Chemosphere 252 (2020) 126569

Contents lists available at ScienceDirect

Chemosphere

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

Assessment of organophosphate flame retardants in Mediterranean *Boops boops* and their relationship to anthropization levels and microplastic ingestion

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HIGHLIGHTS

- OPFRs were analysed in the muscle of bogues from the NW Mediterranean Sea.
- OPFR concentrations were higher in fish from the area off the city of Barcelona.
- No relationship was detected between OPFR levels and microplastic ingestion.
- Tri-n-butyl phosphate (TNBP) was the most abundant OPFR in both studied areas.
- ΣOPFRs ranged from nd to 1,194 ng g^{-1} lipid weight basis.

ARTICLE INFO

Article history:
Received 8 February 2020
Received in revised form
18 March 2020
Accepted 18 March 2020
Available online 19 March 2020

Handling Editor: Myrto Petreas

Keywords:
Bogue
Plasticizers
Plastic litter
OPFRs
Mediterranean sea

ABSTRACT

Plastic litter pollution is increasing in the seas and oceans worldwide, raising concern on the potential effects of plasticizer additives on marine fauna. In this study, muscle samples of 30 bogues (*Boops boops;* Linneaus, 1758) from the North Western Mediterranean Sea were analysed to assess the concentrations of 19 organophosphate flame retardant (OPFR) compounds and to inspect any relationship with microplastic ingestion and relative levels of anthropization. Out of the 19 OPFRs analysed, 6 compounds were detected, being tri-n-butyl phosphate (TNBP), 2-ethylhexyldiphenyl phosphate (EHDPP) and triphenylphosphine oxide (TPPO) the most abundant. As expected, OPFR concentrations were higher in samples collected off the most anthropized area of the city of Barcelona than in those from the Cap de Creus Marine Protected Area, while no significant correlation was detected between OPFR concentrations and microplastic ingestion. The results of this manuscript provide a first evidence of OPFR presence in the muscle of the bogue and identify the coastal area off Barcelona as a possible concentration area for contaminants, further supporting the use of the bogue as an indicator species of plastic pollution in the Mediterranean Sea.

1. Introduction

Marine litter pollution has been raising major concerns in recent years due to its potential impact to marine biodiversity, particularly in the Mediterranean Sea, which is one of the most polluted seas worldwide (Suaria and Aliani, 2014). Microplastics (*i.e.*, plastics < 5 mm; Arthur et al., 2009) of primary origin, or derived from the degradation of larger plastic items, have been found in concentrations up to 115,000–1,050,000 particles km^{-2} in the NW Mediterranean Sea (UNEP/MAP, 2015). Ingestion of macro and microplastics has been reported in various species of marine birds (*e.g.*, Ryan et al., 2016), cetaceans (*e.g.*, Besseling et al., 2015), marine turtles (*e.g.*, Domènech et al., 2019) and fish (*e.g.*, Boerger et al.,

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https://doi.org/10.1016/j.chemosphere.2020.126569
0045-6535/

2010; Garcia-Garin et al., 2019), with consequences ranging from severe injuries, to the obstruction of the digestive tract and, eventually, death by malnutrition (Gall and Thompson, 2015). Moreover, microplastics may act as vectors for the transport of inorganic and organic contaminants, which might cause toxic effects to the organism ingesting them (Rios et al., 2007).

Although plastic is inert (Galgani et al., 2013), the additives used to improve its features (*e.g.,* plasticizers, flame retardants) might modify its reactivity, producing toxic effects (Lithner et al., 2011). Indeed, high concentrations of plasticizers and flame retardants such as phthalates, polybrominated diphenyl ethers (PBDEs) and organophosphate flame retardants (OPFRs) may cause endocrine and carcinogenic effects on marine fauna (Aznar-Alemany et al., 2019; Du et al., 2019; Fossi et al., 2016).

Since the prohibition of PBDEs by the Stockholm Convention in 2009 (Stockholm-Convention, 2010), the use of OPFRs has increased exponentially (Pantelaki and Voutsa, 2019). Occurrence of these compounds has been studied in fresh water, air, sediment, biota, humans and some categories of food for human consumption (Du et al., 2019; Hou et al., 2016; Pantelaki and Voutsa, 2019; Zhao et al., 2019), but data regarding their toxicity is still limited. Some OPFRs, such as tris(chloroethyl) phosphate (TCEP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) were proven to be neurotoxic and carcinogenic (van der Veen and de Boer, 2012). *In vitro* studies on cells of experimental animals also showed tri-n-butyl phosphate (TNBP) and tris(phenyl) phosphate (TPHP) to cause developmental neurotoxicity, as well as adverse transcriptomic, reproductive, endocrine and carcinogenic effects (Bruchajzer et al., 2015; Du et al., 2019; Su et al., 2014; van der Veen and de Boer, 2012). Furthermore, 2-ethylhexyldiphenyl phosphate (EHDPP) showed adverse effects on female reproduction and foetal development in humans (Hu et al., 2017), and cytotoxic and transcriptomic effects in chicken embryonic hepatocytes, altering mRNA expression levels of multiple genes associated with different biological pathways (Shen et al., 2019). *In vitro* tests on human nuclear receptors also showed that several OPFRs may have potential endocrine disrupting effects (Kojima et al., 2013). Other novel OPFRs such as triphenylphosphine oxide (TPPO), whose occurrence has been reported in several environmental matrices (*e.g.*, Wang et al., 2017; Zhao et al., 2019) and biota (*e.g.*, Garcia-Garin et al., 2020) have effects still unknown on biota and humans.

The dramatic increase of production and use of OPFRs urges for a better assessment of their levels in the biota and of their potential effects on human health. The need for monitoring these contaminants is also stressed within the EU Marine Strategy Framework Directive (MSFD), which addresses the issue of marine pollution by litter and chemical contaminants through Descriptors 8, 9 and 10. According to the MSFD requirements, the good environmental status of the Mediterranean Sea would be reached when *concentrations of contaminants are at levels not giving rise to pollution effects* (D8), *contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards* (D9) and *properties and quantities of marine litter do not cause harm to the coastal and marine environment* (D10) (Zampoukas et al., 2014). In response to such requirements, the bogue (*Boops boops*; Linneaus, 1758) has been proposed as an indicator species of microplastic pollution in the Mediterranean Sea (Bray et al., 2019; Garcia-Garin et al., 2019).

The bogue is a benthopelagic species distributed across a wide latitudinal range in the eastern Atlantic Ocean, from Norway to Angola. It is common in the Mediterranean Sea, where it is ubiquitously distributed (FAO, 2020), and it is an edible species, what makes it relatively easy to collect for sampling. Finally, in the relatively small gut of this species, microplastics have been detected at high frequencies (Bray et al., 2019), further supporting the validity of this species as indicator for microplastic pollution.

The present study aims to assess the concentrations of OPFR compounds in the bogue and their relationship with microplastic ingestion and the relative levels of anthropization of the sampling areas, in order to test the potential use of the bogue as an indicator species for OPFR pollution. For this purpose, OPFRs were analysed in the muscle of bogues with known levels of microplastics (MP) in the gastrointestinal (GI) tract (Garcia-Garin et al., 2019), sampled from two areas in the North Western Mediterranean Sea characterized by different levels of urbanization and industrialization.

2. Materials and methods

2.1. Sample collection

The 30 bogues analysed in the current study were selected from a group of 102 individuals previously analysed for microplastic ingestion (Garcia-Garin et al., 2019). The selected sample included half individuals with microplastics in their GI tract and half without.

All bogues were caught during spring 2018 by local fishermen off the Spanish Catalan coast, in two areas characterized by a different degree of industrialization and urbanization: 1) an area in the vicinity of the city of Barcelona ($n = 15$); and 2) a marine protected area "the Cap de Creus (MPA)" ($n = 15$) (Fig. 1). The number of samples analysed for each area was selected to have a balance between the costs of the analysis and to have enough samples to perform statistical analysis. Sample collection was carried out avoiding contact with plastic material. Weight and total length of each fish were measured before storing at −20 °C. (Table 1).

2.2. Microplastic analysis

As previously mentioned, the analysis of microplastic ingestion was performed within the study by Garcia-Garin et al. (2019), which can be referred to for a detailed description of the methods used. Following the protocol developed in the framework of the MEDSEALITTER project (MEDSEALITTER consortium, 2019), the GI tract of the fish was dissected and digested with hydrogen peroxide (15%), and the microplastics in the digested filtrate were counted and classified by size, colour and type. Microplastic abundance was expressed as the number of microplastic items per individual.

2.3. OPFRs analysis

2.3.1. Standards and reagents

Nineteen OPFRs were analysed in the present study (Table S1). Analytical and labelled standards were obtained from different companies, as described in Giulivo et al. (2016). In addition, triethyl phosphate (TEP) and tri-n-propyl phosphate (TnPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-isopropylphenyl diphenyl phosphate (2IPPDPP), 4-isopropylphenyl diphenyl phosphate (4IPPDPP) and bis(4-isopropylphenyl) phenyl phosphate (B4IPPPP) were obtained from Wellington Lab-oratories Inc. (Guelph, ON, Canada).

2.3.2. Sample preparation

Muscle samples of about 15 g were lyophilised during 48 h. After that, samples were prepared according to Giulivo et al. (2016): 0.5 g dry weight (dw) were extracted by sonication using 15 mL of hexane:acetone (1:1) during 15 min. The extraction was carried out twice, and both extracts were combined. Then, the extract was evaporated under a gentle nitrogen stream in order to change the solvent, and it was reconstituted in 5 mL of hexane:methanol (1:3).

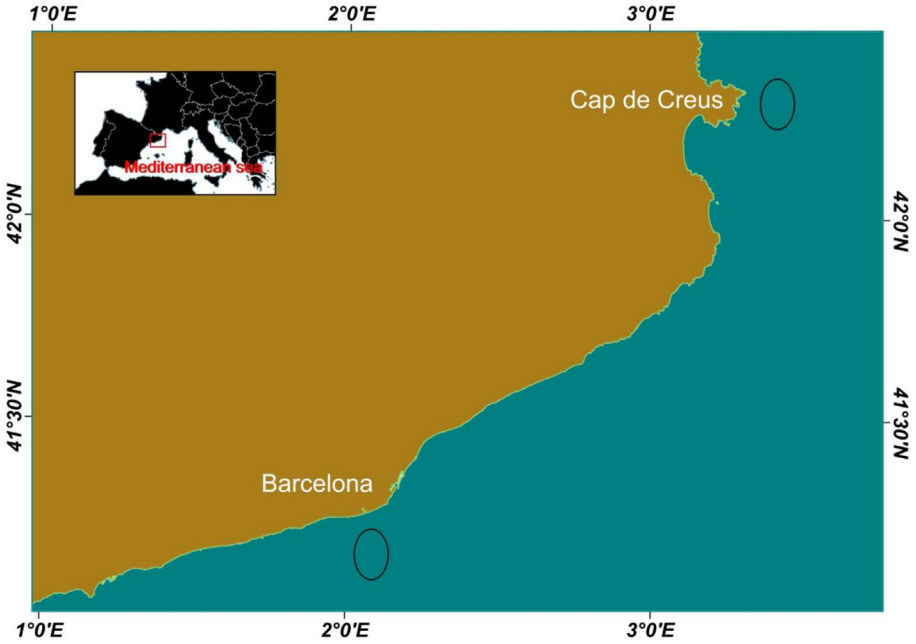

Fig. 1. Study area showing the two sampling areas off Barcelona and Cap de Creus MPA.

Table 1
Sample location, biometric data, number of MP in the GI tract and ΣOPFR concentrations in the muscle of bogue specimens.

ID	Area	Total length (mm)	Weight (g)	MP (items individual^{-1})	ΣOPFR (ng g^{-1} lw)
BB13	Barcelona	155	39.3	4	1,443
BB15	Barcelona	175	54.0	3	710
BB16	Barcelona	185	62.9	3	475
BB12	Barcelona	170	48.9	3	328
BB7	Barcelona	175	59.2	2	309
BB14	Barcelona	160	47.9	2	332
BB4	Barcelona	175	63.4	2	353
BB6	Barcelona	160	43.9	1	242
BB26	Barcelona	210	81.0	0	557
BB8	Barcelona	170	45.8	0	250
BB27	Barcelona	235	135.4	0	876
BB2	Barcelona	195	75.9	0	95.7
BB28	Barcelona	215	83.7	0	2,566
BB24	Barcelona	235	122.4	0	1,310
BB3	Barcelona	185	65.3	0	234
BM11	Cap de Creus MPA	220	146.6	3	162
BM3	Cap de Creus MPA	220	124.7	2	851
BM17	Cap de Creus MPA	235	127.2	2	1,379
BM8	Cap de Creus MPA	300	336.2	2	470
BM5	Cap de Creus MPA	300	411.7	1	904
BM1	Cap de Creus MPA	220	128.0	1	nd
BM12	Cap de Creus MPA	190	98.7	1	210
BM2	Cap de Creus MPA	240	162.9	0	177
BM29	Cap de Creus MPA	240	152.4	0	nd
BM26	Cap de Creus MPA	245	141.6	0	nd
BM19	Cap de Creus MPA	215	105.2	0	nd
BM18	Cap de Creus MPA	220	99.4	0	531
BM27	Cap de Creus MPA	220	126.3	0	319
BM31	Cap de Creus MPA	255	192.0	0	16
BM30	Cap de Creus MPA	260	191.9	0	962

nd: below detection limits.

The solution was centrifuged for 10 min at 4000 rpm and an aliquot of 200 μL was analysed by turbulent flow chromatography (TFC) coupled with LC-MS/MS. Labelled OPFR standards were added prior to analysis. Lipid weight (lw) was determined gravimetrically from the remaining 4.8 mL, after evaporating the solvent using a nitrogen stream and drying the sample in an oven at 90 °C until constant weight was reached.

2.3.3. Instrumental analysis

Purification was done according to Giulivo et al. (2016), and it was performed on-line at the beginning of the instrumental analysis with a Thermo Scientific TurboFlow™ system. Columns used for purification were CycloneTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm). An analytical column (Purosphere Star RP-18, 125 mm × 0.2 mm) was used for chromatographic separation.

The mobile phase was a gradient of water (0.1% formic acid) and methanol (ammonium acetate) at 0.75 mL min^{-1} (Santín et al., 2016). Mass spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source. For all compounds, selective reaction monitoring (SRM) mode was used with two transitions selected for each analyte.

2.3.4. Quality assurance

A blank was included every 10 samples. If its signal did not exceed 10% of the sample batch signals, its OPFR levels were subtracted from the corresponding batch of samples. If, on the contrary, the blank signal was higher than 10% of the sample batch signals, all samples in the batch were re-analysed. All the non-volumetric material was heated at 340 °C and rinsed with the appropriate solvent before use, and no plastic material was used to avoid contamination. Recoveries were 48–102% with RSDs between 0.3 and 24.7%. Limits of detection (LODs) and limits of quantification (LOQs) were 0.2–19.3 ng g^{-1} lw and 1.0–24.8 ng g^{-1} lw, respectively.

2.3.5. Expression of concentrations

OPFRs are lipophilic compounds and, as such, their concentrations are usually expressed on a lipid weight basis to normalize for varying lipid content between species, individuals and tissues (Krahn et al., 2003).However, here we express the concentrations of OPFRs on three bases to allow comparison with previous studies: extractable lipid basis (lw), wet weight basis (ww), and dry weight basis (dw).

2.4. Statistical analysis

The sum of all OPFR compounds (ΣOPFR) was calculated excluding the compounds that were below the limit of detection. The normality and heteroscedasticity of the distribution of ΣOPFR concentrations were tested using the Shapiro Wilk and the Levene test, respectively. ΣOPFR concentrations did not follow a normal distribution ($p < 0.05$, Shapiro Wilk test), although variances were homogeneous ($p = 0.28$, Levene test). Thus, ΣOPFR concentrations were normalized using the square root. Data were checked for possible collinearity between variables and the presence of outliers (Zuur et al., 2010). ΣOPFR concentrations were modelled using GLMs (generalized linear models) fitted with a Gaussian distribution. The explanatory variables used for building the models included: the level of anthropogenic impact, categorized as low (MPA) and high (off Barcelona); the number of microplastics (MP) detected in the fish GI tract; the fish length and the fish weight. Best fitting models were selected using the Akaike information criteria corrected for small sample sizes (AICc; Hurvich and Tsai, 1989) and the corresponding AICc increments (ΔAICc) and weights (AICc wt; Johnson and Omland, 2004). Differences in the OPFR concentrations, which were related to the level of anthropogenic impact and the presence or absence of microplastics in the fish GI tract, were highlighted through a Principal Component Analysis (PCA). The significance level was set at $p < 0.05$. R.3.6.2. statistical software was used for all analyses (R Core Team, 2018).

3. Results

Microplastic abundance ranged from 0 to 4 items $individual^{-1}$ in the bogues sampled in the area off Barcelona and from 0 to 3 items $individual^{-1}$ in those sampled in the Cap de Creus MPA (Table 1). Mean microplastic abundance was slightly higher in the bogues sampled off Barcelona than in those sampled in the Cap de Creus MPA (1.33 ± 1.45 and 0.8 ± 1.0 items $individual^{-1}$, respectively).

OPFRs were detected in all the bogues sampled off Barcelona and in 11 out of the 15 sampled in the Cap de Creus MPA. ΣOPFR concentrations ranged from below LOD (nd) to 2,566 ng g^{-1} lw, and were higher (96–2,566 ng g^{-1} lw) in the bogues sampled off Barcelona than in those sampled in the Cap de Creus MPA (nd to 1,379 ng g^{-1} lw) (Table 1).

The distribution of the different OPFR compounds (lw basis) in each area are depicted in Fig. 2. Among the 19 OPFRs analysed, 6 were detected in the bogues muscle. TNBP, EHDPP and TPPO were the most abundant compounds, detected in 100, 40 and 27% of the bogues sampled off Barcelona and in 67, 7 and 13% of the bogues sampled in the Cap de Creus MPA, respectively. Tri-n-propyl phosphate (TPP) and TPHP were detected in 47 and 7% of the samples from Barcelona, while they were not detected in the samples from the MPA. On the contrary, TDCIPP was detected only in 7% of the samples from the MPA (Table 2).

The mean and standard deviation of the concentration of each compound and of the ΣOPFR are shown in Table 2, grouped by sampling area. Mean ΣOPFR concentration, expressed on a lipid weight basis, was 672 (SD = 657) ng g^{-1} in the samples from Barcelona and 379 (SD = 411) ng g^{-1} in the samples from Cap de Creus MPA (Table 2).

A total of 16 different GLMs were fitted from the combination of the 4 variables (anthropogenic impact, number of microplastics, length and the weight of the fish) plus the level of anthropogenic impact*MP interaction (Table 3). The best fitting model showed a significant correlation of ΣOPFR concentrations with anthropogenic impact and fish length but the correlation with number of ingested microplastics was not significant (Table 4). No correlation between ΣOPFR concentrations and fish weight was found, as the latter was not included in the model (Table 3).

PCA ordination of samples considering the six OPFRs detected in the muscle of bogues and the two factors “area” and “microplastic presence in the GI tract” produced a two-dimensional pattern, with the first two components explaining 54.1% of the total variance (Fig. 3). Despite some overlapping, most of the separation between sampling areas and levels of microplastic ingestion, occurred along PC1 axis, mainly referring to EHDPP (0.60) and TPPO (0.57). On the other side, TNBP (0.71) and TDCIPP (−0.62) determined most of the separation along PC2.

4. Discussion

In this study, the levels of several plasticizers and flame

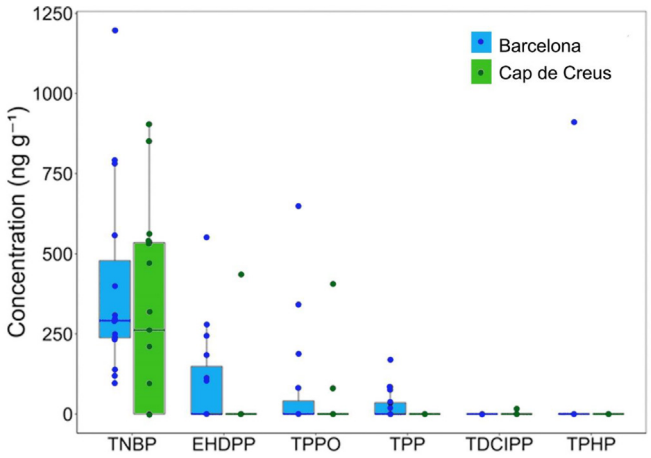

Fig. 2. Box-plots illustrating the OPFR concentrations (lw) detected in the bogues sampled off Barcelona and in the Cap de Creus MPA.

Table 2
Individual OPFR concentrations (mean ± SD, expressed in ng g^{-1} ww, dw and lw) and frequency of detection measured in the muscle of bogues collected from the area off Barcelona and in the Cap de Creus MPA.

	Barcelona				Cap de Creus MPA			
	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})	Frequency of detection (%)	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})	Frequency of detection (%)
TPPO	1.0 ± 1.8	4.1 ± 7.0	84.0 ± 183	27	0.7 ± 1.7	2.0 ± 5.3	40.5 ± 117	13
TPP	0.5 ± 0.5	1.8 ± 2.0	29.2 ± 47.7	47	nd	nd	nd	0
TDCIPP	nd	nd	nd	0	0.1 ± 0.3	0.3 ± 1.1	1.08 ± 4.2	7
TPHP	0.8 ± 3.1	3.4 ± 13.2	60.7 ± 235	7	nd	nd	nd	0
TNBP	6.5 ± 1.6	26.1 ± 6.3	400 ± 307	100	7.0 ± 6.4	25.4 ± 23.1	316 ± 313	67
EHDPP	1.9 ± 2.6	7.3 ± 9.8	98.5 ± 159	40	0.3 ± 1.1	1.1 ± 4.2	29.0 ± 112	7
ΣOPFRs	10.6 ± 4.9	42.6 ± 19.4	672 ± 657	100	7.9 ± 6.5	28.8 ± 23.5	379 ± 411	73

nd: below detection limits.

Table 3
GLM results for ΣOPFR concentrations in the bogues muscle, ranked by the Akaike information criteria corrected for small sample sizes (AICc). The variables included in the models were: level of anthropogenic impact (low and high), number of microplastics in the fish GI tract (MP), fish length (mm) and fish weight (g). The best-fit model is shown in bold. df = number of parameters; ΔAICc = AICc increments, AICc wt = AICc weights.

	Model	df	AICc	ΔAICc	AICc wt
M1	**Level of anthropogenic impact + MP + Fish length**	**5**	**235**	**0.0**	**0.18**
M2	Level of anthropogenic impact + Fish length	4	236	0.5	0.14
M3	Level of anthropogenic impact + MP	4	237	1.3	0.10
M4	Level of anthropogenic impact	3	237	1.4	0.09
M5	Level of anthropogenic impact + MP + Fish length + Fish weight	6	238	2.3	0.06
M6	Level of anthropogenic impact + MP + Fish weight	5	238	2.9	0.04
M7	Level of anthropogenic impact + Fish weight	4	239	3.0	0.04
M8	Level of anthropogenic impact + Fish length + Fish weight	5	239	3.4	0.03
M9	MP	3	239	3.4	0.03
M10	Level of anthropogenic impact * MP + Fish length + Fish weight	7	240	4.3	0.02
M11	Fish length	3	241	5.3	0.01
M12	Fish weight	3	241	5.3	0.01
M13	MP + Fish length	4	242	5.9	0.01
M14	MP + Fish weight	4	242	6.1	0.01
M15	Fish length + Fish weight	4	244	7.9	0.00
M16	MP + Fish length + Fish weight	5	244	8.5	0.00

Table 4
Summary of the outputs of the best-fit GLM, including the variables "level of anthropogenic impact", "MP" and "Fish length" (M1).

Term	Coefficient estimate	Standard error	Z value	Pr(>|z|)
Intercept	−13.89	15.22	−0.91	0.37
Level of anthropogenic impact (Low)	−16.19	5.42	−2.99	<0.01
MP	3.19	1.80	1.77	0.09
Fish length	0.18	0.08	2.38	0.02

retardants of the OPFR family were analysed in the muscle of bogues sampled from two areas subject to different anthropogenic pressures, and the relationship between microplastic ingestion and OPFR concentrations was investigated. Three main results were obtained: 1) mean ∑OPFR concentrations were higher in the most anthropized area off Barcelona, 2) positive correlation was found between ∑OPFR concentrations and fish length, and 3) the correlation between ∑OPFR concentrations and the abundance of ingested MP was not significant.

Flame retardants and plasticizers have been detected in fresh water, air, sediment, humans and biota (Du et al., 2019; Garcia-Garin et al., 2020; Hou et al., 2016; Pantelaki and Voutsa, 2019). As most OPFRs can easily be metabolized (WHO, 1990, 1991, 1997, 1998, 2000), the constant presence of these compounds in all the environmental compartments would point out to permanent emissions and exposure and possibly negative effects to humans and biota.

Information on the presence of OPFRs in aquatic biota is scarce and only in the last few years these compounds have been analysed in fish. Thus, studies reporting OPFR concentration in freshwater fish are few. Giulivo et al. (2017) analysed fish from the Adige, Sava and Evrotas rivers (Mediterranean Sea), finding lower OPFR levels than those reported in the present study. On the contrary, Santín et al. (2016), who analysed OPFR in fish from the Llobregat river, which flows into the Mediterranean Sea near Barcelona, reported concentrations in the same range as our results. Although they found that IPPP (a compound not detected in the bogues analysed in the present study) was the most concentrated OPFR, they also detected EHDPP (63 ± 165 ng g^{-1} lw) as one of the most concentrated OPFR in fish, consistently with our study.

Even more limited information is available on the occurrence of OPFR in marine fish, which, to the best of our knowledge, has been reported only in 5 published articles (Brandsma et al., 2015, Giulivo et al., 2016; Hallanger et al., 2015; Kim et al., 2011; Sundkvist et al., 2010), none of them referring to the Mediterranean Sea. OPFR concentrations in the bogues analysed in the current study are higher than the OPFR concentrations found in herring (*Clupea harengus*) from the sea of Sweden, reported by Sundkvist et al. (2010), in benthic and pelagic fish species from Western Shetland, reported by Brandsma et al. (2015), and in salmon (*Salmo salar*)

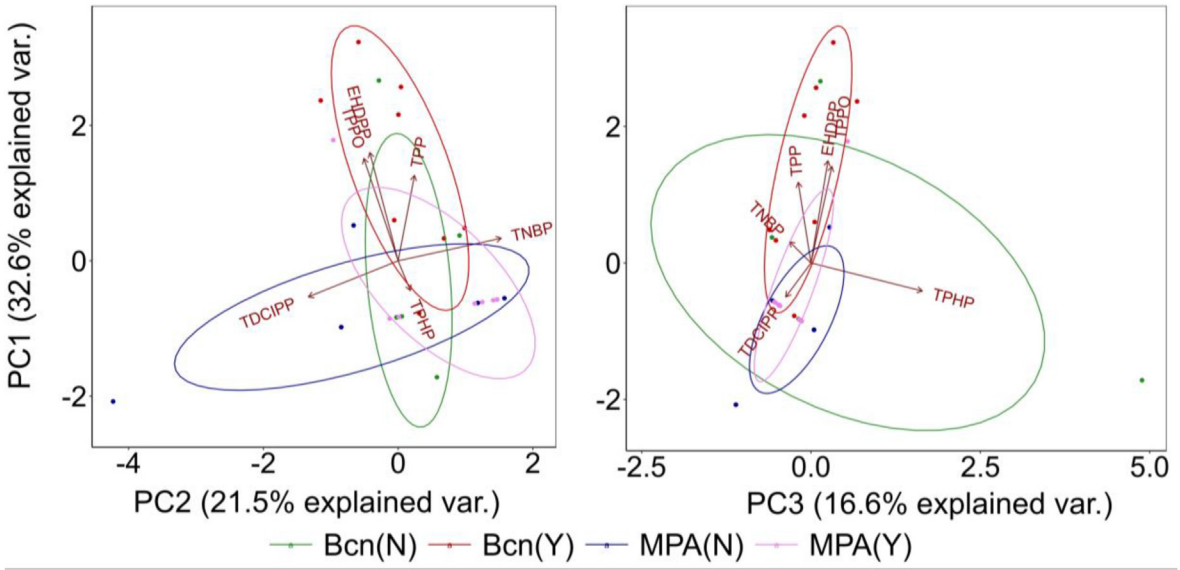

Fig. 3. PCA analysis of OPFR concentrations for area (Barcelona (Bcn) and Cap de Creus MPA (MPA)) and presence (Y)/absence (N) of microplastics inside the bogues GI tract.

from the Atlantic, reported by Giulivo et al. (2016). The above comparisons suggest that the Mediterranean Sea is more polluted in terms of OPFR concentrations than the Baltic Sea, the North Sea or the Atlantic Ocean, which can be indicative of higher plastic inputs in the basin. On the other hand, OPFR concentrations in the bogues sampled from the Barcelona area are in the same order of magnitude as those reported by Kim et al. (2011) in fish samples from the Philippines, in which TNBP was one of the most abundant OPFR, like in the present study. Finally, concentrations of TDCIPP, TPHP and EHDPP in capelin (*Mallotus villotus*) from the high-arctic archipelago of Svalbard (Hallanger et al., 2015) were higher than those found in the Mediterranean bogues. These studies indicate that OPFRs are omnipresent in the marine environment, although their compound composition and concentrations vary among different geographic areas.

∑OPFR concentrations were significantly different in the bogues from the two sampling areas, being higher, as expected, in those collected from the most anthropized area. Barcelona is the second city of the Mediterranean Sea in terms of estimated inputs of plastic, with an estimate annual input of plastic litter of 1,800 tons (Liubartseva et al., 2018). Its littoral hosts many industries, large commercial and tourist ports, and the city is located between the rivers Besòs and Llobregat, where wastewater treatment plants may contribute to the input of plastics in the sea. The importance of sewage plants as source of OPFR contamination was stressed by Sundkvist et al. (2010), who found OPFR concentrations two times higher in fish from lakes close to sewage treatment plants than in fish from other lakes. These factors might explain the higher OPFR concentrations in the bogues sampled from the area off Barcelona than in those sampled from Cap de Creus MPA. Furthermore, PCA analysis revealed that differences between sampling areas were mainly due to TPP and TPHP, which were detected only in Barcelona, and TDCIPP, which was detected only in Cap de Creus MPA. In addition, the concentrations of EHDPP and TPPO also separated the two sampling areas along the PC1 axis. Differences in the concentrations of specific congeners might be due to pollution by different types of plastics in the two sampling areas. However, further investigations are needed to allow relating each OPFR congener to its marine litter source.

Significant positive correlations were detected between ∑OPFR and fish length, although no significant correlation was found between ∑OPFR and fish weight. We might expect a similar relationship of ∑OPFR concentrations with fish length and weight, although fish length and weight are not linearly correlated, but their relationship follow instead an exponential function. While some studies do not report any significant correlation between ∑OPFR concentrations and fish length or weight (Kim et al., 2011; Malarvannan et al., 2015), others do: Choo et al. (2018), similarly to our results, reported positive correlation between TNBP concentrations in the muscle of crucian carp and the fish length and weight, implying that TNBP may accumulate in the muscle as the fish grows. Nevertheless, most OPFRs can easily be metabolized (WHO, 1990, 1991, 1997, 1998, 2000) and further research is needed to understand whether OPFRs accumulate in fish or biomagnify through the food web.

The number of ingested microplastics was included in the best GLM but it was not significantly correlated to the ∑OPFR concentration in the fish muscle. Similarly, PCA analysis did not reveal any difference between the concentrations of OPFR congeners and the presence of microplastics in the bogues GI tract, although one would expect the presence of microplastics to be highly related to the levels of OPFRs, which are widely used as plasticizers (Du et al., 2019). However, microplastics are likely to remain in the fish GI tract only few hours/days, depending on its length and thus on the digestion transit time (German and Horn, 2006), limiting the time during which additives could be absorbed by the fish tissues. In addition, OPFRs can also reach the fish tissues through the direct contact of the gills and the epidermis with the water and not only from microplastic ingestion (Kim et al., 2011). These aspects may explain why the abundance of ingested microplastics was not correlated to the ∑OPFR concentrations in the muscle of the bogues.

There is scarce data on potential toxic effects caused by the OPFRs commonly used as flame retardants and plasticizers. Among the OPFR compounds detected in the bogue samples, the effects related to TNBP, TDCIPP, and TPHP are the most studied. *In vitro* studies of OPFR effects on human cells have shown that TNBP, at concentrations ranging from 0 to 43 μg ml^{-1}, could inhibit cell viability, overproduce reactive oxygen species, induce DNA lesions, and increase the lactate dehydrogenase leakage (An et al., 2016). Other investigations showed that TDCIPP, at concentrations higher than 2 μg ml^{-1}, caused apoptosis in human corneal epithelial cells

(Xiang et al., 2017). On the other hand, *in vivo* toxicological laboratory tests have shown that TNBP, TDCIPP and TPHP were lethal for certain animal species, mainly rats, chick, rabbits, zebrafish and mice, with a median lethal dose (LD_{50}) of over 1,400 μg g^{-1} (Du et al., 2019; van der Veen and de Boer, 2012). ∑OPFR found in the muscle of bogues ranged from nd to 18.2 ng g^{-1} ww, which are values five orders of magnitude lower than those referred to as LD_{50} in laboratory animals. We can conclude that, at the concentration found in our study, the potential toxic impacts of OPFRs on bogues are limited, although the assessment of their effect should be made at a medium – large temporal scale as they may lead to chronic toxicity.

To determine the potential risk caused by the levels of OPFRs reported in fish to the human population, the intake of OPFRs through fish consumption was estimated considering that all edible fish would have concentrations similar to those found in the bogue. In the present study, the mean ∑OPFR concentration detected in bogues was of 9.27 ng g^{-1} (ww), which would imply, considering that the annual fish consumption in Europe is 22.5 kg (FAO, 2018) and the average human body weight is 60 kg, a mean OPFR intake by humans of 9.5 ng kg^{-1} day^{-1}. Such OPFR intake due to the consumption of Mediterranean bogues would be two to three orders of magnitude lower than the reference doses of TNBP and TPHP, which are 2,400 and 7,000 ng kg^{-1} day^{-1}, respectively (Van den Eede et al., 2011). In addition, the total dietary intake of 9.5 ng kg^{-1} day^{-1} is also much lower than the suggested guideline value of 40 μg kg^{-1} day^{-1} for the sum of TNBP, TBEP, TCEP, TCPP, TEHP and TPP (Sundkvist et al., 2010). Nevertheless, this dose refers only to fish intake and we should notice that there is also a large risk of exposure to OPFRs via other kinds of food intake (Li et al., 2019; Zhao et al., 2019) and via inhalation (Marklund et al., 2003). Thus, the concentrations detected in bogues are not negligible, and due to the global increase of plastic litter pollution, OPFR concentrations in marine organisms will likely increase, mostly affecting organisms and ecosystems close to highly anthropized areas. As the fish consumption in the world is constantly rising (FAO, 2018), the potential risks to human health are also expected to rise, urging for further research on the potential toxic effects of OPFRs.

5. Conclusions

The results of this study provide a first evidence of OPFR presence in the muscle of the bogue, identifying the waters off Barcelona as a potential area of concentration for these pollutants. The relationship between OPFR concentrations and microplastic ingestion is unclear, as no significant correlation was found between these two variables. The current OPFR concentrations in the bogue do not seem to be harmful to humans. However, dietary intake estimation should be conducted based on a broader spectrum of foodstuff samples and not just on one fish species. Further research is needed to analyse the occurrence of OPFRs in other edible species and their potential toxic effects in fish and, thus, indirectly, on human health. However, our results contribute to increase the knowledge on the levels of OPFRs in marine biota, highlighting the bogue as good indicator species of OPFR pollution in the Mediterranean Sea.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Odei Garcia-Garin: Conceptualization, Resources, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Morgana Vighi:** Writing - original draft, Writing - review & editing, Supervision. **Berta Sala:** Methodology, Validation, Supervision. **Alex Aguilar:** Supervision, Project administration, Funding acquisition. **Catherine Tsangaris:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Supervision, Project administration, Funding acquisition. **Nikoletta Digka:** Methodology, Validation, Resources, Writing - original draft, Supervision. **Helen Kaberi:** Resources, Writing - original draft, Project administration, Funding acquisition. **Ethel Eljarrat:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Supervision, Project administration, Funding acquisition. **Asunción Borrell:** Conceptualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgements

This study was supported by the Spanish Ministry of Science and Innovation (Project CEX2018-000794-S) and by the Generalitat de Catalunya (Consolidated Research Group Water and Soil Quality Unit 2017 SGR 1404). The authors are grateful to the Confradia de pescadors de Barcelona and the Confradia de pescadors de Roses, which provided the bogues samples. OGG's work was funded through an FPU Ph.D. scholarship granted by the Spanish Government. Constructive feedback from two anonymous reviewers contributed to improve the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126569.

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