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# Does the Clam *Ensis siliqua* Provide Useful Information About Contamination by Polychlorinated Biphenyls and Organochlorine Pesticides Beyond that of Mussel *Mytilus galloprovincialis*?

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**Abstract** Several polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) investigated in soft tissues of the frequently monitored *Mytilus galloprovincialis* were compared to those of *Ensis siliqua*, a highly dispersed and economically important bivalve species, though rarely investigated. Overall PCBs had higher concentrations than OCPs in both species with a prevalence of tri- tetra-and penta-chlorinated biphenyls in *E. siliqua* and a prevalence of hexa- hepta and octa-chlorinated biphenyls in *M. galloprovincialis*. *E. siliqua* emerges as a suitable complement to mussels for monitoring PCBs and OCPs pollution.

**Keywords** Polychlorinated biphenyls · Organochlorine pesticides · *Mytilus galloprovincialis* · *Ensis siliqua* · Bioindicator

The monitoring of organochlorine compounds (OCs) environmental presence is of great importance. Due to high chemical stability and hydrophobicity, OCs tend to persist for many years in ecosystems, and to bioconcentrate as well as biomagnify in the food web. Given the elevated concentrations achieved in high trophic level organisms, injuries to nervous, endocrine, reproductive and immune systems of humans and animals might be induced (El-Shahawi et al. 2010).

There are at least two reasons for studying polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) pollution by means of bivalve molluscs. The first being that, they are sedentary organisms capable of concentrating contaminants as a result of removing suspended particles and filtering large water volumes. The second that, they may warn about potential risks for human health related to seafood consumption, the latter being a major means of human exposure to OCs (Kljaković-Gaspić et al. 2010).

The mussel (*Mytilus galloprovincialis*) is cosmopolitan and abundant, lives attached to rocks and other hard surfaces or ropes at variable distances from the shore and can be easily collected. Differently to fish and crustaceans, the mussel is characterized by a very low activity of enzyme systems responsible for metabolizing OCs, thus the concentration levels of such pollutants are scarcely modified by biotransformation reactions (Sheehan et al. 1995). The mussel is also fairly resistant and insensitive to OCs. Therefore, contaminant concentrations in its tissues reflect the magnitude of environmental pollution more accurately than other marine species, thus providing useful information to investigate temporal and spatial variations of OCs in aquatic systems (Ferrante et al. 2007; Bellas et al. 2011).

The razor clam *Ensis siliqua*, unlike the mussel, living in the sandy seabed near the shore, is much more exposed to contaminants accumulated in the sandy sediments of infralittoral regions. This is the case, for instance, of pesticides such as the OCPs that are directly inputted into the

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sea via the irrigation waters of neighbouring farmed fields. Moreover, immunity of *E. siliqua* to toxic events has been evidenced (Wootton et al. 2003). Finally, *E. siliqua* is widely distributed (Fahy and Gaffney 2001), and it is one of the two most economically important species of razor clams in the European Union (Freire et al. 2008). Together these characteristics make *E. siliqua* a potentially useful biomonitor whose information may complement or substitute that provided by mussels.

Very scarce empirical evidence about OC bioaccumulation, however, is available for *E. siliqua* (Carro et al. 2012). We aimed to fill this gap in the literature. Concentrations of PCBs and OCPs in *E. siliqua* and *M. galloprovincialis* were evaluated and differences related to the microhabitat, sources of pollutants and metabolic capacity of these bivalves were investigated. Samples were collected from the coast of Campania region (Italy), an area that previous studies suggested to be characterized by heavy OCs pollution (see, for instance, Ferrante et al. 2010).

## Materials and Methods

Farmed specimens of *M. galloprovincialis* and wild specimens of *E. siliqua* were collected during the period May–July 2008 within the part of the Tyrrhenian Sea which is adjacent to the coastal area of Castelvolturno (in the province of Caserta). This area is characterised by very scarce manufacturing firms, the main economic activity being buffalo farms and agro-industry. Regarding the aquatic environment, during the sampling period the water temperature is usually in the range 19–24°C while the oxygen saturation is often more than 100 % (see Table 1).Our Mediterranean mussels were collected at 5 m depth from a wooden pike in a rectangular area located about 5 km off the coast (between N41°/03′/43 and N41°/02′/60 and between E13°/51′/30 and E13°/52′/00, respectively, for latitude and longitude). The razor clams were collected by hand in shallow sea waters a few meters away from broad sandy beaches. The specimens, all of commercial size, were immediately refrigerated, and transported to the laboratory.

After washing the surface crust, lengths and weights of the molluscs were measured and recorded. Just after having collected a given group of specimens, the bivalves were cracked through excision of the adductor muscle and the soft tissues were removed and pooled to form our sample unit of about 100 g of weight. The samples of mussels and clams consisted of 40 and 15 units, respectively. Each unit was homogenized and stored at −20°C until chemical analysis.

Concentration levels of 5 OCPs—HCB, dieldrin, *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD—and 20 PCBs—IUPAC nos. 28, 52, 66, 74, 99, 101, 105, 118, 128, 138, 146, 153, 170, 177, 180, 183, 187, 196, 194, and 201—were determined for each sample unit. The extraction and separation of the analytes from the lipid fraction, and the purification of the extracts were carried out adapting the method described by Di Muccio et al. (2002). For each sample unit, an aliquot of about 3 g was cold-extracted with petroleum ether/acetone (1:1, v/v) and the extract was passed through a glass tube packed with anhydrous sodium sulphate to obtain lipid fraction. The lipid content was determined gravimetrically, and a further purification was performed through an *n*-hexane/acetonitrile repartition. The extract was then cleaned up on a glass column packed with activated Florisil; three fractions were obtained and the PCB 209 was added as internal standard. Finally, gas chromatographic analysis was carried out by a Carlo Erba HRGC 5160 Mega Series equipped with a 63Ni electron capture detector and randomly confirmed by GC-mass spectrometry. Two fused silica capillary columns of different polarities coated with a CP-SIL 5CB (25 m × 0.32 mm id, 0.25 μm film thickness) (Varian Inc., UK) and Rtx-1701 (30 m × 0.32 mm id, 0.25 μm film thickness) (Restek, UK) were used to separate and quantify the residues. The ECD was kept at 310°C. Hydrogen and nitrogen were used as carrier gas and make-up gas, respectively.

Data, not corrected for recovery, were expressed as ng $g^{-1}$ lipid weight (LW) soft tissues to avoid inter- and

**Table 1** The environment of the sampling area

| | | | | | |
|---|---|---|---|---|---|
| General characteristics | Heavily populated area, with many buffalo farms and extensive forage crops. Low and sandy coastline with beach and dune behind. Presence of streams and reservoirs | | | | |
| Physico-chemical characteristics of the aquatic environment | Dissolved oxygen (mg/L) | Water temperature (°C) | pH | Secchi depth (m) | Oxygen saturation (%) |
| | 6.8–10.8 | 19–24 | 8 | max 1.5 | 91.6–153.5 |
| Location of the bivalves | *Mytilus galloprovincialis* | Live in the offing not too far from the shore (about 5 km off), fixed on supports at 5 m depth. In contact with resuspended finest particulate | | | |
| | *Ensis siliqua* | Live burrowing in sand, very close to the coast (few meters away from beaches). In contact with sandy bottoms, and fine-medium size sediments | | | |

The physico-chemical characteristics of the aquatic environment are collected during Spring of 2011

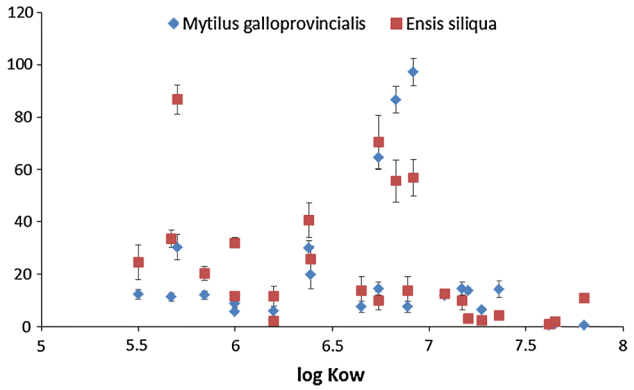


**Fig. 1** PCBs and OCPs concentrations in *Mytilus galloprovincialis* and *Ensis Siliqua*

intra-species variability due to differences in lipid content, and to allow for a more accurate comparison of bioaccumulation in the two bivalve species. For all PCBs and OCPs the detection limits ranged from 0.08 to 0.60 ng $g^{-1}$ LW. Results were reported as not detectable (nd) when the concentrations were lower than the detection limits.

Pure reference standard solutions allowed instrument calibration, recovery determination and quantification (Dr. Ehrenstorfer laboratory). The recovery performance of the method was in the range 80 %–110 %. Certified reference materials (mussel tissues homogenate) supplied by the National Institute of Standards and Technology (SRM 2977 and SRM 1974b) were used for quality control.

Data were preliminary analysed through summary statistics such as mean and standard deviation. To assess the difference in the presence of PCBs between the species, we divided the investigated congeners in four groups based on the chlorine substitutions. The groups were defined as follows. Group-1: the tri- and tetra-chlorinated biphenyls 28, 52, 66, 74; Group-2: the penta-chlorinated biphenyls 99, 101, 118, 105, 146; Group-3: the hexa-chlorinated biphenyls 128, 138, 153, 187; Group-4: the hepta- and octa-chlorinated biphenyls 170, 177, 180, 183, 196, 194, 201. For any group j, the PCB concentration, denoted as ΣPCBs Group-j, corresponds to the sum of concentrations for all congeners which are part of the group. Differences in concentrations of OCs among the two species were evaluated by sample mean-difference test (*t*-Student statistics). When the *p* value was less than 0.05, the difference was considered statistically significant. All analyses and calculations were performed by Stata.

## Results and Discussion

The means of Length and Weight were, respectively, 5.75 cm and 16.03 g for mussels and 12.4 cm and 15.9 g for clams. The lipid content means were, respectively, 2.46 % and 2.59 % for mussels and clams. For both species the OC presence was measured in edible tissues and reported as average concentration across sample units. Detailed information on concentrations are reported in the appendix (Table A1). Figure 1 shows concentrations and error bars of analyzed OCs against relative $logK_{ow}$.

PCBs were found in all sample units. The highest measured concentrations found were, for PCB 153 (97.13 ng $g^{-1}$) in the *M. galloprovincialis*, and PCB 118 (70.40 ng $g^{-1}$) in the *E. siliqua*. In both species, high concentrations were also found for the PCB 138, that is 86.60 and 55.59 ng $g^{-1}$ relative to the *M. galloprovincialis* and the *E. siliqua*, respectively. These amounts account for 20.5 % and 14 % of the corresponding ΣPCBs.

As Table 2 shows, in Group-1 and Group-2, the PCB concentrations were higher in *E. siliqua* than in *M. galloprovincialis*, the difference being highly statistically significant in Group-1 ($p < 0.001$). As noted, congeners which are part of Group-1 are those characterized by the lowest values of $logK_{ow}$. The opposite was true for Group-3 and Group-4, the difference being highly statistically significant for Group-3 ($p < 0.001$). Within Group-4, we noted a large difference between species regarding the concentration of the PCB 180, that is 14.27 ng $g^{-1}$in *M. galloprovincialis* and 4.27 ng $g^{-1}$in *E. siliqua* (however, the *p* value related to the mean-difference test was 0.67). Note that if we compared species in terms of total concentrations of the seven indicator congeners, it followed a statistically insignificant difference. Evidence shown in Table 2 clarifies that this result is related to the different number of chlorine substitutions or values of $logK_{ow}$, which characterize the seven indicators.

The habitat may provide a first explanation for the observed differences between the two species analysed. The saltwater mussel *M. galloprovincialis* thrives in an intertidal habitat characterized by a dynamic environment with changing temperature and salinity, turbidity, as well as regular episodes of exposure to surrounding air due to tides. Because mussels filter large amounts of water for both feeding and respiration, they are directly exposed to contaminants from both dissolved and particulate (mainly finest sediments) phases in the water column. It is likely that tidal movements and other disturbances of the sediment floor tend to resuspend fine size sediments. If the higher chlorinated (or larger $logK_{ow}$) compounds are associated preferentially with the finest fractions of the sediments, as for instance suggested by Piérard and Garrigues (1996), than such compounds (e.g. those of our Group-3 and Group-4) would be more available to *M. galloprovincialis* than *E. siliqua*.

Our evidence is congruent with that by Thompson et al. (1999), who observed the dominant presence of PCB 153

**Table 2** PCB mean-difference test

| | $logK_{OW}$ (range) | *Mytilus galloprovincialis* | *Ensis siliqua* | Difference ($p$ value) |
|---|---|---|---|---|
| ΣPCB Group-1 | (5.67–6.20) | 31.83 | 67.48 | 35.65 (0.000)* |
| ΣPCB Group-2 | (6.38–6.74) | 129.55 | 163.84 | 34.29 (0.157) |
| ΣPCB Group-3 | (6.74–6.92) | 212.75 | 132.07 | −80.68 (0.000)* |
| ΣPCB Group-4 | (7.08–7.80) | 48.05 | 35.94 | −12.11 (0.074) |

The table reports mean-difference tests between species for PCB congeners. For any single congener, the concentration refers to its mean value (expressed as ng $g^{-1}$ on LW) in edible tissues of *M. galloprovincialis* and *E. siliqua*. ΣPCB values are computed as the sum of PCB concentrations of all congeners within the group. Groups of congeners are defined as follows according to the index number (which relates with the number of chlorine atoms). Group-1: 28, 52, 66, 74; Group-2: 99, 101, 105, 118, 146; Group-3: 128, 138, 153, 187; Group-4: 170, 177, 180, 183, 194, 196, 201. Values of $logK_{ow}$ were obtained from Hawker and Connell (1988). The $p$ value associated to each mean-difference test is reported in parenthesis. Statistical significance ($p$ value less than 0.01) is denoted with *. Note that all means are statistically different from zero (5 % significance level)

**Table 3** OCP mean-difference test

| | $logK_{OW}$ | *Mytilus galloprovincialis* | *Ensis siliqua* | Difference |
|---|---|---|---|---|
| $p,p'$-DDD | 5.50 | 12.30 | 24.47 | 12.17 (0.000)* |
| $p,p'$-DDE | 5.70 | 30.27 | 86.67 | 56.4 (0.000)* |
| $p,p'$-DDT | 6.00 | 8.64 | 1.85 | −6.79 (0.223) |
| HCB | 6.00 | 5.67 | 11.52 | 5.85 (0.000)* |
| Dieldrin | 3.70 | 36.33 | 25.46 | −10.87 (0.206) |

The table reports mean-difference tests between species for organochlorine pesticides. The concentration refers to the mean value (expressed as ng $g^{-1}$ on LW) in edible tissues of *M. galloprovincialis* and *E. siliqua*. Values of *log* $K_{OW}$ were obtained from Suntio et al. (1988), Table 4.The $p$ value associated to each mean-difference test is reported in parenthesis. Statistical significance ($p$ value less than 0.01) is denoted with *. Note that all means are statistically different from zero (5 % significance level)

in mussels and the prevalence of PCB 118 in the benthic species. The significantly lower presence of lighter congeners (Group-1) in mussels might be due to the partial volatilization of the not bound fraction of these compounds caused by weathering and other more specific factors, such as aquatic movements occurring during shipping activities (Ailstock et al. 2002).It is clearly evident, however, that PCB congener distributions differ in either water, ingested particles or both water and ingested materials for the two species. Given that the samples, by necessity of habitat, are collected from different locations, we cannot properly tease out whether differences in PCB congener patterns are due to environmental fate characteristics in the two environments or differences in sources.

Regarding the OCPs, the rank of average concentration levels was as follows: dieldrin > $p,p'$-DDE > $p,p'$-DDD > $p,p'$-DDT > HCB, for *M. galloprovincialis*, and $p,p'$-DDE > dieldrin > $p,p'$-DDD > HCB > $p,p'$-DDT for *E. siliqua* (Table 3). In particular, dieldrin was detected in all *E. siliqua* sample units, suggesting a recent use of the pesticide in the fields along the coastal area, and in 92.5 % of *M. galloprovincialis* units. The $p,p'$-DDT was found, respectively, in 55 % and 80 % of the *M. galloprovincialis* and *E. siliqua* units analysed.

By comparing the two species we found larger amounts of $p,p'$-DDE, $p,p'$-DDD and HCB in *E. siliqua* than in *M. galloprovincialis*. HCB was found in 87 % of the mussels and in all the clams analysed, although with low residue levels. For all three compounds the differences were statistically significant ($p$ value <0.000). Oppositely, differences among species in the concentrations of both dieldrin and $p,p'$-DDT were found not statistically different from zero. Not surprisingly, when the concentration averages of DDE and DDT are used to calculate theDDE/DDT ratio, an index useful to assess the chronology of DDT entering the ecosystems, it follows a larger value for the *E. siliqua*.

Overall evidence on OCPs is quite consistent with that on PCBs. In particular, larger amounts of pesticides characterized by lower values of $logK_{ow}$, that is the $p,p'$-DDE and $p,p'$-DDD, were found in *E. siliqua* than *M. galloprovincialis*. This would support the environmental fate explanation of differences in bioaccumulation profiles between species. However, we also note that the *E. siliqua* lives in the sandy seabed near the shore in proximity to the outlet of canals that drain irrigation waters of neighbouring farmed fields. If soils are characterized by more OCPs than sediments, as reported by Iwata et al. (1995), the pesticides are poured into the sea entering into direct contact with the

species living in the sandy seabed near the shore. Thus, we cannot neglect that differences in sources may also matter.

The partitioning factor $K_d$ is exploited to describe the distribution of compounds between particulate and aqueous phases in the equilibrium. Carvalho et al. (2009) used this parameter to explain the differences observed in the OCPs bioaccumulation in oysters and fish. In particular, as the $K_d$ values for dieldrin and *p,p′*-DDT were lower than those for *p,p′*-DDD and *p,p′*-DDE, they concluded in a higher presence of the first two pesticides in the aqueous phase and a higher presence of the second two pesticides in the sediment phase. This is indeed consistent with our evidence of higher concentrations of *p,p′*-DDD and *p,p′*-DDE in *E. siliqua*.

It has been recently suggested that distribution and levels of hydrophobic pollutants in aquatic organisms may also be affected by biotransformations. There are few studies, however, regarding enzymatic activities focusing on the OCPs metabolism in clams as well as few experiments comparing the enzymatic levels of *M. galloprovincialis* with *E. siliqua*, in order to explain the different concentration levels detected as a reduced GSTs presence in razor clams. Thus, even if metabolic biotransformation differences are a possible alternative explanation, there is insufficient data, particularly for the clam, to test this.

With regard to the human risk assessment, for various food products of animal origin the EU legislation (Commission Regulation EU No 1259/2011 of 2 December 2011) has recently determined MRLs which refer to the sum of six indicator-PCBs (the seven indicators but PCB 118). For all mussels and razor clams analysed, concentrations of these six indicator-PCBs never exceeded the relative MRL of 75 ng $g^{-1}$ WW established for muscle meat of fish and fishery products and products thereof. Notice, however, that for most mussels and razor clams investigated (82.5 % and 73.3 % of the total, respectively) such concentrations are higher than the previous limit of 200 ng $g^{-1}$ LW fixed by the EU for terrestrial edible class of food (Commission Decision of 3 December 1999, 1999/788/EC) and used as a reference until now (results not reported). As for OCPs, residue levels of all mussels and clams showed values lower than the MRLs set in Italy (results not reported).

Dealing with *E. siliqua* and *M. galloprovincialis* coming from a coastal area of the Campania region, we showed differences between species for residue levels of several OCs. Such differences may be reasonably related to peculiar factors characterizing the microhabitat of the two bivalves and/or the sources of chemicals. The measured contamination levels suggest that OCs are likely to contribute to the pollution of Campania's aquatic ecosystem, even though PCB residue concentrations are lower than the new limits recently established by the EU to protect the consumer health.

As key conclusion, our analysis suggests that *E. siliqua* is a suitable complement to mussels for a more complete overview of the pollution levels of a study area, or a useful substitute when mussels are not available.

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## Appendix

See Table 4.

**Table 4** Statistics on organochlorine concentrations

| | *Mytilus galloprovincialis* | | *Ensis siliqua* | |
|---|---|---|---|---|
| | % | Mean; SD (range) | % | Mean; SD (range) |
| HCB | 87.5 | 5.67; 4.37 (nd–14.38) | 100 | 11.52; 3.99 (4.49–19.28) |
| Dieldrin | 92.5 | 36.33; 30.09 (nd–142.41) | 100 | 25.46; 21.28 (4.34–83.34) |
| p;p′-DDE | 100 | 30.27; 12.26 (4.36–57.56) | 100 | 86.67; 25.32 (44.39–154.84) |
| p;p′-DDD | 90 | 12.30; 8.64 (nd–32.90) | 100 | 24.47; 7.77 (12.11–41.25) |
| p;p′-DDT | 55 | 8.64; 21.16 (nd–130.07) | 80 | 31.85; 1.48 (nd–5.30) |
| PCB 28 | 75 | 11.26; 9.41 (nd–42.91) | 100 | 33.47; 12.86 (15.30–56.67) |
| PCB 52 | 75 | 12.00; 9.75 (nd–36.09) | 100 | 20.36; 9.92 (8.00–37.17) |
| PCB 74 | 42.5 | 2.70; 4.03 (nd–14.55) | 53.33 | 2.12; 2.85 (nd–8.20) |
| PCB 66 | 37.5 | 5.87; 12.33 (nd–59.57) | 53.33 | 11.53; 14.37 (nd–35.39) |
| PCB 101 | 90 | 29.84; 18.04 (nd–95.81) | 100 | 40.52; 25.57 (17.84–101.12) |
| PCB 99 | 72.5 | 19.86; 34.64 (nd–215.68) | 93.33 | 25.63; 19.63 (nd–74.72) |
| PCB 118 | 97.5 | 64.62; 26.33 (nd–109.16) | 100 | 70.40; 39.81 (30.00–170.81) |
| PCB 105 + 146 | 70 | 15.23; 13.70 (nd–43.75) | 100 | 27.29; 20.80 (10.82–80.84) |

**Table 4** continued

| | *Mytilus galloprovincialis* | | *Ensis siliqua* | |
|---|---|---|---|---|
| | % | Mean; SD (range) | % | Mean; SD (range) |
| PCB 153 | 100 | 97.13; 32.88 (22.96–161.88) | 100 | 56.88; 27.28 (25.33 -118.46) |
| PCB 138 | 100 | 86.60; 31.50 (26.49–171.35) | 100 | 55.59; 31.62 (24.53–126.07) |
| PCB 128 + 187 | 97.5 | 29.02; 15.59 (nd–67.36) | 100 | 19.60; 13.16 (3.04–52.14) |
| PCB 183 | 97.5 | 13.71; 7.39 (nd–33.36) | 86.66 | 3.15; 2.37 (nd–7.75) |
| PCB 177 | 100 | 11.72; 4.40 (1.13–20.99) | 100 | 12.54; 6.35 (5.44–24.83) |
| PCB 180 | 100 | 14.27; 20.53 (3.09–138.50) | 100 | 4.27; 1.68 (1.45–7.08) |
| PCB 170 | 95 | 6.50; 4.67 (nd–20.69) | 73.33 | 2.36; 2.56 (nd–8.33) |
| PCB 201 | 22.5 | 0.55; 2.06 (nd–12.68) | 33.33 | 0.96; 2.15 (nd–7.78) |
| PCB 196 | 55 | 0.85; 1.21 (nd–5.36) | 100 | 1.91; 0.87 (0.84–3.95) |
| PCB 194 | 40 | 0.44; 0.62 (nd–2.31) | 100 | 10.76; 4.86 (4.79–22.97) |

For each analysed bivalve the table reports the percentage of sample units contaminated by each compound; the mean; standard deviation (SD) and range of organochlorine concentrations (ng $g^{-1}$ on LW) in edible tissue. Not detectable values are indicated with nd

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