish species, especially those with fish-eating behaviour like salmonids [6,7].

Polybrominated diphenyl ethers (PBDEs) save lives by serving as flame retardants in a wide variety of commercial and household products [2]. Despite their benefits, PBDEs pose a threat to environmental and human health due to their persistence, bioaccumulation potential, and adverse effects on the nervous, reproductive, and endocrine systems [8,9]. PBDEs are categorized into three technical formulations according to their degree of bromination; penta-, octa-, and deca-BDE commercial mixtures. Penta- and octa-BDE formulations are included in the Stockholm Convention [10]. Even though many countries have banned or restricted the use of two (penta- and octa-) of the three technical mixtures, the presence of lower brominated diphenyl ethers which are predominant in the so-called penta-BDE mixture has been detected in freshwater organisms (invertebrates, fish, birds) collected worldwide [2,9].

Trout are a member of the Salmonidae family, distributed worldwide in cold and temperate aquatic ecosystems, and easily reared in captivity [11,12]. Freshwater wild trout species have characteristics helpful to being included in environmental pollution studies [13,14]. Furthermore, humans and many species of trout inhabit similar ecosystems and are exposed to common climates, food sources, and pollutants [15–17]. To assess the extent of PBDE contamination in freshwater environments, trout present several advantageous biological characteristics (including being at top of the food web, having lipid-rich tissues, pollutant concentration capacity, a wide geographic distribution due to their phenotypic plasticity that allows them to successfully invade new ecosystems), in addition to their sporting and gastronomic worth [18–22]. Therefore, information derived from wild trout may be more useful to the study of PBDE levels and distribution than models of lower aquatic trophic levels [23,24]. Over the last 20 years, there has been a widespread use of wild trout for PBDE assessment in freshwater environments (Table A.1, Fig. 1). This review summarizes this field of research, and considers the implications of the reported data with the aim to explore the feasibility of wild trout as a sentinel species for PBDEs in environmental health assessments.

2. Strategy of this review

Relevant biological features of trout and key results from reports on PBDE levels in wild trout species inhabiting freshwater environments were summarized. Subsequently, certain critical factors were statistically analysed to explore patterns of PBDE levels among trout species worldwide. Since other top predator fish, such as wild anadromous salmon species, have been claimed as a suitable tool for PBDE assessments [6]; this review focuses this issue on freshwater wild trout species. Because free-living wildlife can integrate ecological factors and real world complexities, only field studies within the 1994 to present time frame were referred to. Therefore, studies conducted on farmed trout were not included in this review. In order to capture target publications, the literature was examined using the key words: trout* AND char* AND PBDEs* AND BFRs* AND polybrominated* AND sediment* AND freshwater environment*. Each publication fitting the above criteria was compiled in a database containing the following information: trout species, PBDE levels in different tissue types, including muscle, liver or whole body; lipid content; and trout morphometry. Additionally, PBDE levels in sediment of the studied region were considered. In all cases, the average PBDE levels reported were used. Both, georeferenced sampling sites and the year when samples were taken were included in the database. Most publications provided data in

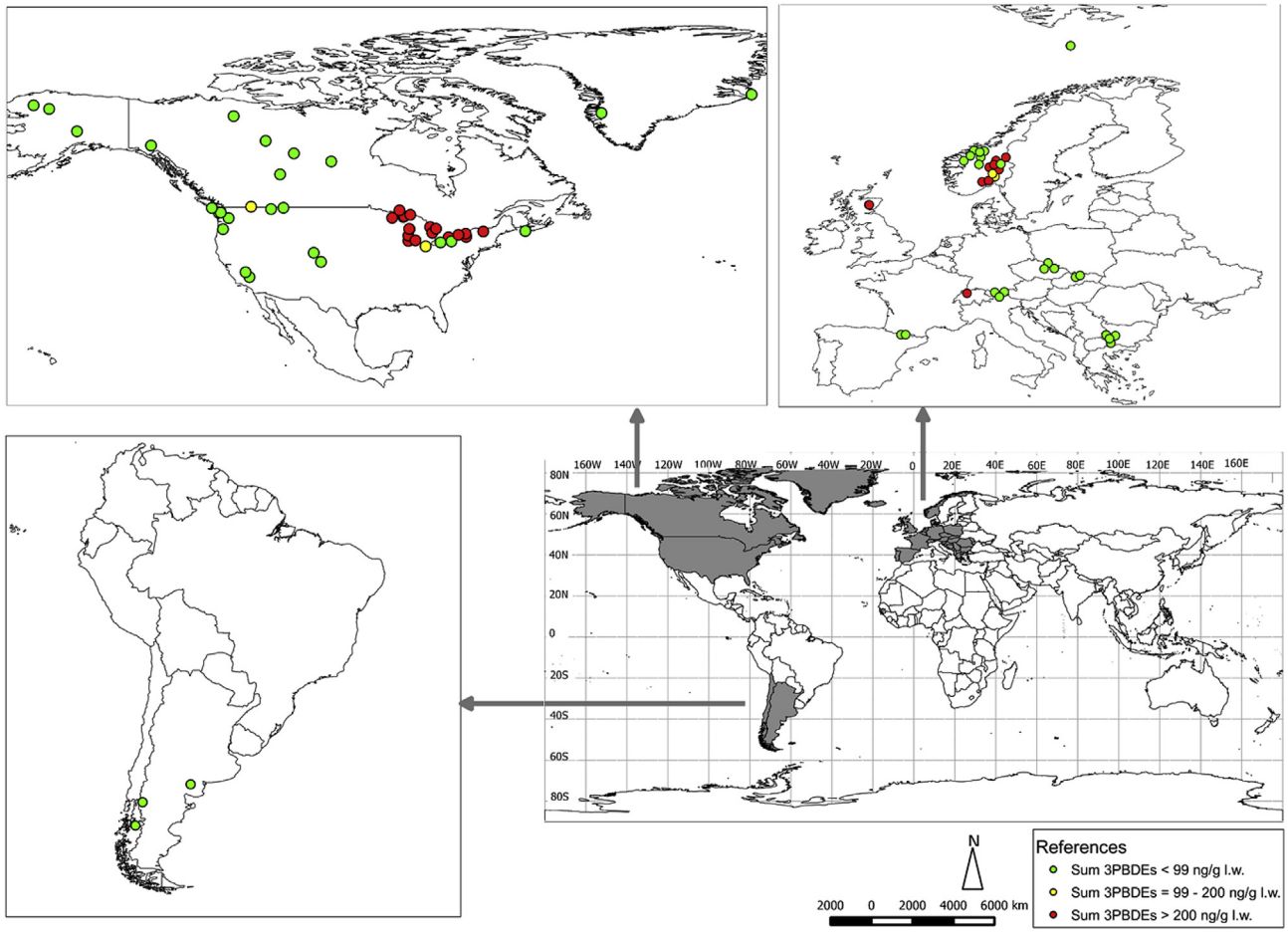

Fig. 1. Global distribution patterns and PBDEs hot-spots based on sum of three major congeners concentrations (i.e. the sum of BDE-47, -99, -100) in wild trout species and geo-referenced sampling locations in Europe, North and South America.

tabular format; however, only in a few cases was the corresponding author contacted to clarify interpretation. Recommendations for future studies to facilitate an objective determination of global benchmarking for local fish contamination are also discussed in following sections.

3. Summary of critical factors – trout biology

Pollutant levels in fish depend on the contaminant's capacity to bioconcentrate and biomagnify in a particular species [24]. Bioconcentration and biomagnifying processes are defined by the chemical's capacity to accumulate in organisms and be transferred between trophic levels, thus leading to a stepwise increase in contamination [25]. Bioconcentration and biomagnification can be determined from field data in which sampled organisms are exposed to chemicals in water and in the diet, respectively [25]. These processes depend not only on the physical–chemical properties of the pollutant, but also on biological factors of importance in the species assessed. The critical factors outlined below include food habits, age, size, lipid content, tissue type, fish sex and reproductive stage, mechanisms of contaminant uptake and metabolism.

3.1. Food habits

Trout are top predators with an opportunistic feeding behaviour and a broad diet [26]. Their primary food items depend in part on the habitat occupied as well as on prey availability [27]. Trout feed on items characteristic of the benthos (organisms associated with the substrate or with other solid materials), and on items from the water column (drifting in streams and zooplankton and nekton in lakes) and from the water surface (animals that have fallen into the water or are swimming). Fry trout restricted to quiet waters feed on small insects and other invertebrates, including nematodes, amphipods, cladocerans, and many types of both terrestrial and aquatic insects [28]. The diet of adult trout is even more diversified, including a wide assortment of fish as major prey items, aquatic and terrestrial insects, other aquatic invertebrates including decapod crustaceans, gastropods and other molluscs, and fish eggs and larvae [27,28]. Knowledge of trout feeding behaviour will enhance assessment of potential pollutant sources and biomagnification processes.

3.2. Fish age and size (weight and length)

Biomagnification and bioconcentration of contaminants in fish are directly related to the age or size of the fish [29]. Age may influence contaminant levels as it is a measure of the time an organism has been exposed to a contaminant. However, this correlation among contaminant levels, chemical exposure time, and organism age is useful until a steady state is reached between the contaminant in the organism and its ambient environment, after which age on its own becomes useless [18,26]. This time to steady state is long for piscivorous fish including trout, which have

slow contaminant elimination rates, and accumulation of PBDEs with age thus exceeds losses due to metabolism and excretion [30–32]. Previous reports have shown significant positive correlations between trout age and liver levels of BDE-47, -99, and -100 [22]. In contrast, this correlation was not observed between age and PBDE levels in muscle. The authors suggested that this difference could reflect that the amounts of PBDEs arriving to high-mountain fish are not yet in steady-state conditions [22].

Size (either length or weight) is used as an alternative to age because these measurements are easier to obtain [18]. However, the relationship between age and size is generally not consistent through time, with growth rate typically greater in younger individuals and decreasing with time and age [33]. Further, any change in environmental conditions over time may influence the organism's growth [34]. In such cases, accounting for size alone would not eliminate the influence of exposure time. Organism size can also have a direct influence on pollutant bioaccumulation which differs from that of age, since it is indicative of piscivory. This means that trout tend to consume only what they can swallow, so larger trout will usually eat larger prey and thus may feed at a higher trophic level than smaller trout. In addition, basal metabolic rate, which can affect contaminant uptake and detoxification rates, may vary more with animal size compared to age at species-specific level [35]. However, it is worthwhile to mention that correlation between PBDE levels and trout size is controversial: while several studies found positive relations between these factors [15,24], other authors reported no clear correlation, highlighting species specificity when using this approach.

3.3. Tissue type

PBDE distributions among tissue types depend on the fish's physiological status (age, sex, reproductive stage, nutritional condition), as well as lipid content in analysed tissues [36]. Generally, major levels of PBDE congeners are expected to be found in liver, which is considered the major detoxification organ in vertebrates [35]. Most of the analysed publications limited their studies to PBDE levels in muscle and liver, these tissues will therefore be mostly discussed in this section. However, there are reports about tissue accumulation patterns which suggest that these might be associated with food habits, metabolism and efficiency of transfer rates via gills (uptake and elimination kinetics), which vary among species [36,37].

The major congener commonly found in muscle and liver of wild trout species is BDE-47 and -99 followed by -100 [7,13,22]. Although less frequently, BDE-28, -153, and -154 might also be found. This distribution is in agreement with those found in environmental samples [13]. Differences in distribution (higher concentration in muscle than liver, as found by Vives et al. (2004)) and in congener predominance (BDE-99 as the major congener) may be pointing to direct contamination by PBDEs from a local source, as suggested by these authors. The close parallelism between PBDE distribution patterns on specific sites and commercial mixtures has also been interpreted to reflect episodes of point-source contamination [22,38]. Hepatic metabolic debromination of more highly brominated congeners was proposed as a plausible explanation for the high BDE-47 concentrations found in salmonids [31,39]. However, experimental studies conducted on rainbow trout support the hypothesis that the BDE-47 concentrations observed may be explained by a higher uptake from the environment [39]. Contaminant concentrations and lipid levels in muscle may be strongly linked to seasonal variation and to the species' biological cycle [40,41]. The use of fat reserve in muscle during spawning (discussed below), lower food availability, and lower temperatures in winter, may induce lipid transport from muscle to other tissues, thus modifying PBDE concentrations and distribution among tissues [18].

3.4. Lipid content

Due to the high hydrophobicity of PBDEs, it is expected to find a positive correlation between fat and PBDE levels in trout samples on a wet weight basis, indicating a preference of these compounds for concentrating in lipid-rich tissues. Several individual studies have demonstrated this pattern [15,18,19] while others reported no clear correlation. There is considerable debate on whether to account for lipid in the reporting of environmental contaminants and over the methods for doing so [18]. The appropriateness of accounting for lipid depends on the objectives of the study. It is not suitable to lipid-normalize concentrations if the objective is to evaluate concern to human and wildlife fish consumers, since they eat the fillet or the whole fish and not just the lipid fraction. In contrast, if the goal is to assess overall environmental contamination, biotransport processes or interspecific comparisons, then consideration of lipid may be justified. This is because, for example, pollutant levels determined could be an artifact of differences in lipid among collection localities, species considered or changes in lipid through time [18]. Differences may exist among sampling localities in terms of food availability and (or) aquatic environment characteristics, which could cause variations in fat content, and hence varying organic pollutant accumulation in a predator fish [2,15,41]. Likewise, the importance of this critical factor in PBDE assessments also resides in the fact that lipid content may depend on the top predator fish species used. For example, in a study addressing PBDE temporal trends conducted in North America's Great Lakes, the collected walleye (Percidae) were relatively lean (whole-body lipid content averaging 9.9 ± 2.3%) compared to lake trout (whole-body lipid content averaging 17.5 ± 3.3%), which might account for some of the difference found in the levels of these lipophilic compounds [13]. It should be noted that concentrations are generally better understood by the public on a wet weight basis. However, given the potential importance of lipid, it is often appropriate to describe trends on both a wet and lipid weight basis and (or) to report lipid content.

3.5. Sex and reproductive stage

Even when fish sex and reproductive stage may influence pollutant concentrations [40,41], this factor is rarely included in PBDE studies. In situations where fish are well advanced in the reproductive cycle, it is expected that several pollutants, such as heavy metals [29,42], will accumulate in gonads due to a higher metabolic activity in these organs [29]. In the Southern Hemisphere, a field study conducted on wild rainbow trout found higher PBDE levels in gonads than in muscle, liver or gills [38]. However, only females were collected and assessed and no comparisons were made between sexes. Other studies that have compared between sexes found differences for specific PBDE congener loads in other tissues tested. For example, Vives et al. (2004) found that male brown trout exhibited higher PBDE levels in liver than did females [22]. For fish, in most cases where sex affects bioaccumulation, females have lower pollutant levels than males due to contaminant losses during spawning and, to a lesser extent, to processes such as lower growth efficiency [43]. However, an opposite pattern was reported in a later study. Hartmann et al. (2007) reported higher concentrations of PBDEs in the liver of female brown trout than in males [44]. The authors argue that such results could be due to an uncertainty in the data as a result of the small size of the samples considered (three samples of male fish) [44]. Several authors have hypothesized that the lack of differences in PBDE levels between

female and male results from the fact that trout were collected during a non-reproductive stage, although no definite information is available [17].

Sexual maturation and spawning are highly energy-demanding processes for which fish lipid reserves are the major source of energy [45,46]. Adult trout and salmon spend 50% or more of their total energy in reproduction [40]. In this way, an increased use of lipid reserves on their way up-river for spawning may induce a rise in bioaccumulation of PCBs in *Oncorhynchus nerka* [41] and *Oncorhynchus mykiss* [11]. After spawning, biochemical changes occur, including build-up of potentially large energy reserves. This fattening process may gradually involve accumulation of lipophilic pollutants [40]. Notwithstanding, there is considerable debate regarding this topic. For example, in the wild white-spotted charr (*Salvelinus leucomaenis*) in northern Japan, organochlorine (OCs) concentrations are related to continuous lipid accumulation during their entire life cycle rather than during spawning time [40]. Complementary studies addressing the role of the reproductive stage of wild trout used as tool in PBDE monitoring research programs are needed to provide more robust conclusions regarding this overlooked factor of importance.

3.6. Mechanisms of contaminant uptake and metabolism

Uptake of contaminants by fish occurs mainly through two pathways: water (gill ventilation) and diet. Each route may be assessed by examining specific tissue types that will accumulate higher contaminant concentrations over the rest of the organism [37,47]. Accumulation in the gills of fish is believed to be associated with available concentrations in the water column, while uptake of contaminants in food or ingested material is reflected by those concentrations found in the liver [48]. However, specifically for wild trout, there is only one report that analysed tissues other than liver and muscle to better understand the uptake pathway. Ondarza et al. (2011), who analysed PBDE content in gills, gonads, liver, and muscle, found the same concentrations in the gills and liver of brown trout from Patagonia (79.2 ± 22.8 vs. 81.3 ± 25.7 ng g^{-1} lipid weight, respectively). These results may indicate the importance of both PBDE uptake pathways at species-specific level. However, other elements might *a priori* indicate that diet is the main source of PBDEs; among these elements, the high hydrophobicity of PBDEs (log K_{ow} values vary between 5.7 and 8.3 [32]). It is strongly proposed that the importance of diet as an exposure pathway generally increases with chemical hydrophobicity and trophic position, with diet as the source of virtually all contaminant biomagnification for hydrophobic chemicals with log $K_{OW} > 5$ in aquatic predators [25]. Further, since trout are considered to be top predators, when species representing different trophic levels are compared, trout show the highest PBDE levels, indicating biomagnification through the food web [24,49]. Consequently, determination of PBDE levels in different tissues (specifically muscle, gills, and liver) of different trout species should be strongly encouraged for a better understanding of the pathways (or pathway) of PBDE uptake [20]. However, both pollutant uptake and detoxification processes may take place simultaneously, and their intensities may differ among the fish species considered [21], which highlights the importance of taking into account the species specificity of the target study system.

Metabolism is another important factor influencing body PBDE loads in fish. Roberts et al. (2011) reported species-specific differences among three teleost fish species (common carp and two salmonids: rainbow trout and chinook salmon) in terms of efficiency of metabolic debromination of dosed congeners (BDEs 28, 47, 49, 99, 100, 153, 154, 183, 203, 208, and 209) [39]. In general, the metabolic products and rates were similar for rainbow trout and chinook salmon. These authors observed that carp could metabolically debrominate BDE-99 to a greater extent than salmonids and that the metabolic products differed between species (BDE-47 was formed in carp and BDE-49 was formed in salmonids). The only congener that was debrominated to similar products in all three fish species was BDE-183. BDE-154 was the dominant metabolite, and BDEs 153 and 149 were minor metabolites of BDE-183 in all species [39]. Future research regarding uptake mechanisms and metabolic PBDE debromination, and also providing a multi-species approach is highly recommended.

4. Analysis of PBDE levels

4.1. PBDE levels in trout, lipid content and morphometry

The set of congeners reported among papers was not consistent. Some papers reported as few as three congeners (mainly BDE-47, -99, -100), while some reported ten or more. As a compromise between these two extremes, only congeners BDE-47, -99, -100 were included here, because concentrations of these congeners were always reported in wild trout studies. Further, the sum of congeners BDE-47, -99 and -100 represents on average 81% of the total PBDE concentration reported for trout in reviewed papers. From this congener-specific database, the units were normalized by converting ng g^{-1} wet weight to ng g^{-1} lipid weight, using reported lipid content via 'the ratio approach' [50]. Summation of n = 3PBDEs: BDE-47, -99, -100 concentration ($\sum_{n=3}$PBDEs), and percentage of congener distribution (e.g. %BDE-47 = BDE-47/$\sum_{n=3}$PBDEs × 100) were then calculated. Once the dataset was compiled and classified, only those publications reporting the above congener-specific information were used to perform a principal component analysis (PCA). This statistical tool allowed identifying whether the usually reported biological factors (weight, length and lipid content) are related to PBDE levels in five wild trout species: lake trout (*Salvelinus namaycush*), brown trout (*Salmo trutta*), rainbow trout (*O. mykiss*), arctic char (*Salvelinus alpinus*), and brook trout (*Salvelinus fontinalis*) collected worldwide within the 1994 to present time frame. Factor loadings > 0.65 were considered significant [51] and used for interpreting PCA patterns. Due to the difficulty of assessing age in wild fish (e.g., otolith measurements involve great effort and expertise), this biological factor of importance is not usually considered in PBDE monitoring studies; therefore it was not included in the PCA analysis in this review.

From the compiled and classified database, it was possible to estimate that BDE-153 was reported in 83% of total cases, whereas BDE-154 and BDE-209 were reported in 30% and 3% of cases, respectively. Subsequently, a supplementary PCA was performed considering those cases reporting congeners BDE-47, -99, -100 and -153 in wild trout. However, due to the requirements of the statistical tool used (PCA), which is based on the analysis of homogeneous data among the cases considered, inclusion of cases reporting BDE-153 would lead to the exclusion of some geographical regions for which there are no reports of BDE-153 in wild trout. The excluded cases in this subsequent approach represent 16% of the classified database, corresponding to the following geographical regions: all of South America, Eastern Greenland, Switzerland, and two rivers in Czech Republic. Results achieved by the supplementary PCA (Table A.2., Fig. A.1) showed grouping patterns comparable to those found in the former PCA, without BDE-153. This could be due to the relatively low significance of BDE-153 levels (ca. 5%) within the overall congener contribution (sum of BDE-47, -99, -100 and -153 levels) of the classified database. Hence, in order not to exclude cases and not to reduce the global scope of both statistical and geographical (hotspot mapping) analysis, it was decided not to include BDE-153 in them. However, a

supplementary PCA considering cases where congeners BDE-47, -99, -100 and -153 were reported in wild trout was included in the Appendix Section.

To test for interspecific morphometric differences, two-way ANOVA and *a posteriori* multiple comparisons analysis (Fisher's LSD tests) were used. Pearson's correlations were used to explore for intraspecific relationships between PBDE levels and lipid content as well as size. Variables were arcsine transformed [52] previous to carrying out all statistical analyses since this raw data did not fit a normal distribution. Normality was assessed after transformation (Shapiro–Wilks W test, $p < 0.05$). All statistical analyses were performed using Statistica Version 6.0 [53]. A p value <0.05 was considered significant, except for Pearson's correlations, which were adjusted by Bonferroni correction, and α error was divided by the number of comparisons (i.e. 5 species). Thus, correlations were all considered significant for $p < 0.01$. To identify PBDE hotspot areas for contaminant accumulation and spatial distribution patterns on an intercontinental scale, the sum concentration of three major congeners in wild trout species and geo-referenced sampling sites were mapped using QGIS software [54].

4.2. Trout-to-sediment ratio of PBDE levels

In order to evaluate the feasibility of an organism to be used as a sentinel for pollutants, it is useful to analyse its capacity to accumulate chemicals. As mentioned above, a suitable sentinel implies that pollutant concentration in its tissues achieves a level that exceeds that found in its major prey items. Likewise, bioaccumulation in biota relative to chemical concentrations in sediment also describes the potential of a species to concentrate pollutants. Bioaccumulation, biomagnification, and biota-sediment accumulation factors have been used for this purpose [25]. It was neither possible to calculate bioaccumulation or biomagnification factors nor to make interspecific comparisons among the trout species considered in this review, since only two studies [24,49] examined PBDE levels in both trout and their major prey items. Because PBDE levels in sediment were reported for several regions where wild trout were studied, the relationship between trout and sediment PBDE levels was explored. Thus, PBDE accumulation capacity of each trout species was assessed. Despite the inherent complexity of each freshwater ecosystem herein reviewed, trout-to-sediment ratio was used as a screening tool to identify potential PBDE bioaccumulation capacity among the trout species considered. Therefore, those species showing a higher value of this ratio potentially represent a more feasible tool for monitoring PBDEs.

PBDE levels in sediment for the above mentioned analysis were collected considering geo-referenced trout collection sites listed in Table A.1. In this sense, reports from the same year, or from five years before or after the year in which trout samples were taken, were considered. Trout-to-sediment ratio was calculated for each trout species as follows:

$\text{T-SR} = \text{BDE}^{\text{Trout}}/\text{BDE}^{\text{Sediment}}$, where T-SR is the calculated ratio for concentration of individual congeners BDE-47, -99 and -100 reported for trout and sediment from a particular study site or country. Hence, $\text{BDE}^{\text{Trout}}$ and $\text{BDE}^{\text{Sediment}}$ represent the concentration of each congener reported for trout species (on a lipid weight basis) and sediment (on an organic carbon basis), respectively. For comparison purposes, PBDE levels in sediment were normalized using total organic carbon (TOC) values reported for each studied site. Correlation between TOC levels and PBDE concentration in sediment was reported in previous studies [25,55], as well as the relevance of the type (degradation degree) of organic matter involved in the sediment [55]. This evidence highlights the relevant role of TOC in accumulating PBDE in sediment.

Table 1
Principal Component Analysis based on levels of three major BDE congeners, percentage of congener distribution, lipid content and morphometric characteristics of 5 trout species collected from 13 different countries. Loading factors (>0.65) for the most heavily weighted variables are shown with an asterisk.

	PC 1	PC 2	PC 3
BDE-47	*0.93	0.06	−0.14
BDE-99	*0.73	0.64	−0.11
BDE-100	*0.90	0.28	0.18
%BDE-47	0.41	−0.61	−0.38
%BDE-99	−0.37	*0.80	−0.22
%BDE-100	0.31	−0.04	*0.90
Lipids	*0.72	−0.32	0.06
Weight	*0.91	−0.03	−0.15
Length	*0.88	0.03	−0.06
Eigen values	4.73	1.60	1.10
Variance (%)	53	18	12
Cumulative variance (%)	53	71	83

5. Results and discussion

5.1. Associations between PBDE concentrations and biological factors

Principal Component Analysis identified three components that accounted for 83% of the data variance (Table 1). The first component accounted for 53% of the variation and was positively associated with levels of BDE-47, -99, -100; and trout length, weight and lipid content (Table 1). The second component accounted for 18% of the variation and was positively associated with percentage of BDE-99 congener distribution. Trout with the highest percentage of BDE-100 congener distribution scored high on the third component, representing 12% of data variance. Principal component 1 is plotted against principal component 2 in Fig. 2 where different colour symbols denote the continental region where trout were collected. The right side of the figure represents fatty and larger trout, with higher PBDE levels. Several of these trout were collected from lakes in eastern Norway (cluster A), close to Oslo and nearby industrialized areas (Figs. 1 and 2). Within this cluster, trout with the highest reported PBDE levels (cluster A′) were collected from Mjøsa, the largest lake in Norway. Textile industrial activity has been reported as the local source responsible for PBDE pollution in Mjøsa Lake [19], discussed in more detail in Section 5.2. The data on trout collected from North America's Great Lakes, on both Canadian and US sides, cluster together (cluster B) and represents a self-consistent data-set: all lake trout data. Trout collected from lakes in European high mountains, lakes in Greenland, and rivers in northern Patagonia all overlap (cluster C). However, the data on Patagonian trout seems to cluster together (cluster C′), and the orange triangles (connected with dashed line, Fig. 2), correspond to trout sampled in western lakes (nearby triangles), far from East Greenland lakes (bottom triangle). Cluster C is somewhat separated from clusters A and B, which may indicate that these trout have different PBDE sources. It is wise to mention that main trout data in cluster C is from regions often referred to as remote world areas by several authors [22,56]. Thus, the distance between these clusters may simply show that remote areas would get PBDE inputs through atmospheric transport while lakes in eastern Norway and North America's Great Lakes are affected by direct local sources. However, it is too early to speculate that one or two PBDE congeners can be used as 'markers' of a particular PBDE source. Consistent quality data from many more locations around the world (e.g. Oceania and Asia) is needed before approaching this issue with even modest certainty.

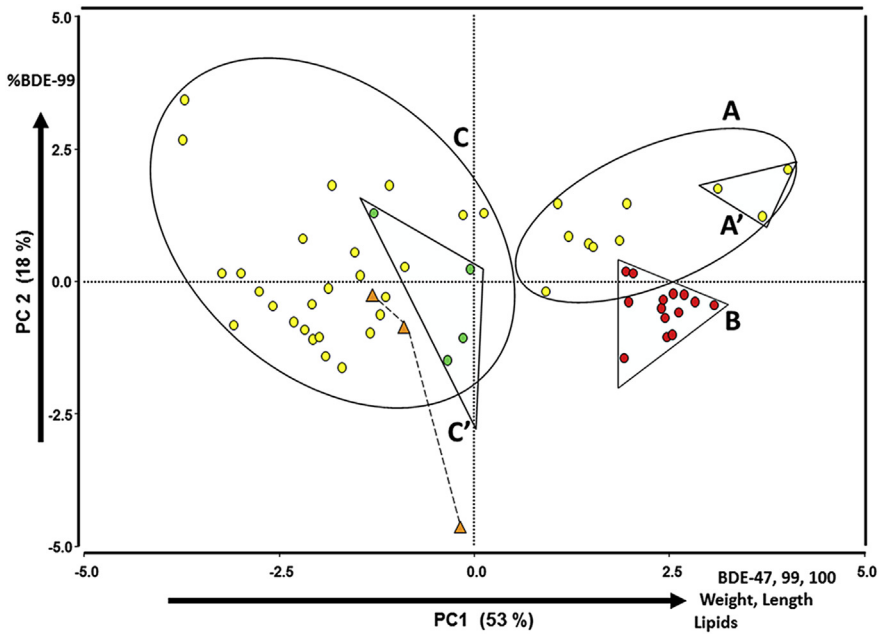

Fig. 2. Position of wild trout data in the plane defined by the first two axes obtained from a principal components analysis carried out with: levels of BDE-47, -99, -100; the percentage of BDE congener distribution; and trout morphometry and lipid content. The yellow dots are for trout collected in Europe, the red for North America, the green for South America, and the orange triangles are for trout collected in Greenland. The clusters A–C are discussed in the text.

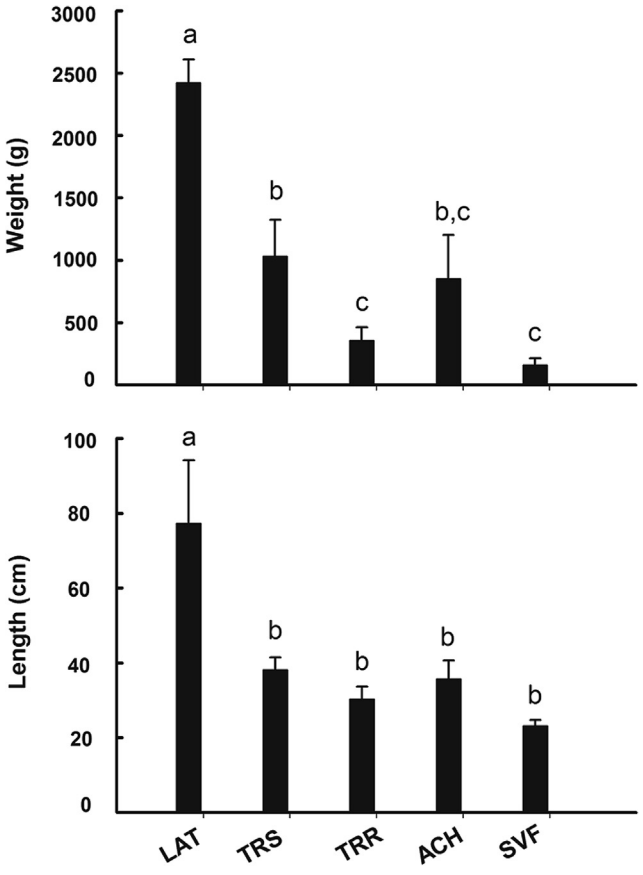

Fig. 3. Weight and length based on the reported data for LAT ($n = 419$), TRS ($n = 387$), TRR ($n = 77$), ACH ($n = 53$), and SVF ($n = 88$). Trout acronyms: LAT (lake trout: *Salvelinus namaycush*), TRS (brown trout: *Salmo trutta*), TRR (rainbow trout: *Oncorhynchus mykiss*), ACH (arctic char: *Salvelinus alpinus*), and SVF (brook trout: *Salvelinus fontinalis*).

Highest PBDE levels have been reported for brown and lake trout (Fig. 2). These elevated PBDE levels may be related to the feeding behaviour and trophic level of brown and lake trout. Both species tend to feed higher in the food web throughout their adult life [19,57] and also grow to be larger individuals (Fig. 3). Weight and length differed significantly (ANOVA, $F_{4,\ 88} = 21.661$, $p < 0.001$; $F_{4,\ 88} = 5{,}037$, $p = 0.001$, respectively) among assessed trout species. Multiple interspecific comparisons (Fisher's LSD tests) revealed that lake trout was the largest one, whereas brook and rainbow trout were smaller and lighter than brown and lake trout (Fig. 3). Except for the highly fatty liver reported for lake trout, lipid contents in muscle and liver were comparable among species (Fig. 4). Because of lack of degrees of freedom, no statistical comparisons were performed for muscle versus liver lipid levels among species (i.e. unlike often reported lipid levels in muscle, lipid levels in liver

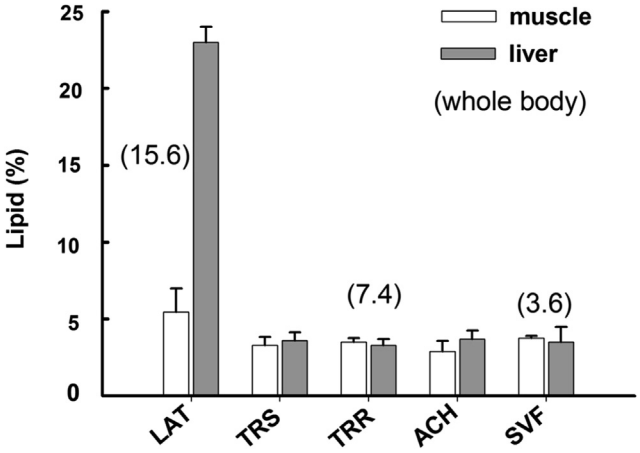

Fig. 4. Muscle, liver or whole body lipid content based on the reported data for LAT, TRS, TRR, ACH, and SVF. Trout acronyms: see Fig. 3 legend.

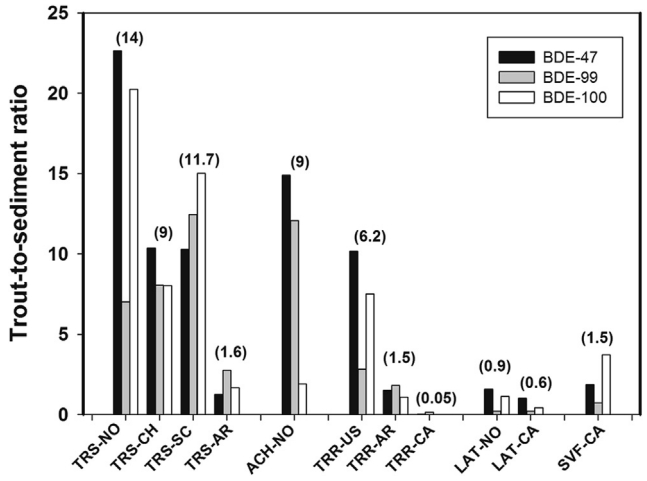

Fig. 5. Trout-to-sediment ratios calculated for individual congeners (BDE-47, -99 and -100) reported for each trout species and sediment. Numbers in brackets denote estimation of trout-to-sediment ratios based on the sum concentration of BDE-47, -99 and -100 for both trout species and sediment. Country acronyms: Norway (NO), CH (Switzerland), Scotland (SC), AR (Argentina), United States (US), and Canada (CA). For trout acronyms see legend of Fig. 3.

were not reported most of the time for lake, rainbow, brook trout or arctic char). Although lipid content found in the whole body was higher in lake trout, this variable has not always been assessed in all other species reviewed, which makes it difficult to consistently establish interspecific comparisons. Reported data on weight and length of brown trout was positively correlated with levels of BDE-47, BDE-99, BDE-100, and ∑3PBDEs (Table A.3). For all other species considered here, reported PBDE levels were not correlated with morphometry, which highlights species specificity when using correlational approaches. Full data sets on lipid content in muscle, liver or whole body reported for each trout species considered were not correlated with BDE-47, -99, -100 congener levels.

5.2. Trout-to-sediment ratio

As it is possible to observe in Fig. 5, there are several cases in which the trout-to-sediment ratio is higher for BDE-100 than for BDE-99 (i.e., 6 of 11 considered cases; Fig 5). Due to the lack of information on this issue this pattern could not be attributed only to the species-specific differences in the metabolic PBDE debromination. To the best knowledge of the authors, there is only one study about metabolic debromination of BDE-99 and -100, among other congeners of PBDEs, in rainbow trout [39]. This species reported only BDE-49 as metabolite of BDE-99; while metabolites of BDE-100 were not observed in this study. For the remaining fish species considered in this review, there is no available information about the metabolic debromination of congeners BDE-99 or BDE-100. The observed distribution pattern might be due to multi causal mechanisms and environmental factors, including: species-specific differences in the metabolic PBDE debromination, physical-chemical as well as microbiological conditions in sediments; and environmental processes (e.g. effect of light and temperature).

Regardless of the different world regions where trout were collected, brown trout generally showed higher trout-to-sediment ratios, which is suggestive of a relatively greater capacity of this species to concentrate PBDEs related to the sediment (Fig. 5). Brown trout have a wide geographic distribution and can be found in freshwater ecosystems of Europe, North and South America, Oceania, Asia and East Africa [28]. As mentioned before, brown trout tend to feed higher in the food web and grow to reach large sizes [28,58], representing the second largest species after lake trout (Fig. 3). A literature survey also indicated that juvenile brown trout typically grow faster than other trout species [58]. Taken together, these results suggest that, besides pollution level in the studied area, the target species in PBDE studies is a critical factor that should not be overlooked.

5.3. PBDE spatial distribution patterns at intercontinental scale – global comparison

European, North and South American sampling locations, trout species, tissues or whole body, sampling year, and the sum concentration of three major congeners (i.e. the sum of BDE-47, -99, -100) in wild trout species are summarized in Table A.1. Among countries, trout from North America's Great Lakes (Michigan, Ontario, Erie, Huron and Superior), Norwegian lakes (Eikern, Losna, Heddalsvatn, Ellasjøen, and largest Lake Mjøsa), a high-mountain lake in Scotland (Lochnagar), and several Swiss rivers in Europe had the highest PBDE levels (Table A.1, Fig. 1). Moreover, brown trout from Lake Mjøsa present the highest PBDE levels reported thus far for wild salmonid species [19]. This is the second largest lake in Europe and it was later confirmed that its elevated PBDE levels were mainly caused by direct local release of PBDEs into the watercourse by a textile factory [19]. Instead, trout collected from Alaska, western U.S. National Parks, Canadian subarctic watercourses, high-mountain lakes located in Austria, Norway, Bulgaria, Czech Republic, France, Slovakia, and Greenland had the lowest PBDE levels (Table A.1, Figs. 1 and 2). Likewise, in South America, low PBDE levels were found in wild trout from Patagonia (Fig. 1), which represents one of the most pristine locations on the planet and has been designated as a Biosphere reserve [56].

Taken together, results based on reported PBDE levels in wild trout have revealed that their accumulation potential is considerably higher in certain areas compared to others (so-called 'hotspot areas for contaminant accumulation' according to AMAP 2009 [59]). Several hotspots were identified in freshwater systems in the Northern Hemisphere (Fig. 1). PBDE hotspots found in North America's Great Lakes and in one of the largest European lakes (Mjøsa Lake) may be due to high industrial activity, which is suggestive of a local point source. For the high PBDE loads found in trout from a high-mountain lake (Lochnagar, Scotland. Fig. 1), atmospheric transport was proposed by authors as plausible explanation for PBDE inputs [22]. Total PBDE loads reported for wild trout from industrialized and anthropized areas in Europe and North America were twenty times higher than in trout species collected from less anthropized world regions. Hence, on average, total PBDE loads reported for wild trout from European and North American great lakes were in the order of ~1250 ng g^{-1} l.w., while ~60 ng g^{-1} l.w. corresponds to trout from South America and remote regions in Europe, Greenland, and North America. This is a solid and consistent pattern observed in this review.

6. Conclusions

This review summarizes field studies with an underlying objective of determining PBDE pollution levels and environmental threat. Critical biological factors and environmental surrounding aspects (i.e. PBDE levels in sediment) of target species were put forward and argued. The analysis of PBDE levels was also successfully achieved and has exhibited the-state-of-the-art in both, PBDE levels in a top predator freshwater fish and spatial trends of these pollutants on an intercontinental scale. In this study, PCA identified

an association pattern among trout size, lipid content and levels of major PBDE congeners (BDE-47, -99, -100). Individual correlation analysis showed that the data on fish size was clearly correlated with the concentrations of BDE-47, -99, -100 for brown trout. Further, this species generally showed higher trout-to-sediment ratios than other species. Overall, results suggest that adult wild trout could be useful as a PBDE bioindicator. Several biological factors such as size, tissue lipid content and feeding behaviour are critical when choosing freshwater fish as sentinels for PBDE pollution.

Global PBDE hotspots identified call for intensive pollutant monitoring research to determine environmental threat in the Northern Hemisphere. Results also provide evidence that some regions support trout that are unsafe to eat, presumably a response to uncontrolled and unregulated waste disposal into watercourses. The wide geographic distribution of this freshwater fish species assists in establishing benchmarks for PBDE levels among trout populations around the world. However, constructing comparable datasets is critical to the success of biomonitoring programs. Future PBDE assessments could be improved if several tissues (gill, gonads, liver, and muscle) are assessed, allowing for examination of mechanisms of contaminant uptake by adult wild trout. Likewise, full reports on sampling sites, dates, capture methodology, morphological measurements, sex and reproductive stage of individuals are also recommended. Hence, meaningful comparisons can be made, particularly in response to restoration efforts implemented by environmental managers. Our work may improve future comparisons among studies involving not only trout species, but other freshwater fish species as well.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.emcon.2015.08.002.

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