

Article

Evaluation of Environmental Quality of Mediterranean Coastal Lagoons Using Persistent Organic Pollutants and Metals in Thick-Lipped Grey Mullet

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Abstract: The evaluation of past and present anthropogenic impacts affecting the ecological quality status of transitional ecosystems is crucial from the perspective of protecting them from further deterioration, and to evaluate remediation and restoration measures. Contamination patterns of thick-lipped grey mullet from two Mediterranean coastal lagoons within a protected area in Italy were assessed and compared in order to evaluate their overall quality status and to collect information that can provide useful feedback on management choices aimed at enhancing environmental quality and biodiversity conservation. The quality status of the two lagoons was evaluated by an environmental assessment methodology based on indicators of direct and indirect human pressures, while a broad range of analyses were carried out to determine the presence and concentration of persistent organic pollutants (POPs) and metals in fish muscle tissue. A good quality status resulted for both lagoons, and an overall limited anthropogenic impact in the surrounding area. This could account for POPs and metal contamination levels found in mullet, although limited, and relating to their patterns. The overlap of results achieved with the two evaluation approaches can provide support for management choices in Mediterranean lagoon environments, especially for those committed to the protection and conservation of biodiversity.

Keywords: POPs; metals; lagoon ecosystem; bioindicator; *Chelon labrosus*; Italy; environmental quality

1. Introduction

Coastal lagoons are shallow aquatic environments which span from freshwater to hypersaline conditions depending on the water balance [1,2], and that show significant ecological gradients due to their intermediate position between the continental and marine domain. They support a high biodiversity of habitats suitable for birds, fish and invertebrate species [3], many of which are commercially or recreationally harvested all over the world, namely in the Mediterranean region [4–6]. The exploitation of living resources in these highly resilient and productive ecosystems has been

providing livelihood to humans since ancient times [3,6,7]. Other valuable ecosystem services [8–11], such as shore protection by coastal erosion, harbor and navigation facilities, flood control, waste and pollution assimilation, are also provided by coastal lagoons, related to their strategic position in the coastal area as well as to their ecological features [12,13].

Several threats are increasingly affecting lagoons as a direct consequence of the multiple uses of inland and coastal aquatic ecosystems, relating to habitat degradation and loss. These have been progressively occurring in most coastal lagoons, in the Mediterranean and elsewhere, as a consequence of the additive effects of many anthropogenic impacts [14]. Among these, water pollution due to the input of persistent organic pollutants (POPs) and toxic metals, as well as land use and reclamation, overexploitation of resources, introduction and spread of invasive species are the most relevant [7,13,15]. This has raised concerns for the ability of coastal lagoons and wetlands to further support the provision of valuable ecosystem services [10,14,16]. Therefore, the need to evaluate anthropogenic impacts affecting the overall status and quality of transitional ecosystems has become imperative from the perspective of protecting them from further deterioration, and to evaluate remediation and restoration measures.

The use of fish as a bioindicator for the evaluation of the environmental status of aquatic ecosystems and to assess habitat alterations is an issue that was raised long ago, and there are many reasons why fish are widely used [17]. The inclusion of fish as a Biological Quality Element (BQE) in the implementation of the Water Framework Directive (WFD) 2000/60 [18] has added further interest, especially for transitional waters [19–22]. The proposed methods for the assessment of the ecological status of transitional waters, based on fish as BQE, rely on the use of multi-metric indices that take into account the fish assemblage of the lagoon as a whole, and supplementary information to evaluate anthropogenic pressures acting on the lagoon [23,24]. Nevertheless, the approach based on the use of a single fish species as a biological indicator remains valid [25], and can rely on the use of fish condition, health and contamination patterns. With reference to the latter, long-living organisms such as fish are sensitive to complex mixtures of chemicals in specific ecosystems, and therefore tissue concentrations of chemicals are indicators of the environmental loads of specific toxicants, also integrating the information over time and space. Many pollutants may persist for decades and accumulate in soils and sediments, from which they can be remobilized because of the changing physico-chemical condition of the water body, or management interventions directly involving bottom and sediments. Hence, fish contamination can integrate information on changes occurring over time and space in environmental conditions and the status of a wide variety of aquatic ecosystems [19,26–29].

Among the potential environmental indicators or sentinel species for such ecosystems, many mullet species have been taken into account [30–33]. Grey mullets (Mugilidae) are one of the most ubiquitous teleost families in transitional and coastal waters [34], with five species dwelling in coastal lagoons in the Mediterranean region, and one of these is the thick-lipped grey mullet, *Chelon labrosus* (R. 1827). This marine migrant species enters lagoons at the juvenile stage, during which it displays a planktonic diet, and dwells in these environments in the adult phase, mainly with detritivorous habits [32–35], ingesting large amounts of the sediment organic matter (detritus, benthic diatoms and algae, small invertebrates, and crustaceans). After a growth period of variable duration (2–3 years males and 3–4 years females), adults return to marine coastal waters between December and February for spawning [34]. The life cycle then takes place mostly in transitional waters, and the feeding behavior, both as detritivore and water-column filter-feeder, makes *C. labrosus* potentially interesting as a biological indicator of chemical contamination since it can integrate possible contamination of both water and sediment matrices [33].

In this study, contamination patterns in the thick-lipped grey mullet from two Mediterranean coastal lagoons are evaluated, based on concentrations of a broad range of organochlorine compounds and metals analyzed in fish muscle tissue. Along with this, an assessment of the environmental quality status of the two lagoons is performed, based on descriptors of direct and indirect human pressures acting on these lagoon ecosystems. The aim is to assess the environmental quality of the lagoons, and to compare the results obtained with the two study approaches, in order to evaluate

if the use of the thick-lipped grey as bioindicator can give feedback useful to direct management choices for safeguarding habitat environmental quality, from the perspective of supporting biodiversity conservation in Mediterranean coastal lagoons.

2. Materials and Methods

2.1. Study Sites

The coastal lagoons of Fogliano and Caprolace are included within the Circeo National Park (Figure 1), that addresses species and habitats conservation, fisheries in the lagoons being banned since 2007, and carries on the environmental management of the lagoons in collaboration with the Forestry Unit of the Carabinieri Force. Their main ecological features are given in Table 1. Each lagoon is connected to the sea by a tidal channel, occasionally dredged to maintain water exchanges when silting occurs. Freshwater supplies (provided originally to Fogliano by a river and Caprolace by some adjacent channels) were cut off during the 80s because of their poor water quality, which frequently led to water eutrophication [36]. The whole area lies within a reclamation plain [37], and land use is directed mainly at intensive agriculture and livestock, while industrial activities are reduced in the whole area.

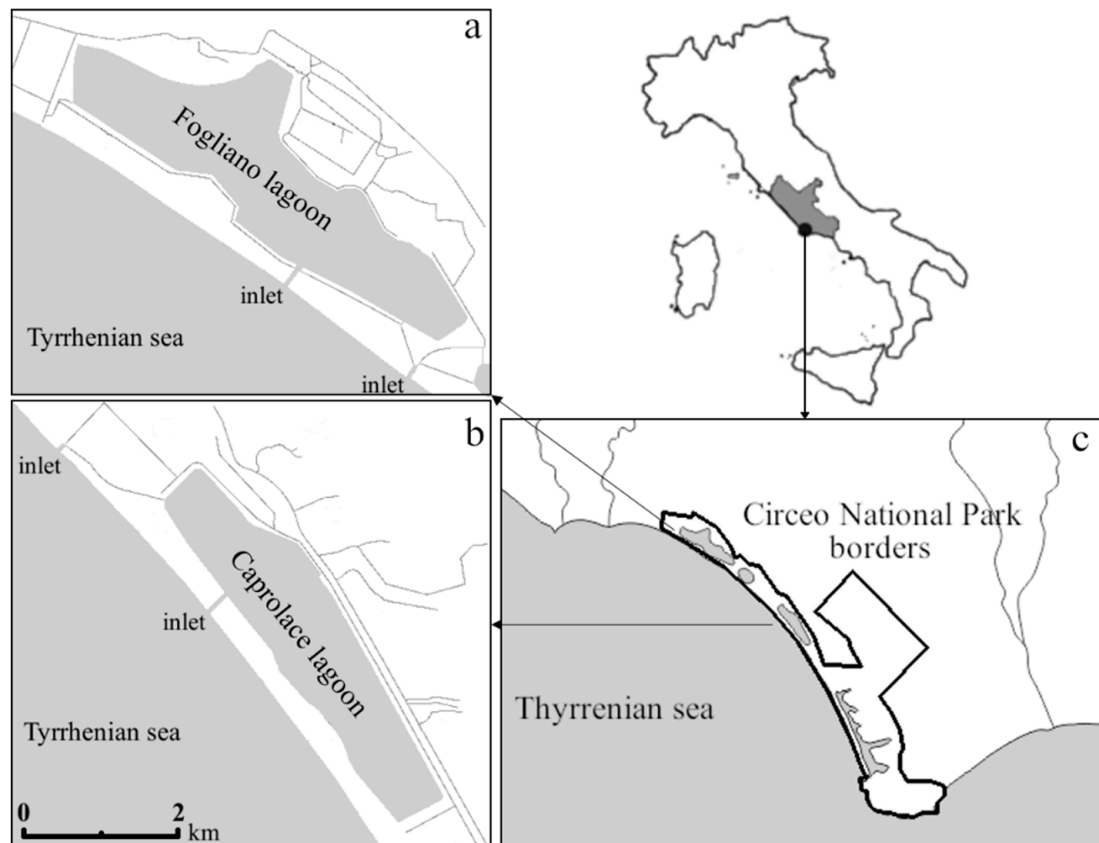


Figure 1. Study sites: the two coastal lagoons of Fogliano (a) and Caprolace (b) in the Circeo National Park, Central Italy (c).

Table 1. Geo-morphological and hydrographic features of the two coastal lagoons of the Circeo National Park, Fogliano and Caprolace. Both lagoons fall in the category of non-tidal (<50 cm), euhaline lagoons (average 30 psu) of the Mediterranean ecoregion according to the classification of the transitional water under Directive 2000/60 EC (WFD).

	Fogliano Lagoon	Caprolace Lagoon
Latitude	41°24'	41°21'
Longitude	12°54'	12°58'
Perimeter (km)	11.2	8.4
Surface (ha)	404	226
Max Depth (m)	2.0	2.9
Mean Depth (m)	0.9	1.3
Volume (m ³)	3,616,000	2,923,783
n° of tidal inlets	2	2
n° of tributaries	0	0
Tidal range (m)	0.23	0.21
Rate of water exchange (days)	60	90
Annual average water temperature (°C)	20.3	19.4
Annual average Salinity (psu)	40.6	39.1

2.2. Environmental Quality Assessment

A methodology based on an expert judgement approach [38], developed for the ecological evaluation of transitional waters (WFD, 2000/60) with special reference to lagoon ecosystems in the Mediterranean area has been set to assess the environmental quality of Fogliano and Caprolace lagoons [39]. This method relies on the preliminary selection of several environmental descriptors of both direct and indirect human pressures acting on lagoon ecosystems. Hydro-morphology and the quality of water, as well as the use of the lagoon resources and the surrounding landscape, are taken into account and quantified for the calculation of indices. A detailed description of the selection of pressure indicators, their attribution to specific categories and quantification, data sources and methods of calculation of indices are given in Capoccioni et al. [39].

2.3. Lipid Level and Contaminant Determination

A total of 32 thick-lipped grey mullets, 16 from each lagoon, were collected during winter 2016 by trammel nets. At capture, fish were anaesthetized on ice and painlessly sacrificed (National Park authorization n° PNC/DIR/2016/4773).

In the lab, individuals were weighed (to the nearest g) and measured for total length (to the nearest mm) and dissected for macroscopic gender assessment. Scales were taken and examined for ageing according to [40]. Skinned muscle tissue samples of approximately 15 g were collected from the dorsal part of the body, weighed, and freeze-dried (water loss during this process was registered).

2.4. Fish Collection and Tissue Sampling

A wide range of POPs was measured in muscle samples of individual fish. Contamination by polychlorinated biphenyls (PCBs) was assessed considering 29 congeners, including seven PCB indicators: 28, 52, 101, 118, 138, 153, and 180. Organochlorine pesticides (OCPs), such as chlordanes, dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorobenzene (HCB) and hexachlorocyclohexane isomers (HCHs), were measured as well as polybrominated diphenyl ethers (PBDEs) and methoxylated brominated diphenyl ethers (MeO-BDEs). The following metals were also measured: zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), cadmium (Cd), and mercury (Hg). Muscle sample processing, lipid content measurements, POP and metals analysis are described in detail in Section S1 of the Supplementary Material.

Concentrations of POPs were reported as ng g^{-1} on both a lipid weight (lw) and a wet weight (ww) basis. Metals were reported in $\mu\text{g g}^{-1}$ ww.

2.5. Quality Assurance/Quality Control

The extraction, clean up, and fractionation steps were evaluated by measurement of the absolute recoveries of the internal standards (70–115%). The peaks were quantified as targeted compounds if (1) the retention time matched that of the standard compound within ± 0.1 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. The limits of quantification (LOQs) were calculated as three times the standard deviation of the blank measurements mean. Procedural blanks were analyzed simultaneously within each batch of seven samples to check for interferences or contamination from solvent and glassware. Procedural blanks were consistent (RSD < 30%) and therefore the mean value was calculated for each compound and subtracted from the sample values. The analytical procedures for POPs were validated through the analysis of certified material SRM 1945 (organics in whale blubber) for which deviations from certified values of less than 20% were recorded.

Samples with concentrations below LOQ were calculated as $\text{df} \times \text{LOQ}$, with “df” being the fraction of samples above LOQ. POP limits of quantification (LOQs) ranged between 0.1 and 0.3 ng g^{-1} ww.

As far as toxic metals were concerned, Certified Reference Materials (CRM) were purchased from the National Research Council, Canada (DORM-4, Fish protein for trace metals). Metals LOQs ranged between 0.001 and $0.078 \mu\text{g g}^{-1}$ ww.

2.6. Data Analysis

Mean length and weight ($\pm \text{SE}$) were calculated for mullets grouped by gender and site, as well as mean ($\pm \text{SE}$) age and annual growth rate (GR, cm year^{-1}), estimated according to [41] to allow for growth comparison between sites. Body condition was assessed individually using Le Cren condition factor (CF), calculated by the formula $(100 \times W)/W'$, where W is the mass of an individual fish and W' the predicted mass of a fish of the same total length [42], mean values ($\pm \text{SE}$) were calculated for each sampling site. Statistical analyses were performed for each category of contaminants (i.e., $\sum_6 \text{PCBs}$, $\sum_7 \text{PCBs}$, $\sum \text{PCBs}$, $\sum \text{Chlordane}$, $\sum \text{DDTs}$, HCB, $\sum \text{HCHs}$, $\sum \text{PBDEs}$, and $\sum \text{MeO-BDEs}$) and metals (Zn, Cu, Pb, Cr, Cd, and Hg), looking for differences between sites and gender. POPs data treatment has been done using lipid weight values since bioaccumulation of hydrophobic chemicals is influenced by lipid content of muscle tissue [43]. Conversely, to compare contamination levels found in this study with those observed in other Mediterranean locations and the Atlantic coast of Europe, data on a wet weight basis were also considered.

A two-way ANOVA was used to test for significant differences in contaminants concentration among sites and gender. Differences between groups of contaminants in all analyses were tested for significance using non-parametric methods (Kruskal–Wallis test) adjusted by Bonferroni correction.

3. Results and Discussion

3.1. Environmental Quality Assessment

The environmental status assessment performed for Fogliano and Caprolace accounts for a generally good quality status in both lagoons, and an overall limited anthropogenic impact (Figure 2). Fogliano and Caprolace have shown nevertheless to be sensitive to specific indicators of anthropogenic pressure (Figure 2). In particular, the state of maintenance of the tidal channels, and hence water renewal, and the inputs due to the use of surrounding land affect the lagoons, bringing about increased levels of Dissolved Inorganic Nitrogen (DIN) and chlorophyll-a concentration, and lower dissolved oxygen. Such outcomes on water quality are more pronounced in Fogliano, that experiences at a higher degree the consequences of reduced water exchange with the sea. This entails a reduced turnover (Table 1) and therefore a slower dilution run-off water nutrient input. In Caprolace, the highest impact is detected for pressures related to the use of land and resources, because of the larger share of the

surrounding land affected by anthropogenic use, mostly for agriculture and livestock. In both lagoons, the presence of some non-priority pollutants (e.g., pesticides, PAHs, PCBs, and metals such as arsenic) and the presence of priority pollutants, such as nickel, lead, cadmium, and mercury, has been detected in the water column, albeit never over threshold values [44–46] and therefore never substantially affecting water quality.

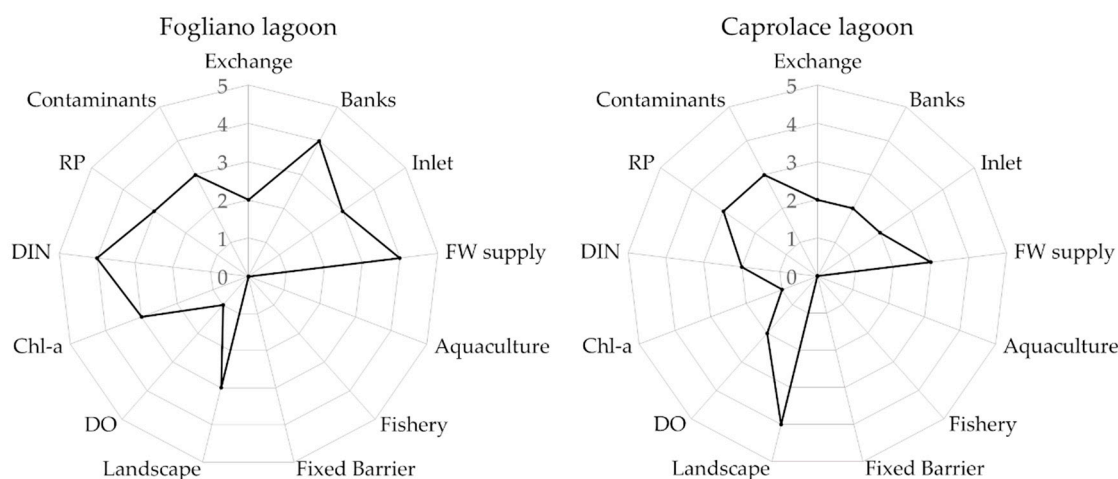


Figure 2. Environmental assessment of the lagoons: score values of indicators of anthropogenic pressure acting on Fogliano and Caprolace. Vectors represent: Water Exchange, Banks, Efficiency of Tidal Inlets and Freshwater supply (CPI-Morpho); Aquaculture, Fishery, Fixed barrier and Landscape (CPI-Use); Dissolved Oxygen (DO), Chlorophyll- α (Chl- α); Dissolved Inorganic Nitrogen (DIN), and Reactive Phosphorus (RP) CPI-Qual.

3.2. Biological Features

Mullet's biological characteristics are shown in Table 2. Data have been grouped by site and irrespective of gender (sex 50.0% and 60.3% of females respectively in the two lagoons) since biological parameters did not significantly differ for any attribute between males and females (Two-way ANOVA, $p > 0.05$, Table S1). Total length, weight, and condition factors showed no significant differences between sites. At Fogliano, mullets exhibit a significantly faster mean annual growth, amounting to $21.0 \text{ cm year}^{-1}$ vs. $11.3 \text{ cm year}^{-1}$ observed at Caprolace (Mann–Whitney U-test, $p < 0.01$) ($H = 7.364$, $p < 0.01$), as well as a higher lipid content (2.2% vs. 1.3% in Caprolace, $H = 5.549$, $p < 0.05$). Such differences, along with a mean age significantly lower than those from Caprolace (Mann–Whitney U-test, $p < 0.05$), could be due to the higher trophic status documented by the habitat quality assessment in Fogliano lagoon (see DIN and Chl- α scores, Figure 2).

Table 2. Mean \pm SE of total length, weight, lipid content, age, annual growth rate, and of Le Cren's relative condition factor of thick-lipped mullet samples from Fogliano and Caprolace.

	Fogliano Lagoon (n = 16)		Caprolace Lagoon (n = 16)	
	Mean	\pm SE	Mean	\pm SE
Females (%)	50.0		60.3	
Length (cm)	34.7	1.3	33.6	1.0
Weight (g)	744.8	63.9	573.1	36.6
% Lipids (on ww basis)	2.2 **	0.2	1.4	0.2
Age (year)	2.0	0.0	3.0 *	0.0
Growth rate (cm year^{-1})	21.0 **	2.5	11.3	0.7
Le Cren's CF	1.0	0.1	1.0	0.0

Significant differences between the two lagoons are marked by a single ($p < 0.05$) or double asterisk ($p < 0.01$).

3.3. Organic Contaminants

In most cases, compounds concentrations in fish muscle showed very low values in both sites, standing for a general low contamination pattern that agrees with the environmental quality assessment (Table 3). Two-way ANOVA (see Table S2) did not show a significant interaction effect of site and gender on contaminants ($p > 0.05$), therefore further comparisons were carried out irrespective of sex, and evidenced some statistical differences for specific contaminants in mullets from the two lagoons (Table 3). These can be interpreted based on each specific environmental context and compared with results available in the scientific literature, dealing with grey mullet species contamination in Mediterranean and Atlantic transitional and marine habitats.

PCBs were found in all fishes from both lagoons. Median and range values of total PCBs amounted to 219 ng g⁻¹ lw (61.7–6134) and 212 ng g⁻¹ lw (81.7–945) in Fogliano and Caprolace, respectively, with no significant difference between sites (Table 3). The congeners that mostly contributed to the total PCB loads were PCB 153 (18.5% vs. 22.2% respectively for Fogliano and Caprolace) and PCB 138 (12.3% vs. 10.9%). These percentages are in line with those observed in other fish species, such as the eel from Belgium [47,48] and from transitional environments in Italy [39,49,50]. In 1994, in the two coastal lagoons under study, Casini et al. [51] found, in muscle tissues of four species of grey mullets (*C. aurata*, *C. saliens*, *C. ramada*, and *M. cephalus*), similar total PCB concentrations. Overlapping results after nearly twenty years, despite the ban of PCBs since the 70s, can be explained by the persistence of this compound in Fogliano and Caprolace lagoons water and sediments. Results of the lagoon quality status carried out in this study highlighted the peculiar hydrological condition of this lagoon, with a reduced water exchange with the sea as well as occasional silting of the tidal channels. Dredging operations periodically carried out in the two lagoons to overcome this problem might easily remobilize contaminants no longer in use but accumulated in the sediments [52,53]. This seems to be testified by the fact that persistent contaminants such as PCBs, banned from several years, are still present in fish tissue in both lagoons.

Among the other organochlorine compounds (Tables 3 and S2), total Chlordane median concentrations in mullet muscle were 6.0 (3.0–191.0) ng g⁻¹ lw and 8.2 (3.9–60.0) ng g⁻¹ lw, respectively in Fogliano and Caprolace, with no significant difference between sites (Mann–Whitney U-test, $p > 0.05$). Median concentrations values of HCB were 3.2 (2.0–8.1) and 4.7 (2.9–8.4) ng g⁻¹ lw in mullets from Fogliano and Caprolace respectively, the latter showing a significantly higher concentration for Fogliano (Mann–Whitney U-test, $p < 0.05$). The level of Σ HCH was on the other hand significantly higher in fish from Caprolace (Mann–Whitney U-test, $p < 0.01$), with a median value of 8.8 (3.3–13) ng g⁻¹ lw concerning 4.6 (2.3–13) ng g⁻¹ lw in mullets from Fogliano. As for HCH isomers, mean levels of β -HCH and γ -HCH (which are, respectively, the most persistent and the most toxicological active HCH isomers [54]) contributed to Σ HCH levels 13–13% and 71–73%, respectively in Fogliano and Caprolace, γ -HCH being, therefore, the dominant isomer. Such a difference in HCH compounds, prone to accumulate in the sediments through adsorption processes and direct sedimentation of suspended particulate matter [55], could be due to their accumulation to a greater extent by mullets in Caprolace, older than those in Fogliano, as a function of the exposure time. Additionally, the pressures related to the use of land and resources, mostly for agriculture and livestock, more pronounced in the area surrounding the southernmost of the two lagoons, could support this hypothesis.

Σ HCH levels found in Caprolace mullets were generally comparable with those reported in mullets from other Mediterranean lagoons [54] (Table 4). In contrast, results from Fogliano were lower than those reported by Naso et al. [56] and Ameer et al. [54] (mean values, 24.1 and 6.7 ng g⁻¹ lw, respectively) for mullets from Mediterranean coastal waters (Table 4).

Total PBDE median concentrations were not significantly different in fish from the two lagoons, amounting to 1.9 ng g⁻¹ lw (range, 1.0–410.8) in the specimens from Fogliano and 3.2 ng g⁻¹ lw (range, 1.2–83.7) in those from Caprolace. Although both exceeded the EQSBIOTA threshold (Biota Environmental Quality Standard: 0.0085 ng g⁻¹ ww) set by Directive 2013/39/UE and by National Decree 172/2015 (Table S4), the detected values do not suggest significant contamination by

PBDEs. It has been stated that the EU EQS limit should be revised [57] as they are too low given that more than 70% of the analyzed fish at the European aquatic systems level had results exceeding the EQSBIOTA [58]. As far as PBDE congeners are concerned, most of them showed low concentrations: BDE 47, BDE 100 and BDE 154 made up about 96% and 86% on average of the total PBDE load respectively for Fogliano and Caprolace. The dominant compound was BDE-47 (47%), which is usually the most abundant compound in fish, as a result of its highest bioaccumulation factor [59]. As expected, several authors made the same observations in previous studies in fish and other organisms [60–63]. As for the ratio between PBDE-99 and PBDE-100, considered an indicator of the organism metabolic capacity [60], fish from the two lagoons displayed a comparable ratio of 1:9 and 1:4 (Fogliano and Caprolace respectively), in line with the mean BDE-99: BDE-100 ratio in fish from marine coastal areas that are not excessively polluted [63].

Concerning MeO-PBDEs, a significantly higher mean concentration was found in Fogliano, but in any case levels observed in mullet muscle tissue from both lagoons are noticeably higher than those found in the same lagoons in the European eel [39]. Such a difference can be accounted for by the different trophic behavior of the grey thick-lipped mullet compared to the eel. The filter-feeding habits of this mugilid encompasses ingestion of detritus, algae and cyanobacteria, which are the natural sources of these compounds [64,65]. Therefore, the high MeO-PBDEs levels, that have no anthropogenic origin, in mullets from Fogliano could be linked to a more intense primary production that follows the nutrient input loads. Such higher loads in this lagoon, coupled with a reduced water renewal, is an issue that has been highlighted by the environmental assessment (Figure 2). High concentrations of MeO-PBDEs in urchins from the Bizerte lagoon have been found with site-specific differences, in turn related to different levels of agricultural inputs [66], while seasonal variations in their levels in blue mussels in the Baltic Sea have been explained by summer peaks of algal production [67].

As for DDTs, a significant difference was detected between Fogliano and Caprolace thick-lipped grey mullets ($p < 0.01$), with the former showing higher median values ($58.0 \text{ ng g}^{-1} \text{ lw}$, range: 25.7–949.9) than those from Caprolace ($49.9 \text{ ng g}^{-1} \text{ lw}$, range: 31.1–292.0). Median concentrations expressed on a wet weight basis (1.3 and 0.5 ng g^{-1} , respectively) are much lower than the EQSBIOTA limit ($100 \text{ ng g}^{-1} \text{ ww}$, Table 3) established under National Decree 172/2015 [46] for fish with $<5\%$ lipid. Σ DDT values in mullets from the two study sites, and the higher values in fish from Fogliano, are of the same order of magnitude as those reported for grey mullets from other Mediterranean transitional waters (Table 4), such as the Bizerte lagoon in Tunisia [54] and the Ebro delta in Portugal [68], while they are sensibly lower than those reported for marine habitats such as the Marmara Sea in Turkey [69] and from the coastal waters of Italy [56]. It is not surprising that the banned DDT and its degradation products DDE and DDD are found in fish and other organisms because their presence in sediments persists even for decades. They can return to the water column or surface sediments and in the food chain in case of disturbance or handling of sediments such as those due to dredging [52,53]. Such intervention, as already pointed out before, occurs in Fogliano, and probably accounts for the higher DDT levels found in mullet from this lagoon.

More specifically for DDT and its metabolites, Hitch and Day [70] and Zhou et al. [71] highlighted that the ratio (DDE/DDD)/DDTs can be used to assess the chronology of DDT inputs to aquatic ecosystems. In general, a ratio higher than 0.5 indicates long-term biotransformation of DDT to DDE and DDD, while a ratio of less than 0.5 may indicate the recent input of DDT [70,71]. In this study, observed ratios (Fogliano = 0.79; Caprolace = 0.82) stand for past DDT pollution, likely used in agriculture in the area surrounding the two lagoons. The Agro Pontino plain, where both are located, has become a reclaimed area since the 20s, where a progressively intensified agriculture and livestock has been occurring. Furthermore, the history of this territory should be taken into account, where an extensive program for malaria eradication was performed since the end of the Second World War, in the period 1944–1965, making use of DDT spread with airplanes [72,73]. Therefore, the presence of DDTs in fish in this area [39,74] testifies to the still on-going contamination, as a consequence of past massive use and diffused pollution from intensive agriculture [53].

Table 3. Detection frequencies, medians, mean concentrations (\pm SD) and ranges of organic compounds (ng g^{-1} lw) analyzed in *C. labrosus* muscle from Fogliano and Caprolace.

Substance	Fogliano Lagoon (n = 16)						Caprolace Lagoon (n = 16)					
	Det. Freq.	Median	Mean	SD	Min	Max	Det. Freq.	Median	Mean	SD	Min	Max
PCB 28	0%	0.00	0.00	0.00	0.00	0.00	0%	0.00	0.00	0.00	0.00	0.00
PCB 52	56%	4.73	11.74	7.45	0.75	122.40	38%	0.75	5.14	1.84	0.75	23.13
PCB 49	19%	0.40	3.91	2.57	0.40	40.79	31%	0.40	3.55	1.42	0.40	19.04
PCB 74	0%	0.05	0.05	0.00	0.05	0.05	6%	0.05	0.69	0.64	0.05	10.31
PCB 95	56%	3.77	8.92	5.52	0.45	90.40	56%	4.33	6.64	2.06	0.45	23.51
PCB 101	100%	15.39	38.69	22.68	4.48	376.72	100%	17.05	24.37	4.45	10.64	59.46
PCB 99	100%	18.29	28.98	10.08	7.50	175.81	100%	14.25	20.06	3.01	10.54	48.66
PCB 110	38%	0.28	15.24	12.43	0.28	201.08	31%	0.28	5.99	2.93	0.28	35.22
PCB 105	100%	3.85	13.73	9.25	1.00	151.70	100%	5.41	7.75	1.59	2.51	22.63
PCB 118	100%	16.17	41.78	24.00	4.95	399.01	100%	16.06	23.52	4.62	8.07	63.10
PCB 151	6%	0.03	10.35	10.33	0.03	165.29	0%	0.03	0.03	0.00	0.03	0.03
PCB 149	75%	13.69	18.86	6.76	0.80	113.48	56%	10.24	17.79	5.96	0.80	68.90
PCB 146	100%	10.90	21.13	9.59	3.82	162.94	100%	6.88	10.84	2.29	2.86	34.03
PCB 153	100%	42.16	104.25	54.92	17.11	920.43	100%	41.74	58.30	10.97	21.13	171.78
PCB 138	81%	22.91	68.37	41.63	0.63	687.24	75%	25.71	38.70	9.88	0.63	124.38
PCB 187	100%	14.88	37.68	19.82	5.69	331.65	100%	11.66	19.60	4.78	4.89	70.07
PCB 183	100%	3.59	15.29	10.49	1.17	172.15	100%	3.59	6.37	1.65	1.48	22.90
PCB 128	100%	3.48	11.27	6.89	1.12	113.86	100%	4.16	6.58	1.50	1.92	19.57
PCB 174	88%	2.07	5.80	3.01	0.75	50.14	100%	2.63	4.76	1.34	0.95	17.11
PCB 177	88%	1.88	7.98	5.36	0.68	87.97	81%	2.67	4.91	1.47	0.68	18.39
PCB 171	75%	1.49	6.72	4.81	0.58	78.76	69%	1.53	2.74	0.73	0.58	9.78
PCB 156	69%	1.54	6.85	4.96	0.60	80.91	81%	2.01	2.99	0.71	0.60	9.59
PCB 180	100%	12.78	62.51	44.99	4.14	735.65	100%	13.07	22.46	5.86	4.54	81.22
PCB 170	100%	5.20	26.73	19.64	1.62	320.59	100%	5.44	9.49	2.47	2.08	32.74
PCB 199	88%	2.12	13.50	10.12	0.68	164.83	81%	2.63	4.73	1.31	0.68	17.18
PCB 196/203	88%	2.18	15.62	12.28	0.70	199.46	88%	2.61	4.85	1.33	0.70	18.34
PCB 194	100%	3.04	13.01	8.68	0.99	142.59	100%	3.31	5.25	1.32	0.99	18.84
PCB 206	38%	0.33	2.90	2.13	0.33	34.81	44%	0.33	1.12	0.29	0.33	3.85
PCB 209	25%	0.28	1.34	0.82	0.28	13.32	44%	0.28	1.18	0.29	0.28	3.30
Σ_6 PCBs		100.10	285.55	171.52	27.09	2842.44		104.54	148.98	31.44	38.06	436.05
Σ_7 PCBs		117.45	327.33	195.50	32.04	3241.45		122.94	172.50	35.92	46.13	491.51
Σ PCBs		219.15	613.19	370.27	61.68	6134.03		212.36	320.38	71.97	80.68	944.48

Table 3. Cont.

Substance	Fogliano Lagoon (n = 16)						Caprolace Lagoon (n = 16)					
	Det. Freq.	Median	Mean	SD	Min	Max	Det. Freq.	Median	Mean	SD	Min	Max
oxy-chlordane (OxC)	31%	0.43	1.15	0.46	0.43	7.82	75%	1.50*	1.55	0.23	0.43	3.69
trans-chlordane (TC)	75%	1.18	1.99	0.73	0.65	12.70	88%	1.73	2.15	0.39	0.65	6.99
cis-chlordane (CC),	63%	1.30	4.47	3.23	0.63	52.90	94%	1.34	2.36	0.71	0.63	12.61
trans-nonachlor (TN)	94%	1.81	8.37	5.68	0.78	92.96	100%	2.23	4.33	1.59	1.55	27.51
cis-nonachlor (CN)	75%	1.17	2.77	1.46	0.50	24.47	50%	0.73	1.64	0.56	0.50	9.22
∑Clordane		5.92	18.75	11.53	2.98	190.86		8.16	12.02	3.33	3.90	60.03
HCB		3.18	3.62	0.41	1.97	8.14		4.72 *	4.77	0.35	2.97	8.40
a-HCH	44%	0.50	0.87	0.11	0.50	1.63	69%	1.31	1.14	0.13	0.50	2.16
b-HCH	19%	0.50	0.68	0.10	0.50	1.62	44%	0.50	1.06	0.23	0.50	3.94
g-HCH	100%	3.10	3.77	0.60	1.25	10.54	100%	6.88 **	6.08	0.63	2.26	9.72
∑HCH		4.36	5.32	0.72	2.25	12.50		8.81 *	8.28	0.83	3.26	12.84
PBDE 28	6%	0.06	0.48	0.42	0.06	6.81	25%	0.06	0.49	0.20	0.06	2.06
PBDE 47	100%	1.23	22.02	20.37	0.39	327.53	100%	1.55	9.34	4.15	0.61	57.21
PBDE 100	44%	0.21	4.54	3.99	0.21	64.28	63%	0.59	2.31	0.89	0.21	10.14
PBDE 99	31%	0.18	0.63	0.35	0.18	5.79	56%	0.53	0.74	0.17	0.18	2.19
PBDE 154	31%	0.15	0.74	0.39	0.15	6.36	44%	0.15	1.52	0.66	0.15	9.78
PBDE 153	0%	0.03	0.03	0.00	0.03	0.03	13%	0.03	0.23	0.16	0.03	2.41
PBDE 183	100%	0.00	0.00	0.00	0.00	0.00	100%	0.00	0.00	0.00	0.00	0.00
∑PBDE		1.85	28.45	25.50	1.01	410.79		3.23	14.63	6.09	1.23	83.67
2-MeO-BDE68	100%	6.37	7.84	1.54	1.52	22.54	100%	3.55	4.16	0.60	1.10	8.17
6-MeO-BDE47	100%	108.82 **	105.25	14.76	8.50	181.03	100%	25.03	33.58	6.13	10.80	108.74
∑MeO-BDE		116.55 **	113.10	15.72	10.02	203.57	0%	29.79	37.74	6.20	12.66	110.78
op-DDD	100%	0.00	0.00	0.00	0.00	0.00	100%	0.00	0.00	0.00	0.00	0.00
op-DDT	100%	0.00	0.00	0.00	0.00	0.00	100%	0.00	0.00	0.00	0.00	0.00
pp-DDE	100%	46.78	94.16	39.90	20.18	680.60	100%	41.40	65.58	15.82	22.13	245.06
pp-DDD	13%	0.10	4.99	3.76	0.10	58.16	0%	0.10	0.10	0.00	0.10	0.10
pp-DDT	100%	11.71	26.37	12.61	4.00	211.08	100%	11.64	14.19	2.90	0.06	46.79
∑DDT		58.00 **	125.51	56.07	25.69	949.85		49.86	79.87	18.33	31.14	291.95

∑₆PCBs is the sum of the six indicators 28, 52, 101, 138, 153, and 180. ∑₇PCBs 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 to distinguish them in the table from the single pollutants.

Table 4. Concentration of organochlorine compound ranges or mean values (\pm SD where available) (ng g^{-1}) in grey mullet (*Mugilidae*) muscle tissue available in the scientific literature of other Mediterranean locations, in the Atlantic coast of Europe and from the present study.

Country	Site	Year	Species	lw/dw/ww	Σ PCBs	Σ_6 PCBs	Σ_7 PCBs	Σ DDT	HCB	Σ HCHs	Σ PBDE	Σ MeO-PBDEs	Σ Chlordane	Source
Portugal	Douro Estuary	2002	MC	dw	311.0 \pm 58.0			65.0 \pm 35.0						[78]
Portugal	Mondego	2008	CL	ww		0.6								[79]
Portugal	Mondego	2008	LA	ww		1.0								[79]
Portugal	Mondego	2008	LR	ww		5.0								[79]
Spain	Ebro Delta	1992	MC	lw			271.0 \pm 111.0	546.0 \pm 226.0	1138.0 \pm 410.0					[68]
France	Gironde Estuary	nr	LR	dw			108.0 \pm 60.0							[80]
France	Vilaine estuary	2005	LR	dw			125.9	19.5		1.5	6.7			[81]
France	Vilaine River	2005	LR	dw			203.4	23.8		4.8	10.1			[81]
France	Vaccare's Lagoon	2002–2005	<i>Mugilidae</i> spp.	dw					0.3–9.1	171.8–112.4				[82]
Italy	Fogliano	1994	LA	ww	12–23			0.6–2.8	0.0–0.1	0.0–0.0				[51]
Italy	Fogliano	1994	LS	ww	60–150			2.0–4.0	0.02–0.0	0.0–0.1				[51]
Italy	Fogliano	1994	LR	ww	11–50			0.8–4.8	0.1–0.1	0.0–0.0				[51]
Italy	Fogliano	1994	MC	ww	7–31			0.4–1.4	0.0–0.0	0.0–0.0				[51]
Italy	Caprolace	1995	LA	ww	3–150			1.5–31.5	0.0–2.0					[51]
Italy	Fogliano	2016	CL	lw	613.1 \pm 1481.8	285.6 \pm 171.5	327.3 \pm 782.0	125.5 \pm 224.3	3.6 \pm 1.7	5.3 \pm 2.9	141.5 \pm 94.1	113.1 \pm 15.7	18.8 \pm 6.1	Present study
Italy	Caprolace	2016	CL	lw	320.4 \pm 287.9	149.1 \pm 31.4	172.5 \pm 143.7	79.9 \pm 73.3	4.8 \pm 1.4	8.3 \pm 3.3	52.4 \pm 39.4	37.7 \pm 6.2	12.0 \pm 13.3	Present study
Italy	Med Sea	nr	MC	lw	1042.0			596.0		24.1				[56]
Italy	Tyrrhenian Sea	2004	MC	ww			9.0							[83]
Italy	Adriatic Sea	2005	MC	lw	1210 **									[84]
Tunisia	Radès	nr	LA	lw	96.0 *		2350.0							[85]
Tunisia	Raoued	nr	LA	lw	52.0 *		961.0							[85]
Tunisia	Bizerte	2010	MC	lw	249.0 \pm 56.7		220.8 \pm 89.3	95.4 \pm 30.5		6.7 \pm 6.1	45.3 \pm 31.1	100.7 \pm 137.4 ***		[54]
Tunisia	MedSea	2010	MC	lw	109.0 \pm 13.7		97.6 \pm 12.6	15.4 \pm 11.0		1.8 \pm 0.65	7.8 \pm 2.4	293.1 \pm 179.9 ***		[54]
Turkey	Marmara Sea	2003	MC	lw			404.0	731.4		224.7				[69]

Species: *Mugil cephalus*, MC; *Liza ramada*, LR; *Liza aurata*, LA; *Liza saliens*, LS; *Chelon labrosus*, CL. nr = not reported; * ww; ** sum of PCB52, PCB60, PCB77, PCB101, PCB105, PCB118, PCB138, PCB153, PCB156, PCB180; *** sum of 2—MeO-BDE-68, 5—MeO-BDE-47, 6—MeO-BDE-47.

3.4. Metals

All the considered metals, except for Hg, showed very low contamination levels (Table 5), also in comparison with results from other Mediterranean transitional and marine environments (Table 6). The same as metals, two-way analysis of variance (ANOVA, see Table S3) did not evidence significant interaction effects of site and gender on contaminants ($p > 0.05$), and therefore further comparisons were carried out irrespective of sex (Table 5).

Table 5. Detection frequencies, medians, mean concentrations (\pm SD), and ranges of metals ($\mu\text{g g}^{-1}$ ww) detected in *C. labrosus* muscle from Fogliano and Caprolace.

Substance	Fogliano Lagoon (n = 16)						Caprolace Lagoon (n = 16)					
	Det. Freq.	Median	Mean	\pm SD	Min	Max	Det. Freq.	Median	Mean	\pm SD	Min	Max
Cu	100%	0.367	0.732	0.266	0.105	4.567	0%	0.312	0.341	0.050	0.114	0.689
Zn	100%	5.189	5.463	0.466	1.658	9.822	0%	5.871	6.106	0.635	2.985	11.858
Cr	25%	0.000	0.002	0.001	0.000	0.019	38%	0.000	0.003	0.001	0.000	0.015
Cd	50%	0.000	0.000	0.000	0.000	0.000	69%	0.000 *	0.001	0.000	0.000	0.005
Pb	38%	0.000	0.002	0.001	0.000	0.011	31%	0.000	0.003	0.002	0.000	0.024
Hg	100%	0.036	0.050	0.010	0.010	0.168	0%	0.080 **	0.095	0.014	0.031	0.240

Significant differences between lagoons are marked by a single ($p < 0.05$) or double asterisk ($p < 0.01$) (Mann–Whitney U tests Bonferroni corrected).

Specifically, for mercury, values found in mullet muscle tissues were high in both sites ($0.04 \mu\text{g g}^{-1}$ ww and $0.08 \mu\text{g g}^{-1}$ ww, for Fogliano and Caprolace respectively), with samples from Caprolace being significantly higher than in Fogliano ($p < 0.01$). Such concentrations are above the EQSBIOTA limits for fish (i.e., $0.02 \mu\text{g g}^{-1}$ ww). This may likely be attributed to an accumulation of this metal in the sediments [75,76], but in the case of Fogliano and Caprolace, its origin cannot be explained based on known past or present activities or on impacts in the area. The higher Hg concentration in Caprolace compared to Fogliano mullets, on the other hand, is probably due to a longer permanence of fish in the lagoon, established by the larger body size associated with age, and thus to prolonged exposure [77].

Table 6. Metal mean concentration (\pm SD where available) ($\mu\text{g g}^{-1}$) in grey mullets (*Mugilidae*) muscle tissue available in the scientific literature relative to sites from other Mediterranean locations, the Atlantic coast of Europe, and from the present study.

Country	Site	Year	Species	ww/dw	Zn	Cu	Hg	Cr	Cd	Pb	Source
Portugal	Rosário	nr	CL	dw	23.30 \pm 7.80	1.60 \pm 0.10			0.90 \pm 0.10	3.70 \pm 0.60	[86]
Portugal	Hortas	nr	CL	dw	22.50 \pm 4.00	0.94 \pm 0.20			0.40 \pm 0.02	1.50 \pm 0.40	[86]
Portugal	S. Joao da Talha	nr	CL	dw	24.70 \pm 4.30	1.50 \pm 0.30			0.30 \pm 0.01	1.10 \pm 0.10	[86]
Spain	Guadalquivir estuary	1998	LR	ww	8.50 \pm 5.90	0.50 \pm 0.30			<0.02		[87]
Spain	Bacuta reservoir	2002	LA	ww	6.07	0.50	0.01	0.04	0.03	0.03	[88]
Spain	Liebre reservoir	2002	LA	ww	8.41	0.60	0.01	0.03	0.02	0.03	[88]
Spain	San Carlos reservoir	2002	LA	ww	3.87	0.20	0.01	0.03	0.01	0.04	[88]
Spain	San Juan reservoir	2002	LA	ww	3.10	0.40	0.01	0.04	0.02	0.05	[88]
France	Gironde estuary	2001	LR	ww	4.20 \pm 0.50		0.10 \pm 0.01		<0.07		[89]
Italy	Adriatic Sea	2003	LA	ww	6.59 \pm 1.14	0.93 \pm 0.14	0.04 \pm 0.02	0.15 \pm 0.06		0.04 \pm 0.02	[84]
Italy	Adriatic Sea	2003	LA	ww			0.05				[84]
Italy	Adriatic Sea	2003	CL	ww	6.90 \pm 0.16	0.84 \pm 0.17	0.05 \pm 0.02	0.16 \pm 0.05		0.04 \pm 0.02	[84]
Italy	Ligurian Sea	2008	MC	ww			<0.01		0.01	0.06	[90]
Italy	Caprolace lagoon	2016	CL	ww	6.11 \pm 2.54	0.34 \pm 0.20	0.10 \pm 0.06	0.00 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.01	Present study
Italy	Fogliano lagoon	2016	CL	ww	5.46 \pm 1.86	0.73 \pm 1.01	0.05 \pm 0.04	0.00 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	Present study
Tunisia	Ghar El Melh	2004	MC	dw			0.34		0.02	0.20	[91]
Tunisia	Sfax	2014	LA	ww	45.26 \pm 0.81		0.12 \pm 0.02		0.10 0.01	0.06 \pm 0.00	[92]
Tunisia	Gulf of Gabes	2015	MC	dw	0.77 \pm 0.09	6.276 \pm 3.845				5.21 \pm 1.43	[93]
Egypt	El-Mex Bay	2003	MC	ww	6.75	1.22		0.21	0.47	0.73	[94]
Egypt	Manzala lake	nr	MC	dw	32.42	4.62			1.94	3.15	[31]
Egypt	Manzala lake	nr	LR	dw	31.05	5.36			1.07	2.65	[31]
Egypt	El Serv	nr	LR	dw	23.47 \pm 6.23	2.9 \pm 2.83				4.97 \pm 6.17	[95]
Egypt	Manzala lake	nr	LR	dw	16.62 \pm 3.02	1.47 \pm 0.32			0.05 \pm 0.1	4.55 \pm 6.19	[95]
Egypt	Med Sea	nr	LR	dw	20.19 \pm 3.5	2.13 \pm 1.02			0.82 \pm 1.23	9.39 \pm 10.06	[95]
Turkey	Karataş	2003	MC	dw		9.83			1.45	6.33	[96]
Turkey	Iskenderun Bay	1999	MC	ww	47.25 \pm 18.77	1.39 \pm 0.80		1.71 \pm 0.92		6.42 \pm 2.82	[97]
Turkey	Camlik lagoon	2001	MC	dw	88.9				0.07		[98]
Turkey	Camlik lagoon	2015	LA	ww	4.92 \pm 0.35	0.88 \pm 0.09		0.81 \pm 0.05	0.64 \pm 0.06	0.45 \pm 0.06	[99]
Turkey	Camlik lagoon	2015	LS	ww	4.21 \pm 0.31	0.67 \pm 0.11		0.49 \pm 0.12	0.36 \pm 0.05	0.98 \pm 0.21	[99]
Turkey	Camlik lagoon	2015	MC	ww	6.13 \pm 0.57	0.70 \pm 0.19		0.27 \pm 0.05	0.13 \pm 0.02	0.63 \pm 0.12	[99]
Turkey	Tuzla lagoon	2001	MC	dw	39.60	0.53			0.09	0.92	[100]
Turkey	Tuzla lagoon	2015	MC	ww	21.20 \pm 7.08	0.64 \pm 0.13		0.39 \pm 0.04	0.18 \pm 0.02	0.26 \pm 0.05	[99]
Turkey	Tuzla lagoon	2015	LA	ww	7.80 \pm 1.03	1.14 \pm 0.12		1.05 \pm 0.17	0.35 \pm 0.07	0.37 \pm 0.04	[99]
Turkey	Tuzla lagoon	2015	LS	ww	15.00 \pm 2.61	0.95 \pm 0.29		0.62 \pm 0.12	0.06 \pm 0.01	0.78 \pm 0.14	[99]

Species: *Mugil cephalus*, MC; *Liza ramada*, LR; *Liza aurata*, LA; *Liza saliens*, LS; *Chelon labrosus*, CL.

4. Conclusions

The results of the present study allow for some conclusive considerations. Contamination levels found in the thick-lipped mullet at both Caprolace and Fogliano lagoons were generally low, mostly in line or lower than levels recorded in mullets and other fish species in transitional habitats in the Mediterranean region. Based on the high number of contaminants and congeners examined, it has been possible nevertheless to highlight differences in contamination patterns between the two adjacent lagoons. These share a common geographical position, and have undergone similar changes in the surrounding landscape, in their hydro-morphology and in their management framework. The presence of persistent contaminants or their metabolites, some of which banned many years ago, testify long standing pollution, while others are more directly related to the current situation of each of the two lagoons. Differences found in some contaminants can be related to site-specific differences in the ecological conditions of the two lagoons, such as water management and their renewal, or to the different duration of the fish permanence in each lagoon, as ascertained by mullet age estimation.

The quality status of the two lagoons under study, evaluated taking into account anthropogenic pressures inside and around the water bodies, is good. This is attributable to the fact that anthropogenic pressures related to the lagoon resources exploitation (fisheries, aquaculture) are today reduced, owing to the present management of the lagoons under the National Park Authority, mainly focused on habitat and resource conservation. On the other hand, such a management framework does not address issues such as solving the structural problems of the lagoons first of all nor the maintenance of exchanges with the sea by the tidal channels. This is the crucial factor for the correct functionality of coastal lagoons, both hydrological (water renewal) and biological (fish recruitment). Occasional interventions, such as dredging, can have adverse effects, recirculating contaminants which then accumulate along trophic chains, and therefore posing threats to the lagoon living resources. Results of this study can therefore provide support to management choices in Mediterranean lagoon environments, especially those committed to the protection and conservation of biodiversity.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4441/12/12/3450/s1>. Methods of determination of persistent organic pollutants (POPs) and metals; 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 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 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. 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.

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