Supplementary Materials

Determination of persistent organic pollutants (POPs) and metals

Lipids and POPs were measured in the muscular tissues of 32 thick-lipped grey mullets. The following POPs were targeted: 29 PCB congeners (IUPAC numbers: −28, −49, −52, −74, −95, −99, −101, −105, −110, −118, −128, −138, −146, −149, −151, −153, −156, −170, −171, −174, −177, −180, −183, −187, −194, −199, −196/203, −206 and −209), dichlorodiphenyl-trichloroethane (DDT) and its metabolites, hexachlorocyclohexanes (alpha-, beta- and gamma-HCHs), hexachlorobenzene (HCB), PBDEs (BDE 28, 47, 99, 100, 153, 154 and 183) and MeO-PBDEs (2-MeO-BDE 68 and 6-MeO-BDE 47).

Analyses of POPs in thick-lipped grey mullet samples were performed according to the methods described elsewhere [1-3] with slight modifications. Briefly, a homogenized sample of approximately 1g pooled fish muscle was weighed, mixed with anhydrous Na2SO4 and spiked with internal standards (CB 143, BDE 77, BDE 128, and γ-HCH). Further, the samples were extracted twice by using 6 mL of a hexane:acetone mixture (3:1, v/v) and applying ultra-sonication twice for 20 min, with vortexing for 5 min between each sonication period. After each sonication, the mixture was centrifuged at 3500 RPM, and the supernatants were combined afterwards. The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was further evaporated to dryness, reconstituted in 0.5 mL of hexane, and cleaned on ~8 g acidified silica (44%) and eluted with 20 mL hexane and 15 mL dichloromethane. After having evaporated to incipient dryness, the extracts were re-dissolved in 100 μL iso-octane. Quantification of POPs was done by GC-ECNI/MS. Abbreviations are expressed as follows: PBDEs as the sum of 14 congeners, PCBs as the sum of 29 congeners and DDTs as the sum of 5 compounds. The sum of six indicator PCBs (28, 52, 101, 138, 153 and 180; further abbreviated as Σ6PCBs), considered by EFSA as an appropriate indicator for occurrence and humans, was calculated. The sum of PCB 28, 52, 101, 118, 138, 153 and 180 (further abbreviated as Σ7PCBs) was also calculated as these substances are commonly found in the environment and are considered as indicators of contamination degree [4].

Zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), cadmium (Cd) and mercury (Hg) were extracted by Milestone Ethos 900 microwave for the mineralization phase using 64% Nitric Acid, hydrogen peroxide and ultrapure water. Pb, Cr and Cd were determined by atomic absorption method with Perkin Elmer graphite furnace Analyst 600, following the standard Perkin Elmer procedures. Zn and Cu were determined by Perkin Elmer AAS3100 atomic absorption flame furnace. For Hg, the FIMS100 Perkin Elmer atomic absorption method was used with carrier HCl 3% and reducing solution of NaBH4 0.2%; NaOH 0.05%.

Table S1. Results of analyses of variance (F statistic and level of significance p) carried out to test the effects of site and gender factors on biometric and biological features of thick-lipped mullet samples from Fogliano and Caprolace.

<table>
<thead>
<tr>
<th>Site</th>
<th>Gender</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT (cm)</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>W (g)</td>
<td>5.435</td>
<td>*</td>
</tr>
<tr>
<td>% lipids on w.w basis</td>
<td>7.513</td>
<td>*</td>
</tr>
<tr>
<td>Age</td>
<td>5.911</td>
<td>*</td>
</tr>
<tr>
<td>GR (cm yr⁻¹)</td>
<td>12.760</td>
<td>**</td>
</tr>
<tr>
<td>Le Cren’s CF</td>
<td>0.006</td>
<td>0.939</td>
</tr>
</tbody>
</table>

Significant differences are marked by a single (p < 0.05) or double asterisk (p < 0.01) (Tukey’s post-hoc tests).

Table S2. Results of analyses of variance (F statistic and level of significance p) carried out to test the effects of site and gender factors on contaminants on lw basis.

<table>
<thead>
<tr>
<th>Site</th>
<th>Gender</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>7PCB</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>0.593</td>
<td>0.448</td>
<td>1.344</td>
</tr>
<tr>
<td>Substance</td>
<td>Median</td>
<td>Mean (± SD)</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>PCB 28</td>
<td>0.000</td>
<td>0.000 (± 0.000)</td>
</tr>
<tr>
<td>PCB 52</td>
<td>0.03*</td>
<td>0.119 (± 0.046)</td>
</tr>
<tr>
<td>PCB 49</td>
<td>0.010</td>
<td>0.036 (± 0.018)</td>
</tr>
<tr>
<td>PCB 74</td>
<td>0.005</td>
<td>0.027 (± 0.022)</td>
</tr>
<tr>
<td>PCB 95</td>
<td>0.146*</td>
<td>0.157 (± 0.029)</td>
</tr>
<tr>
<td>PCB 101</td>
<td>0.470**</td>
<td>0.567 (± 0.115)</td>
</tr>
<tr>
<td>PCB 99</td>
<td>0.522**</td>
<td>0.488 (± 0.054)</td>
</tr>
<tr>
<td>PCB 110</td>
<td>0.147**</td>
<td>0.201 (± 0.071)</td>
</tr>
<tr>
<td>PCB 105</td>
<td>0.138**</td>
<td>0.184 (± 0.050)</td>
</tr>
<tr>
<td>PCB 118</td>
<td>0.455**</td>
<td>0.616 (± 0.124)</td>
</tr>
<tr>
<td>PCB 151</td>
<td>0.000</td>
<td>0.063 (± 0.060)</td>
</tr>
<tr>
<td>PCB 149</td>
<td>0.367**</td>
<td>0.412 (± 0.032)</td>
</tr>
<tr>
<td>PCB 146</td>
<td>0.266**</td>
<td>0.306 (± 0.049)</td>
</tr>
<tr>
<td>PCB 153</td>
<td>1.283**</td>
<td>1.484 (± 0.276)</td>
</tr>
<tr>
<td>PCB 138</td>
<td>0.768**</td>
<td>0.988 (± 0.209)</td>
</tr>
<tr>
<td>PCB 187</td>
<td>0.410**</td>
<td>0.512 (± 0.102)</td>
</tr>
<tr>
<td>PCB 183</td>
<td>0.126**</td>
<td>0.163 (± 0.058)</td>
</tr>
<tr>
<td>PCB 128</td>
<td>0.116**</td>
<td>0.150 (± 0.038)</td>
</tr>
<tr>
<td>PCB 174</td>
<td>0.010</td>
<td>0.052 (± 0.021)</td>
</tr>
<tr>
<td>PCB 177</td>
<td>0.015*</td>
<td>0.080 (± 0.032)</td>
</tr>
<tr>
<td>PCB 171</td>
<td>0.002</td>
<td>0.031 (± 0.029)</td>
</tr>
<tr>
<td>Compound</td>
<td>PCB 156</td>
<td>PCB 180</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>oxy-chlordane (OxC)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>trans-chlordane (TC)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>cis-chlordane (CC)</td>
<td>0.005</td>
<td>0.031</td>
</tr>
<tr>
<td>trans-nonachlor (TN)</td>
<td>0.010</td>
<td>0.075</td>
</tr>
<tr>
<td>cis-nonachlor (CN)</td>
<td>0.010</td>
<td>0.018</td>
</tr>
<tr>
<td>(\Sigma)Clordane</td>
<td>0.025</td>
<td>0.124</td>
</tr>
<tr>
<td>HCB</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>a-HCH</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>b-HCH</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>g-HCH</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>(\Sigma)HCH</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PBDE 28</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PBDE 47</td>
<td>0.048</td>
<td>0.204</td>
</tr>
<tr>
<td>PBDE 100</td>
<td>0.005</td>
<td>0.040</td>
</tr>
<tr>
<td>PBDE 99</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PBDE 154</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PBDE 133</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PBDE 183</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>(\Sigma)PBDE</td>
<td>0.053</td>
<td>0.245</td>
</tr>
<tr>
<td>2-MeO-BDE68</td>
<td>0.092</td>
<td>0.151</td>
</tr>
<tr>
<td>6-MeO-BDE47</td>
<td>1.024</td>
<td>1.832</td>
</tr>
<tr>
<td>(\Sigma)MeO-BDE</td>
<td>1.146</td>
<td>1.983</td>
</tr>
<tr>
<td>op-DDD</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>op-DDT</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>pp-DDE</td>
<td>1.233</td>
<td>1.450</td>
</tr>
<tr>
<td>pp-DDD</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>pp-DDT</td>
<td>0.060</td>
<td>0.246</td>
</tr>
<tr>
<td>(\Sigma)DDT</td>
<td>1.293</td>
<td>1.696</td>
</tr>
</tbody>
</table>

\(\Sigma\)PCBs is the sum of the six indicators 28, 52, 101, 138, 153 and 180. \(\Sigma\)PCBs is the sum of the seven indicators 28, 52, 101, 118, 138, 153, and 180.

Significant differences between the two lagoons are marked by a single \(p < 0.05\) or double asterisk \(p < 0.01\)
(Mann–Whitney U tests, Bonferroni corrected. In bold are reported the groups of contaminants in order to distinguish them in the table from the single pollutants.)
References


