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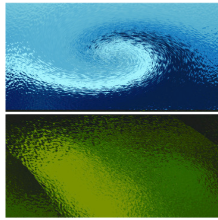

RESEARCH ARTICLE

Persistent organic pollutants and enzyme activities in European eel (Anguilla anguilla) from Orbetello lagoon

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Abstract

1 - The aim of the present study was to obtain insights into the environmental quality of Orbetello lagoon using the European eel (*Anguilla anguilla*) as bioindicator organism.

2 - Levels of POPs, including seven polychlorobiphenyls (PCBs) (known as markers of Aroclor 1260), three coplanar PCBs, ∑DDT (*op*-DDD, *pp'*-DDD, *op*-DDT, *pp'*-DDT, *op'*-DDE, *pp'*-DDE), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH: α-HCH, β-HCH, γ-HCH, δ-HCH), polychlorodibenzo-p-dioxins (PCDDs), polychlorodibenzo-*p*-furans (PCDFs) and polybromodiphenylethers (PBDEs), were investigated in eel muscle to study bioaccumulation of toxic compounds.

3 - EROD MROD and UDPGT activities were measured in liver microsomal fraction to investigate eel detoxifying/metabolic capacity.

4 - Ovaries were analyzed by light microscopy to evaluate potential risk for eel reproduction. Muscle concentrations of PCBs, DDTs, *pp'*-DDE, HCB and HCHs were in the ng/g lipid weight (l.w.) range while coplanar PCBs were in the pg/g wet weight range.

5 - PCDD/Fs were below the detection limit. Toxic equivalents (TEQs) calculated for mono- and non-*ortho* PCBs were below the risk thresholds established by the European Union.

6 - EROD, MROD and UDPGT activities suggested low exposure to chemical inducers in line with the low concentrations of contaminants observed in muscle. Principal components analysis (PCA) showed a potential role of DDTs, especially *pp'*-DDE, in inducing EROD, MROD and UDPGT activities.

7 - All specimens analysed were females; oocytes in advanced stages of development showed normal morphology, whereas those in early stages of development showed histological anomalies.

Keywords: European eel, POPs, CYP450, UDPGT, Mediterranean lagoon

Introduction

Lagoons are environments of great ecological value, being the best natural habitat for juveniles and adults of various aquatic species (Moore *et al.*, 1994). These ecosystems are increasingly threatened by anthropogenic stressors which have detrimental effects on ecological and biological components (Corsi *et al.*, 2005).

The European eel was once a prominent species of coastal lagoons. A decline in eel stocks has been documented since 1980 (Robinet and Feunteun 2002), but the causes of this reduction are still unclear. Hypotheses include habitat loss, impeded migration from rivers to sea, climate change of oceans and overfishing (Dekker 2004). Eels also have a life cycle and physiology which make them sensitive and vulnerable to environmental contamination. Eels breed in the Sargasso Sea and migrate to Europe in the larval stage, where they metamorphose into the more recognisable elvers. The 'yellow eel'

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stage grows in freshwater, where males and females spend 6 to 12 and 9 to 20 years of their lives, respectively. Towards the end of this time, they become sexually mature, turn a silvery colour ('silver eel' stage) and migrate back to the sea on dark, moonless and stormy nights. On returning to the sea, common eels live in mud, crevices, and under stones. Spawning occurs in winter and early spring in the Sargasso Sea (Cognetti & De Angelis, 1980; *www.fishbase.org*). Juvenile or yellow eels, common in rivers, estuaries and coastal lagoons, are relatively stationary and territorial and since they feed on sediment and benthic invertebrates, they are threatened by sediment-associated contaminants (Larsson *et al*., 1991).

During migration to the coasts of Europe, North Africa and North America, leptocephali (eel larvae) come into contact with domestic and industrial effluent rich in lipophilic contaminants (Palstra *et al*., 2005). Forty percent of the body weight of the European eel is composed of lipids, found mainly in muscle and to a lesser extent in liver and around viscera. This is why eels accumulate remarkable concentrations of lipophilic xenobiotics such as γ-HCH, certain PCBs, PCDDs and PCDFs (Larsson et al., 1991; De Boer *et al*., 1994; Schlezinger and Stegeman *et al*., 2000). During their long migration, eels use their fat deposits as energy reserves, so lipophilic contaminants accumulating in these tissues are released into the bloodstream and transferred to offspring, posing a serious threat for survival of the species (Larsson *et al*., 1991; Schlezinger and Stegeman 2000; Robinet and Feunteun 2002). Since the 1960s, eels have declined greatly in Mediterranean lagoons and particularly in Orbetello lagoon, where the species was once a major economic resource.

The aim of the present study was to obtain insights into the environmental quality of Orbetello lagoon using the European eel (*Anguilla anguilla*) as bioindicator organism. Orbetello lagoon is one of the largest lagoons in the western Mediterranean and its shallow brackish water, poor circulation, low water volume with limited turnover and partial isolation from the sea drastically reduce the dilution of any organic input, nutrients from urban effluent and aquaculture plants, agricultural waste water and anthropogenic contaminants. The lagoon has suffered periodic algal blooms and severe dystrophic crises which have caused fish die-offs and a drastic reduction in biodiversity. Benthic communities have shown poor recovery. Our previous studies showed the presence of environmental pollutants such as PCBs, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides and PBDEs in fish tissue and underlined the need for continuous monitoring of lagoon health status. Eels were identified as a suitable bioindicator species for monitoring studies (Corsi et al., 2005; Mariottini *et al*, 2005; Mariottini *et al*., 2008).

In the present study we measured levels of POPs including mixtures of polychlorobiphenyls (PCBs), three coplanar PCBs, dichlorodiphenyltricholroethane (DDT: *op*-DDD, op-DDE, *pp*'-DDD, *op*-DDT, *pp'*-DDE, *pp'*-DDT), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH: α-HCH, β-HCH, γ-HCH, δ-HCH), polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzo-*p*-furans (PCDFs) and polybromodiphenylethers (PBDEs) in eel muscle. Risk for the eel population of the lagoon was assessed by analysis of Toxic Equivalent Factors (TEFs). Biomarker responses including cytochrome P4501A, 7-ethoxy/7-methoxy resorufin-*O*-deethylase (EROD and MROD) and UDP glucuronyltransferase (UDPGT) activities, were assayed in liver to determine the detoxifying/metabolic capacities of eels. To assess reproductive risk, gonads were examined by light microscopy.

Materials and methods

Study area and sampling

Orbetello lagoon (Figure 1) is a major Mediterranean lagoon with an area of 27 Km2 situated on the southern coast of Tuscany (42°30'N 11°10'E) (Di Bitetto *et al.*, 1998). Ten yellow eels (juveniles) were collected using fish traps in the western basin (UTM coordinates WGS84: 4703040; 0681365) in April 2006. The site chosen for biomonitoring was identified as one of the most contaminated parts of the lagoon in previous studies (Focardi *et al.*, 2009) on the basis of concentrations of POPs (e.g. lindane) in organisms and sediment. It was not possible to choose a control site outside the lagoon, as juveniles are only found in the lagoon. The specimens were shipped to the laboratory in oxygenated coolers with aerated collection-site water. They were measured and weighed individually; mean (± s.d.) length was 131 ± 61 (cm) and weight was 46.7 ± 2.5 (g). After light anaesthesia with clove oil the eels were sacrificed and dissected for tissue analysis. A portion of muscle was stored at -20°C until chemical analysis. Livers were excised, flash frozen in liquid nitrogen and stored at -80°C until enzyme assay. Ovaries were excised and embedded in Bouin solution for histological analysis.

Figure 1. Map of Orbetello lagoon and sampling site.

Contaminant analysis

About 2 g of muscle was freeze dried and taken to the marine ecology laboratory in Orbetello, where it was extracted in a Dionex accelerated solvent extractor with hexane/acetone (1:1) according to La Rocca *et al*. (2004). PCDD/Fs were determined by isotope dilution according to EPA 1613 (US EPA 1994) using specific PCDD/F standard ($^{13}C_{12}$, 99%) (Cambridge Isotope Laboratories). An aliquot of extract (10%) was used for gravimetric determination of lipids. The lipid weight of eel muscle tissue was determined since bioaccumulation of hydrophobic chemicals is influenced by this parameter (van der Oost *et al*.,1996). The extract was then evaporated, made up to 9 ml with hexane and purified by Power-Prep™ (Fluid Management Systems Inc.) as described by Pirard *et al*., 2002.

PCBs and OC pesticides

PCB congeners and chlorinated pesticides were identified and quantified by GC/MS with ion trap detector (ThermoFinnigan Trace™ GC 2000/GCQ plus) using a Restek Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm), splitless injection mode and helium as carrier gas. Injector temperature was 250°C. Oven temperature ramping was 100°C (hold 2 min), to 140°C at 20 C°/min (hold 0 min), to 200°C at 4 C°/min (hold 13 min) and to 300°C at 4 C°/min (hold 10 min). The mass spectrometer operated with an EI+ source (200°C), a transferline temperature of 280°C and 70 eV filament energy. Other conditions are reported in Thomas *et al*. (1998). A mixture of seven PCBs (PCB-28, -52, -101, -118, -138, -153, -180), known as markers of Arochlor 1260, and 11 pesticides was used as calibration standard. Congeners PCB-30 and PCB-141 ($^{13}C_{12}$, 99%, Cambridge Isotope Laboratory) were added as internal standard, and a mixture of $^{13}C_{12}$-labelled PCB congeners (Cambridge Isotope Laboratories) was added as recovery standard before extraction (recovery > 60%). Concentrations of individual congeners were summed to obtain total PCB concentrations (lipid weight l.w.).

Coplanar PCBs and PCDD/Fs

A Polaris GC/MS with ion trap detector coupled to a Trace™ GC 2000 gas chromatograph with AS3000 autosampler (ThermoFinnigan) and a Restek Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm) was used for quantitative analysis and confirmation of coplanar PCBs (PCB-77, -81, 126, -169, Wellington Laboratories Inc.) and 17 PCDD/F congeners. Two µl of sample made up to final volume with a solution of 1,2,3,4-TCCD and 1,2,3,7,8,9-HxCDD (13C12, 99%) (Cambridge Isotope Laboratory) was injected in splitless mode at 250°C. Oven temperature ramping was: 110°C for 1 min, to 220°C at 20 C°/min (hold 2 min) and to 300°C at 10 C°/min (hold 5 min). Filament energy was 70 eV for coplanar PCBs and 50 eV for PCDD/Fs. MS/MS conditions of coplanar PCBs are reported in Pirard *et al*., 2002, and those for PCDD/Fs in Grabic *et al*., 2000. The six-point calibration curve was provided by Cambridge Isotope Laboratory. Recovery, calculated with PCDD/F standard specific for EPA 1613 method ($^{13}C_{12}$, 99% Cambridge Isotope Laboratories), was over 70%.

TEQs

Toxic equivalent (TEQ) concentrations were obtained by multiplying the concentration of each toxic PCB and PCDD/F by its assigned toxic equivalent factor (TEF). TEFs (van den Berg *et al*., 1998) were used to calculate TEQs with respect to 2,3,7,8-TCDD. The contribution of mono, non-*ortho* substituted PCB and PCDD/F congeners to total toxic

quantitative analysis and confirmation of PBDEs in samples. Two µl of sample made up to final volume with PBDE-139 internal standard ($^{13}C_{12}$, 99%) in nonane (Wellington Laboratories Inc.) was injected in splitless mode at 275°C. Oven temperature ramping was: 80°C for 2 min, to 200°C at 25 C°/min (hold 0 min), to 300°C at 4 C°/min (hold 10 min). MS/MS conditions are described in Mariottini *et al*. (2008). A mixture of nine PBDEs (BDE-28, -47, -66, -85, -99, -100, -138, -153, -154) from Cambridge Isotope Laboratory was used as calibration standard. Mean recovery was 70%.

Quality assurance and quality control

Quality assurance and quality control (QA/QC) of the procedure were conducted by analyzing two replicates of Certified Reference Material WMF-01 (freeze-dried fish tissue) from Wellington Laboratories Inc. Recovery results agreed well with certified values with an average error of 5% for PCBs, 4% for pesticides, 7% for PBDEs and 10% for PCDD/Fs. Detection Limits (LODs) calculated as mean blank +3SD were 0.2 and 0.6 ng/g lipid weight (l.w.) for PCBs and OCPs, respectively, and 0.9 ng/g l.w. for PBDEs. LODs for non-*ortho* PCBs and PCDD/Fs were 2 and 0.4-1 pg/g l.w., respectively. A blank prepared by the same procedure as for the samples was included every five samples and the results were blank-corrected. Our laboratory complies with ISO 9001:2000 and ISO 14001 standards for ecotoxicological analysis of sediments and organisms (reg. no. IT33804).

Biochemical assays

Eel livers were individually homogenized 1:4 (w/v) with sucrose buffer (50 mM K_2HPO_4, 0.75 M sucrose, 1 mM EDTA, 0.5 mM DTT, 400 µM PMSF, pH 7.5) using a Potter-Elvenjem glass/Teflon homogenizer at 2000 rpm. Microsomes were obtained by differential spinning in a Sorvall RC28S ultracentrifuge. Homogenates were first centrifuged at 9000xg for 20 minutes to remove nuclei, mitochondria, lysosomes and cell debris and the resulting supernatants were collected and centrifuged at 100,000xg for 1 h. The microsomal pellets were resuspended 1:2.6 (w/v) in Tris-(base) buffer (10 mM Tris, 20% p/v glycerol, 0.5 mM DTT, 400 µM PMSF, pH 7.5). All procedures were carried out at 4°C as previously described (Corsi *et al*., 2003). Liver microsomal EROD and MROD activities were assayed according to the fluorimetric methods of Burke and Mayer (1974) using a Perkin-Elmer LS50B luminescence spectrofluorimeter. EROD and MROD assay conditions in the reaction mixture (final volume 2.25 ml) were as follows: pH 7.5, 30°C, in a fluorimeter cuvette containing Tris-HCl, MgCl2·6H2O, 10 µl NADPH and about 50-100 µl of eel liver microsomal fraction. 7-ethoxy/methoxy-resorufin (10 µl 0.1 mg/ml in DMSO) was used as substrate. The reaction was started by adding NADPH and the progressive increase in fluorescence was recorded for 4 minutes at λ_{EX}=522 nm/λ_{EM}=586 nm. The amount of resorufin produced was calculated from a pure resorufin standard calibration curve with a detection limit of 0.05 pmol min^{-1} mg $prot^{-1}$. EROD and MROD activities were expressed as picomoles of resorufin produced per minute per milligram of total microsomal protein (pmol min^{-1} mg $prot^{-1}$). UDPGT activity was measured according to the spectrofluorimetric microplate assay of Collier *et al*., 2000. The reaction mixture consisted of 15 µl of microsomes or buffer (blank) and 0.1 mM 4-methylumbelliferon (4-MU) in 0.1 M Tris buffer, 5 mM $MgCl_2$, 0.05% BSA (pH 7.4). The cofactor UDP-glucuronic acid (UDPGA) 2 mM was added to start the reaction. The reaction was allowed to run for 10 minutes in a Victor 3 1420 Multilabel Counter (Wallak)

at 30°C (λ_{EX} 355 nm/λ_{EM} 460 nm). UDPGT activity was expressed in nmolmin^{-1}mg prot^{-1} calculated from a standard calibration curve generated with 4-MU. Microsomal protein concentrations were measured as described by Bradford (1976) in a Shimadzu UV-160A visible recording spectrometer using bovine serum albumin as standard. Assays were carried out in triplicate.

Histological analysis

For histological studies, left gonads were dissected out, reduced to small pieces and fixed with Bouin solution; after dehydration, the tissue was embedded in hydroxyethyl methacrylate (Technovit 7100, Heraeus Kulzer GmbH, Wehrheim, Germany), sectioned at 3-5 µm with an ultratome and stained with Mayer emallume and eosin (Bio-Optica; Culling *et al.*, 1985). Sections were observed under an Olympus BX 51 light microscope and images were taken with an Olympus digital camera (DP 50). Oocytes were staged by a method modified from Guraya *et al.*, 1975: I - oogonium; II - previtellogenic oocyte (oocyte with chromatin nucleolus or perinucleolus with strongly basophilic cytoplasm and no or few oil droplets); III - cortical alveolus stage (containing peripheral yolk granules); IV - midvitellogenic oocyte (containing peripheral yolk granules and central yolk platelets).

Statistical analysis

Correlations between the enzyme activities were determined by the correlation coefficient of Pearson using the software Statistica 6.0 (StatSoft, USA).

The joint distribution of the variables (biometric parameters, biomarkers and contaminant concentrations) was analyzed by Principal Components Analysis (PCA). Data were log-transformed and principal components were extracted from the correlation matrix because of variables with different units. PCA results were assessed by the percentage of variance explained by eigenvalues and interpreted by eigenvectors (Chatfield and Collins 1980; Quinn and Keough 2004). A correlogram was produced for a bi-plot of variables on the first two principal components in order to show the weight of each original variable on the two major sources of variations (Chessel *et al.*, 2005). PCA was carried out using R (Copyright 2006, The R Foundation for Statistical Computing Version 2.3.0 (2006-04-24) ISBN 3-900051-07-0), a free programme available at *http://www.r-project.org*, ADE-4 package (Chessel *et al.*, 2005).

Table 1 - POPs mean concentrations (ng g-1 l.w.) ± standard deviation (s.d.) in muscle of eels from Orbetello lagoon.

	Mean conc. ± s.d.
∑PCB	12.7 ± 4.3
∑ DDT	30.8 ± 1.7
pp'-DDE	18.6 ± 19.8
∑HCH	4.9 ± 19.1
HCB	0.8 ± 1.2
∑PCDD/F*	<1
∑PBDE	2.1 ± 3.7

*PCDD/Fs: pg g-1 l.w.

Results

Contaminants

Concentrations of POPs ranked in the following order ΣDDT > ΣPCB > ΣHCH > ΣPBDE > HCB > coplanar PCB > ΣPCDD/F (Table 1). The predominant PCB congeners were PCB-153 > PCB-138 > PCB-118 > PCB-128 > PCB-180. The prevalent isomer classes were moderately chlorinated: hexa-Cl 74%, penta-Cl 13%, tri-Cl 8% and hepta-Cl 5%. More than 50% of coplanar PCBs were PCB-77, followed by PCB-169 and PCB-81.

Total TEQs indicated a low toxic potential of about 0.008 pg/g (w.w) (Table 2).

The prevalent DDT isomer was *pp'*-DDE 56%, whereas *op'*-DDE was only 2%. The prevalent isomer of HCH was γ−HCH 90% of ΣHCH followed by δ-HCH 5%. α-HCH and β-HCH were LOD.

PCDD/F concentrations were below LOD (1 pg/g l.w.) in all eel samples. PBDE concentrations were also low (Table 1). BDE-47 was the most abundant congener, followed by BDE-99; BDE-100 and BDE-153 showed the lowest concentrations. The most prevalent PBDE isomers were penta-BDE (51%), tetra-BDE (42%) and hexa-BDE (7%).

Biotransformation enzymes

Enzyme activity measurements are reported in Table 3. All activities were low and showed small differences between individuals. Positive correlations between EROD and MROD ($r = 0.88$ $p<0.01$) and EROD and UDPGT activities were observed ($r = 0.71$ $p<0.05$).

Histological analysis

All eels were females. Macroscopic observation showed well formed ovaries of normal colour and shape. They contained previtellogenic oocytes and cortical alveolus stages II and III. The morphology of perinucleolar oocytes was anomalous, being partly shrunken with irregular nuclear shape and evident chromatin and having perinucleoli with strongly basophilic

Table 2 - TEFs, mean concentration and TEQs (pg g-1 w.w.) of mono- and non-ortho PCBs measured in muscle of eels from Orbetello lagoon.

PCBs	TEFs	mean	TEQs
mono-*ortho*			
118	0.000005	0.1	0.0000005
non-*ortho*			
81	0.0005	8.9	0.0045
77	0.0001	31.2	0.0031
126	0.005	<LOD	0
169	0.00005	16	0.0008
Sub-Total		**56.1**	**0.008**
Total		**56.2**	**0.008**

Table 3 - Enzymes activities measured in liver of eels from Orbetello lagoon.

Enzyme activities	mean ± s.d.
EROD pmol $min^{-1} mg\ prot^{-1}$	45.06 ± 18.37
MROD pmol $min^{-1} mg\ prot^{-1}$	17.14 ± 5.67
UDPGT nmol $min^{-1} mg\ prot^{-1}$	2.76 ± 0.51

cytoplasm (Figure 2 a, b arrow).
Oocytes in cortical alveolus stage had normal morphology with oil droplets at the oocyte periphery and nuclei with few nucleoli (Figure 2 c,d arrow).

Principal Components Analysis (PCA)
The first two principal components explained 55% of the total variance, the first three explained 75%. The first axis explained 30% of total variance. As shown in Figure 3, the

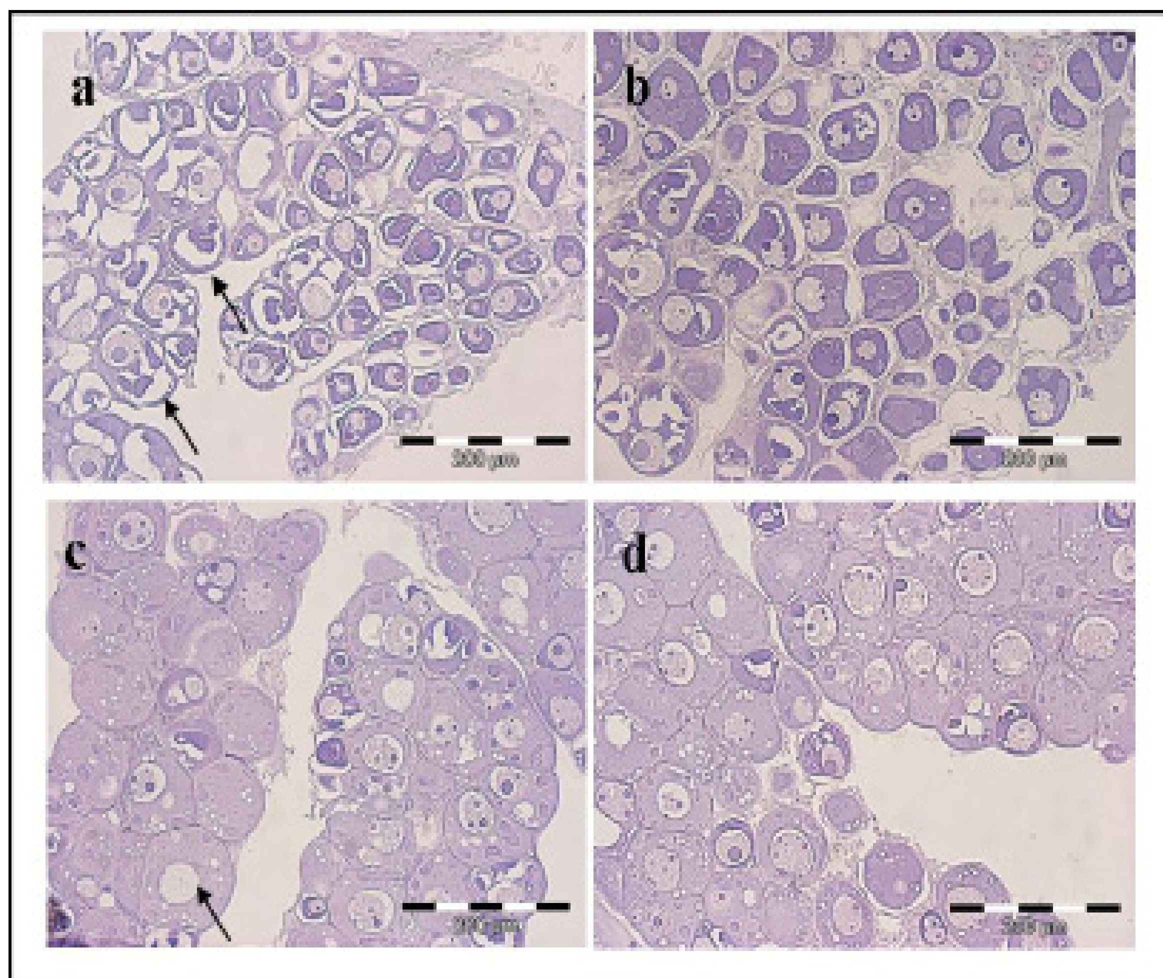

Figure 2. Previtellogenic oocytes in stage II showing perinucleolus with strongly basophilic cytoplasm (a,b); more advanced oocytes with lipid vesicles at onset of cortical alveolus stage III, with oil droplets in the cytoplasm but not strongly basophilic cytoplasm (c,d).

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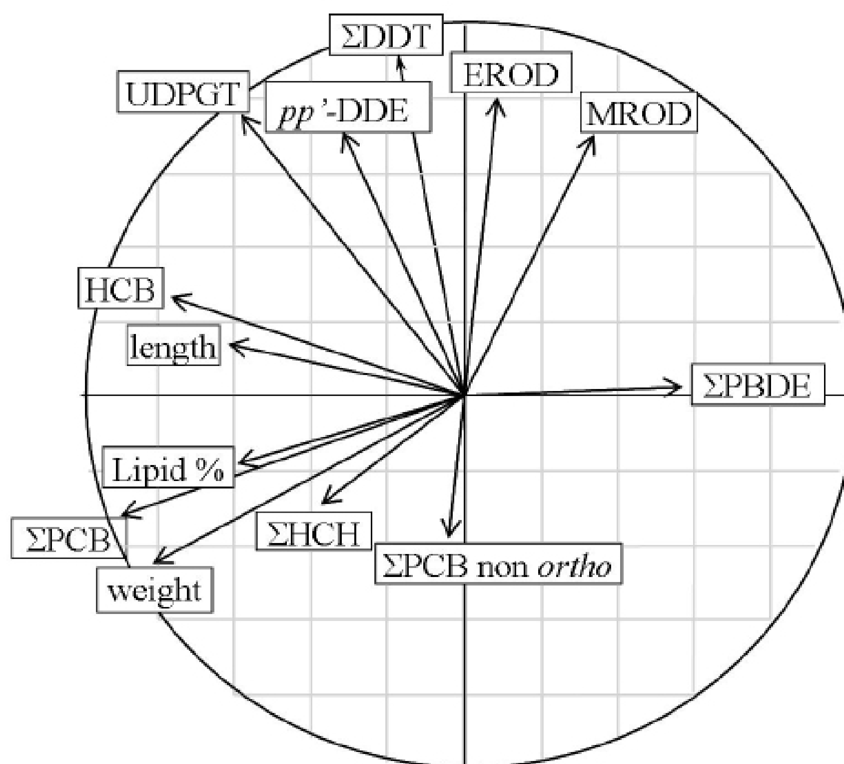

Figure 3. PCA canonical graph.

main variables defining the axis were PCBs and HCHs in the negative portion, positively correlated with each other and with weight and lipid content, and PBDEs in the positive portion, though with less weight. The second axis explained 25% of the variance and its main variables were EROD, MROD and UDPGT activities which showed a positive correlation with DDTs and *pp'*-DDE concentrations.

Discussion

Many studies have reported decline in eel populations in different parts of the world (Robinet and Feunteun 2002; Dekker 2004). In Orbetello lagoon, eel decline has also been documented recently, probably related to intensive fishing, fish farming and man-made pollution. Large amounts of chemicals from agriculture and small industries, as well as fish farm effluent, are discharged into the lagoon and have been detected in fish (Corsi *et al*., 2003; Corsi *et al*., 2005; Mariottini *et al*., 2005; Mariottini *et al*., 2008). The present research confirmed that levels of environmental contaminants in Orbetello lagoon are low, so pollution does not seem to play a role in eel decline.

Comparison of our results with data in the literature (Table 4) showed that organochlorine levels in muscle of Orbetello eels are lower than those reported in muscle of eels from other parts of Europe. PCB levels were lower than those reported for eels from other Mediterranean areas characterised by high human pressure (Bressa *et al*., 1997; Bordajandi et al., 2003; Quadroni *et al* 2008). Among PCBs, the PCB-153 proved to be the

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predominant congener, in line with other studies in the same species (Bordajandi et al., 2003; Mariottini *et al.*, 2005; Mariottini *et al.*, 2006; Quadroni *et al.*, 2008). Percentages of moderately chlorinated compounds and isomer classes were in line with a monitoring study previously performed in the same area (Mariottini *et al.*, 2006).

The TEQ (about 0.008 pg/g w.w.) was below the 4 pg TEQ/g w.w. limit set for muscle by the European Community (Council Regulation 2001), suggesting that there is no major threat to eels in Orbetello lagoon, as suggested also by Mariottini *et al.* (2006). TEQs were also lower than those reported for freshwater eels from the river Turia in Spain (Bordajandi *et al.*, 2003) (Table 4).

∑DDT and *pp'*DDE levels in muscle of eels also proved comparable with those recorded in low-impact areas (Table 4). The presence of DDT in the lagoon is therefore presumably historically related to extensive use of this pesticide against malaria in the last 50 years (Della Croce *et al.*, 1997). *pp'*DDE was found to be the predominant DDT isomer, in line with previous findings in eels from the lagoon (Corsi *et al.*, 2005). The high percentage of *pp'*-DDE found in the eels is compatible with dechlorination of DDT (which was low) to DDE in the course of time, indicating that only this metabolite of DDT remains in the environment.

γ-HCH was the most abundant HCH isomer and this, too, is in line with previous studies on eels from Orbetello (Corsi *et al.*, 2005) and Vaccarès (Table 4). This contaminant is presumably derived from its current use in agriculture to treat soil before sowing and also as a pesticide on cereal crops.

Concentrations of HCB in eel muscle were lower than those reported in the literature (Table 4). Although HCB is now banned, it is still found in many environmental matrices due to its high persistence. The low levels detected in eels from Orbetello lagoon suggest an absence of local input into the lagoon.

PBDE concentrations were low and similar to those recorded in eels collected in a previous study (2002) from Orbetello lagoon (Mariottini *et al.*, 2008). Similar levels were also reported by Akutsu *et al.* (2001) in eels from Japan (Table 4). Among PBDEs, BDE-47 was predominant, in line with the known general trend of its abundance in aquatic biota. This congener is a major component of Bromkal 70-5DE, a synthetic formulation widely used for industrial purposes (WHO 1994). Other dominant isomers were tetra-, penta- and hexa-BDE, as found in other studies (Akutsu *et al.*, 2001; Eljarrat *et al.*, 2004; Mariottini *et al.*, 2005, Mariottini *et al.*, 2008). Penta-BDEs accounted for 51% of total PBDEs, being more volatile, soluble and bioavailable than more brominated BDEs, such as BDE-209, which tends to accumulate near sources of release (Watanabe and Sakai 2003).

Measurement of biological responses such as induction of biotransformation enzymes provides information about detoxification process occurring in fish. Our results indicate that eels from Orbetello lagoon are subject to low chemical stress. Both phase I and phase II enzyme activities such as EROD, MROD and UDPGT were similar to those reported in eels collected from areas with low environmental impact (Doyotte *et al.*, 2001; van der Oost *et al.*, 1996; Gorbi *et al.*, 2005; Buet *et al.*, 2006;). EROD activities were higher than those reported in eels collected in the same area of the lagoon in 2002 (32.54 in Corsi *et al.*, 2005) indicating that chemicals inducing the CYP450 system are still present in the lagoon.

Regarding phase I enzymes, the high correlation observed between EROD and MROD activities ($r=0.88$ $p<0.001$) indicates active involvement of both enzymes in cell detoxification pathways. MROD proved

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Table 4 - Reference mean concentrations and ranges of POPs in muscle of eels from Orbetello lagoon.

Compound	Concentrations	Area	Reference
∑ PBDE	0.9-14.14 ng g^{-1} l.w.	Orbetello lagoon	Mariottini et al. 2008
∑ PCB	162.14±23.31 ng g^{-1} l.w.	Orbetello lagoon	Mariottini et al. 2006
∑ PCB	97.94-191.3 ng g^{-1} l.w.	Orbetello lagoon	Corsi et al. 2005
HCB	1.02-2.95 ng g^{-1} l.w.		
γ-HCH	6.33-707.3 ng g^{-1} l.w.		
∑ DDT	22.93-98.4 ng g^{-1} l.w.		
∑ PCB	190 ng g^{-1} w.w.	Spain (1983-84)	De Boer et al. 1994
γ-HCH	20 ng g^{-1} w.w.		
pp'-DDE	156-2176 ng g^{-1} l.w.	Netherlands (1996)	van der Oost et al. 1996
ΣHCH	33-1701 ng g^{-1} l.w.		
HCB	57-2418 ng g^{-1} l.w.		
∑ PCB	265±9.3 µg kg^{-1} w.w	Po delta (Italy)	Bressa et al. 1997
∑ DDT	18.5±12.7 µg kg^{-1}.w.w		
∑ PCB	68.1-163.7 ng g^{-1}d.w.	Vaccarès lagoon (France)	Roche et al. 2000
pp'-DDE	4.5-8.09 ng g^{-1}d.w.		
γ-HCH	59.2-212.4 ng g^{-1}d.w.		
HCB	3.1-9.1 ng g^{-1} d.w.		
BDE-47	1.2-38 ng g^{-1} l.w.	Japan	Akutsu et al. 2001
∑ PCB	9.22 - 126 ng g^{-1}d.w.	Turia River (Spain)	Bordajandi et al. 2003
∑ TEQ	0.019 - 0.316 ng g^{-1}d.w.		
∑ DDT	45.3 - 136 ng g^{-1}d.w.		
pp'-DDE	1.25 - 15.9 ng g^{-1}d.w.		
∑ DDT	0.02 - 2.6 ng g^{-1}d.w.	New Zealand	Holqvist et al 2006
∑ PCB	259-313 ng g^{-1}w.w.	Lesina lagoon (Italy)	Storelli et al. 2007
pp'-DDE	12-28 ng g^{-1}w.w.		
∑ DDT	14-34 ng g^{-1} w.w.		

significantly lower than EROD ($p<0.001$), confirming the different sensitivity of MROD to contamination suggested by other authors (Schlezinger and Stegeman. 2000; Mahata *et al.*, 2003).

The positive correlation between EROD, MROD and UDPGT indicated synergistic activity of phase I and II in cell responses to pollutants in eels.

PCA analysis identified an interesting multivariate correlation between contaminants and biological responses. The first component indicated that only PCBs and HCHs were correlated with body weight and lipid content and did not seem to influence biotransformation activities. This is presumably related to the recently reported overall reduction in PCB levels in biota. In fact, the low levels of total and coplanar PCBs and pesticides found in eel muscle probably originated from past contamination (Corsi *et al.*, 2003; van der Oost *et al.*, 2003). These compounds are stored in fatty tissue but are not metabolically active.

The second component described a correlation between all biotransformation enzymes and DDTs and its metabolite *pp'*-DDE. DDT is a CYP1A inducer (van der Oost *et al.*, 1996) and the presence of *pp'*-DDE in fish tissue suggests that these compounds are still bioavailable and metabolically active in the organism.

Regarding PBDEs, several authors report induction of CYP1A by specific congeners (BDE-100, BDE-126, BDE-119) (Chen *et al.*, 2001). Other congeners have high inhibitory potential towards CYP1A (BDE-47, BDE-99, BDE-153) (Kuiper *et al.*,2004; Peters *et al.*, 2006). We detected both inducers (BDE-100) and inhibitors (BDE-47, BDE-99, BDE-153) of CYP1A, which could explain why no positive correlations were found between these contaminants and enzyme activities.

Regarding ovarian histology, the individual moderate concentrations of contaminants recorded are difficult to reconcile with the morphological anomalies observed in stage II oocytes. However, the cause could be synergy among a number of contaminants present in low concentrations. Later stages of oocyte development did not show any morphological or structural anomalies, suggesting that reproductive success of the progeny may not be threatened.

Our results underline the need for caution in interpreting results based on these enzymes when complex mixtures of pollutants with confounding effects on enzyme responses are investigated. Although the results of this study suggest low chemical impact on eels of the lagoon, the simultaneous presence of several POPs with well known toxic properties suggests a potential threat for the species. In fact, the combined effects of different pollutants even at very low concentrations could have detrimental effects on eels.

Conclusions

The results of this study show low levels of POPs in muscle together with low detoxification activities in eels from Orbetello lagoon, confirming a low impact of man-made contamination in the lagoon. The TEQ calculated for mono- and non-*ortho* PCBs was below the limit set by the European Community and a clear decreasing trend with respect to past studies of lagoon organisms was observed.

The overall results of this study suggest that the eel population of the lagoon is at low risk due to chemical impact and pollutants do not seem to have a role in Orbetello eel decline. Indeed, slight anomalies in oocyte morphology were observed in early developmental stages, though reproductive success of the progeny did not seem to be threatened.

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