



Legacy and emerging pollutants in marine bivalves from the Galician coast (NW Spain)



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ABSTRACT

The presence of pollutants in estuary and oceanic systems is a global problem and a serious concern to human and environmental health. Usually, environmental monitoring studies consider classical persistent organic pollutants (POPs). However, the lists of POPs keep continuously growing and new POPs and other emerging pollutants should be considered in new monitoring programs. So, this study aimed to investigate the distribution and profile of classical POPs (polychlorinated biphenyl (PCBs), organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs)), new POPs and emerging pollutants (polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), novel halogenated flame retardants (NFRs) and UV filters) in bivalve mollusc samples (both raft-cultivated and wild mussel, *Mytilus galloprovincialis*; cockle, *Cerastoderma edule*; and clam, *Ruditapes decussatus*) collected in nine Galician Rias during the period February 2012 to February 2013. A predominance of PAHs (6.8–317 ng/g dry weight (dw)) followed by PCBs (0.47–261 ng/g dw), UV filters (1.4–157 ng/g dw), PFCs (0.53–62 ng/g dw), OCPs (0.07–29 ng/g dw), PBDEs (0.31–6.6 ng/g dw) and NFRs (0.07–3.2 ng/g dw) was found in the studied bivalves, being the UV filter octocrylene the compound found at the highest concentration (141 ng/g dw in a cockle sample), while the PAHs chrysene and benzo(b)fluoranthene were the compounds with the highest average concentration (20 and 14 ng/g dw, respectively). Inter-species, temporal and geographical variations on pollutants concentration were assessed by multifactorial analysis of variance. Statistically significant differences among the type of mollusc were observed for levels of organochlorinated and organobrominated pollutants considered (PCBs, OCPs and PBDEs), which were detected at higher concentrations in wild mussel. On the other hand, the main PFCs and UV filters showed a higher detection frequency in cockle samples. Location played significant role for PAHs, PCBs and the main PBDEs, being the most polluted rias those more industrialized and populated, i.e. A Coruña, Ferrol and/or Vigo. Finally, sampling timepoint was also a significant factor for most of the families considered but with different profiles. Thus, PAHs and PCBs showed higher concentrations in both February 2012 and 2013 and lower in August 2012, while the main PBDEs were measured at higher concentrations in November 2012 and lower in February 2012; and the main NFRs, PFCs and UV filters were present at lower levels in February 2013.

1. Introduction

The presence of persistent organic pollutants (POPs) in estuarine and oceanic systems is a global problem. They are ubiquitous and long-range transported, being found in many biotic and abiotic media. This property is derived from their persistence in the environment. POPs are semivolatile and hydrophobic compounds, having a great bioaccumulative capacity (Jones and de Voogt, 1999). Due to their lipophilicity and low chemical degradation rate, they tend to concentrate in lipid

rich tissues of organisms and to biomagnify through food webs (Guzzella et al., 2005). Many POPs are listed as possible carcinogens and are of serious concern to human and environment health. Organochlorine compounds (OCs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs) are important groups of POPs.

The list of POPs keeps, however, continuously growing. Different groups of brominated flame retardants have been commercialized to prevent the development of fire (Wilford et al., 2004), among them,

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polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes and tetrabromobisphenol A. The commercial formulations of PBDEs includes penta, octa and deca-BDE technical mixtures. As classical POPs, these compounds have several toxicological effects and a great bioaccumulation potential (Strid et al., 2013). Because of that, the usage of PBDEs has been regulated as regards commercialized mixtures and articles in Europe and USA (CSA, 2003; EC, 2003; ECR, 2008). Furthermore, several congeners of the penta-BDE and octa-BDE mixtures have been added to POPs list of the Stockholm Convention (UNEP, 2009). As a consequence, these flame retardants are being replaced by other compounds, generally called “novel” flame retardants (NFRs) (Betts, 2008). Some of the most important representative NFRs are decabromodiphenylethane (DBDPE) as a substitute of deca-BDE and with similar physicochemical properties, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) replacing octa-BDE, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) used mostly in PVC, bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (DEHBTP) which are used in replacement of penta-BDE, and tris(tribromoneopentyl)phosphate (TTBPP). Another compound which is considered as hazardous to the environment and that has not yet been regulated is Dechlorane plus (DP). It has been used during decades but had received little attention until it was detected in different compartments of the environment (Gauthier and Letcher, 2009; Hoh et al., 2006; Tomy et al., 2007). DP is a chlorinated compound that can be included in the term NFRs. These NFRs are not regulated for their use or production, nor even regarding maximum tolerance levels in foodstuffs, being their presence in seafood literature scarce.

Perfluorinated compounds (PFCs) comprise a diverse group of chemicals including perfluoroalkyl sulphonates (PFASs), such as perfluorooctane sulphonate (PFOS), and perfluorinated carboxylic acids (PFCAs), such as perfluorooctanoic acid (PFOA). These compounds are constituted by a hydrophobic alkyl chain of varying length (typically C4 to C16) and a hydrophilic end group (de Voogt and Saez, 2006). PFCs have been used in different commercial and industrial applications, such as: surfactants and surface protectors in paper, leather, carpets, upholstery, paints, lubricants, polishers, food packaging, and fire-fighting foams including aqueous film forming foams (Kissa, 1994). PFCs are resistant to hydrolysis, photolysis, biodegradation, and metabolism. These characteristics explain the environmental persistence and bioaccumulative potential of PFCs, of which PFOS and PFOA are the two most commonly reported in the environment (Powley et al., 2005; Tseng et al., 2006). Due to these characteristics the EU banned most uses of PFOS and related compounds in 2008 (EC, 2006b). Furthermore, in May 2009, PFOS was included in Annex B of the Stockholm Convention on POPs (UNEP, 2009).

Ultraviolet (UV) filters are compounds used in sunscreens and cosmetics to prevent chemical degradation and skin damage under sunlight irradiation (Manova et al., 2013). They are considered as emerging environmental pollutants. Their worldwide usage, ubiquitous presence in water samples and endocrine disruption activity of certain species are some of the issues which have raised the concern about the long-term impact of UV filters in the environment (Giokas et al., 2007; Giraldo et al., 2017; Krause et al., 2012; Paredes et al., 2014; Ramos et al., 2015).

Galicia in north-west Spain, is the second largest mussel producer in the world; with a production that has surpassed 200,000 t annually (Caballero Miguez et al., 2009). This causes that Galician administrative authorities have the duty to control and ensure the quality of shellfish that is produced in its coast. Moreover, bivalve molluscs are an important filter feeding organisms. They have been used as bioindicators in environmental monitoring programs due to several characteristics such as, resistance to stress, sessile behaviour, tolerance to salinity changes and capacity to accumulate contaminants at levels higher than found in marine water (Philips and Rainbow, 1993). They are considered as an indicator of environmental contamination in several phases, as they are exposed to seawater and sediment. Thus, it is of

wide interest the monitoring of levels and trends of classical and emerging pollutants, as the above mentioned ones. For instance, since 1986 the U.S. National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Mussel Watch Program carries out a yearly nationwide sampling to collect bivalves, *Mytilus edulis*, or similar species, as sentinel organism, from sites along the USA coast to assess the status and long-term trends of selected contaminants (approximately 140 analytes) in coastal marine environments (Bricker et al., 2014). More recently, to expand the utility of the Mussel Watch Program, several agencies in California partnered with NOAA to design a pilot study that targeted contaminants of emerging concern, such as PBDEs and PFCs (Dodder et al., 2014; Maruya et al., 2014a; Maruya et al., 2014b).

Classical POPs distribution has been previously studied in the Galician coast (Bellás et al., 2014; Bellás et al., 2011; Carro et al., 2014; Carro et al., 2010, 2015; Fernandez et al., 2013). However, until now only a few works have been published dealing with concentration and distribution of PBDEs in Galician molluscs (Bellás et al., 2014). Regarding NFRs, PFCs and UV filters, no data is available for these geographical area, and only few published papers focused in the development of analytical methodologies, have reported some concentrations (Negreira et al., 2013; Villaverde-de-Saa et al., 2012; Villaverde-de-Saa et al., 2013). In fact, the worldwide number of studies on the distribution of such chemicals (particular if studied together) is also very scarce.

Bearing that in mind, this study aimed to investigate the distribution and profile of PBDEs and NFRs, PFCs and UV filters in bivalve mollusc samples (*Mytilus galloprovincialis*, *Cerestoderma edule* and *Ruditapes decussatus*) collected in nine Galician Rias during the period February 2012 to February 2013. Classical POPs, PCBs, OCPs and PAHs, have also investigated not only to study their distribution but for comparison to emerging pollutants.

2. Material and methods

2.1. Samples

The analysed bivalve mollusc samples were chosen from selected locations in the Galician coast, shown in Fig. 1, as to consider the main production areas in the Galician coast. Table S1 shows an overview of the main anthropic pressures on the different rias.

Raft mussels (*Mytilus galloprovincialis*), placed on the rope at 1, 5 and 10 m of depth, were obtained in five sampling timepoints (February, May, August, November 2012 and February 2013) from 8 locations, identified as 6, 10, 12, 15, 17, 18, 20 and 21 in Fig. 1. They cover the 5 Rias where mussels are cultivated. One location is sampled in the rias of Ares-Betanzos and Muros-Noia, and two locations in Arousa, Pontevedra and Vigo, since these last three rias have a larger production.

Wild mussels (*Mytilus galloprovincialis*) were collected in three sampling timepoints (February and August 2012 and February 2013) from 10 locations with codes 1, 3, 5, 7, 9, 11, 13, 16, 19 and 22.

Cockles (*Cerestoderma edulis*) and clams (*Ruditapes decussatus*) were collected in five sampling timepoints (February, May, August, November 2012 and February 2013). However, due to ecological characteristics, not all species could be collected at each location and during every season. Cockles were collected from 10 locations, codifies as 2, 4, 5, 8, 9, 11, 14, 16, 19 and 22, while clams were collected from only 4 locations: 2, 8, 14 and 16.

Wild mussels, cockles and clams were collected manually during low tides. Analysis of bivalve molluscs were made in pools of soft tissue (30 individuals), in the case of raft mussel ten individuals by each depth, 1,5 and 10 m. After removing the shell, molluscs were stored at -30°C before being freeze-dried. After lyophilization, samples were homogenised by means of a mixer mill with zirconium oxide balls and stored at room temperature until analysis.

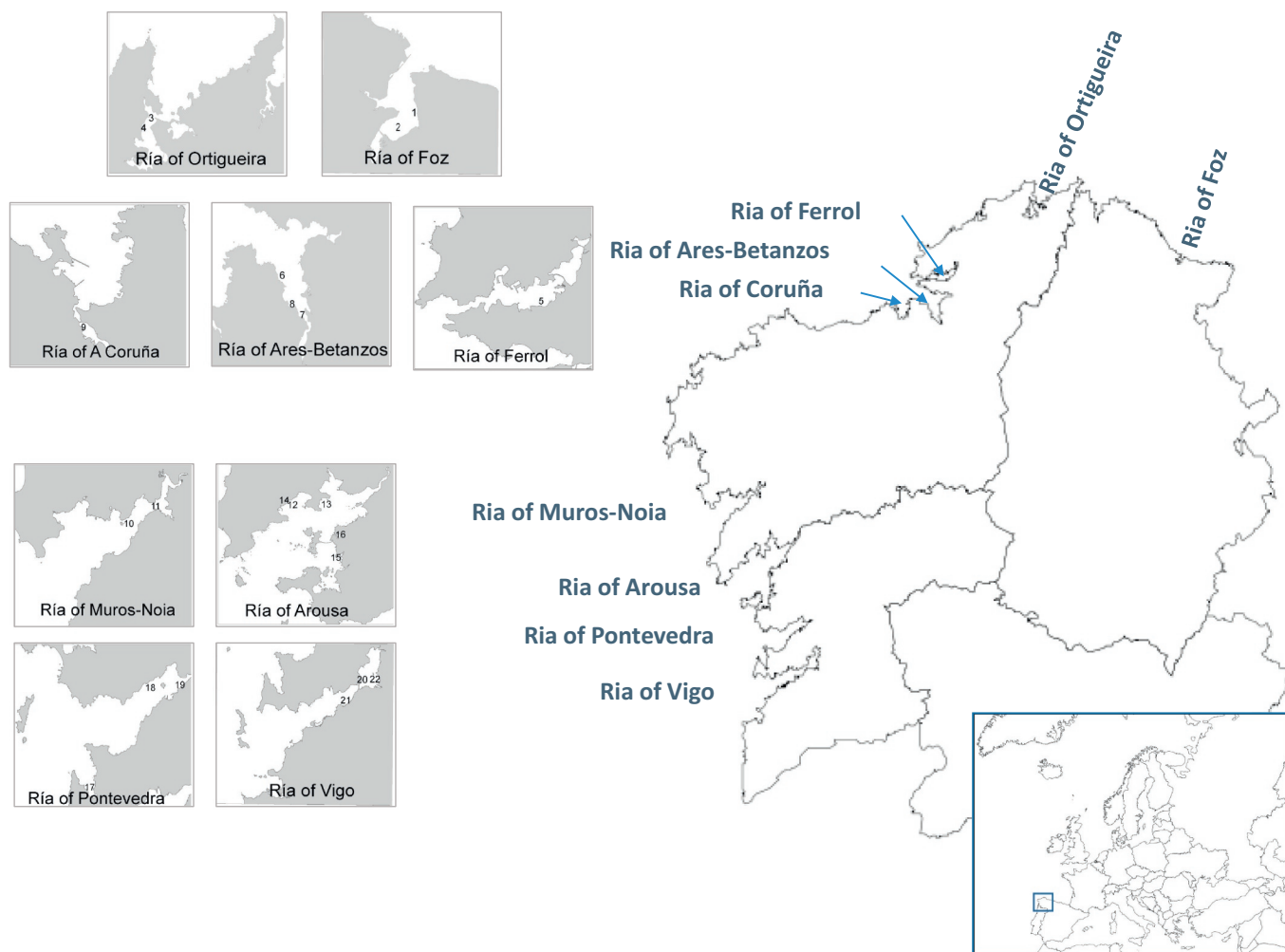


Fig. 1. Map showing sampling locations.

2.2. Reagents and standards

Sources of chemicals are provided in Supplementary Information Text S1.

2.3. Instrumental analysis

Instrumental analysis information is provided in Supplementary Information Text S2.

2.4. Analyte determination procedure and QA/QC

The analytical methods to measure OCs, PAHs, PBDEs, NFRs, PFCs and UV filters and the QA/QC procedure are detailed in Supplementary Information Text S3.

2.5. Statistical analysis

For each individual compound, the estimation of the average was performed according to the recommendations of the USA EPA guidance EPA QA/G-9S (EPA, 1996) depending on the percentage of samples which were below the detection limit (non detects). Hence, if < 15% of the samples were non detects, these were replaced with half the detection limit, when non detects were between 15% and 40%, the Cohen's method (Cohen Jr, 1959; EPA, 1996) using the Hass and Scheff (Hass and Scheff, 1990) empirical equation for λ estimation has been applied, and, finally, when the number of non detects was > 40%, the

average was not calculated.

The above mentioned strategy is not feasible for estimating the total concentration of each family of pollutants since it is contributed by different chemicals with different detection limits. So, the sum of concentrations was calculated by replacing those samples below the detection limit (non detects) applying three different scenarios, i.e. either: detection limit, $\frac{1}{2}$ detection limit and 0. Those compounds below the detection limit (non detects) in all the samples analysed were not included.

Statistical analysis was performed with Statgraphics XVII statistical package by considering as quantitative variables (chemical variables) the concentration of contaminants expressed in ng/g dry weight (dw) after logarithmic transformation. Statistical analysis was only performed for those compounds with a detection frequency above 85% (i.e. < 15% of the data are non-detects). The data from clam samples was not considered for statistical analysis since only 8 samples are considered in this study due to its low availability. In order to consider the possible relationship between composition of contaminants and biological parameters, lipid content, condition index were also included as quantitative variables (biological variables). Species, sampling time and location were considered as qualitative variables.

First, inter-species, temporal and geographical variations on biological parameters were assessed by multifactorial analysis of variance (ANOVA). Secondly, since the accumulation of contaminants by bivalve mollusc species depends on differences in exposure routes and on the distinct types of habitat and feed (Dominguez et al., 2011), multifactorial ANOVA considering the effect of mollusc species, location

(Ria) and sampling timepoints on the concentration of each family of contaminants and each individual contaminant was performed.

For factors that show significant p-values in the ANOVA analysis, a further analysis was performed by the Fisher's Least Significant Difference (LSD) test.

2.6. Environmental risk assessment

Risk assessment of legacy pollutants was performed on the basis of different well-established criteria such as European Water Framework Directive (EU, 2013) and assessment criteria by OSPAR (OSPAR, 2009). An environmental risk assessment for emerging pollutants was performed according to the Technical Guidance Document of European Commission on risk assessment (JRC, 2003). Toxicity and bioaccumulation data were collected from peer-reviewed literature and databases (ECOTOX and ECHA). Missing experimental data were estimated by ECOSAR. EC₅₀ (effect concentration for 50% of individuals) values for standard test organism (algae, invertebrates and fish) were used (compiled into Table S2). Predicted no-effect-concentrations (PNECs) were calculated for each chemical by dividing the lowest available EC₅₀ by an assessment factor (AF) of 1000. Three different values of Risk Quotients (RQ) have been considered: 0.01, 0.1 and 1. Subsequently, the water environmental concentrations corresponding to these RQ were obtained by multiplying them by PNECs. Finally, the equivalent mussel environmental concentrations were obtained by considering the bioaccumulation factor (BAF) values. The lowest available BAF value was applied, as the worst-case scenario. Hence these mussel concentration thresholds can be compared to experimentally measured values in order to assess the risk, which is established into four levels (Hernando et al., 2006; Sang and Leung, 2016; Tsui et al., 2014), viz.: unlikely risk (RQ < 0.01), low risk (0.01 ≤ RQ < 0.1), medium risk (0.1 ≤ RQ < 1), and high risk (RQ > 1).

3. Results and discussion

3.1. Biological parameters

The inter-species, temporal and geographical analysis on biological parameters (lipid content and condition index, CI) is detailed in the Supplementary Information (Text S4 and Fig. S1). Briefly, the main conclusions are that mussel samples (average 7.4% and 6.9%, for raft and wild mussel, respectively) presented a significantly higher lipid content than cockle (average 3.1%), while in the case of CI the highest values were obtained for raft mussels (0.43).

3.2. Levels and distribution of contaminants

It is important to note that all levels found in these samples for those compounds regulated in seafood products (i.e. PAHs, PCBs and OCPs) were below the allowable limits for human consumption (EC, 2005, 2006a; EU, 2011a, 2011b).

The detection frequency (percentage of samples above the detection limits), percentiles, maximum and average concentrations, expressed in ng/g dw of pollutants for bivalve molluscs coming from the Galician coast are shown in Table 1. The average values were calculated as explained in Section 2.5. Moreover, the sum of log-scaled concentrations for each family of compounds is presented in Fig. 2 and average concentrations found at each sampling point in Table S3 (Supplementary Information). As shown in Fig. 2, in terms of total concentrations, a predominance of PAHs followed by PCBs, UV filters, PFCs, DDs (i.e. p,p'-DDT, o,p'-DDT and their metabolites, p,p'-DDD and p,p'-DDE), PBDEs and NFRs was found in the studied molluscs, which indicates the importance of hydrocarbon pollution in the Galician Rias, caused by shipping oil spills, land run off, industrial wastewater and city sewage drainage discharges.

It is noteworthy that UV filters are ranked third in terms of total

concentrations, given the fact that they represent the group of chemicals studied with lower persistence, since, for instance, some UV filters are biodegradable or easily photodegraded (Sánchez-Quiles and Tovar-Sánchez, 2015; Volpe et al., 2017). This may be partly explained by the fact that modelled dispersal of UV filters in the Galician Rias predicts between 3 and 8 h of time since UV filters are emitted from the source (coastline or wastewater effluent outfalls) to the rafts, which may be too low for degradation to take place (Lindo-Atichati et al., 2019). Furthermore, even the most polar UV filter analysed in this study, BP-4, has been shown to have a BAF of 905 L/Kg in mussel (Vidal-Liñán et al., 2018).

3.2.1. PAHs

The concentration of ΣPAHs (8 PAHs) in this study varied between 6.8 and 318 ng/g dw (Fig. 2). Such values are in accordance with previous findings of PAHs in bivalves from other marine environments, such as the US coast (O'Connor and Lauenstein, 2006). Valavanidis et al. in 2008 (Valavanidis et al., 2008) found comparable or slightly greater levels in *Mytilus galloprovincialis* from the coastal area of the Saronikos Gulf (Greece).

The analysed PAHs were found above the LOD in almost 100% of the samples: detection frequency higher than 98%, except DBaH (88%), Table 1. The main PAHs in decreasing order of abundance were CHR (28.4% of total PAHs), BbF (20.3% of total) and IcdP (13.3% of total), Fig. 3a. This pattern is similar to that found by Leon et al. in bivalves from a Mediterranean coastal lagoon in 2013 (Leon et al., 2013).

Significant differences in ΣPAHs levels were found among location (p-value < 0.0001), Fig. 4a, and also for all the individual PAHs. The samples from Ria of A Coruña had the highest concentrations of PAHs (average ΣPAHs concentration of 194 ng/g dw), followed by the samples from Ria of Ares-Betanzos (average ΣPAHs concentration of 108 ng/g dw). The samples from Rias of Muros-Noia (average ΣPAHs concentration of 18 ng/g dw) and Foz (average ΣPAHs concentration of 30 ng/g dw) had the lowest load of PAHs. Furthermore, significant differences were observed in samples from the Northern area of the Ria de Arousa (locations 12, 13 and 14) and the Southern area (locations 15 and 16), see Table S3, as higher concentrations of PAHs were detected in the Southern area of the ria (average 85 and 67 ng/g dw, respectively).

Several authors, among which Soriano et al. (Soriano et al., 2006), established that levels of PAHs below 50 ng/g dw could be considered as a background pollution, whereas values above 200 ng/g dw might reflect the vicinity of industrial hydrocarbon sources, and values above 500 ng/g dw were assigned to an oil spill. So, the high values found in the Ria of A Coruña (and to a minor extent Ares-Betanzos) could be attributed to the vicinity of an urban area and of petrochemical industry that might cause chronic hydrocarbon pollution. Furthermore, PAHs have been included in the European Water Framework Directive and an environmental quality standard (EQS) has been set at 5 ng/g wet weight (ww) for BaP in biota (EU, 2013). Only one cockle sample of May 2012 from location 4 (Ria of Ortigueira) exceeds this EQS (BaP concentration 6.9 ng/g ww).

On the basis of the traffic light criteria proposed by OSPAR (OSPAR, 2009) to indicate the status of the marine environment using mussels' data, Arousa and Foz rias can be classified as Blue (concentration below the background assessment criteria (BAC) for the 5 PAHs included by OSPAR, i.e. BaA, CHR, BaP, BghiP and IcdP) and also in Ria de Ortigueira for BghiP, which indicates that concentrations in these rias are close to background or zero and the status is acceptable. The other rias could be classified as Green for those compounds with established environmental assessment criteria (EAC) (concentration below EAC) i.e. BaA, BaP and BghiP, or Amber (concentration above BAC) for those compounds without EAC (CHR and IcdP). Green status means that concentrations of contaminants are at levels where it can be assumed that little or no risks are posed to the environment and its living

Table 1

Overview of the limits of detection and quantification and concentrations (ng/g dw) of the analytes studied in this work.

Compound	LOD (ng/g dw)	LOQ (ng/g dw)	Detection frequency (%)	25th percentile	50th percentile	75th percentile	Maximum (ng/g dw)	Average (ng/g dw)
CB31	0.01	0.03	95	0.02	0.11	0.28	1.9	0.20
CB28	0.01	0.03	97	0.02	0.10	0.26	1.5	0.19
CB52	0.01	0.03	95	0.02	0.11	0.37	2.0	0.26
CB101	0.01	0.03	100	0.74	1.35	2.73	18	2.3
CB118	0.01	0.03	99	0.28	0.69	2.0	14	1.6
CB153	0.01	0.03	100	2.32	4.81	15.3	112	12
CB105	0.01	0.03	97	0.02	0.12	0.49	4.8	0.42
CB156	0.01	0.03	97	0.02	0.23	1.42	33	1.8
CB138	0.01	0.03	100	1.19	3.33	7.97	85	6.9
CB180	0.01	0.03	100	0.35	0.74	2.19	16	1.6
γ-HCH	0.01	0.03	97	0.02	0.02	0.27	1.9	0.21
HCB	0.01	0.03	97	0.03	0.05	0.08	1.9	0.11
p,p'-DDE	0.01	0.03	100	0.37	1.07	2.19	24	2.0
o,p'-DDT	0.01	0.03	98	0.02	0.02	0.25	1.7	0.20
p,p'-DDT	0.01	0.03	98	0.02	0.43	0.97	7.0	0.69
Dieldrin	0.01	0.03	98	0.02	0.02	0.12	3.0	0.35
p,p'-DDD	0.01	0.03	98	0.08	0.73	1.53	5.7	0.99
BaA	0.27	0.90	99	3.4	6.3	9.8	38	7.8
CHR	0.12	0.40	100	8.0	16.0	26.7	113	20
BbF	0.23	0.77	100	6.0	10.5	17.7	62	14
BkF	0.26	0.87	99	2.5	4.7	7.4	24	5.9
BaP	0.35	1.17	99	1.7	3.3	5.9	44	5.9
DBahA	0.14	0.47	88	0.29	0.58	0.94	7.5	0.91
BghiP	0.19	0.63	100	2.5	4.3	7.2	36	6.0
IcdP	0.21	0.70	100	3.3	7.0	10.3	51	9.2
BDE-47	0.005	0.017	100	0.2	0.38	0.64	4.4	0.51
BDE-100	0.004	0.013	73	0.002	0.14	0.21	1.5	0.13
BDE-99	0.006	0.020	97	0.19	0.28	0.38	1.5	0.31
BDE-85	0.01	0.03	34	0.005	0.005	0.12	2.1	–
BDE-154	0.01	0.03	78	0.05	0.2	0.39	1.8	0.23
BDE-153	0.01	0.03	50	–	–	0.13	1.6	–
BDE-183	0.02	0.067	50	–	–	0.14	0.65	–
BDE-197	0.009	0.030	2	–	–	–	0.31	–
BDE-209	0.07	0.23	66	–	0.32	0.7	2.7	0.33
EHTBB	0.07	0.23	72	0.035	0.18	0.39	2.5	0.23
BTBPE	0.03	0.10	8	–	–	–	2.7	–
DEHTBP	0.03	0.10	6	–	–	–	2.9	–
Syn-DP	0.003	0.010	63	–	0.014	0.025	0.25	0.01
Anti-DP	0.004	0.01	62	–	0.08	0.15	1.2	0.05
TTBPP	0.6	2	0	–	–	–	–	–
DBDPE	0.6	2	0	–	–	–	–	–
PFHxA	0.08	0.27	60	0.04	0.5	4	35	–
PFHpA	0.08	0.27	58	0.04	0.28	1.45	15	–
PFOA	0.2	0.67	76	0.30	1.46	3.0	17	2.4
PFNA	0.3	1.00	36	0.15	0.15	0.65	14	–
PFDA	0.05	0.17	26	0.025	0.025	0.5	3	–
PFUnA	0.09	0.30	57	0.045	0.2	1.8	10	–
PFDoA	0.2	0.67	35	0.1	0.1	0.54	21	–
PFOS	0.05	0.17	35	0.025	0.025	0.60	17	–
4-MBC	0.5	1.67	36	0.25	0.25	8.0	49	–
BP-3	1	3.33	17	0.5	0.5	0.5	63	–
EHMC	0.2	0.67	30	0.1	0.1	5.0	94	–
OC	0.1	0.33	79	2.4	9.0	18.0	141	9.4
ODPABA	0.05	0.17	17	–	–	–	12	–
BP-4	1	3.33	23	–	–	0.54	87	–

resources at the population or community level while amber indicates that concentrations are lower than dietary limits for shellfish and above background but the extent of risks of pollution effects is uncertain.

The sampling timepoint was also a significant factor for ΣPAHs (p-value = 0.0001, Fig. 5a), showing lower concentrations in August 2012 than in February 2012 or 2013.

The factor species was not statistically significant for ΣPAHs (p-value = 0.1268). However, for some individual PAHs differences among species were statistically significant (p-value < 0.0151), showing higher levels in cockle samples for BaP, DBahA and IcdP (the compounds with the lowest water solubility, Fig. S2). This pattern is repeated in some recent works (Leon et al., 2013) and it is related to the ease of ingesting particulate matter due to its habitat, just under the surface of the sand and mud bottoms. On the other hand, raft mussels

showed higher levels than cockle samples for CHR and BbF.

3.2.2. PCBs

PCBs are the second group of pollutants in terms of total concentration (Fig. 2). ΣPCBs (sum of 10 congeners) concentrations ranged from 0.47 (a clam sample from location 11 collected during February 2013) to 261 ng/g dw (a wild mussel sample from location 9 collected in February 2012). These levels are in agreement with those found in molluscs, especially mussel, coming from other zones, such as the Adriatic Sea in the Italian littoral (Bayarri et al., 2001), Istanbul strait in Turkey (Okay et al., 2009), Adriatic coast in Croatia (Kljakovic-Gaspic et al., 2010) and in the Galician coast (Fernandez et al., 2013).

The major congeners in all studied species were the hexachlorinated biphenyls, CB 153 (43.4% of total) and CB 138 (25.6%), followed by

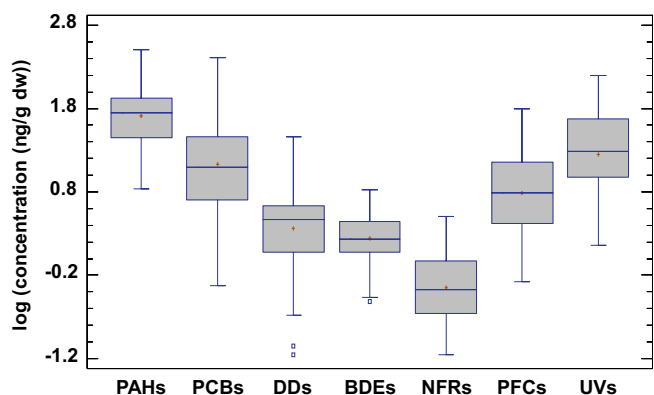


Fig. 2. Average log-scaled concentration (ng/g dw) for each family of compounds (PAHs, PCBs, DDs, PBDEs, NFRs, PFCs, UV filters).

the pentachlorinated congener CB 101 (8.4% of total) and the hexachlorinated CB 156 (6.8% of total) (Fig. 3b). The high prevalence of CBs 153 and 138 has been previously detected in several bivalves (Deudero et al., 2007; Leon et al., 2013) because they are the main constituents in Aroclor 1260 and Aroclor 1254 industrial mixtures. CB-153, CB-138, CB-101 and CB-180 were found in all the samples analysed. The other congeners, CB-31, CB-28, CB-52, CB-118, CB-105 and CB-156, were found above de LOD at least in 95% of the samples analysed.

Significant differences in ΣPCBs levels were found among species (p-value < 0.0001, Fig. 6). The ΣPCBs concentration average was of 27 ng/g dw for raft mussel, 54 ng/g dw for wild mussel and 12 ng/g dw for cockle. A similar pattern was observed for the individual PCBs congeners, the factor species being significant for all them. This fact demonstrates the great power of accumulation of PCBs in mussel in relation to other mollusc species. Significant correlation between fat content and log-scaled PCBs concentration was also observed (p-value = 0.0001). Thus, the higher concentration in mussels could be related to the highest percentage of lipid content in this specie (see text S4 in Supplementary Information), in agreement with the literature (Waszak and Dabrowska, 2009).

The location (Ria) was also a significant factor (p-value < 0.0001) for ΣPCBs and most individual PCBs (except CB-28 and CB-31). Fig. 4b presents the ΣPCBs 95% confidence intervals in each Ria. The highest levels of PCBs were found in samples from the most populated and industrialized Rias (see Table S1 and S3) such as, Ria of A Coruña, Ria of Ferrol and Ria of Vigo (ΣPCBs average 81 ng/g dw, 71 ng/g dw and 52 ng/g dw, respectively), where industrial discharges and domestic sewage can occur more often. Moreover, significant differences were observed in raft mussel samples in Ria of Pontevedra from location 17 and 18, showing higher concentration of PCBs in the samples from the inner area of the ria than in the outer area (average 13 and 8.3 ng/g dw respectively, Table S3).

In accordance with the traffic light criteria proposed by OSPAR (OSPAR, 2009), most stations would be classified as Blue

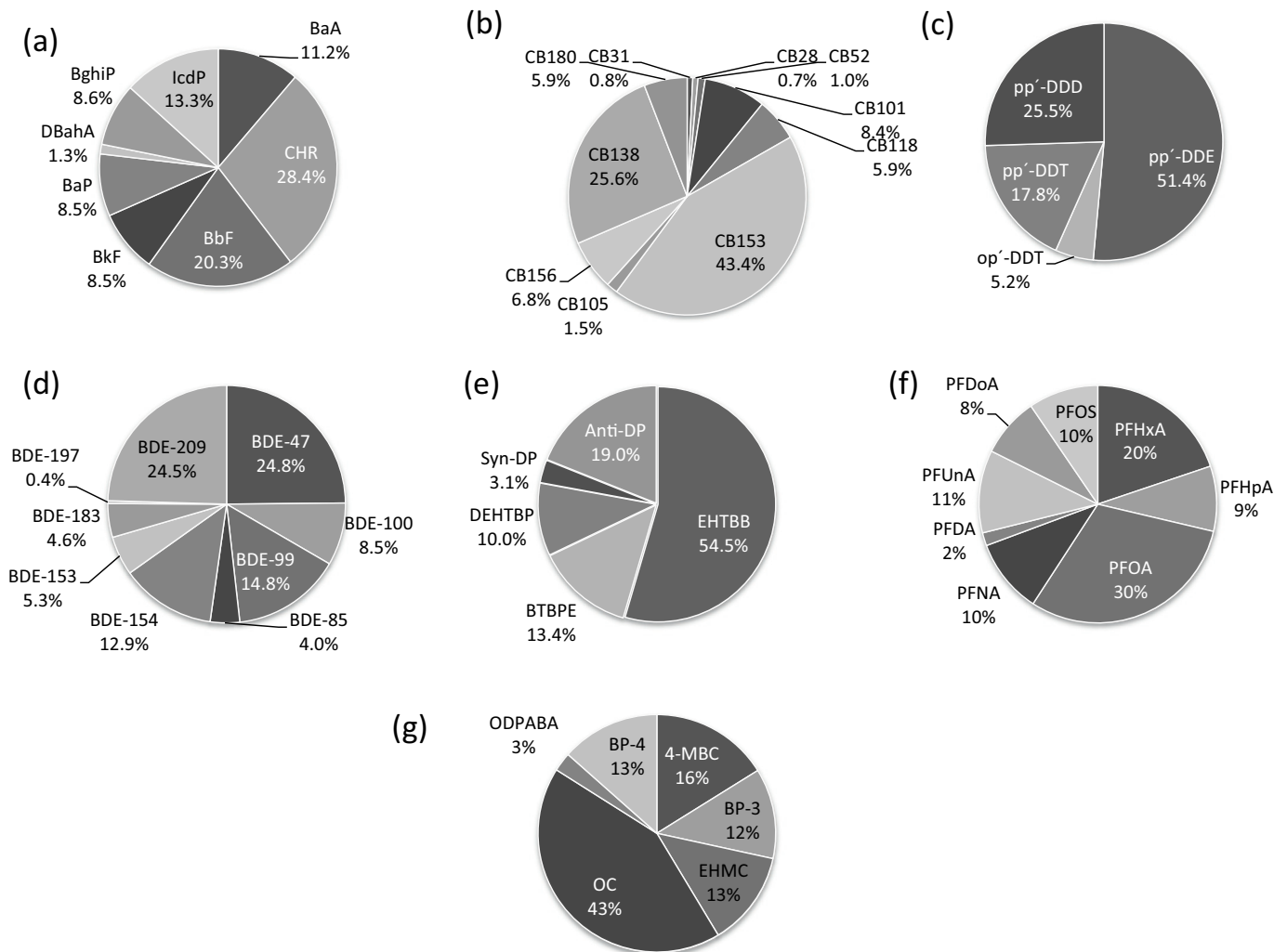


Fig. 3. Distribution of different compounds within each family of chemicals: (a) PAHs; (b) PCBs; (c) DDs; (d) PBDEs; (e) NFRs; (f) PFCs; (g) UV filters.

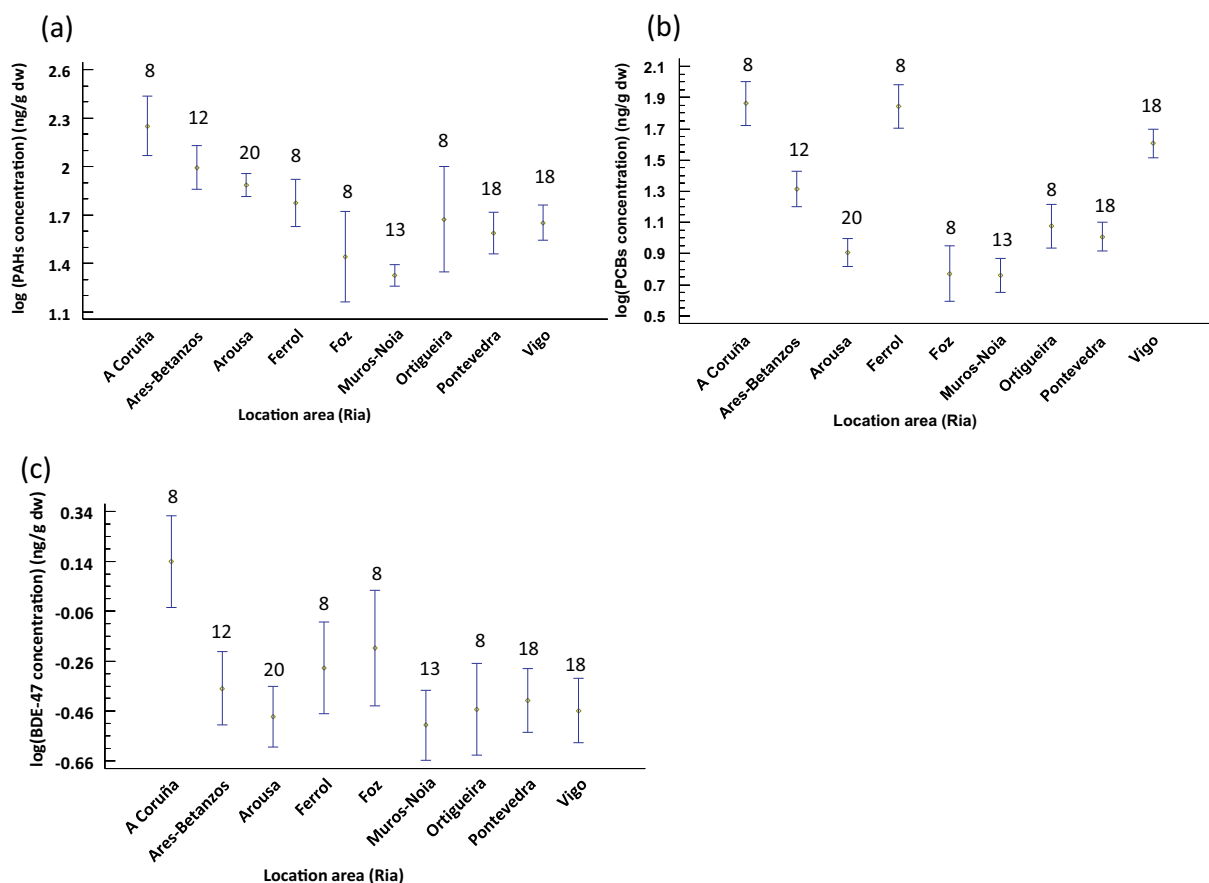


Fig. 4. Average and 95% confidence intervals for the different Galician Rias: (a) ΣPAHs, (b) ΣPCBs (c) BDE-47. The numbers on top of the bars represent the number of samples plotted per category.

(concentration below BAC) or Green (concentration below EAC) for the 7 PCBs considered by OSPAR (i.e. CB28, CB52, CB101, CB118, CB138, CB153 and CB180), except for mussel samples coming from Ria of A Coruña, Ferrol and Vigo which would be classified as Red (concentration above EAC) for CB101, CB118 and CB138, indicating that concentrations of contaminants are at levels where a risk to the environment and its living resources at the population or community level should be assumed. So, their status could be considered unacceptable. In addition, for CB118 some locations in Ria de Muros-Noia and Arousa (sites 11 and 16) for wild mussels would be classified as also Red (concentration above EAC).

Finally, the sampling timepoint was a statistically significant factor for ΣPCBs in raft mussels (p -value = 0.0335) and most individual PCBs, similar to PAHs (Fig. 5a) rendering higher concentrations in the sampling timepoints taking place in February 2012 and February 2013 (Fig. S3, Supplementary information).

3.2.3. OCPs

ΣDDs levels (sum of DDTs and their metabolites, p,p' -DDD and p,p' -DDE) ranged from 0.07 (cockle from location 2 collected in November 2012) to 29 ng/g dw (raft mussel from location 15 collected in February 2012), see Table S3 and Fig. 2. These levels are in the same order of magnitude as those found in other geographical areas (Choi et al., 2010; Kljakovic-Gaspic et al., 2010; Sericano et al., 2014). The most prevalent OCP of this family was p,p' -DDE found in all the samples analysed, while p,p' -DDD, p,p' -DDT and o,p' -DDT were found in the 98% of the samples. The ratios among p,p' -DDT and o,p' -DDT and their metabolites varies from sample to sample with an average of 51.4% of p,p' -DDE, 25.5% of p,p' -DDD, 17.8% of p,p' -DDT and 5.2% of o,p' -DDT (Fig. 3c). The predominance of p,p' -DDE and p,p' -DDD in the studied samples

indicates that there was no new input of DDT on the Galician coast. In accordance with the traffic light criteria proposed by OSPAR (OSPAR, 2009) for p,p' -DDE in blue mussel, all sites would be classified as amber (concentration above BAC for those compounds without EAC).

Species was the only statistically significant factor on ΣDDs levels (p -value < 0.0001), see Fig. S4. As for PCBs (Fig. 6), the highest ΣDDs average concentration was found for wild and raft mussel (7.3 ng/g dw and 4.4 ng/g dw, respectively) followed by cockle (1.2 ng/g dw). Significant correlation between fat content and log-scaled concentration of DDs was also observed (p -value < 0.0001). So, the higher concentration in mussels could be related to the highest percentage of lipid content in mussels as in the case of PCBs.

In relation to the remaining OCPs (γ -HCH, HCB and Dieldrin), they were detected at lower concentrations. γ -HCH was above the quantification limit (0.033 ng/g dw) in 30% of the samples analysed and above the detection limit (0.010 ng/g dw) in 97% of the samples with a maximum concentration of 1.9 ng/g dw and an average of 0.21 ng/g dw. HCB was present in the 69% of the samples above LOQ (average concentration 0.11 ng/g dw, maximum concentration 1.9 ng/g dw) while Dieldrin was only found in the 29% of the samples above LOQ (average concentration 0.35 ng/g dw, maximum concentration 3.0 ng/g dw). However, both compounds were found in 97% and 98% of the samples above the LOD (0.010 ng/g dw). Species was a statistically significant factor for γ -HCH and HCB. For γ -HCH the highest levels was observed in wild mussel samples (average of 0.39 ng/g dw), although it was only detected above the LOQ in the sampling campaign in February 2013 (33% samples), followed by raft mussel (average of 0.24 ng/g dw, 53% of the samples). For HCB, mussels showed the higher concentrations with an average of 0.16 and 0.11 ng/g dw, respectively.

For γ -HCH and HCB, significant correlation between fat content and

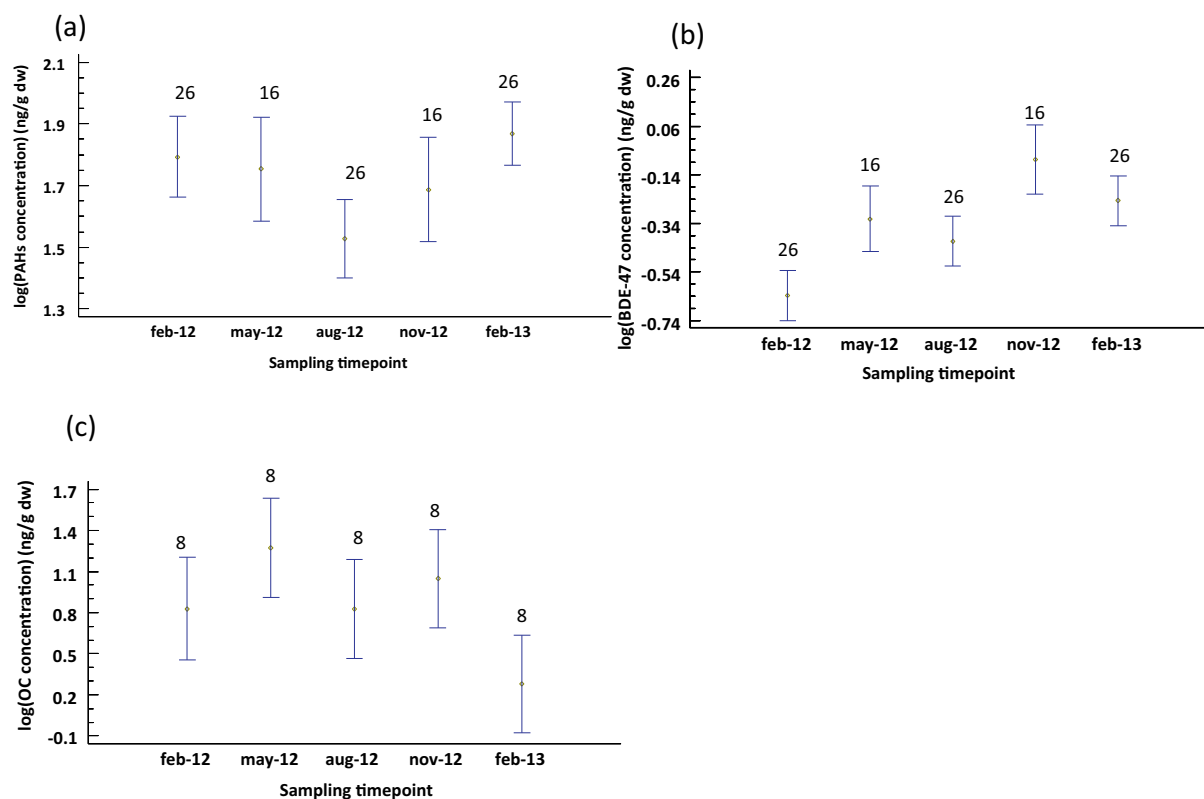


Fig. 5. Average and 95% confidence intervals for the different sampling timepoints: (a) ΣPAHs, (b) BDE-47 and (c) OC in cockle samples. The numbers on top of the bars represent the number of samples plotted per category.

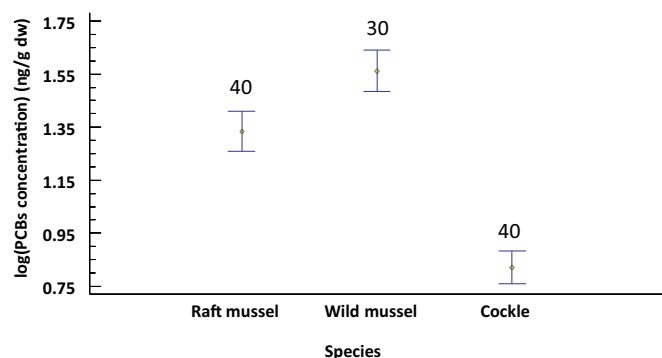


Fig. 6. Average and 95% confidence intervals of ΣPCBs for the different mollusc species. The numbers on top of the bars represent the number of samples plotted per category.

concentration was also observed (p-value = 0.0046 and 0.0001, respectively). So, the higher concentration in mussels could be related to the highest percentage of lipid content in mussels (see text S4), as for DDs and PCBs.

All location would be classified as Blue (concentration below BAC) for γ -HCH and HCB using the traffic light criteria proposed by OSPAR (OSPAR, 2009).

3.2.4. FRs

ΣPBDEs (sum of 9 congeners: BDE-47, 85, 99, 100, 153, 154, 183, 197 and 209) ranged from 0.31 to 6.6 ng/g dw (Fig. 2), represented mainly by BDE-47 (tetrabrominated congener, 24.8%), BDE-209 (decabrominated congener, 24.5%), and BDE-99 (pentabrominated congener, 14.8%) (Fig. 3d). The most frequently detected congeners were BDE-47 (100% of the samples analysed) followed by BDE-99, BDE-154, BDE-100 and BDE-209 (97%, 78%, 73%, 66% of the samples analysed,

respectively) and BDE-183, BDE-153 and BDE-85 (50%, 50% and 34%, respectively) (Table 1). BDE-197 was only found above LOQ in two samples of raft mussel.

PBDEs levels found in mussels were comparable or lower than those reported in other marine systems, such as Hong Kong marine waters (Liu et al., 2005), French coast sites (Johansson et al., 2006), Bo Sea in China (Wang et al., 2009), the Japanese coast (Ueno et al., 2010) and N-NW Spanish coast (Bellas et al., 2014). However, the sum of the 5 congeners considered in the European Water Framework Directive (BDE-47, 99, 100, 153, 154; BDE-28 was not considered in this study), ranged from 0.026 to 0.77 ng/g ww. Thus, they are above the EQS. (0.0085 ng/g ww) established by the European Water Framework Directive (EU, 2013) in all the samples. However, as mentioned, this is actually a worldwide issue.

As in the case of PCBs (Fig. 6), BDE-47 (p-value < 0.0001) were found at slightly higher levels in wild mussel (Fig. S5a) than those in raft mussel (average of BDE-47 1.01 and 0.69 ng/g dw, respectively). Infaunal mollusc (cockle) had the lowest concentrations (average 0.30 ng/g dw). Significant correlation between fat content and concentration of BDE-47 was also observed (p-value = 0.0004). So, the higher concentration in mussels could be related to the highest percentage of lipid content in mussels. Similarly, BDE-100 and BDE-154 were below LOD in most of the cockle samples, but were detected in of 94 and 98% in the mussel samples, respectively. For BDE-99, the species was not a significant factor.

The location was also a significant factor for the distribution of BDE-47 and BDE-99 (p < 0.0009). The highest levels were found for BDE-47 in the Ria de A Coruña (average 1.67 ng/g dw) (Fig. 4c) and for BDE-99 in Ria de Ferrol (average 0.62 ng/g dw) (Fig. S5b), derived from the greater anthropic activity in these areas (Table S1). The location was also an statistically significant factor for BDE-100 for mussel samples being found at lower levels in Ria de A Coruña and Ortigueira (Fig. S5c). Moreover, significant differences were observed in samples from the

Northern area of the Ria of Arousa (locations 12, 13 and 14) and the Southern area (locations 15 and 16), showing again (as in PAHs) higher concentration of BDE-47 in samples from the Southern area (average 0.69 and 0.3 ng/g dw, respectively).

The sampling timepoint was a significant factor for BDE-47 (p -value < 0.0001), showing a higher concentration in November 2012 (Fig. 5b). This temporal trend differs from that observed for PAHs and PCBs with higher concentrations in February 2012 and February 2013. BDE-209 was observed with concentration values below LOD in February 2012 but with a frequency of detection of 85% in the other sampling timepoints. Considering the sampling timepoints between May 12 and February 13, this factor was statistically significant for BDE-209 (p -value = 0.0006), showing, as BDE-47, higher concentrations in November 2012. Although there is no clear explanation for this behaviour, it clearly reflects that PBDE sources are different from the other classical POPs mentioned already.

NFRs is mainly represented by EHTBB (54.5%) (Fig. 3e), whose concentration (average 0.23 ng/g dw) is about half of the two main BDEs (average 0.51 and 0.33 ng/g dw for BDE-47 and BDE-209, respectively). The most prevalent NFRs were EHTBB, *syn*-DP and anti-DP, (73%, 63% and 62% of the samples analysed, respectively) followed by BTBPE and DEHTBP (only 8 and 6%, respectively, Table 1). TTBP and DBDPE were not detected in any sample. Few studies have been conducted to date in marine organisms and more specifically in mussel. The concentrations found in Galicia were at the same order of magnitude than levels found in other aquatic biota such as, mussel coming from Lake Winnipeg (Law et al., 2006) and muscle tissue of common sole coming from the French coast (Munschy et al., 2007) (Table S4).

For EHTBB, a low detection frequency was observed in February 2013, whereas between February 2012 and November 2012 its detection rate was 85%. So, the statistical analysis was performed removing the sampling timepoint of February 2013. The seasonal variation was still statistically significant (p -value = 0.0199), showing different profile to those reported previously for PAHs and PCBs or PBDEs with higher concentration in May 2012 (Fig. S6). On the other hand, no differences were observed among species and location areas.

In the case of anti-DP and *syn*-DP, most of the samples from the sampling timepoints of February 2012 and May 2012 were reported as non-detected, being the frequency of detection in the rest of sampling timepoints of 94 and 87%, respectively. After removing these sampling timepoints, no statistically significant differences were observed among species, location areas or sampling timepoints.

3.2.5. PFCs

ΣPFCs ranged from 0.53 (wild mussel from location 7 in February 2013) to 62 ng/g dw (clam from location 16 in February 2013, Table S3 and Fig. 2). The most prevalent PFCs was PFOA found in 76% of the samples, followed by PFHxA, PFHpA and PFUnA found in the 62, 58 and 57% of the samples respectively. PFOS was found above the LOD in 32% of the samples (Table 1). Concerning concentrations, the main PFCs were PFOA (30% of total PFCs) and PFHxA (20% of total PFCs). PFOS, PFNA, PFHpA and PFDoA represented each one around a 10% of total PFCs, while PFDA represented < 2% (Fig. 3f). Published studies show that the composition profiles of the PFCs in fish and shellfish are different. Whereas in fish the predominant PFC is PFOS, in shellfish the predominant one, as in this study, is PFOA (Pan et al., 2010; Wu et al., 2012). Concentrations of PFOS and PFOA in the literature are similar to this work, in the lower ng/g, although higher levels (tens of ng/g) have also been reported (a detailed comparison is presented in Table S5). In 2012, EFSA published an occurrence study performed in 13 European countries during the period 2006–2012. The reported concentrations in molluscs for PFOS and PFOA were in the range 0.02–2.9 ng/g ww and 0.03–0.98 ng/g ww, respectively (EFSA, 2012). Those values are similar to the obtained in this work, 0.005–2.3 ng/g ww and 0.005–2.0 ng/g ww for PFOS and PFOA, respectively.

Although PFOS has been included in the European Water

Framework Directive with an EQS set at 9.1 ng/g ww in biota (EU, 2013), none of the samples exceeded that threshold.

Due to the low detection frequency, an ANOVA analysis could not be performed. However, differences were visually observed among the detection frequency in the different species. For PFHxA, PFHpA and PFOA, higher detection frequency was observed in cockle (95% for PFOA, and 75% for PFHpA and PFHxA) followed by raft mussel and wild mussel (Fig. S7). PFNA and PFDA showed also higher detection frequency in cockle while for PFUnA and PFDoA no differences were observed. On the other hand, PFOS showed higher detection frequency in raft mussel and cockle. Especially, the mussels collected in February 2013 showed very low detection frequency for all the compounds (below 10%).

3.2.6. UV filters

ΣUV filters ranged from 1.4 (raft mussel in location 10) to 157 ng/g dw (raft mussel in location 21), thus representing a relevant class of chemicals in terms of concentration (Fig. 2). In order of abundance, the main UV filter is OC which represents 43% of the total concentration, followed by 4-MBC, BP-4, BP-3 and EHMC which are between 16 and 12% of the total concentration, while ODPABA only represents 3% (Fig. 3). The most prevalent UV filter is also OC, found in 79% of the samples. Far from this compound, 4-MBC was found in 36%, EHMC in 30%, BP-4 in 23% and ODPABA and BP-3 in 17% of the samples (Table 1).

The concentrations found are significantly lower than those reported by Bachelot et al. (Bachelot et al., 2012) in marine molluscs from the French coast, up to 200 ng/g dw EHMC and up to 7000 ng/g dw for OC, and by Picot Groz et al. (Picot Groz et al., 2014) in mussels from beaches in the Southern Portuguese coast, up to 4000 ng/g dw, 1700 ng/g dw and 800 ng/g dw for OC, EHMC and ODPABA, respectively (for a detailed comparison, see Table S6). However, OC is the pollutant found at highest concentration in a single sample (above the concentrations found for POPs) in this study.

When visually comparing detection frequencies, differences were observed among species (Fig. S8). For OC the detection frequency in cockle reaches 95% of the samples, thus ANOVA could be performed to evaluate the distribution of OC in this species. Considering only the samples of cockle, statistical differences were observed among the sampling timepoints (p -value = 0.0061), showing lower levels in February 2013 (Fig. 5c). This is due to the fact that sunscreens are used predominantly in spring and summer during the outdoor activities and beach season. In addition, cockles live buried in surface sediments of the beach, where they can be exposed to OC used in many sunscreens. No differences were observed among sampling locations.

3.3. Environmental risk assessment of the emerging pollutants

As explained in Section 2.6, three different threshold values were obtained for RQs of 0.01, 0.1 and 1 (Table 2), first in terms of water concentration and then into mussel equivalent concentration (dw). In this way, the experimentally measured concentrations in bivalves, which are therefore used as bioindicators, can be compared to these thresholds. The data gathered to derive such values is compiled into Table S2. In the case of NFRs, effects have not been observed experimentally nor predicted at higher concentrations than their aqueous solubility values (Table S2), hence no risk assessment was performed for these chemicals. Mussel concentrations found in the samples were categorized in the four risk categories (see 2.6) and the results are summarized in Fig. 7, as percentage of the total number of samples analysed.

For PFDoA the LOQ is above the concentration corresponding to RQ = 1, therefore all the samples above the LOQ (22%) would represent a high risk. Similarly, BP-3 and PFNA LOQs are above RQ = 0.1 concentrations. Therefore, a medium (21% of the samples for PFNA) or high (3% and 13% for PFNA and BP-3, respectively) risk of all the

Table 2
Environmental risk assessment of emerging pollutants.

	AF	NOEC (µg/L) ^a	PNEC (ng/L)	Water environmental concentrations (ng/L)			BAF (L/kg dw) ^a	Mussels concentration (ng/g dw)		
				RQ = 0.01	RQ = 0.1	RQ = 1		RQ = 0.01	RQ = 0.1	RQ = 1
				PFHxA	1000	42,300		42,300	423	4230
PFHpA	1000	7850	7850	78.5	785	7850	323	25	254	2536
PFOA	1000	594	594	5.94	59.4	594	15	0.09	0.89	8.9
PFNA	1000	195	195	1.95	19.5	195	46	0.09	0.90	9.0
PFDA	1000	78	78	0.78	7.8	78	105	0.08	0.82	8.19
PFUnA	1000	8	8	0.08	0.8	8	454	0.04	0.36	3.63
PFDoA	1000	1.4	1.4	0.014	0.14	1.4	370	0.005	0.052	0.518
PFOS	1000	33	33	0.33	3.3	33	378	0.12	1.25	12.47
4-MBC	1000	38	38	0.38	3.8	38	801	0.30	3.04	30.44
BP-3	1000	14	14	0.14	1.4	14	252	0.04	0.35	3.53
EHMC	1000	75	75	0.75	7.5	75	20,592	15	154	1544
OC	1000	68	68	0.68	6.8	68	2210	2	15	150
ODPABA	1000	30	30	0.3	3	30	19,473	6	58	584
BP-4	1000	25,000	25,000	250	2500	25,000	905	226	2263	22,625

^a The lowest available value compiled from the Table S2.

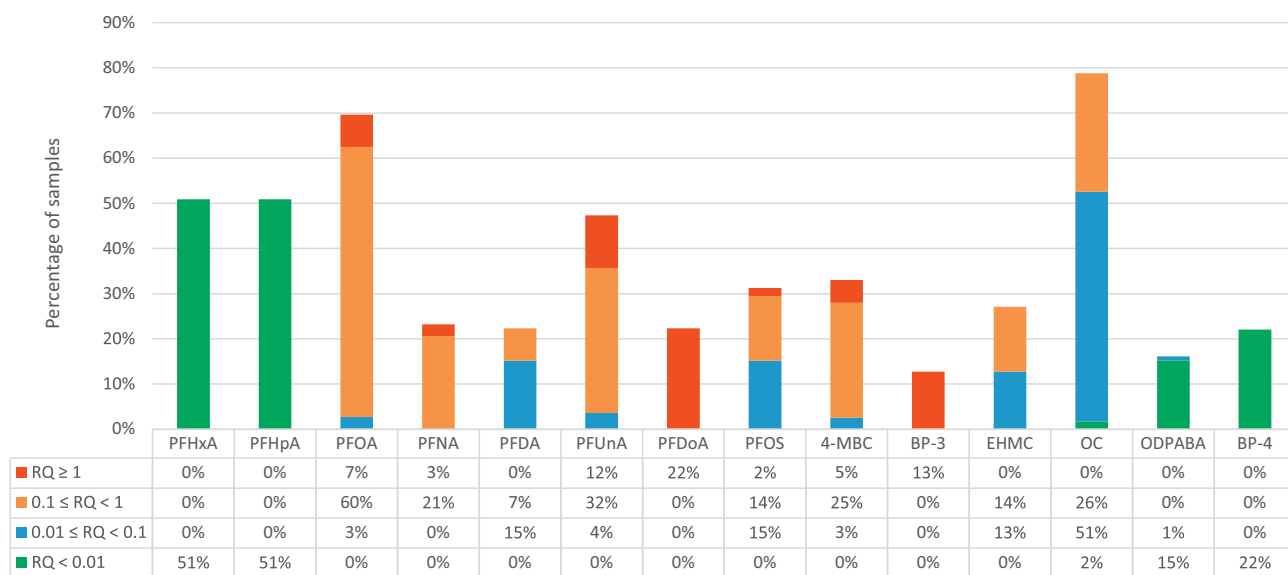


Fig. 7. Percentage of the total number of samples analysed categorized in the four risk categories: unlikely risk (RQ < 0.01), low risk (0.01 ≤ RQ < 0.1), medium risk (0.1 ≤ RQ < 1), and high risk (RQ > 1).

samples above LOQ would be represented. Risk quotient above 1 has been also estimated for BP-3 in the literature (Paredes et al., 2014; Sang and Leung, 2016). Due to a lower toxicity and/or BAF, an “unlikely risk” (RQ < 0.01) would be obtained for PFHxA, PFHpA, ODPABA and BP-4. In the case of BP-4, this result agrees with the work of Fent et al. (Fent et al., 2010), who obtaining a risk quotation of 0.02 considering the highest measured environmental concentration reported in the literature. A medium risk would be obtained for PFUnA, PFOS, 4-MBC, EHMC and OC in between 14 and 32% of the samples, reaching up to 60% of the samples for PFOA. Moreover, a high risk was estimated for PFOA, PFUnA and 4-MBC in 7%, 12% and 5% of the samples, respectively.

4. Conclusions

The present work provides new data about levels of some pollutants, including POPs (PCBs, OCPs, PAHs), new POPs (PBDEs, PFCs) and emerging pollutants (NFRs, UV filters) in mussel, cockle and clam from the Galician Rias. PAHs was the family found at higher concentration level, however the individual compound found at the highest concentration level was the UV filter OC in a cockle sample. According to

environmental risk assessment, PCBs and PBDEs were the families of legacy pollutants most relevant, among the emerging pollutants, PFDoA, PFUnA and BP-3 followed by PFOA, 4-MBC, PFNA and OC were the compounds posing the highest risk. The distribution profile observed for PBDEs is similar to that for PCBs in terms of inter-species and geographical variations, however it differs in the temporal pattern. In the case of NFRs, PFCs and UV filters the distribution profile do not match those observed for the classical POPs pointing to different pollution sources.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.05.018>.

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