

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 μ L 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 Σ_6 PCBs), 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 Σ_7 PCBs) 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.

	Site		Gender		Interaction	
	F	p	F	p	F	p
LT (cm)	0.402	0.531	0.139	0.712	0.024	0.877
W (g)	5.435	*	1.097	0.304	0.232	0.634
% lipids on ww basis	7.513	*	0.066	0.800	3.873	0.059
Age	5.911	*	1.085	0.307	0.293	0.593
GR (cm yr ⁻¹)	12.760	**	0.673	0.419	0.236	0.631
Le Cren's CF	0.006	0.939	0.053	0.820	0.035	0.853

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.

	Site		Gender		Interaction	
	F	p	F	p	F	p
7PCB	0.593	0.448	1.344	0.256	0.183	0.672

PCB	0.590	0.449	1.403	0.246	0.176	0.678
DDT	0.589	0.449	1.689	0.204	0.067	0.798
HCH	7.819	**	0.099	0.755	4.025	*
HCB	4.775	*	0.001	0.971	3.317	0.079
PBDE	11.440	**	0.308	0.584	0.228	0.637
Chlordane	0.310	0.582	1.745	0.197	0.075	0.787
γ HCH	7.129	*	0.100	0.754	2.212	0.148
2MeOBDE68	5.434	*	3.302	0.080	0.244	0.626
6MeOBDE47	21.920	**	0.773	0.387	2.890	0.100

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

Table S3. 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 ww basis.

	Site		Gender		Interaction	
	F	p	F	p	F	p
Hg	7.032	*	2.737	0.109	0.151	0.701
Cu	2.204	0.149	2.186	0.150	1.860	0.184
Zn	0.634	0.433	0.377	0.544	0.075	0.786
Cr	0.782	0.384	0.452	0.507	0.227	0.637
Cd	2.853	0.102	0.147	0.705	0.227	0.638
Pb	0.404	0.530	0.583	0.452	0.004	0.949

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

Table S4. Medians, mean concentrations (\pm SD) and ranges of organic compounds (ng g^{-1} ww) detected in *C. labrosus* muscle from Fogliano and Caprolace.

Substance	Fogliano					Caprolace				
	Median	Mean	\pm SD	Min	Max	Median	Mean	\pm SD	Min	Max
PCB 28	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
PCB 52	0.03 *	0.119	0.046	0.025	0.717	0.03	0.025	0.000	0.025	0.025
PCB 49	0.010	0.036	0.018	0.010	0.239	0.01	0.010	0.000	0.010	0.010
PCB 74	0.005	0.027	0.022	0.005	0.358	0.01	0.005	0.000	0.005	0.005
PCB 95	0.146 **	0.157	0.029	0.038	0.530	0.04	0.047	0.007	0.038	0.121
PCB 101	0.470 **	0.567	0.115	0.246	2.208	0.13	0.167	0.019	0.104	0.367
PCB 99	0.522 **	0.488	0.054	0.216	1.030	0.17	0.185	0.019	0.078	0.356
PCB 110	0.147 **	0.201	0.071	0.028	1.178	0.03	0.040	0.009	0.028	0.133
PCB 105	0.138 **	0.184	0.050	0.040	0.889	0.04	0.043	0.004	0.026	0.107
PCB 118	0.455 **	0.616	0.124	0.252	2.338	0.13	0.159	0.019	0.075	0.334
PCB 151	0.000	0.063	0.060	0.003	0.969	0.00	0.003	0.000	0.003	0.003
PCB 149	0.367 **	0.412	0.032	0.259	0.665	0.05	0.097	0.019	0.053	0.300
PCB 146	0.266 **	0.306	0.049	0.118	0.955	0.06	0.093	0.011	0.041	0.199
PCB 153	1.283 **	1.484	0.276	0.611	5.394	0.39	0.448	0.043	0.242	0.819
PCB 138	0.768 **	0.988	0.209	0.474	4.027	0.23	0.219	0.039	0.063	0.447
PCB 187	0.410 **	0.512	0.102	0.205	1.943	0.13	0.147	0.017	0.055	0.275
PCB 183	0.126 **	0.163	0.058	0.030	1.009	0.03	0.031	0.001	0.030	0.043
PCB 128	0.116 **	0.150	0.038	0.030	0.667	0.03	0.030	0.000	0.030	0.030
PCB 174	0.010	0.052	0.021	0.010	0.294	0.01	0.010	0.000	0.010	0.010
PCB 177	0.015 *	0.080	0.032	0.015	0.516	0.02	0.015	0.000	0.015	0.015
PCB 171	0.002	0.031	0.029	0.003	0.462	0.00	0.003	0.000	0.003	0.003

PCB 156	0.005	0.041	0.030	0.005	0.474	0.01	0.005	0.000	0.005	0.005
PCB 180	0.442 **	0.685	0.245	0.226	4.311	0.13	0.134	0.015	0.070	0.251
PCB 170	0.178 **	0.288	0.108	0.045	1.879	0.05	0.053	0.005	0.045	0.105
PCB 199	0.012 **	0.102	0.059	0.013	0.966	0.01	0.013	0.000	0.013	0.013
PCB 196/203	0.015 **	0.121	0.071	0.015	1.169	0.02	0.015	0.000	0.015	0.015
PCB 194	0.101 **	0.126	0.049	0.023	0.836	0.02	0.023	0.000	0.023	0.023
PCB 206	0.002	0.015	0.013	0.003	0.204	0.00	0.003	0.000	0.003	0.003
PCB 209	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
Σ_6 PCBs	2.966 **	3.843	0.878	1.653	16.656	0.97	0.993	0.100	0.562	1.888
Σ_7 PCBs	3.543 **	4.458	0.999	1.905	18.994	1.09	1.152	0.118	0.657	2.222
Σ PCBs	6.137 **	8.013	1.940	3.316	36.224	1.75	2.012	0.188	1.232	3.647
oxy-chlordane (OxC)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
trans-chlordane (TC)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
cis-chlordane (CC),	0.005	0.031	0.020	0.005	0.310	0.005	0.000	0.005	0.005	0.005
trans-nonachlor (TN)	0.010	0.075	0.036	0.010	0.545	0.010	0.000	0.010	0.010	0.010
cis-nonachlor (CN)	0.010	0.018	0.008	0.010	0.143	0.010	0.000	0.010	0.010	0.010
Σ Clordane	0.025 *	0.124	0.063	0.025	0.998	0.025	0.000	0.025	0.025	0.025
HCB	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
a-HCH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b-HCH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
g-HCH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Σ HCH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PBDE 28	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
PBDE 47	0.048 **	0.204	0.118	0.011	1.919	0.01	0.011	0.000	0.011	0.011
PBDE 100	0.005	0.040	0.023	0.005	0.377	0.01	0.005	0.000	0.005	0.005
PBDE 99	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
PBDE 154	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
PBDE 153	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
PBDE 183	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
Σ PBDE	0.053 **	0.245	0.142	0.016	2.296	0.02	0.016	0.000	0.016	0.016
2-MeO-BDE68	0.092	0.151	0.044	0.025	0.562	0.07	0.101	0.022	0.025	0.302
6-MeO-BDE47	1.024	1.832	0.475	0.055	5.919	0.63	0.946	0.217	0.099	2.765
Σ MeO-BDE	1.146	1.983	0.514	0.080	6.467	0.74	1.047	0.234	0.124	2.997
op-DDD	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
op-DDT	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
pp-DDE	1.233 **	1.450	0.211	0.570	3.988	0.46	0.499	0.056	0.260	0.822
pp-DDD	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000
pp-DDT	0.060 **	0.246	0.084	0.060	1.237	0.06	0.072	0.012	0.060	0.254
Σ DDT	1.293 **	1.696	0.291	0.630	5.225	0.52	0.571	0.061	0.320	0.999

Σ_6 PCBs is the sum of the six indicators 28, 52, 101, 138, 153 and 180. Σ_7 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

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