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Perfluorinated compounds: Levels, trophic web enrichments and human dietary intakes in transitional water ecosystems

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ABSTRACT

The results of a study on levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), analyzed in terms of HPLC-ESI-MS in water, sediment, macrophyte, bivalve, crustacean and fish samples, are reported here. The aim of the research is to define, for the first time, PFOA/S levels in a heavily human-stressed transitional water ecosystem (Orbetello lagoon, Italy) and evaluate trophic web enrichments and human dietary intakes. The results obtained show that: (i) levels significantly higher than those reported in the literature were found in mussels, clams and crabs; (ii) the river is a significant pollution source; (iii) although absolute levels are relatively low, macroalgae proliferation contributes to redistribute pollutants from river-affected areas throughout the entire lagoon basin; (iv) to the best of our current knowledge, water-filtering species considered in this study are the most exposed to PFOA/S pollution; (v) human daily dietary intakes of PFOA/S through Slow Food-endorsed product consumption are below maximum tolerable levels suggested by the EFSA.

1. Introduction

Perfluorinated organic compounds (PFCs) are emergent persistent organic pollutants widely used for industrial and commercial applications including adhesives, agrochemicals, fire-fighting retardants, foams propellants, food packaging, lubricants, medicines, paints, polishes, refrigerants, and surfactant production (Renner, 2001). In particular, fire-fighting use for accident prevention during high-risk military procedures, routine fire-fighting training exercises and airport activities are the main direct sources of pollution of soil, fresh water and groundwater (Moody and Field, 2000), but leaching from discarded food packaging and runoff from discarded painted objects also produce significant indirect emissions (Renzi, 2012 and citations therein).

These emission sources release significant quantities of PFCs into the aquatic environment. In Europe for the year 2007, PFOS and PFOA discharges along the entire European river network to coastal areas have been estimated to be around 20 and 30 tons/year, respectively (Pistocchi and Loos, 2009).

Although in widespread use for a variety of purposes, these chemicals are hazardous substances which could affect the health of ecosystems and organisms due to their endocrine-disrupting activity, of which relatively little is described in the literature (Richardson and Ternes, 2005; Renzi, 2012 and citations therein). Recently, some studies documented PFCs in wildlife tissues (Kannan et al., 2002; Olivero-Verbel et al., 2006; Perra et al., 2010) and human body fluid samples (Yeung et al., 2006; Guerranti et al., 2013), suggesting a significant wide-ranging diffusion in the environment and a concrete exposure risk for human populations (Midasch et al., 2006).

A general lack of knowledge on environmental levels and bio-enrichment dynamics is reported for transitional water ecosystems as well as river effluents or lagoons.

This study, carried out in the Orbetello lagoon (Italy), aims to: (i) provide data on PFC levels in a large number of different environmental and biological matrices; (ii) compare observed levels with values reported in the literature for other aquatic ecosystems both in Europe and worldwide; (iii) evaluate the occurrence of enrichments throughout the lagoon trophic web; (iv) evaluate daily dietary intakes for humans related to the consumption of Slow Food-endorsed products from the lagoon.

2. Materials and methods

2.1. PFC physical–chemical features

PFCs are anionic fluorine-containing surfactants soluble in both water and oil and characterized by a half-life of more than two months in water and more than six months in sediments/soils

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(UNECE, 1998). Their physical-chemical properties favor long-range transport. Atmospheric conveyance of volatile precursor compounds and ocean currents have an important role in the global distribution of PFCs (Simcik, 2005a,b; Prevedouros et al., 2006), which are more volatile than chlorine or bromine analogues. Perfluorooctanoic acid (PFOA) and perfluoroctanesulfonic acid (PFOS) are the specific PFCs considered in this study; their physical-chemical features are summarized in Table 1. The carboxylic acid surfactant PFOA is much more volatile and soluble in water than PFOS and is measurable in environmental matrices (Corsolini et al., 2012; Senthilkumar et al., 2007), while PFOS is found predominantly in biota (Senthilkumar et al., 2007).

2.2. Study area

The Orbetello coastal lagoon (Central Tyrrhenian sea, Fig. 1) was selected for this study on the basis of general scientific knowledge developed in previous research (Renzi et al., 2013). Its principal meteorological, geomorphologic, hydrological and ecological characteristics are summarized in Table 2. The Albegna river ensures freshwater inputs throughout the Fibbia canal (W-basin) and represents a source of nutrients (ARPAT, 2007a,b) and pollutants due to human activity (Renzi, 2007; Specchiulli et al., 2008; Perra et al., 2009; Renzi et al., 2009, 2013) for the lagoon ecosystem. Direct fishing and aquaculture are important commercial resources in this ecosystem, principally based on eel (*Anguilla anguilla* L., 1758), sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus auratus* L., 1758), and sole (*Solea vulgaris*).

2.3. Experimental design

PFOA/S levels were measured in water, sediment, and biota collected in six sampling stations located in the Orbetello lagoon (Fig. 1). Organisms living at different trophic levels were collected, including primary producers, herbivores, filter feeders, and carnivores. Sampled species were: macrophytes (*Alsidium corallinum*, *Chaetomorpha linum*, *Cymodocea nodosa*, *Ruppia cirrhosa*), bivalves (*Mytilus galloprovincialis*, *Ruditapes decussatus*), crustaceans (*Palaemon serratus*, *Carcinus aestuarii*), and fish (*Parablennius* sp., *Zosterisessor ophiocephalus*, *Atherina* sp., *Gobius niger*). Samples were sized to reduce Type *I* and Type *II* errors according to a logical model (Underwood, 1994; Underwood and Chapman, 2003; Benedetti-Cecchi, 2004) based on a nested hierarchical design developed on three fixed *versus a priori* randomly defined factors: matrix (three levels, fixed), internal spatial variability (six levels, fixed), sampling replicates (three, random). To reduce sampling error, the geographical locations of sampling replicates were randomly extracted from a squared subsample grid of $1 \times 1\ km^2$ (Cochran, 1977), and extracted coordinates were localized *in situ* using a Global Positioning System (Garmin, mod. *e*-trex legend). The number of sampling replicates varied depending on the matrix considered. Abiotic matrices (water and sediments) and macrophytes were collected in triplicate and analyzed separately to include low-range spatial fluctuations, while animal species were sampled in statistical replicates considered representative of the entire lagoon population. As far as the ecological behavior of each species is concerned, not all of the six sites were sampled. To obtain sufficient quantities of tissues, analytical pools were prepared mixing equal wet weight of corresponding anatomical parts excised from ten organisms of the same species, obtaining a real $n = 30$ per each of the six selected sampling stations. Variability due to sediment grain size was *a priori* excluded: sediments characterized by a high silt content fluctuating within a narrow range (80–90% d.w.) were considered. It is well known that in water, POP levels tend to increase with body size as a function of exposure time; to reduce age-dependant variability, analyses were performed on a narrow-range distribution of body length (Renzi et al., 2012). Aquatic exemplars were sampled using movable trap nets (*bertovelli*) set within each sampling area in May 2008 to standardize seasonal-based temporal variability of pollution, biological phenomena linked to life cycle stages and sexual activity.

2.4. Sampling and laboratory pre-treatment of matrices

Water was sampled 5 cm under the surface and transferred after *in situ* filtration into a pre-cleaned HDPE polypropylene bottle

Table 1
Physical-chemical characteristics of studied molecules. Substance identification (extended names and international classification numbers), principal molecular properties, and related risks of PFOA (perfluorooctanoic acid perfluorooctanoate) and PFOS (perfluoroctanesulfonic acid) are summarized in table. Specific references: PFOA records were extracted from the GESTIS Substance Database from the IFA (last access on 5th November, 2008), Prevedouros et al., 2006.

	PFOA	PFOS
Extended name	Perfluorooctanoic acid	Perfluoroctanesulfonic acid
Other names	Perfluorooctanoate Perfluorocaprylic acid FC-143 F-*n*-octanoic acid	1-Perfluorooctanesulfonic acid Heptadecafluoro-1-octanesulfonic acid Perfluoro-n-octanesulfonic acid
Substance identification		
CAS numb	335-67-1	1763-23-1
Pubchem	9554	74483
EC number	206-397-9	217-179-8
Molecular properties		
Molecular formula	$C_8HF_{15}O_2$	$C_8HF_{17}O_3S$
Molecular mass	414.07 g mol^{-1}	500.13 g mol^{-1}
Boiling point	189–192 °C	133 °C (6 torr)
Appearance (25 C, 100 kPa)	Colorless liquid	White powder
Vapor pressure	4.2 Pa (25 °C)	3.31×10^{-4} Pa (20 °C)
Melting point	40–50 °C	>400 °C
Solubility in water	3400 mg L^{-1}	519 mg L^{-1} (20 ± 0.5 °C) 680 mg L^{-1} (24–25 °C)
Solubility in other solvents	Polar organic solvents	56 mg L^{-1} (octanol)
Acidity (p*K*a)	2–3	Calculated value of −3.27
Related risks		
S-phrases	S36, S37, S39	S61
R-phrases	R22, R34, R52/53	R61, R20/21, R40, R48/25, R64, R51/53

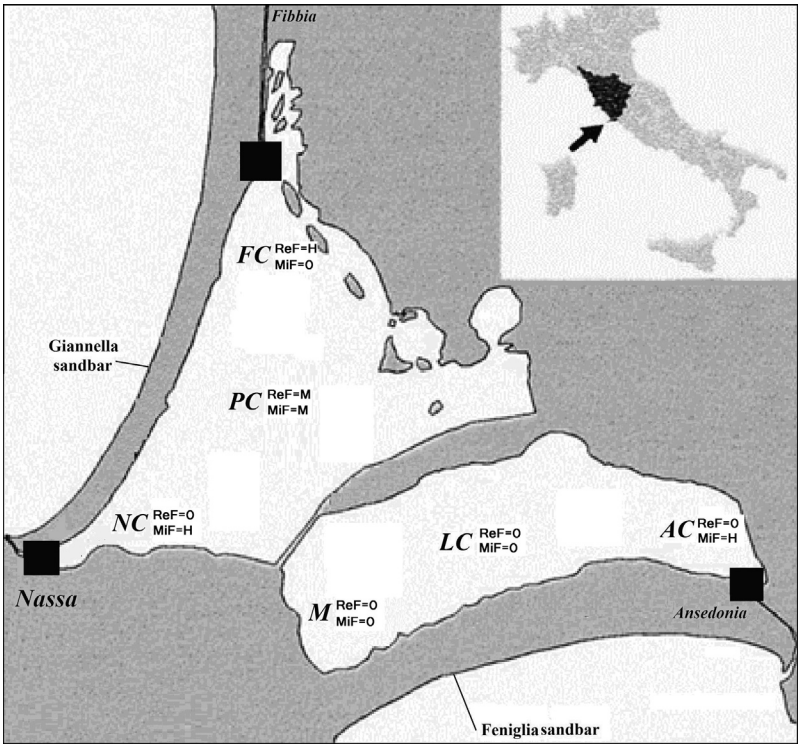

Fig. 1. Study area: the Orbetello lagoon. Georeferenced localization of sampling stations selected to evaluate the effect of the internal spatial variability factor is evidenced. *A priori* defined ranking classifications describing both the river (ReF) and marine (MiF) influences are made on the basis of bibliographical data reported for lagoon water concerning average water salinity (Specchiulli et al., 2008) and hydrodynamics (Giusti et al., 2010). Legend: NC = Nassa Channel, AC = Ansedonia Channel, M = Mines, FC = Fibbia Channel, LC = Eastern basin Centre, PC = Western basin Centre. ReF = ranked classification for the factor river influence, MiF = ranked classification for the factor marine influence. Three levels are defined for both ReF and MiF factors: H = high, M = medium, 0 = no effect.

Table 2
Principal characteristics of studied ecosystems. Principal general features of studied ecosystems are summarized in table.

Feature	Orbetello lagoon
Geographical district	Central Tyrrhenian
Geographical localization	Southern Tuscany
Gauss–Boaga coordinates	42.29°N; 11.17°E
Total surface (ha)	2700
Average depth, range (m)	1 (0.30–1.70)
Lagoon structure	The lagoon is divided by a dam into two communicating areas named Western (W, 15.25 km^2) and Eastern (E, 10.00 km^2) basins
Seawater exchanges	Managed by artificial pumping. Two inflow canals (Nassa and Fibbia W-basin), and one outflow (Ansedonia)
Freshwater inputs ($m^3\ s^{-1}$)	Albegna river: 15[a]
Temperature (°C)	4–11 in winter[b]
Salinity (‰)	20–35[b]
Sediment structure	Dominated by fine-grained sediments with the clay component prevailing in the central area and the silty component covering the entire south-eastern side of the basin[c]
Economic relevance and impacts	Intensive fish farming, summer tourism. Effluents of various human activities such as municipal wastewater treatment plant, industrial activities and urban settlement[d]. Significant persistent organic pollutants are recorded in eels' tissues[e]

References, as indicated in square brackets:
[a] ARPAT (2007a,b)
[b] Specchiulli et al. (2008).
[c] Renzi et al. (2007)
[d] Giovani et al. (2010)
[e] Renzi et al. (2013).

wrapped in aluminum foil. Filtration was performed using disposable nylon filters (0.2 μm Ø pores) fixed onto a 500 mL syringe. Surface sediments (0–5 cm) were collected using a stainless steel box-corer (15 × 15 × 15 cm). The central part of the sediment core was sampled with a stainless steel spatula and placed in a pre-cleaned large-mouth amber bottle. Samples collected were immediately taken to the laboratory and stored on ice at +4 °C. Sediment samples were lyophilised and accurately homogenized to reduce matrix variability. Large components, if present, were removed mechanically by sieving using *a* >1 mm Ø steel test sieve (DIN EN ISO, 9001). Macrophytes were sampled in triplicate using a sampling surface of 9 m^2 (3 × 3 m) standardizing the number of replicates as per the literature (Giovani et al., 2010, in press). Biomass within the sample area was collected, stored in polypropylene large-mouth bottles, and transferred to the laboratory where macrophytes were carefully washed using synthetic marine water (ISO 10253:1995, E) to remove extracellular water and sediment particles. Epiphytes were manually removed from leaves. Different species were cleaned and classified and identification confirmed by means of optical microscopy (Optika, mod. SZR10). Collecting

procedures and treatment of animals differed according to both their ecology and to the feeding habits of predators, including humans. Crustaceans and fish were caught by authorized fishermen using appropriate fixed nets, sacrificed and homogenized after a rapid rinse with synthetic marine water to remove external elements. *M. galloprovincialis* was sampled on solid substrates using a knife to remove it from rocks; *R. decussatus* was collected from soft mobile lagoon bottoms using a rake; and both were excised from valves before analysis.

2.5. Chemical analyses

2.5.1. Extraction of water

Analyses were performed using the method adopted by Senthilkumar and colleagues (2007). A known amount of the ^{13}C-PFOA was added as an internal standard for quality assurance.

2.5.2. Extraction of solid matrices

The analytical procedure for the extraction of sediments and tissues was performed according to methods detailed by Corsolini et al. (2008, 2012), Guerranti et al. (2013).

2.5.3. Quantification of selected molecules

Measurements were performed by means of high-performance liquid chromatograph (HPLC) equipped with an electro-spray ionization (ESI) tandem mass spectrometry (Finnigan Surveyor Plus HPLC System) interfaced to a Finnigan LTQ linear ion trap mass spectrometer (Thermo Electron Corporation) operating in negative electro-spray mode. Quantifications were optimized as reported by Guerranti et al. (2013). Standards for the four point calibration curve were prepared by progressive dilution with methanol from a standard solution purchased from Chiron (Trondheim, Norway). Concentrations were evaluated in comparison to a non-extracted standard curve composed of 5 dilutions of PFOS/A standards.

2.6. Quality assurance and Quality control (QA/QC)

Laboratories complied with ISO 9001:2000 and ISO 14001 standards for ecotoxicological analysis of sediments and organisms (IT33804). Chemicals and reagents were analytical grade, and glassware was carefully washed to avoid sample cross- contamination. Data quality assurance and quality control protocols included matrix spikes, laboratory blanks, and continuing calibration verification. The accuracy and precision of the procedures were tested for each matrix by analyzing laboratory-made spiked matrices in statistical replicates ($n = 3$) for each type of sample to calculate average and standard deviation (SD) of recoveries. Standard solution additions containing 0.5 ng L^{-1}, 1.5 ng L^{-1}, and 3.0 ng L^{-1} of PFOA and 0.02 ng L^{-1}, 0.10 ng L^{-1}, 0.20 ng L^{-1} of PFOS were performed for water samples (mean errors: 4% for PFOA, 5% for PFOS). Dried sediments were added with 10 ng g^{-1} of both PFOA and PFOS, while dried macrophytes and animal biomasses were added by 1 ng g^{-1} of both PFOA and PFOS. Due to the high percentages of recoveries in sediment (>98% PFOA/S) and tissues (>93% for PFOA, and >89% for PFOS in *A. corallinum* and *C. nodosa*, >89% for PFOA/S in *C. aestuarii*, *M. galloprovincialis*, >90% for PFOA and >85% for PFOS in *Z. ophiocephalus*), analytical concentrations were not corrected. The instrumental limit of detection (LOD) was determined as three times the signal-to-noise (*S*/*N*) ratio and was 0.01 ng L^{-1}. Limit of quantification (LOQ) were 0.01 ng L^{-1} in water, 0.50 ng g^{-1} d.w. in sediment and 0.40 ng g^{-1} in tissues. Blanks were analyzed with each set of five tissue samples as a check for possible laboratory contamination and interferences. Data were expressed as mean (max–min) ± SD. Levels in sediments were expressed as concentration per dry weight (d.w.) whereas, data measured in tissues were calculated both on a dry weight (d.w.) basis and on a wet weight (w.w.) basis for the enrichment factors and TDI calculation.

2.7. Statistical analyses

Pearson's correlations were applied to explore relationships between the variables analyzed, using a raw data matrix ($p < 0.01$), with GraphPad Prism (GraphPad Software, San Diego California USA, www.graphpad.com) package. Significant segregations among groups were examined using the non-parametric Mann-Whitney test (confidence interval 95%, $p < 0.01$). Multivariate analyses were run using the Primer v6.0 software package (Primer-E Ltd., Plymouth Marine Laboratory, UK) as per Clarke and Warwick (2001). Principal Component Analysis (PCA) was applied after square root ($\sqrt{}$) and $\log(x + 1)$ transformation and normalization of data. The dissimilarities observed were confirmed by a one-way ANOSIM (ANnalysis Of SIMilarities) R statistic test, which tests hypotheses of differences between groups of samples according to factors defined *a priori*, using permutation/randomization methods on a resemblance matrix and performing 9999 runs. Further details on methodological criteria applied in multivariate approaches are reported in Benedetti-Cecchi (2004), whereas statistical procedures adopted are detailed in Renzi et al. (2013).

2.8. C/N ratios

Concerning sediments, *C*/*N* mean (min–max) atomic ratios were estimated to evaluate the principal origin of the organic matter available for the detritus-feeder component in each sampling station. *C*/*N* ratios were calculated on the basis of total organic carbon (TOC) and total nitrogen (TN) concentrations measured in sediments sampled from the same sites by Renzi et al. (2007); unpublished data.

2.9. Bio-enrichment factors

Biological enrichment indices were calculated to evaluate water and sediment contributions to bioaccumulation of pollutants both in specific species and throughout the trophic web. BEFs (Bio-Enrichment Factors) were calculated as $BEF_{(b/s)} = [PFOS_{(t)}]:[PFOS_{(s)}]$ and $BEF_{(b/w)} = [PFOA_{(t)}]:[PFOA_{(w)}]$, where: $BEF_{(b/s)}$ are Bio-Enrichment Factors biota/sediment, $PFOS_{(t)}$ are PFOS levels measured in tissues, $PFOS_{(s)}$ are PFOS levels measured in sediments, $BEF_{(b/w)}$ are Bio-Enrichment Factors biota/water, $PFOA_{(t)}$ are PFOA levels measured in tissue and $PFOA_{(w)}$ are PFOA levels measured in water.

2.10. Estimated Daily Ingestion

Estimated Daily Ingestion (EDI) due to dietary intake was calculated by multiplying both the average and maximum (worst case) contaminant concentrations (w.w.) in edible parts of species by the estimated daily intake for the general population (38.8 g day^{-1}) and fish consumers (71.0 g day^{-1}) in Italy (Leclercq et al., 2009). Obtained values were used to evaluate the daily intake per kg of body weight, considering an average body weight of 50 kg for women and 70 kg for men. Results were compared with the tolerable daily intakes (TDIs) of 1500 ng kg^{-1} b.w. and 150 ng kg^{-1} b.w. established respectively for PFOA and PFOS (EFSA, 2005, 2012). When the contaminant concentrations were under the respective LODs, daily intakes were calculated assuming that respective values would be equal to one-half of the LOD.

3. Results

Mean levels of PFOA/S and their measured ranges in lagoon water are reported for each sampling station in Table 3a. Higher PFOA/S levels were measured in sampling station FC, whereas PC and LC stations show lower values; the PFOA trend is: PC < LC < M < AC < NC < FC, while the PFOS trend is: LC = PC < M < NC = AC < FC.

Concerning surface and freshwater in Europe, the ranges of PFOA and PFOS levels recorded are, respectively, 0.33–57.0 ng L^{-1} (IFA, 2001) and 0.01–56.0 ng L^{-1} (EFSA, 2008), while drinking water in Europe showed a lower range 0.40–8.10 ng L^{-1}. Municipal wastewaters are sources of PFOS (0.041–5.29 ng L^{-1}), whereas surface water is within the <0.01–0.138 ng L^{-1} range. Hansen and colleagues (2002) evidenced that fluorochemical manufacturing plants contribute to both PFOA (140–598 ng L^{-1}) and PFOS (74.8–144.0 ng L^{-1}) levels in river water.

With regard to the marine environment, between 2002 and 2004, Yamashita and colleagues (2005) measured PFOA levels in the Pacific Ocean (0.015–0.142 ng L^{-1}, $n = 19$), the South China Sea and the Sulu Sea (0.076–0.510 pg L^{-1}, $n = 5$), the North and Mid-Atlantic Oceans (0.100–0.439 ng L^{-1}, $n = 12$), and the Sea of Japan (0.137–1.070 ng L^{-1}, $n = 20$), PFOS levels they measured in the same areas were lower than PFOAs, respectively: 0.001–0.078 ng L^{-1}, <0.017–0.113 ng L^{-1}, 0.009–0.073 ng L^{-1}, and 0.040–0.075 ng L^{-1}. Coastal seawater from several Asian countries (Japan, China, Korea) showed PFOA levels in the following ranges: 1.800–19.200 ng L^{-1} (Tokyo Bay), 0.673–5.450 ng L^{-1} (Hong-Kong), 0.243–15.300 ng L^{-1} (China), and 0.239–11.350 ng L^{-1} (Korea), while PFOS in the same sites were measured as: 0.338–57.700 ng L^{-1} (Tokyo Bay), 0.070–2.600 ng L^{-1} (Hong-Kong), 0.023–9.680 ng L^{-1} (China), and 0.039–2.530 ng L^{-1} (Korea). In Japanese water, PFOA concentrations were 7.9–110 ngL^{-1} and PFOS were <5.2–10 ng L^{-1} (Senthilkumar et al., 2007).

In Europe, initial evidence of the sources and loads of perfluorochemicals (PFCs) in the NW Mediterranean Sea was reported by Sánchez-Avila et al. (2010). These authors evidenced total PFCs levels ranging from 0.07 to 13.0 ng L^{-1}. A successive study performed in the Cantabrian Sea (North Spain) reported similar values (0.06–10.9 ng L^{-1}) evidencing higher levels in wastewater treatment plant effluents and port waters than in submarine emissaries (Gómez et al., 2011).

In Table 3b, PFOA/S concentrations in Orbetello lagoon sediments are reported, and are notably higher than those found in the lagoon's water. Mean PFOA is highest in station NC and lowest in LC. In contrast, LC, PC, and FC stations evidence higher PFOS concentrations. The general PFOA trend is: LC < M < AC < FC < NC<<PC, while the PFOS trend is: M = AC < NC < FC < LC.

Few studies report PFOA/S levels in sediments. In a study of the Kyoto river, Senthilkumar et al. (2007) measured PFOA levels of 1.3–3.9 ng g^{-1} and PFOS values <LOD. PFOA levels measured in the Orbetello lagoon range from comparable to higher than those found in the Kyoto river, whereas PFOS concentrations are notably higher. A recent study reports total PFCs levels in European marine sediments ranging from 0.01 to 0.13 ng g^{-1} d.w. (Gómez et al., 2011).

In this study, only the LC sediment sampling site revealed a PFOA/PFOS ratio of 10 (PFOA 10 times less than PFOS), as reported by Becker and colleagues (2008), but enrichment observed in sediment is neither correlated to the total organic carbon contents nor to total nitrogen (Table 3b).

TOC and TN values were used to calculate *C*/*N* ratios to evaluate the origin of organic matter at each sampling station. Reported TOC and TN levels are typical of a eutrophic ecosystem characterized by high nutrient inputs (Innamorati and Mellilo, 2004). Reported *C*/*N* atomic ratios ranged from 3.7 (LC) to 28.8 (AC), typical values for organic contributions due to macrophyte decomposition.

Univariate statistics performed on PFOA/S levels measured in species are reported in Table 4.

PFOA levels in analyzed taxa differ greatly. Plants showed values < LOQ, as did red macroalgae (*A. corallinum*) and phanerogams (*C. nodosa*, *R. cirrhosa*). Green algae *C. linum* showed an average value of 0.54 ng g^{-1} d.w. The mussel *M. galloprovincialis* showed the highest PFOA concentration, while the clam *R. decussatus* showed lower values. PFOA levels in crustaceans (*P. serratus, C. aestuarii*) are comparable. In fish, average values show the following trend: *G. niger* < *Atherina spp.* < Parablennius *spp.* < Z. *ophiocephalus*.

PFOS in plants shows the same behavior as PFOA: measured values are <LOQ in *A. corallinum, C. nodosa,* and *R. cirrhosa,* whereas *C. linum* shows an average of 0.52 ng g^{-1} d.w.

Concerning bivalves, levels measured in *M. galloprovincialis* are higher than those for PFOA. Even in this case, *R. decussatus* shows lower values than the mussel. In crustaceans, PFOS levels differ significantly, while in fish, the average trend is: *G. niger* = *Parablennius spp.* < *Atherina spp.* < *Z. ophiocephalus*.

Table 3
Levels in abiotic matrices: water (a) and sediments (b). In (a and b) mean levels of perfluorooctanoic (PFOA) and perfluoroctanesulfonic (PFOS) acids are reported in abiotic matrices sampled from the Orbetello lagoon sampling stations (Ref. Fig. 1). Means are calculated on three sample replicates ($n = 3$) for each sampling station; range of variations expressed as minimum and maximum values (*min–max*) are reported.

		NC	AC	M	FC	LC	PC
(a)							
PFOA (ng L^{-1})	Mean	1.65	1.33	1.12	2.03	0.93	0.76
	Min–Max	*1.41–1.86*	*0.98–1.65*	*0.92–1.32*	*1.91–2.15*	*0.84–1.03*	*0.61–0.92*
PFOS (ng L^{-1})	Mean	0.09	0.09	0.05	0.11	<LOQ	<LOQ
	Min–Max	*0.07–0.11*	*0.08–0.09*	*0.04–0.06*	*0.09–0.12*	–	–
(b)							
PFOA (ng g^{-1} d.w.)	Mean	5.33	2.19	1.84	2.52	0.98	9.38
	Min–Max	*5.26–5.84*	*2.12–2.54*	*1.83–1.98*	*2.33–2.74*	*0.87–1.12*	*9.37–9.56*
PFOS (ng g^{-1} d.w.)	Mean	3.12	2.19	2.14	4.17	7.20	5.09
	Min–Max	*3.02–3.25*	*2.18–2.23*	*2.06–2.35*	*4.04–4.21*	*7.19–7.32*	*5.01–5.12*
TOC (% d.w.)	Mean	3.75	0.85	2.85	1.54	2.90	2.12
	Min–Max	*3.62–3.84*	*0.74–0.91*	*2.71–2.93*	*1.43–1.55*	*2.86–2.94*	*1.94–2.19*
TN (% d.w.)	Mean	0.61	0.06	0.56	0.29	0.85	0.35
	Min–Max	*0.57–0.69*	*0.03–0.12*	*0.47–0.64*	*0.25–0.31*	*0.78–0.92*	*0.29–0.36*
C/*N*	Mean	7.2	16.5	5.9	6.2	4.0	7.1
	Min–Max	*6.5–7.4*	*8.8–28.8*	*5.3–6.7*	*5.8–6.7*	*3.7–4.3*	*7.1–7.8*

Notes: NC = Nassa Channel, AC = Ansedonia Channel, M = Mines, FC = Fibbia Channel, LC = Eastern basin Centre, PC = Western basin Centre. Limits of quantification (LOQ) were: (a) 0.01 ng L^{-1}; (b) 0.50 ng g^{-1} d.w. Total organic carbon (TOC) and total nitrogen (TN) literature values (Renzi et al., 2007; unpublished data) are reported for each sampling replicate.

Table 4
Levels in lagoon taxa from different trophic levels. Univariate statistics on levels of perfluorooctanoic (PFOA) and perfluoroctanesulfonic (PFOS) acids are reported (ng g^{-1} d.w.) for each taxa as mean, standard deviation (SD), minimum (Min), 25thpercentile, Median, 75th percentile and maximum (Max) values. Species diffusion within the lagoon is dependent on its ecological features, for example, sessile species are present and collectable only in correspondence to solid substrates or close to the human artefacts at linked channels. The number of sites accounting for the internal spatial variability collectable for each species is reported as SR ranging from 3 (only channels) to 6 (all sites), whereas *n* expresses the total number of sample replicates *per* species. WC is the average water content of analysed tissue expressed as percentage. Limit of quantification (LOQ) was 0.40 ng g^{-1} (d.w.).

PFOA	SR (n)	WC %	Mean	SD	Min	25th Percentile	Median	75th Percentile	Max
A. corallinum	6 (18)	87	<LOQ	–	–	–	–	–	–
C. linum	6 (18)	93	0.54	0.05	<LOQ	<LOQ	0.54	0.58	0.61
C. nodosa	6 (18)	88	<LOQ	–	–	–	–	–	–
R. cirrhosa	6 (18)	89	<LOQ	–	–	–	–	–	–
C. aestuarii	5 (15)	81	0.91	0.17	0.67	0.75	0.94	1.06	1.07
P. serratus	5 (15)	82	0.98	0.23	0.60	0.81	1.01	1.14	1.22
R. decussatus	6 (18)	76	0.59	0.08	0.51	0.52	0.57	0.68	0.71
M. galloprovincialis	3 (9)	75	2.90	1.65	1.02	1.02	3.65	4.04	4.04
G. niger	3 (9)	72	0.58	0.08	0.51	0.51	0.56	0.66	0.66
Atherina spp.	3 (9)	76	0.60	0.01	0.59	0.59	0.60	0.61	0.61
Z. ophiocephalus	3 (9)	76	1.43	0.76	0.91	0.91	1.08	2.30	2.30
Parablennius spp.	3 (9)	75	0.73	0.18	0.61	0.61	0.64	0.94	0.94
PFOS									
A. corallinum	6 (18)	87	<LOQ	–	–	–	–	–	–
C. linum	6 (18)	93	0.52	0.04	<LOQ	<LOQ	<LOQ	0.54	0.61
C. nodosa	6 (18)	88	<LOQ	–	–	–	–	–	–
R. cirrhosa	6 (18)	89	<LOQ	–	–	–	–	–	–
C. aestuarii	5 (15)	81	1.43	0.35	0.87	1.10	1.57	1.69	1.70
P. serratus	5 (15)	82	0.51	0.01	0.51	0.51	0.51	0.51	0.52
R. decussatus	6 (18)	76	0.66	0.17	0.49	0.52	0.61	0.79	0.95
M. galloprovincialis	3 (9)	75	3.11	0.92	2.18	2.18	3.14	4.02	4.02
G. niger	3 (9)	72	0.57	0.06	0.51	0.51	0.56	0.63	0.63
Atherina spp.	3 (9)	76	0.58	0.03	0.56	0.56	0.58	0.61	0.61
Z. ophiocephalus	3 (9)	76	0.65	0.04	0.63	0.63	0.63	0.70	0.70
Parablennius spp.	3 (9)	75	0.57	0.05	0.52	0.52	0.57	0.63	0.63

Considering the humidity percentage (Table 4) of tissue from each considered species, some comparisons were performed using data from the literature. A recent study of total PFC levels measured in mussels from the Cantabrian Sea (North Spain) reported a range from 0.01 to 0.06 ng g^{-1} w.w. (Gómez et al., 2011). Comparing to European data, values measured in taxa sampled in the Orbetello lagoon are comparable to PFOA concentrations reported in fish and fishery products in Europe (0.05–5.00 ng g^{-1} w.w.; van Leeuwen et al., 2006) while levels in crustaceans (0.80–0.90 ng g^{-1} w.w.; van Leeuwen et al., 2006) and mollusks (0.95–1.20 ng g^{-1} w.w., van Leeuwen et al., 2006) are notably higher. In Europe, PFOS levels in fish muscles or entire bodies ranged from 0.60 to 230 ng g^{-1} w.w. (EFSA, 2008), in crustaceans range from 8.30 to 319 ng g^{-1} w.w. (van Leeuwen et al., 2006) and in mollusks the ranges are 0.80–79.80 ng g^{-1} w.w. (Cunha et al., 2005).

Multivariate statistical analysis performed on the whole database evidences that water levels are affected (Global R value = 0.732; $p < 0.01$) by the factor ReF (river influence) while PFOA/S concentrations in biota are not clearly affected by the river distance.

Univariate and multivariate statistics performed on collected data with the aim of evaluating the occurrence of enrichments due to the matrix are represented in Figs. 2–5. In particular, relationships among levels of: (i) PFOA measured in biota and water (Fig. 2), (ii) PFOA measured in biota and TOC in sediments (Fig. 3), (iii) PFOS measured in biota and sediments (Fig. 4), (iv) PFOS measured in biota and TOC in sediments (Fig. 5) are evidenced.

Data on *A. corallinum*, *C. linum*, *C. nodosa*, *R. cirrhosa*, *Carcinus aestuarii*, *Parablennius* sp., *Atherina* sp., *G. niger* are not represented in Figs. 2–5 due to their low significance.

PFOA levels in biota are strongly related to values measured in water for *M. galloprovincialis* and *Z. ophiocephalus* (Fig. 2), while no significant relationships are recorded among PFOA levels in biota

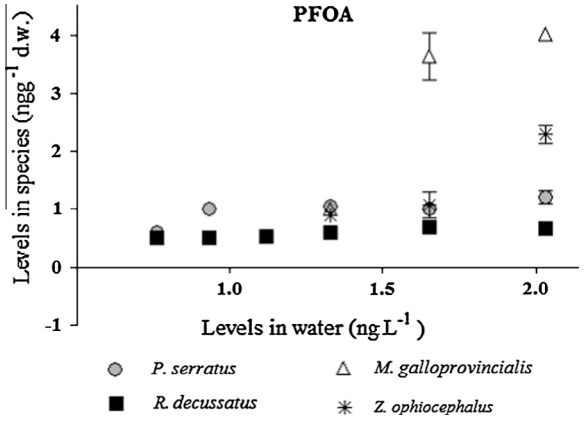

Fig. 2. Comparison between PFOA levels: biota *vs* water. PFOA levels in biota related to water concentrations are reported for most significant species (*P. serratus*, *M. galloprovincialis*, *R. decussatus*, *Z. ophiocephalus*).

and TOC values in sediments (Fig. 3). Statistical analyses performed show significant correlation ($p < 0.01$) among PFOS levels in species and sediments only for *M. galloprovincialis* (Fig. 4), whereas a significant difference among values measured in *C. aestuarii* and other species not correlated to levels in sediments is observed. PFOS differences are not related to TOC levels in sediments, as shown in Fig. 5.

Concerning species evidencing higher statistical correlations (*M. galloprovincialis* and *Z. ophiocephalus*), single relationships between levels measured in sediment/water and concentrations recorded in tissues are calculated. Linear correlations calculated between pairs of significantly correlated variables are reported in Table 5. Statistics associated with calculated relationships are also indicated.

Biological enrichment factors (BEF) calculated on collected data are reported in Figs. 6 and 7. Concerning PFOA, BEF > 1 is observed

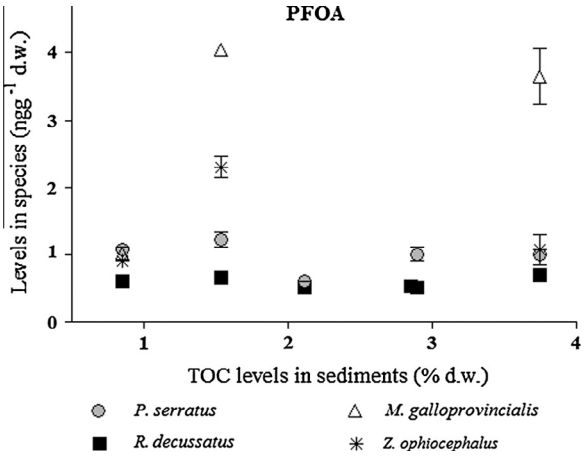

Fig. 3. Comparison between PFOA levels: biota *vs* TOC in sediments. PFOA levels in biota related to total organic carbon (TOC) concentrations in sediments are reported for most significant species (*P. serratus*, *M. galloprovincialis*, *R. decussatus*, *Z. ophiocephalus*).

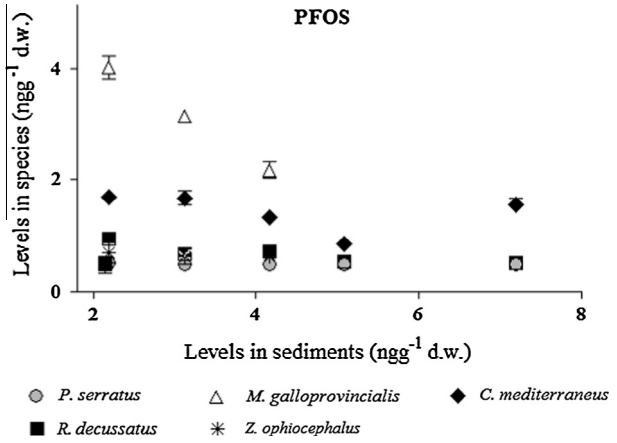

Fig. 4. Comparison between PFOS levels: biota *vs* sediments. PFOS levels in biota related to sediment concentrations are reported for most significant species (*P. serratus*, *M. galloprovincialis*, *R. decussatus*, *Z. ophiocephalus*).

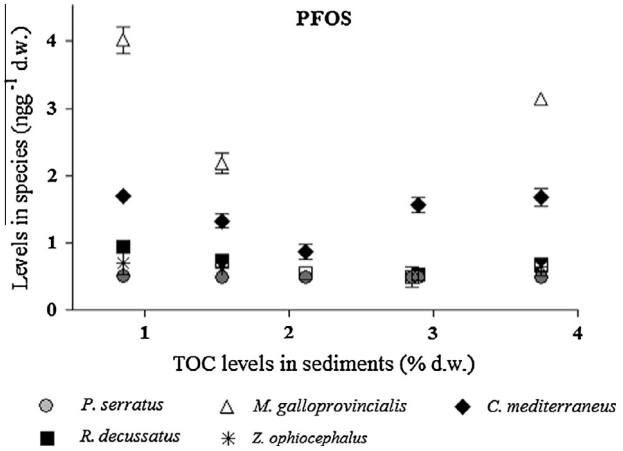

Fig. 5. Comparison between PFOS levels: biota *vs* TOC in sediments. PFOS levels in biota related to total organic carbon (TOC) concentrations in sediments are reported for most significant species (*P. serratus*, *M. galloprovincialis*, *R. decussatus*, *Z. ophiocephalus*).

for *M. galloprovincialis* both in the NC and FC stations. *Z. ophiocephalus* evidenced BEF > 1 only for the FC station, while *P. serratus* and *C. aestuarii* evidenced BEF > 1 in LC (Fig. 6). On the contrary, PFOS enrichments were found only for *M. galloprovincialis* in the AC and NC sampling stations (Fig. 7).

4. Discussion

4.1. Legislative guidelines for PFOA/S pollution assessment

In European Countries, acceptable limits of concentration are defined for potentially hazardous chemicals in water and sediment. In Italy, reference values are indicated by the Central Institute for Applied Marine Research, however, maximum levels in biota are not defined from a specifically environmental point of view. Limits are available only for some species of concern for human safety purposes. However, cited legislative decrees do not include PFOA/S. In fact, PFOA/S are a new-generation class of persistent organic pollutants of recent environmental concern, and little data are available in the literature on their levels and ecotoxicological effects in aquatic ecosystems.

4.2. Water, sediment and biota pollution

In the Orbetello lagoon previous studies have been performed on the distribution of endocrine disruptors (Renzi et al., 2013), but PFOA/S have never been measured before.

Water PFOA levels in the lagoon are notably higher than oceanic values reported in the literature for the Pacific, the North and Mid-Atlantic Ocean and the Sea of Japan (Yamashita et al., 2005). On the contrary, PFOS levels are very low, often inferior to LOQ. Comparisons performed with freshwater values evidence that PFOS levels are comparable to values measured in Europe for freshwater and drinking water.

Results show that higher PFOA/S levels in water are recorded in station FC, supporting the likelihood of an active input of these chemicals from the Albegna river inflow. Higher values found in NC and AC stations are due to the presence of significant human activities close to the Nassa and Ansedonia canals. Furthermore, multivariate statistical analysis evidences a strong relationship between levels in water and sampling stations' distance from the river. This result is consistent with the literature which indicates that water transport phenomena are the principal dynamic affecting PFOA/S dispersion from emission sources (Simcik, 2005a,b; Prevedouros et al., 2006).

Transitional water ecosystems accumulate POPs discharged by human sources (Focardi et al., 2009) due to their high affinity with finer sediment particles and organic substances (TOC, TN), which tend to adsorb and accumulate charged molecules and ions. Data on PFOA/S sedimentary levels reported by a recent study evidenced that total PFCs measured in sediments of the Orbetello lagoon are notably higher compared to marine ones (Gómez et al., 2011). In sediments, the observed trend follows those described for water, supporting the hypothesis of a water-conveyed inflow of PFOA/S into the Orbetello lagoon ecosystem even though a clear relationship with river distance is not indicated.

This study represents the first attempt to quantify PFOA/S in plants in transitional water ecosystems. *C. linum* is shown to contain higher PFOA/S values than other plants. This is probably due to its biological structure, characterized by structural simplicity and high water content. Phanerogam uptakes are low, due to the importance of root-driven adsorption, which could produce different PFOA/S distribution within the plant, producing low levels in leaves. The presence of measurable PFOA/S levels in macroalgae produces important ecological effects in this ecosystem. Our

Table 5
Linear regressions linking PFOA/S levels in abiotic matrices and taxa. Significant linear regressions and associated statistics linking PFOS levels in sediments or PFOA levels in water and biotic tissues are summarized. Relationships are calculated on data expressed as part per billion (ppb) concerning water and part per million (ppm) concerning sediment and biota. Notes: *s* = sediment, *o* = organism, *w* = water.

	PFOS(s,o) M. galloprovincialis	*PFOA(w,o) Z. ophiocephalus*	*PFOA(w,o) M. galloprovincialis*
Best-fit values			
Slope	−0.93 ± 0.06	2.03 ± 0.34	4.21 ± 0.82
Y-intercept when *X* = 0.0	6.05 ± 0.18	−1.96 ± 0.57	−4.14 ± 1.40
X-intercept when *Y* = 0.0	6.51	0.97	0.98
1/slope	−1.08	0.49	0.24
95% Confidence intervals			
Slope	−1.06 to −0.79	1.23 to 2.82	2.27 to 6.16
Y-intercept when *X* = 0.0	5.61 to 6.48	−3.31 to −0.61	−7.44 to −0.84
X-intercept when *Y* = 0.0	6.05 to 7.11	0.45 to 1.23	0.32 to 1.28
Goodness of Fit			
r^2	0.97	0.84	0.79
Sy.x	0.14	0.29	0.71
Statistics			
F	271.9	36.3	26.2
DFn. DFd	1.000. 7.000	1.000. 7.000	1.000. 7.000
P value	<0.0001	0.0005	0.0014

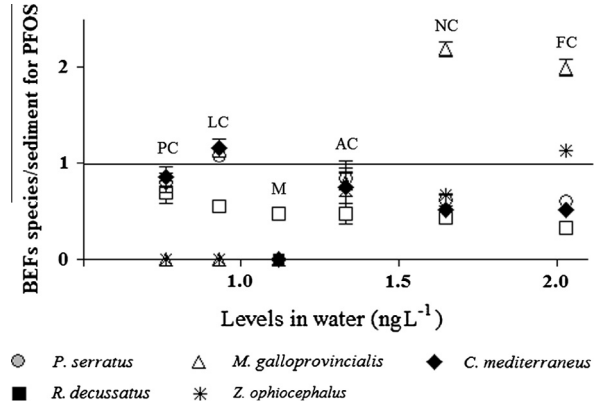

Fig. 6. Biological enrichment factors (BEF) calculated on PFOA data.

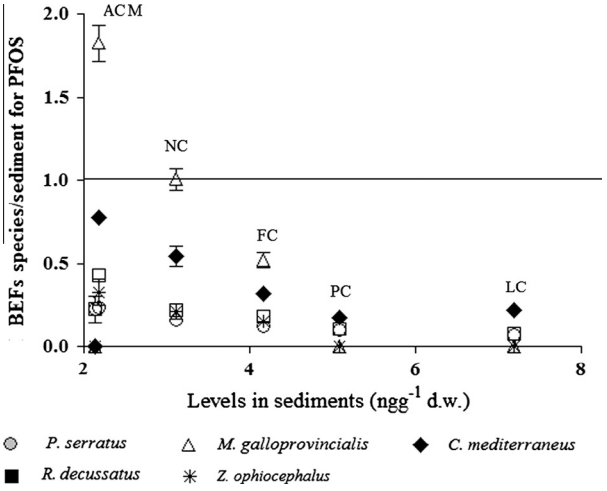

Fig. 7. Biological enrichment factors (BEF) calculated on PFOS data.

research does not show relationships among PFOA/S levels in sediments and organic substance concentration. This is probably due to the autochthonous origin of the organic substance itself which, as supported by TOC/TN atomic ratios, is principally due to primary producer decomposition phenomena. Observed exceptions in sediments are probably the result of a redistribution pollution phenomenon due to macroalgae biomasses which uptake PFOA/S from water and carry them on the wind (Giusti et al., 2010). In the Orbetello lagoon, stations M and PC represent geographical areas particularly impacted by intense proliferation of *C. linum* (Giovani *et al.*, *in press*), whereas *A. corallinum* proliferations are recent (Lenzi et al., 2012). To better understand how the local decomposition of algal biomasses could be responsible for higher accumulation levels of PFOA/S in sediments, a simple evaluation of quantities due to macroalgae decomposition could be performed considering that in Orbetello lagoon the annual macroalgae production is about 6774 tons (Renzi et al., in press). According to average levels measured in *C. linum*, about 3.66 kg y^{-1} of PFOA/S are adsorbed and carried by macroalgae within the lagoon ecosystem each year.

Concerning other species, the presence of a more complex mechanism of pollutant distribution linked to the trophic web is supported by the absence of significant statistical correlations with the river distance factor.

R. decussatus is a filter-feeding lamellibranch mollusk living on rocks or solid substrates, while *M. galloprovincialis* lives burrowed in soft bottoms and feeds on detrital organic matter (Prato et al., 2010). A first hypothesis was that observed differences could be due to the different ecological behaviors of the bivalve species; in fact, total daily litres of water filtered by the two species are not similar, thus assumption of water-conveyed pollution by the mussel is higher than that of clams, which are much more affected by pollutants conveyed by particulate and organic matter. Nevertheless, a recent study shows that the bivalves (oyster and zebra mussel) do not accumulate PFCs, unlike insect larvae, fish and crabs (Fernández-Sanjuan et al., 2010). For this reason, different causes explaining the observed behavior must be hypothesized, as well as a different species-specific mechanism of detoxification/accumulation of pollutants in *M. galloprovincialis* as compared to oysters and zebra mussels (Fernández-Sanjuan et al., 2013).

In crustaceans, differences observed could be due to a dietary contribution: both species are omnivores, but *P. serratus* eats tiny benthonic crustaceans and macroalgae (Guerao, 1993–1994) while *C. aestuarii* prefers invertebrates and tiny fish (Chen et al., 2004).

Concerning fishes, the absence of correlation with water or sediments from a given sampling site within the Orbetello lagoon is probably due to fish ecology. In fact, fish species selected for this study could move throughout the whole ecosystem.

Measured PFOA/S levels in water, sediments, and biota are generally similar to values reported for aquatic ecosystems in Europe,

with some significant exceptions concerning PFOA in water, mollusks and crustaceans. Possible reasons for the observed differences among values recorded in this study and those found in the literature could be due to: (i) ecological and structural differences between the considered ecosystems (marine and freshwater *versus* transitional ones), (ii) different bioaccumulation rates among considered species: different mollusks and crustaceans may show differential accumulation or detoxification rates, or could evidence different levels of exposure due to different ecologies of the species (diet, reproductive cycle, habitat), (iii) statistical artifacts due to the different numbers of individuals considered as representative of the entire population; (iv) different exposure to PFOA/S pollution and pollution sources of considered ecosystems.

4.3. Enrichments throughout the trophic web

Defining levels of toxic pollutants throughout the lagoon trophic web is an important aspect in ecology, crucial in evaluating possible risks for species marked for conservation. Trophic relationships among the considered species are determined on the basis of their ecological characteristics and allow us to better define the trophic web structure in the Orbetello lagoon. In Table 6, the principal feeding behaviors of considered species are reported. Primary producers considered in this study pertain to macroalgae (*A. corallinum*, *C. linum*) and phanerogam (*C. nodosa*, *R. cirrhosa*) groups characterized by different behaviors and biological cycles. The Rhodophyte *A. corallinum* usually prefers the sea rather than transitional waters. In the sea, thalli are attached to rocks, whereas in lagoons they float and take on a spherical form. Boudouresque (1984) classified them in the ecological group of the Photophilous, Infralittoral, Thermophilous species (PhITs). This species produces the pigment caulerpin (Caballero Ortega and Maguregui de Echevarrieta, 2007), which has anti-feeding properties for certain herbivorous gastropods. Due to its production of this anti-feeding substance, *A. corallinum*, is probably not eaten by the herbivorous species considered in this study. Substances, including pollutants, are actively adsorbed by thalli in macroalgae and by roots (in large part) and leaves (to a lesser degree) in phanerogams. The efficiency of pollutant adsorption is both pollutant- and species-dependent. This ecological difference results in the possible adsorption of sediment-linked pollutants by phanerogams and the greater efficiency of water pollutant adsorption by macroalgae.

The same differences are observed for the considered mollusk species. *M. galloprovincialis* is principally affected by water-pollutants, whereas *R. decussates,* which lives burrowed in soft bottoms (Cannas, 2010), is principally affected by sedimentary pollution. Concerning fish, *Parablennius spp.* is omnivorous and eats principally macroalgae, porifera, and tiny invertebrates (Zander and Berg, 1984), *G. niger* and *Z. ophiocephalus* are carnivorous, eating mainly crustaceans, mollusks and tiny fish (Miller, 1986; Franco et al., 2002), while Atherina spp. are opportunistic, feeding on plankton-like tiny crustaceans as well as benthonic ones (Vizzini and Mazzola, 2005). Herbivores tend to accumulate lower levels than water- filtering and detritus-feeding types. In particular, water feed-filtering species are the most exposed to PFOA/S pollution. An unclear relationship between PFOA/S levels in fish and their dietary habits is observed. This is probably due to the fact that in lagoon ecosystems, the fish's diet is frequently opportunistic and species are omnivorous. Further studies are needed to clarify the complex trophic inter-connections among fish in lagoon ecosystems to better relate observed PFOA/S levels in biota to the species trophic level within the trophic web. A possible future application to evaluate these aspects could be isotopic research (Vizzini and Mazzola, 2005).

In Fig. 8 a flowchart summarizing the most probable transfer routes of PFCs in the food chain studied is presented.

4.4. Risk assessment regarding the conservation of wildlife species

Investigated compounds could have numerous adverse effects on ecosystems and organisms due to the fact that PFOA/S are toxic hazardous substances which also act as endocrine disruptors. Risk assessment evaluation related to the toxicological effects induced by exposure to observed PFOA/S levels in Orbetello lagoon water and sediments could be performed through ecotoxicological test results as reported in the literature. Unfortunately, literature reporting ecotoxicological responses due to PFOA/S exposure in aquatic environments is still lacking and needs to be improved. Although some results on similar species are available, to the best of our knowledge, studies on the species considered in this research have never been performed, and extrapolations are difficult to make due to the species-specificity of toxic responses. Concerning aquatic plants, *Selenastrum capricornutum* (unicellular green algae) shows EC_{50} of 71 mg L^{-1} (end point: biomass) and 126 mg L^{-1} (end point: growth rate) and NOEC of 48 mg L^{-1} for both of the end points considered in this study after 96 h of PFOS exposure (3M, 2000, 2003). Regarding mollusks, acute effects are evidenced after 96 hours of PFOS exposure at 2.1 mg L^{-1} (end point: oyster shell deposition). *Crassostrea virginica* shows acute toxicity after 96 hours of exposure at a similar level to *M. bahia* (EC_{50} of 3.0 mg L^{-1}; NOEC of 1.9 mg L^{-1}). In shrimp, subchronic/chronic effects are observed at 0.25 mg L^{-1} after 35 days of exposure (*Mysidopsis bahia*), while acute effects are evidenced at 4.0 mg L^{-1} (96 h EC_{50}) and

Table 6
Ecology and trophic level of considered species.

Species	Feeding behavior	Preys description
Cymodocea nodosa (Ucria) Asch. (1870)	Primary producer	–
Ruppia cirrhosa (Petagna) Grande	Primary producer	–
Alsidium corallinum C.Agardh 1827: 639	Primary producer	–
Chaetomorpha linum (O.F.Müller) Kützing, 1845	Primary producer	–
Mytilus galloprovincialis Lamarck 1819	Filter feeder	Bacteria, zooplankton, phytoplankton, detritus (1)
Ruditapes decussatus	Filter feeder	Bacteria, zooplankton, phytoplankton, detritus (2)
Palaemon serratus (Pennant, 1777)	Omnivorous	Algae and little benthonic crustaceans (3; 4)
Carcinus aestuarii	Omnivorous, benthivorous	Invertebrates and little fishes (5)
Parablennius spp.	Omnivorous, grazer	Algae, porifera, little invertebrates (6)
Zosterisessor ophiocephalus	Predator	Zoobenthos (crustaceans, bivalves, polichaetae), Little fishes (Gobius spp.) (7)
Atherina spp.	Opportunistic predator	Little benthonic and planktonic crustaceans (8)
Gobius niger	Predator	Zoobenthos (crustaceans, molluscs, polichaetae), Rarely little fishes (7)

References: (1) = Prato et al., 2010; (2) = Cannas, 2010; (3) = Guerao, 1993; (4) = Guerao, 1994; (5) = Chen et al., 2004; (6) = Zander and Berg, 1984; Zander, 1986; (7) = Miller, 1986; (8) = Vizzini and Mazzola, 2005.

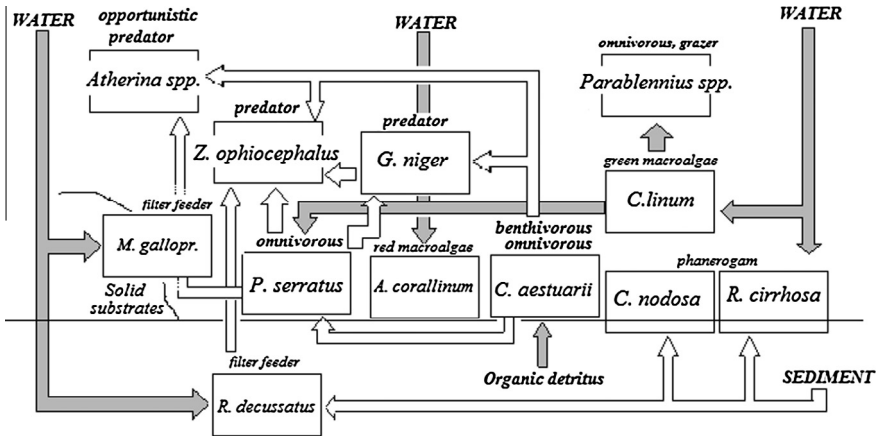

Fig. 8. Flowchart indicating the most probable transfer routes of PFCs in the food chain studied. White arrows indicate PCFs route towards a species while grey ones indicate predominant way of assumption.

1.2 mg L^{-1} (96 h NOEC). In fish, LC_{50} after 96 h of PFOS exposure is measured at 10 mg L^{-1} for *Pimephales promelas* (fathead minnow) and 7.8 mg L^{-1} for *Lepomis macrochirus* (bluegill sunfish), while NOEC in the same species are, respectively, 3.6 mg L^{-1} and 4.5 mg L^{-1}. Chronic exposure tests (42-days) performed considering survival as an end point showed LOEC of 0.65 mg L^{-1} and NOEC of 0.33 mg L^{-1} (3M, 2000, 2003) in *Pimephales promelas*.

Results obtained in this study evidenced PFOS levels in water far below LOEL and NOEC values reported in ecotoxicological studies, thus levels in the Orbetello lagoon could be considered relatively safe for purposes of conservation of wild species. Nevertheless, caution is necessary due to the significant lack of knowledge concerning the ecotoxicological responses of the studied species to PFOA/S exposure and the extrapolation of exposed *in vitro* results to a real environment.

4.5. Assessment of risk associated with human consumption

Many of the species considered are basic elements of the local population's diet and are also important food industry products which are exported worldwide. Some of the tested products are Slow Food® *presidia* and are distributed on the national and international market. Crabs are a typical local seafood industry product and, from November to January, about 10,000 kg per year of *C. aestuarii* are caught and commercialized under the moniker *Femminelle*. Well-known traditional local dishes include a pasta dish with crustaceans, mussels and clams, and there is an important locally-based commerce in bivalves. Bivalves are generally consumed cooked, algae are occasionally served fried, and crustaceans and small fish (*Parablennius* sp., *Z. ophiocephalus*, *Atherina* sp., *G. niger*) are often eaten fried whole, with carapaces and livers left intact and eaten.

For these reasons, the definition of PFOA/S levels in products intended for human consumption including mussels, clams, crabs, shrimp and fish and the evaluation of daily dietary intakes for humans related to their consumption are important aspects for study. Tolerable daily intakes (TDIs) calculated both for women and men show values in all considered species that are far below EFSA maximum levels of 1500 ng kg^{-1} b.w. for PFOA and 150 ng kg^{-1} b.w. for PFOS. In fact, considering PFOA, the maximum TDI values (worst case) recorded for *M. galloprovincialis* were 5.74 ng kg^{-1} b.w. for women and 4.10 ng kg^{-1} b.w. for men. PFOS evidenced similar levels, with maximum TDI values (worst case) recorded for *M. galloprovincialis* of 5.71 ng kg^{-1} b.w. for women and 4.08 ng kg^{-1} b.w. for men.

4.6. European guidelines for transitional ecosystems

Many of Italy's 75,000 lagoon hectares are Ramsar sites that host wildlife listed on the IUNC red list of threatened species. Furthermore, many Italian lagoons include Sites of Community Importance as defined by the European Commission Habitats Directive (92/43/EEC) and/or Special Protection Areas as designated under the European Union Directive for the Conservation of Wild Birds. In spite of all this, lagoons are the most economically exploited ecosystems worldwide (Costanza et al., 1987), supporting urban settlements, tourism activities and human economies based on fishing and aquaculture (Renzi et al., 2011). Nevertheless, little data is available on dynamics involving emerging pollutants (Renzi, 2012), so the definition of PFC levels is a key objective.

The Water Framework Directive (2000/60/EC) plays an important role in EU environmental policy and the development of new conservation and management strategies for aquatic ecosystems. Ecosystem quality is evaluated in terms of the responses of ecological communities, and the evaluation of pollution levels represents an important element to link observed biological responses to environmental conditions (Zaldívar et al., 2008). Among aquatic ecosystems, coastal lagoons, acting as a sort of natural filter for pollutants coming from inland by means of river and freshwater inputs, could represent significant basins of new-generation chemical compounds, as well as PFOA/S.

In these ecosystems, rivers contribute significantly to water-conveyed pollutant inputs. Furthermore, chemicals accumulated in sediments could be transferred into the trophic web and could engender a significant risk for the ecosystem and human health, as well as producing negative effects on local fishery-based economies. In spite of all of this, to the best of our knowledge, studies quantifying PFOA/S levels in the environment and in wildlife have never been performed in these ecosystems (Renzi, 2012).

The EU Commission also establishes specific environmental quality standards for priority substances and other selected pollutants in transitional water sediments, but although some pollutants derived from human activities are included in the Water Framework directive, new-generation xenobiotics are neither included nor monitored, and data concerning levels and behavior of such compounds in coastal systems are not available in the scientific literature.

In particular, PFOA/S levels in sediments could be affected by macroalgae biomass accumulation and decomposition phenomena conveyed by winds (Giusti et al., 2010) and by species ecology and habits (i.e. macroalgae wind-driven distribution, animal aggregation areas). For these reasons, the Water Framework Directive

should be implemented and should include a PFOA/S quality standard for both water and sediments.

Results obtained in this study demonstrate how dynamics of pollutant distribution within a semi-enclosed lagoon basin could be biota-mediated. Furthermore, opportune and sizeable management actions must be planned in such ecosystems both for environmental safety and primary pollution source identification.

5. Conclusions

To the best of our knowledge, this study is the first to describe PFOA/S levels in a wide variety of ecosystem components of a transitional water ecosystem (the Orbetello lagoon, Italy). In general, PFOA/S levels measured were similar to values reported in Europe for fresh and marine waters, with the exception of mussels and crabs, which showed higher values than European counterparts. Results showed PFOA/S levels in *A. corallinum*, *C. nodosa* and *R. cirrhosa* inferior to LOD, while *C. linum* evidenced measurable levels. This species, which is the principal biomass produced in this ecosystem, contributes to determining PFOA/S distribution in sediments within the lagoon basin. The highest PFOA/S levels are recorded in feed-filtering (*M. galloprovincialis*) and detritus feeding (*R. decussatus*, *C. aestuarii*) species, supporting the hypothesis that fresh water contributes to Orbetello lagoon ecosystem pollution due to Albegna river inflows. Relationships among fish pollution and trophic levels within the lagoon trophic web (*P. serratus*, *Parablennius* sp., *Z. ophiocephalus*, *Atherina* sp., *G. niger*) are not completely clear and require further research, but the environmental risk assessment shows relatively safe levels in terms of ecosystem conservation. Risk assessment of human exposure through dietary intakes suggests that levels that could be assumed through diet are notably lower than the maximum tolerable daily intakes suggested by the EFSA. The results obtained could provide an important reference base to develop further research on new-generation chemical pollutants and larger experimental studies in transitional water areas and to improve national and international regulations on environmental conservation. In particular, this research could represent a useful starting point for the correct implementation of the WFD concerning transitional water pollution.

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