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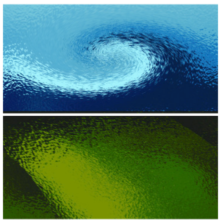

RESEARCH ARTICLE

Occurrence of POPs in sediments and tissues of European eels (*Anguilla anguilla* L.) from two Italian lagoons: Varano and Orbetello

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Abstract

1 - Total levels of persistent organic pollutants (polychlorinated biphenyls, organochlorine pesticides, and polycyclic aromatic hydrocarbons) in sediments and edible tissues (muscle and liver) from a fish species of great local commercial interest (*Anguilla anguilla* L., yellow phase) were determined in Varano and Orbetello lagoons, Italy.

2 - The aim of this study was to improve knowledge on relationships occurring among levels of chemicals in sediments and fish tissues relating them reciprocally and to different intensities of human pressure.

3 - Studied ecosystems were selected due to the notable scientific knowledge acquired by previous detailed research on meteorology, geomorphology, hydrodynamics, types and distribution of local factors linked to different sources of human-made pollution. Samplings were performed in July 2009 according to a logic model based on a priori defined factors of interest and obtained results were statistically analysed in order to evaluate the significance of observed data segregation related to the selected factors.

4 - Concerning levels measured in sediments, significant differences were observed between lagoons in terms of ΣPAHs and ΣOCPs. According to National and international recognised sediment quality guidelines, results evidenced the occurrence of non-critical POPs values in sediments.

5 - Results on sediments are associated to very high levels in eel's tissues. Concerning eels, Orbetello lagoon is characterized by significant higher values of ΣOCPs than Varano, evidencing the presence of an important OCPs local source.

6 - Different human pressure levels produce significant differences in both sediments and eel's tissues in Varano and Orbetello lagoons.

Keywords: Coastal lagoons, PCBs, OCPs, PAHs, sediments, European eel.

Introduction

Coastal lagoons are highly stressed ecosystems characterized by wide natural fluctuations of physico-chemical variables (Carrada, 1990). Their great primary and secondary productivity is strictly linked to system general features (i.e. latitude, geomorphology, local meteorology, hydrodynamics) as well as natural and human-due nutrient enrichments caused, as an example, by agricultural soil drainages or discharges from urban and industrial effluents (Renzi *et al.*, 2011). These features are also linked to the occurrence of higher levels of pollution by chemicals than other aquatic ecosystems (Kim *et al.*, 2006; Specchiulli *et al.*, 2008). In fact, organic pollutants from human origin reach coastal lagoons throughout local or long-range emission sources and, due to their affinity for organic matter, accumulated in sediments through direct adsorption processes or sedimentation of suspended particulate matter (Wainright and Hopkinson, 1997). Sediments, and in particular particles with a diameter less than 63 µm, could accumulate high levels of chemicals of ecotoxicological concern. Pollution levels in abiotic and biotic matrices sampled in European coastal lagoons have been previously well described (Belpaire and Goemans, 2007a). Nevertheless, a synthetic analysis based on a multivariate statistical approach aimed at evaluating the significance of the relationships among human-related factors and the distribution dynamics of persistent organic pollutants (POPs) has never been reported at the best of our knowledge.

Among the relative large number of fish species of any commercial interest living in these ecosystems, the yellow phase of the European eel (*Anguilla anguilla* L., 1758) was selected in this research due to the specific high commercial interest on a local base, and the scientific knowledge acquired on physiology, anatomy, biology, and ecological features of this species (Feunteun *et al.*, 2003; Belpaire and Goemans, 2007a and references therein). Furthermore, the European eel is considered a specific euryhaline bioindicator of chemical pollution being significantly exposed to chemical releases from sediments and due to its diet (carnivorous), feeding habits, and territoriality (Langston *et al.*, 2002; Belpaire and Goemans, 2007b). This species, adopted for coastal lagoon biomonitoring programs in previous research (Oliveira *et al.*, 2005; Mariottini *et al.*, 2006; Renzi *et al.*, 2012), is resistant to high pollutants concentrations (Ashley *et al.*, 2003; Linde *et al.*, 2004), integrates environmental and prey pollution levels (Robinet and Feunteun, 2002) accumulating chemicals lifelong due to its low depuration rates and ecological behaviour (Daverat *et al.*, 2006). In particular, this species during its yellow-sedentary phase lives burrowed into the sediments, allowing to obtain reliable information on a narrow area (Ashley *et al.*, 2003; Linde *et al.*, 2004). The high fat content compared to other fish species (Larsson *et al.*, 1991) makes the European eel able to accumulate in edible tissues high levels of hydrophobic chemicals such as POPs (Bressa *et al.*, 1997; Robinet and Feunteun, 2002; Belpaire and Goemans, 2007a and references therein). These aspects, also, determine a notable risk both related to species conservation and human consumption. Some research related the worldwide European eels' decline occurred over the last two decades to the increasing pollution of coastal ecosystems (Dekker, 2003; Stone, 2003). Robinet and Feunteun (2002) evidenced that the additive stress induced by chemical pollution at sub-lethal doses determines a significant decrease of the energy storage efficiency during the yellow phase which could produces, as consequence, higher susceptibility to diseases, failure of transoceanic migrations and/or reproduction impairment caused by lower efficiency of spawning and gamete maturation. In particular, POPs may alter the lipid metabolism by upsetting thyroid function (Robinet and Feunteun, 2002). Concerning the possible risks related to the human consumption of this species, previous studies performed in Italian coastal lagoons evidenced a moderate to high risk even if integrated evaluations are not reported (Mariottini *et al.*, 2006; Renzi *et al.*, 2012)

The European Food Safety Authority (EFSA) evidences that human exposure to the highest levels of non-dioxin-like PCBs is associated to the consumption of products derived from aquatic animals and, in particular, to the consumption of eel muscle (average level reported of 223 μgkg^{-1} w.w.). Furthermore, higher levels are associated to wild caught eels rather than to farmed ones (EFSA, 2012). Considering two different coastal lagoon ecosystems, the aim of this paper is to provide more detailed knowledge on relationships occurring among levels of some POPs chemicals in sediments and fish tissues (muscle, liver) and different anthropic pressures. Among the great number of POPs, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) were selected. This choice was related to the following considerations on selected chemicals: **i**) they are ubiquitous potential hazardous molecules derived from both natural sources (relevant for PAHs) and human activities; **ii**) they are present at measurable levels also in remote and not polluted Earth areas such as the Antarctic (Weber and Goerke, 2003); **iii**) they evidence a particular relevance in highly stressed areas such as harbours (Birch and Taylor, 2002), estuaries and other shallow coastal zones (Specchiulli *et al.*, 2011) exposed to anthropogenic inputs (Lipiatou *et al.*, 1997; Baumard *et al.*, 1998; Rogers, 2002); **iv**) they are cited by the US Environmental Protection Agency (US-EPA) as priority pollutants to be monitored in the framework of environmental quality control; **v**) they are commonly determined in Italy during preliminary characterizations performed to obtain the legal authorization for sediment dredging (National Decree n. 319 published on 24th January 1996); **vi**) in Europe, they are included in the list of priority pollutants by the 2000/60/CE Directive.

Materials and Methods

Study areas

Two coastal lagoons, Orbetello (Central Tyrrhenian sea) and Varano (Southern Adriatic sea), characterized by different intensities and typologies of human pressures are considered in this study (fig. 1). The selection of these ecosystems was based on the general scientific knowledge acquired from previous research (Specchiulli *et al.*, 2008; 2010; Renzi *et al.*, 2012). Principal meteorological, geomorphologic and hydrological features of these lagoons are summarized in table 1. In both systems, European eel is fished and directly commercialized representing an important economical resource which could expose local inhabitants to possible health risk derived from a diet based on frequent eel's consumption.

Experimental design

The experimental design was focused to evaluate, on a statistical basis, the significance of selected factors (*lagoon ecosystem*, and *local impact*) in segregating samples related to measured POPs levels. Samplings were sized to reduce Type I and Type II errors (Underwood 1994; 2003; Benedetti-Cecchi, 2004) and developed considering specific statistical targets *a priori* defined. In each lagoon, levels of measured variables (see par. 2.4. chemicals of interest) were quantified in sediments and edible tissues (muscle and liver) of the selected fish species (*Anguilla anguilla*, L.). Sediment samplings were performed in July 2009 according to a logic model following the criteria reported. In each ecosystem, on the basis of previous research, three different areas characterized by different types of local human impacts were selected as evidenced in figure 1 with the aim to include high impacted and reference sites. At each selected area, a number ranging within 1-3 referred to the level of *local impact* was

Figure 1. The georeferenced localization of sediment sampling stations selected in each lagoon is reported. Different types of local impacts due to human activities are indicated as "impact factor, IF" and numbered, related to the intensity, as reported: IF-1 = low level; IF-2 = medium level; IF-3 = high level.

Table 1 - Principal general features of studied ecosystems are summarized, bibliographic references are indicated in square brackets. List of references: [1]Spagnoli et al., 2002; [2]ARPAT 2007; [3]Specchiulli et al., 2008; [4]Caroppo, 2000; [5]Specchiulli et al., 2010; [6]Renzi et al., 2007; [7]Manini et al., 2005; [8] Giovani et al., 2010.

Feature		Varano	Orbetello
Geographical district		Southern Adriatic	Central Tyrrhenian
Geographical localization		National Park of Gargano	Southern Tuscany
Gauss-Boaga coordinates		41.88 °N, 15.75 °E	42.29 °N, 11.17 °E
Total surface	*hectares (ha)*	6,500	2,700
Average depth (range)	*meters (m)*	4	1 (0.30-1.70)
Seawater exchanges (artificial canals)		Throughout two canals: Capoiale (Western side) Varano (Eastern side)	Human managed by artificial pumping. Two inflow canals: (Nassa and Fibbia), and one outflow (Ansedonia) ones
Freshwater inputs	m^3s^{-1}	Antonino and S. Lorenzo canals: 1.01[1]	Albegna river: 15[2]
Temperature	°C	8-30	4-11 in winter[3]
Salinity	‰	23-29[3; 4]	20-35[3]
Sediment structure		Dominated by fine-grained sediments[5]	Dominated by fine-grained sediments[6]
Economic relevance and impacts		Intensive fish and mussel farming[7]	Intensive fish farming, summer tourism. Effluents of various human activities such as municipal wastewater treatment plant, industrial activities, urban settlement[8]

associated. Codifications assigned are the follows: 1 = low human impact associated to areas located close to marine/lagoon communicating canals; 2 = medium human impact associated to inner lagoon/basin areas; 3 = high human impact, associated to urban effluents, industrial activities or mines discharges. Further details related to the codification of the factor *local impact* are included in figure 1. Concerning sediments *(n* = 72 records), the logic model developed was based on four factors: *lagoon* (two levels, fixed: Varano *versus* Orbetello), *local impact* (three levels, fixed and orthogonal: from 1 to 3), *local impact replicates* (four levels, random), *sampling replicates* (three levels, random). To avoid interferences due to grain-size differences among sediments collected, only samples characterized by a silt content ranging within 80-90% (unreported data) were reported and discussed in this study. Results were statistically analysed in order to evaluate significant segregations among variables of interest linked to the lagoon and local impact factors. Specimens of *A. anguilla* in its yellow phase were sampled in both lagoons using local caught systems settled within each sampling area. The sedentary benthonic phase of this fish species was chosen in order to give a better description of statistical links occurring among POPs levels and local studied factors. Concerning fishes, the logic model applied was based on four factors: *lagoon* (two levels, fixed: Varano versus Orbetello), *tissue* (two levels, fixed and orthogonal: muscle *versus* liver), *local impact* (three levels, fixed and orthogonal: from 1 to 3), and *sampling replicates* (twenty levels, random) for a total of 120 records per **tissue**. Samplings were performed excluding seasonal-based temporal variability. Statistical analyses were performed on obtained results with the aim to evaluate the significance of observed segregations related to the *lagoon* and *tissue* factors.

Samplings and field activities

Based on the results obtained in previous studies performed in Orbetello (Specchiulli *et al.*, 2008) and Varano (Spagnoli *et al.*, 2002; Specchiulli *et al.*, 2011; Renzi *et al.*, 2012), superficial sediment samples (0-5 cm) were collected in both lagoons in 12 sampling stations georeferenced by the global positioning system technique (fig. 1). The sampling strategy applied in this research aimed at representing the spatial variability of pollutants distribution inside each basin due to different structural factors, such as lagoon morphology, sediment grain-size, and local pollution sources. Sediments were sampled using a stainless steel box-corer (15x15x15 cm) with a stainless steel spatula, placed in pre-cleaned vials, stored in dark and refrigerated (4 °C) conditions during transport to the laboratory (within 12 h). At the laboratory, sediments were air-dried and carefully homogenized before the analyses. At each *local impact replicate* (*n* = 3) sediment samples were analysed for a total of 72 observations on sediments. *A. anguilla* specimens in its yellow phase (n = 120) were sampled in each lagoon (mean length: 45.5 ± 5.0 cm). It is well known that pollutants in fish specimens tend to increase with body size (directly related to the animal age) as a function of the time of exposure to the environmental pollution. Performing the analyses on a similar and narrow length range distribution allowed us to reduce the age-dependant variability. Fishes were caught by authorized fishermen using fixed eel nets located within each area (1-3) and sacrificed immediately after samplings. An equal weight (10 g) of the dorsal muscles (*n* = 20) and the whole liver (*n* = 20) were removed and stored deep-frozen until assaying.

Chemicals of interest

In each matrix, PCB-28, PCB-52, PCB-101, PCB-118, PCB-153, PCB-138, and PCB-180 congeners were determined singularly and expressed as ΣPCBs. OCPs measured were: *2,4-*; 4,4-DDD, *2,4-*; 4,4-DDT, *2,4-*; 4,4-DDE, HCB, *α*-, *β*-, *γ*-HCH, *α*-, *γ*-chlordane, heptachlor, and Mirex. The concentrations of individually resolved peaks are added to obtain total amounts expressed as ΣOCPs. The PAHs molecules analyzed in this study were: naphthalene (2-rings), acenaphthene (3-rings), acenaphthylene (3-rings), fluorene (3-rings), phenanthrene (3-rings), anthracene (3-rings), fluoranthene (4 rings), pyrene (4-rings), chrysene (4-rings), benz[*a*] anthracene (4-rings), benzo[*b*]fluoranthene (5-rings), benzo[*k*]fluoranthene (5-rings), benzo[a]pyrene (5-rings), dibenzo[*a,h*] anthracene (5-rings), benzo[*g,h,*i]perylene (6-rings), and indeno[*1,2,3*-cd]pyrene (6-rings); total ΣPAHs levels represent the sum of the previous cited compounds. Data were expressed as mean (max-min) ± standard error of the mean (SEM). Levels in sediments are expressed as ngg^{-1} dry weight (d.w.) whereas, data measured in tissues are normalized to the lipid weight (l.w.) and expressed as ngg^{-1} calculated on a dry weight (d.w.) basis.

Laboratory analyses

About 10 g of homogenised sample were exactly weighted (accuracy ± 0.0001 g) in pre-cleaned vessels. Concerning PCBs and OCPs, analyses were performed by a preliminary Accelerate Solvent Extraction according to the US-EPA method 3545A followed by a GC-MS quantification. Identification and quantification of peaks were performed by GC-MS equipped with a ion trap detector (ThermoFinnigan, mod. Trace™ GC 2000/GCQ plus) using a Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm, Restek), split-less injection mode, and using helium as carrier gas (Corsi *et al.*, 2005). A calibration mix of each considered PCB congener and OCP was used. $^{13}C_{12}$-labelled congener 141 was added as internal standard. PCB-30 was used as recovery standard and recovered levels ranged within 60-104%. PAHs were extracted from tested matrices and quantified according to methods reported in Specchiulli *et al.* (2011). Samples were Soxhlet extracted with 250 mL of dichloromethane (16 h). The extracts were reduced to about 2 mL under a gentle stream of nitrogen and then cleaned up on a n-hexane previously conditioned micro-column filled with silica gel and anhydrous sodium sulphate (Na2SO4) activated at 120 °C for 24 h. PAHs were eluted from the column using 15 mL of hexane/dichloromethane solution 1:1 (v/v). The solvent of this fraction was removed and the residue was analysed using a High Performance Liquid Chromatography (HPLC, Waters) system. The chromatographic separation was performed on a Supelcosil™ LC-PAH HPLC chromatographic column (250×4.6 mm i.d., particle size 5 µm, Supelco) and the mobile phase was carried out in the following conditions: acetonitrile/water gradient of 60:40 for 40 min using a linear gradient and finally acetonitrile/water 100:0 for 10 min, with a flow rate of 1.5 $mLmin^{-1}$. The maximum elution time was 50 min. Acenaphthylene was identified and quantified using a photodiode series detector (Waters, mod. PDA 996), while all the other PAH compounds were analysed using a scanning fluorescence detector (Waters, mod. SFD 474). The quantitative analysis was performed using a three-point linear calibration of a PAHs solution obtained by dilution of the TLC PAH mix composed by 16 certified standard mixture (Polynuclear Aromatic Hydrocarbon Mix, Supelco). A quite satisfactory linearity was obtained, with values of the r correlation coefficient above 0.99.

Quality assurance and Quality control (QA/QC)

The laboratory complies with the standard ISO 9001:2000 and ISO 14001 for ecotoxicological analysis of sediments and organisms (reg. no. IT33804). Chemicals and reagents were analytical grade and glassware was carefully washed to avoid sample cross-over contamination. The accuracy and precision of the procedure were tested for both sediments and organic matrices by analysing Certified Reference Materials (CRM) purchased from the National Institute of Standards and Technologies (NIST - Estuarine Sediment SRM1646a), the UK Department of Trade and Industry, as part of the UK's National Measurement System (SRM - Harbour sediment), and the National Research Council, Canada (CARP-2, and HS-6 harbour sediments). CRM were analysed in statistical replicates (n = 3) to calculate the averages and standard deviation (SD) of recoveries. Results of two replicates were highly consistent with the certified values (mean errors: 4% for ΣPCBs; 5% for each OCP; and 6% for ΣPAHs). Due to the high percentages of obtained recoveries (over than 90% for each PCB and OCP molecule, and within 70-80% for PAHs), analytical concentrations were not recovery corrected to avoid the introduction of any additional mathematical error. Limits of detection (LOD) were checked for each compound by analysing procedural blanks for every analytical batch. LOD were calculated as the average blank (n = 10) plus three standard deviations (SD). LOD in sediments were: 0.5 ngg^{-1} d.w. sediment for each compounds. On biological tissues, LOD were: 0.2 ngg^{-1} lipid based (l.b.) for PCBs, 0.6 ngg^{-1} lipid based (l.b.) for OCPs, and 0.5 ngg-1 lipid based (l.b.) for PAHs. Quality control procedures applied to instrument detection were performed both for GC-MS and HPLC. For this reason, one solvent/matrices blank was checked every 5 samples, to evaluate the level of accuracy of instrument detection and the obtained results were blank-corrected. The precision for the PAHs analysis in a real sample and expressed in terms of relative standard deviation, ranged between 4.3% (5-rings) and 18.5% (2-rings) and, in most cases, was below 10%.

Statistical analyses

Statistical analyses were performed using univariate and multivariate methods. Pearson's (1894) correlations were applied to explore relationships between couples of variables from a raw data matrix ($p < 0.01$) using GraphPad Prism (GraphPad Software, San Diego California USA, www.graphpad.com) package. Significant segregations were tested using the non-parametric Mann-Whitney test (confidence interval 95%, $p < 0.02$). Multivariate analyses were performed using the Primer v6.0 (Primer-E Ltd., Plymouth Marine Laboratory, UK) software package (Clarke and Warwick, 2001). Further details on methodological criteria applied in multivariate approaches are reported in Benedetti-Cecchi (2004), whereas statistical procedures adopted to analyse sediment and eel's levels of chemicals in this study are similar to techniques reported in Renzi *et al.*, 2012.

Results

Results obtained by the univariate statistical analyses on ΣPCBs, ΣOCPs, and ΣPAHs levels measured in each sampled matrix are reported respectively in table 2, 3, 4, and in figure 2. Results are grouped separately *per* each considered matrix (sediment, muscle, liver) according to the factor *lagoon* (Orbetello versus Varano). Comparing values of measured chemicals in sediments, the observed trend is: ΣPAHs >> ΣPCBs > ΣOCPs in both considered lagoon ecosystems even if measured levels in Orbetello sediments are higher than Varano ones. On the contrary, considering levels in eels' tissues, observed trends are quite different. In Varano lagoon, levels measured in livers evidence the following trend: ΣPCBs >> ΣOCPs > ΣPAHs with absolute maxima recorded for ΣPCBs that are about two orders of magnitude higher than other chemicals. A similar trend is observed in muscles even if ΣOCPs ≈

Table 2 - Levels (ng g^{-1}) of PCB-28, PCB-52, PCB-101, PCB-118, PCB-153, PCB-138, PCB-180 expressed as ΣPCBs are reported separately grouping them both considering the factor *lagoon* (Varano *versus* Orbetello) and the factor *matrix* (sediment, muscle, and liver). Data are reported as mean (max-min) ± standard error of the mean (SEM). Notes: d.w. = dry weight, l.b. = lipid base.

System	Matrix	Minimum	Maximum	Mean	SEM
Varano	Sediment (d.w.)	<0.5	29.8	14.9	8.6
	Liver (d.w.l.b.)	119.7	204.5	152.7	18.4
	Muscle (d.w.l.b.)	9.0	36.4	21.9	6.4
Orbetello	Sediment (d.w.)	1.2	66.1	33.6	18.7
	Liver (d.w.l.b.)	117.1	530.8	261.9	79.6
	Muscle (d.w.l.b.)	24.9	110.7	54.7	16.4

Table 3 - Levels (ng g^{-1}) of *2,4*-; *4,4*-DDD, *2,4*-; 4,4-DDT, 2,4-; 4,4-DDE, HCB, *α*-, *β*-, *γ*-HCH, *α*-, *γ*-chlordane, heptachlor, and Mirex expressed as ΣOCPs are reported. Data are expressed separately as mean (max-min) ± standard error of the mean (SEM) grouping them both considering the factor *lagoon* (Varano *versus* Orbetello) and the factor *matrix* (sediment, muscle and liver). Notes: d.w. = dry weight, l.b. = lipid base.

System	Matrix	Minimum	Maximum	Mean	SEM
Varano	Sediment (d.w.)	0.1	4.3	2.2	1.2
	Liver (d.w.l.b.)	<0.6	29.0	13.5	6.6
	Muscle (d.w.l.b.)	<0.6	1.9	0.9	0.4
Orbetello	Sediment (d.w.)	<0.5	10.2	5.2	2.9
	Liver (d.w.l.b.)	1,541.7	12,741.9	5,702.5	2,202.4
	Muscle (d.w.l.b.)	777.8	10,322.6	4,405.1	1,894.2

Table 4 - Levels (ng g^{-1}) of naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*] pyrene, dibenzo[*a,h*]anthracene, benzo[*g*,*h*,*i*]perylene, and indeno[*1*,*2*,*3-cd*]pyrene expressed as ΣPAHs are reported. Data are expressed separately as mean (max-min) ± standard error of the mean (SEM) grouping them both considering the factor *lagoon* (Varano *versus* Orbetello) and the factor *matrix* (sediment, muscle and liver). Notes: d.w. = dry weight, l.b. = lipid base.

System	Matrix	Minimum	Maximum	Mean	SEM
Varano	Sediment (d.w.)	6.8	55.1	31.0	13.9
	Liver (d.w.l.b.)	3.5	5.8	4.4	0.5
	Muscle (d.w.l.b.)	0.6	1.3	0.9	0.2
Orbetello	Sediment (d.w.)	3.8	250.0	126.9	71.1
	Liver (d.w.l.b.)	10.1	36.9	19.1	5.1
	Muscle (d.w.l.b.)	1.0	4.7	2.3	0.7

ΣPAHs. In Orbetello lagoon the observed trend is clearly different but similar both in muscle and liver: ΣOCPs >> ΣPCBs > ΣPAHs. It is to notice that in eels' tissues mean values of ΣOCPs are about three order of magnitude higher than ΣPCBs and ΣPAHs. The Wilcoxon-Mann-Whitney test performed on collected data evidences significant differences ($p < 0.01$) among studied systems related to ΣPAHs and ΣOCPs in sediments, whereas a slight but not significant difference is recorded for ΣPCBs. Concerning levels measured in both eels' tissues (muscle and liver), the Orbetello lagoon evidenced significant higher values compared to Varano ones as confirmed by the Wilcoxon-Mann-Whitney test ($p < 0.01$).

In figure 2, results obtained grouping data separately for each tested matrix according to the factor *local impact* are reported. As evidenced by the figure, a clear trend to directly increase measured level of chemicals with the assigned *local impact* value is observed for all the tested matrices. This relationship is particularly evident for ΣPCBs and ΣPAHs in sediments and for ΣOCPs in eels' tissues. Principal Component Analysis, PCA, evidences that the first three components (PC1, PC2, PC3) account for the 100% of the total variance (respectively 54.8%, 32.5%, and 12.7%) (fig. 3). Coefficients in the linear combinations of variables making up PC's which show the correlations of the first three axis are, also, reported in the same figure. Eigenvectors results (fig. 3) evidence a significant positive correlation among the first axis and ΣPCBs, ΣOCPs. The second axis is negatively related to ΣPAHs. Results obtained by the cluster analysis are superimposed to the PCA in figure 3 evidencing a statistical distance higher than 1.0 between chemical levels in eels' tissues from Varano compared to those from Orbetello lagoon. Sediments sampled in both systems evidence higher similarity related to the factor *local impact* 2-3 while sediments

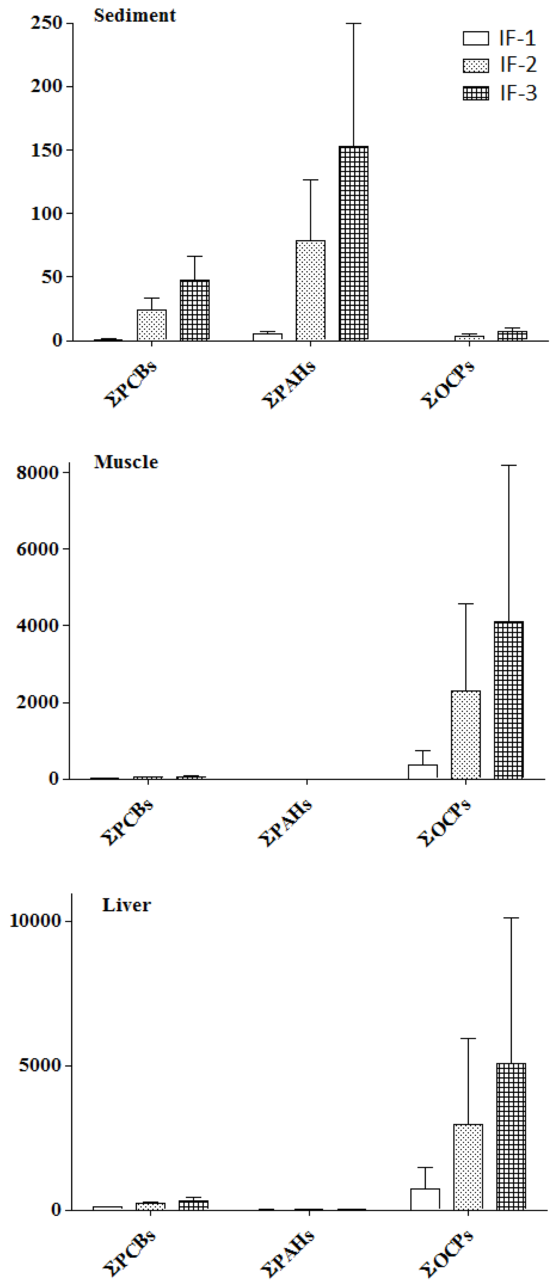

Figure 2. Levels of ΣPCBs, ΣOCPs, ΣPAHs are reported as mean (ng g-1) + standard deviation grouping them both considering the factor matrix and the factor local impact a priori defined as indicated in figure 1. Values represented are not scaled per matrix: levels in sediments are expressed as dry weight, while in tissues values are reported as dry weight corrected for the lipid base.

related to the factor *local impact* 1 evidence a clear segregation from others. The one-way ANOSIM test performed on the factor *lagoon* (Orbetello *versus* Varano) confirms significance related to segregations observed (Global R = 0.266, with a significance level of sample statistic of 1% and a number of permuted statistics greater than or equal to Global R, NPS of 99). Performing the ANOSIM test on the factor *local impact* a low value of Global R was obtained (Global R = 0.132, with a significance level of sample statistic of 8.6% and a number of permuted statistics greater than or equal to Global R, NPS of 860). Nevertheless, the Pairwise tests run to evaluate differences among couples of *local impact* evidences high significant differences between the couple 1-3 (Global R = 0.269, with a significance level of sample statistic of 0.9% and a number of permuted statistics greater than or equal to Global R, NPS of 9). The two-way ANOVA test conducted separately for Varano and Orbetello showed that observed chemical behavior is significantly affected by the considered matrix. In fact, in Varano lagoon, the matrix explains the 18.35% of the total variance (F = 15.84, P = 0.0001), whereas considered chemical variables explained the 28.77% (F = 24.83, P < 0.0001). On

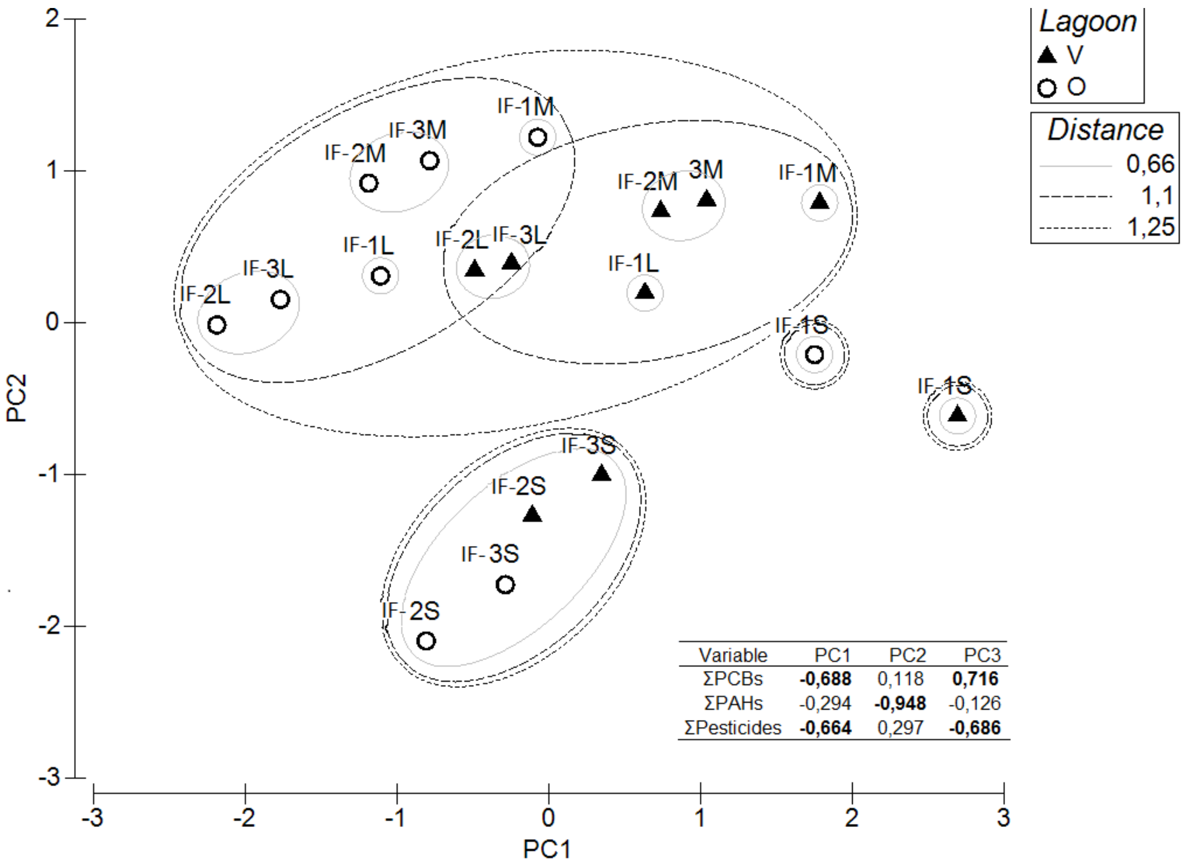

Figure 3. Principal component analysis based on contaminant levels in sediments and fish tissues grouped. In this figure, the following factors of interest are represented: *lagoon* (Varano = triangle, Orbetello = circle), *matrix* (S = sediment, L = liver, M = muscle), *local impact* (IF-1 = low level, IF-2 = medium level, IF-3 = high level, values *a priori* defined). Eigenvectors calculated from PCA for each variable are evidenced (in bold values associated to a significance levels p <0.01). Results obtained from the single-linkage (p < 0.5) cluster analysis are overlaid to the PCA and distances are, also, reported in figure.

the contrary, in Orbetello lagoon the matrix evidences a not significant effect (total explained variance = 9.81%, $F = 2.50$, $P = 0.110$) while a very significant different behavior among considered chemicals was observed (total explained variance of 35.39%, $F = 9.03$, $P = 0.002$).

Discussion

The natural water eutrophic feature of paralic ecosystems, such as coastal lagoons, produces in bottom sediments a consequent enrichment in organic matter (Zaldívar *et al.*, 2008). Finer grain-sized bottom sediments rich in mud and clay tend to accumulate organic compounds more than sandy ones. In paralic ecosystems, the literature evidences the occurrence of a strong linear relationship between the content of particles with a diameter minor than 63 µm and the observed levels of total organic carbon. Due to their physico-chemical characteristics, in aquatic environments POPs tend to be rapidly accumulated onto organic particulate matter through chemical and physical complex mechanisms of adsorption which favour POPs deposit and accumulation in silty nutrient-enriched bottom sediments. Due to the natural high content of silt in bottom sediments, Orbetello and Varano lagoons represent natural accumulating areas for considered chemicals.

Considering measured values in sediments, results on ΣPAHs confirm literature data, in fact Specchiulli *et al.* (2011) evidenced similar levels also observing the highest exposure to these chemicals in Orbetello lagoon. Measured concentrations are shown to be substantially lower than those obtained in urbanised and/or industrial Italian sites or Mediterranean areas such as Venice lagoon (54,000–160,000 ngg^{-1} d.w.; Wetzel and Van Vleet, 2003), Naples (9–31,774 ngg^{-1} d.w.; Sprovieri *et al.*, 2007), Ravenna harbour (300–87,000 ngg^{-1} d.w.; Vassura *et al.*, 2005), the coastal lagoon of Marsala (72–18,381 ngg^{-1} d.w.; Culotta *et al.*, 2006), French and Spanish coasts (1–8,000 ngg^{-1} d.w.; Baumard *et al.*, 1998), Gulf of Fos area, France (2,500–70,000 ngg^{-1} d.w.; Mille *et al.*, 2007), and Tunisia (113.2–10,720 ngg^{-1} d.w.; Zaghden *et al.*, 2007).

POPs levels measured in sediments are compared to national Sediment Quality Guidelines defined for paralic harbour ecosystems by the Central Institute for Applied Marine Research (APAT-ICRAM, 2007). In this guideline, two different ranges of concentrations are defined for each chemical. The first one includes values within the limit of quantification (LOQ) and the natural concentration limit (LCB), while the second includes values within LCB and the critical concentration limit (LCL). As a consequence, three classes of quality for sediments are defined indicating: *i*) natural range, Class 1 (LOQ < measured levels < LCB), *ii*) enrichment, Class 2 (LCB< measured levels < LCL), *iii*) pollution, Class 3 (measured levels > LCL). Concerning PCBs and PAHs, defined values for LCB and LCL are reported as total levels, whereas pesticides limits are defined for single compound. In Varano lagoon, mean ΣPCBs level exceeds LCB (5 ngg^{-1} d.w.) and maximum values are about one order of magnitude lower than LCL (189 ngg^{-1} d.w.). In the Orbetello lagoon a similar behaviour is observed even if the maximum value is minor than about an half the LCL limit. According to the sediment classification proposed by APAT-ICRAM (2007), both systems are classified in "Class 2, enriched concerning ΣPCBs". In both ecosystems ΣPAHs measured values exceed neither LCB (900 ngg^{-1} d.w.) nor LCL (4,000 ngg^{-1} d.w.) and sediments are classifiable in "Class 1, natural range concerning ΣPAHs".

ΣPCBs results could be related to the natural very high silt content of lagoon sediments (within 80-90% of the total sediment weight). In fact relating to POPs, APAT-ICRAM guidelines do not propose any differences in LCB based on silt percentage. As a consequence, in paralic ecosystems that are naturally characterized by higher levels of mud and clays compared to marine ones, levels of chemicals usually exceed LCB even if not direct sources of these pollutants are observed on a local basis. For this reason, *ad hoc* LCB values specifically calculated for paralic ecosystems and taking into account their natural silt enrichment should be proposed.

Even if it does not take into any account the silt content of sediments, a first step towards the definition of specific limits for POPs in lagoons is represented by the "Protocol of agreement on sediments" signed on April 8th in 1993 by Italian Ministry of Environment, Veneto Region, Venice Province, and Venice and Chioggia towns which reports "Guidelines for Venice channel sediment dredging, dredged materials transport, and re-employment". In this Protocol, sediments are classified on the basis of POPs levels in four quality groups. In particular, if pollutants levels are lower than values defined for Class A, sediments are suitable for engineering purposes including re-filling of lagoon marginal areas and direct or indirect water-sediment exchanges are allowed, whereas if chemicals exceed levels defined as maximum acceptable for the Class A but are lower than values allowed for Class B, sediments are also usable but have to be confined to avoid water-sediment exchanges. Orbetello and Varano sediments evidence levels of ΣPCBs (0.01 and 0.2 mgkg^{-1} d.w.) and ΣPOCs (0.001 and 0.02 mgkg^{-1} d.w.) higher than maximum values of Class A, but lower than maximum levels defined for Class B, so they are included in Class B. ΣPAHs values are lower than limits of Class A (1 mgkg^{-1} d.w.). The use of empirically-derived sediment quality guidelines for marine and estuarine ecosystems is recommended by the literature (Long and MacDonald, 1998). Measured concentrations are compared with sediment quality guidelines recognized at international level as well as threshold effects level (TEL) and probable effect levels (PEL) proposed by Long *et al.* (1995), MacDonald *et al.* (1996), and Long and MacDonald (1998). TEL represents the concentration below which adverse effects are expected to occur only rarely, on the contrary, PEL is the concentration above which adverse effects are expected to occur frequently (Smith *et al.*, 1996). TEL and PEL values are available only for ΣPCBs. Measured values in both ecosystems are significantly below the PEL level (277 ngg^{-1} d.w.). Sediments from Varano lagoon evidence average (14.9 ngg^{-1} d.w.) and maximum (29.8 ngg^{-1} d.w.) values lower than TEL (34.1 ngg^{-1} d.w.) level. On the contrary, sediments from Orbetello lagoon evidence average value (33.6 ngg^{-1} d.w.) closed to TEL and maximum (66.1 ngg^{-1} d.w.) higher than TEL. Nevertheless, observed values are always less than 70 ngg^{-1} d.w. that is the lowest effect level (LEL). According to Persaud *et al.* (1993), sediments which PCBs values lower than LEL are considered to be clean to marginally polluted and no effects on the majority of sediment-dwelling organisms are expected below this concentration.

Comparing levels measured for all considered chemicals, a common behavior of these molecules in Varano and Orbetello sediments is evidenced. In fact, in both studied ecosystems, ΣPAHs are higher than other considered chemicals (ΣPCBs, ΣOCPs) which evidence, on the contrary, relative lower and quite similar total levels. The ΣPAHs dominance in sediments could be related to the major exposure of considered ecosystems to hydrocarbon pollution local sources rather than to ΣPCBs, ΣOCPs. PAHs are originated by natural processes such as low to moderate temperature diagenesis of sedimentary organic materials to form fossil fuels (i.e. coal, crude petroleum) and biosynthesis by biota. Nevertheless human activities (e.g. wastes from industrialised and urbanised areas) represent a relevance. PAHs are directly or indirectly spread in aquatic ecosystems within industrial and domestic effluents, throughout surface runoff from land, deposition of airborne particulates, and spillage of petroleum and petroleum by-products (Neff, 1979). Among exposed sources, direct spillage of crude petroleum and refined petroleum products and dry-air depositions from urban areas represent a relevance for coastal lagoons due to the considerable boat traffic, long water renewal times, and the presence of considerable surrounding urbanized areas. In fact, considering crude oil, ΣPAHs content ranges within 7-34 weight% of the total weight, while refined oil products contain lower values ranging within 0.3-3.7%. A detailed research performed on PAHs levels in four Italian coastal lagoons (Orbetello, Varano, Lesina, and S. Giusta) evidenced a dominance of urban wastewaters and boats' exhausts of high-molecular-weight PAHs (4-5-rings) in all sediment samples from Orbetello and Varano. It was also shown that pollution coming from the traffic of boats was widely distributed over the lagoons (except for Varano), while domestic wastewaters prevailed close to the urban centres (Specchiulli *et al.*, 2011). In the Orbetello lagoon ΣPAHs indicate a low to moderate level of PAH pollution and a heterogeneous distribution of these molecules inside the lagoon basin (Specchiulli *et al.*, 2011).

Results obtained in this research evidence that, related to the factor *local impact*, data are evidently grouped according to the *a priori* classification proposed. Furthermore, IF-2 and IF-3 evidence a similar and quite different behaviour compared to IF-1. Concerning sediments, the multivariate analysis performed evidences that the major differences among mean values are principally related to the factor *local impact* rather than to the ecosystem considered. Even if measured levels are not critical, sediments can be considered as a pollution reservoir from which POPs may once again be released into the environment (Salomons *et al.*, 1987). In fact, sediment resuspension could determine secondary, important, releases towards the biota. In particular the European eel in its yellow phase, due to its ecological features, could be particularly exposed. Comparing ΣPAHs levels measured in different matrices (sediment, muscle, liver), results obtained in this study confirm the behaviour attended for these compounds from the literature. In fact, due to their properties, levels in marine ecosystems are generally reported to be higher in sediments, intermediate in aquatic biota, and lower in water column (Neff, 1979). PAHs evidence toxicological effects on adults, juvenile phases or early life stages, gametes and embryos on marine species (Shahidul Islam and Tanaka, 2004). Moreover the bioconcentration, bioaccumulation, and biomagnification properties of some of these molecules and their related toxicological effects on algae, invertebrates, and vertebrates are, also, evidenced by the literature (Mackay and Fraser, 2000; Ritcher and Nagel, 2007). The intensity of these phenomena is related not only to levels, but also to different co-factors such as: external and local environmental features, exposure time, species considered and its trophic level, sex, and the occurrence of synergic effects with other chemicals (Bacci, 1994). ΣPAHs levels are similar in muscles of both ecosystems and higher in liver sampled in eels from the Orbetello lagoons, even if measured values are lower than levels attended from the literature (Neff, 1979). These results evidenced that the total content of PAHs stored in sediments is not efficiently transferred and/or accumulated in eels' tissues. Considering levels measured for all chemicals in tissues, a quite different trend from those observed in sediments is recorded. In fact, in Varano lagoon, levels measured in liver evidence the following trend: ΣPCBs >> ΣOCPs > ΣPAHs, with absolute maxima recorded for ΣPCBs that are about two orders of magnitude higher than other chemicals. A similar trend is observed in muscles even if ΣOCPs ≈ ΣPAHs. In Orbetello lagoon, measured levels evidence a clear different trend which is quite similar in both tissues: ΣOCPs >> ΣPCBs > ΣPAHs. It is to notice that in eels' tissues mean values of ΣOCPs are about three orders of magnitude higher than ΣPCBs and ΣPAHs levels. This occurrence is probably due to differences in pollutant dynamics occurring in these ecosystems. PCBs have been used for a variety of industrial purposes and were released as Arochlor mixtures into the environment. They are the most widely-studied class of POPs due to their ubiquity, potential for magnification in the food chain and harmful ecotoxicological effects (Safe, 1992). Mariottini *et al.* (2006) evidenced in European eel an active uptake over time for PCBs recording mean levels in muscles about twice than values recorded in this study. This occurrence could be related to a general global-basis reduction of PCBs air-dry depositions from active sources. Higher levels of ΣOCPs in European eel's tissues from the Orbetello lagoon could be, on the contrary, related to the presence of a significant source of bioavailable pesticides: the Albegna river which drains the whole inland agricultural area of the Amiata Mountain (Giovani *et al.*, 2010 and references therein). The occurrence of different dynamics involving pollutants behaviour in abiotic and biotic matrices in Orbetello and Varano lagoons is, also, confirmed by the multivariate analysis. In fact, differences among mean values in eels' tissues are majorly reliable to the factor *lagoon*. Eels sampled in Orbetello evidenced a significantly different chemical assessment compared to Varano ones and differences are principally due to ΣPCBs and ΣOCPs behaviours. An evident relation to the matrix is observed and a not strong relationship with the factor *local impact* is recorded even if in each lagoon systems and for each matrix, a difference between IF-1 and IF-2/3 is always observed. This results evidences as the analysis of the yellow phase of this species could allow to efficiently distinguish local differences of pollutants distribution, also within a low-dimension lagoon basin. This is due to the explicit behaviour and movements restricted to a few hundred meters of this phase (Geeraerts and Belpaire, 2010). Even if ΣOCPs levels in sediments are not excessively high, the observed high levels in eels' tissues could be originated both from high uptake rates from sediments and from the exposure to high levels in water column due to the Albegna river inputs. The overall decline in eels' stocks may be due above all to pollution levels; therefore gaining a comprehensive overview of the quality of the eel population in Europe represents an essential and urgent objective for European eel management (Geeraerts and Belpaire, 2010). In this context, these results could represent a recent improvement of knowledge on POPs levels in eels' tissues both in recent (Varano) and long-time (Orbetello) monitored ecosystems.

As a further consideration, obtained results evidenced that an integrated approach based on abiotic and biotic matrices characterization allows to improve knowledge on ecosystem dynamics which could affect decisions taken only on the basis of a single approach (frequently using only data collected on sediments). In fact, in the Orbetello lagoon even if sediments do not indicates criticises, the occurrence of a significant ΣOCPs source for biota is well evidenced by the analyses performed on eels. The inclusion of the yellow stage eel in the list of species indicated for monitoring the chemical status of waters could represents an important resource for improving knowledge on lagoon ecosystems.

Conclusions

This research evidences that: *i*) levels of POPs in sediments are below critical values defined by National and International sediment quality guidelines; *ii*) statistical differences among sediments are more related to the factor *local impact* than to the factor *lagoon*; *iii*) concerning levels in eel's tissue, recorded values highlight high concentrations of POPs in livers; eels sampled in Orbetello evidenced a significantly different chemical assessment compared to Varano ones and differences are principally due to ΣPCBs and ΣOCPs behaviours; *iv*) concerning eels, a difference between level IF-1 and IF-2-3 is always observed considering both *matrix* and *lagoon* separately; *v*) the analysis of the yellow phase of this species could increase knowledge on pollutant dynamics in coastal lagoons; *vi*) integrated monitoring programs which include both abiotic and biotic characterizations allow to improve knowledge on pollutants dynamics in aquatic ecosystems.

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