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Organochlorine pesticides in *Muraena helena* L. 1758 from the Eastern Adriatic Sea

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Summary

This work examines the bioaccumulated organochlorine compounds in *Muraena helena* to draw attention to this species as a potential bioindicator organism. The morays were caught in the East Adriatic Sea, Elaphite Islands, near Dubrovnik, Croatia (lat: 42°45′38.8″; long: 17°45′53.6″) at the same location in summer (August, 2009) and winter (January, 2010), at depths from 5 to 10 m. Nine fish were caught by longline hooks per each field trip (total in summer and winter, N = 18). No single fish was free of organochlorines (OC), but not all pesticides were present in every fish. The concentration level was: Endrin > ppDDE > Heptachlor epoxide > Heptachlor > Aldrine > Lindane > βHCH > δHCH > Endrin aldehyde). Endrin was the only pesticide approaching the maximum concentrations proposed by the European Union and Croatia (summer max. concentration = 14.75 μg kg^{-1}; winter max. concentration = 8.35 μg kg^{-1}). All other pesticides ranged from 0.1 to 0.7 μg kg^{-1}. Heptachlor was absent in winter and δ-HCH and Endrin aldehyde in summer, while Lindane and pp-DDE had higher ($P \leq 0.05$) concentrations in summer. Other residues had similar seasonal concentration levels. The p-DDT was similar as in other biota from the Adriatic or Mediterranean. HCH concentrations corresponded to those found in organisms from the Italian area of the Adriatic. The endocrine and reproductive disrupting potential of the detected OCs are bases for future studies. Results show that morays are a good bioindicator model, with slight interspecies and seasonal variability in OC bioaccumulation as a consequence of diverse food consumption.

Introduction

Persistent organic pollutants, especially organochlorine pesticides (OC), are known to spread globally because of their lipophilic properties and resistance to decomposing. Global monitoring of these xenobiotics is a priority imposed by the Stockholm Convention and other related documents, especially because of their contribution to endocrine and reproductive disruption, and inducement of cancer and other diseases (Tateya et al., 1988; Duffy et al., 2002; Smith and Gangolli, 2002; Porta et al., 2009). Environmental redistribution leads to a progressive transfer of the terrestrial organochlorine load to the sea (Islam and Tanaka, 2004). Once released into the marine environment, these chemicals biomagnify in the food web, thus organisms at higher trophic levels can accumulate significant concentrations.

Researchers have utilized various marine organisms as monitoring species in order to understand the distribution and accumulation characteristics of OC in coastal waters (Yamada et al., 1997; De Metrio et al., 2003; Frignani et al., 2004; Perugini et al., 2004; Stefanelli et al., 2004; Storelli et al., 2004, 2005, 2006a). Benthic organisms and benthic fish are exposed to sedimentary xenobiotics via direct contact with sediments, respiration of interstitial water and incidental ingestion of sediment and other benthic organisms (Storelli et al., 2009; Takeo et al., 2009) and hence lead to a buildup of pollutants in narrow site-specific locations.

Moray eel as an omnivorous generalist top-order predator and semi-sedentary species might represent a good animal model for monitoring pollutant bioaccumulation; hence any screening for pollutants would reflect the pollutant burden composition in the examined location. We found only one report of OC concentrations in a single moray among other species analyzed in the work of Giam et al. (1978). Based on the results of our previous study on the feeding habits of morays (Matić-Skoko et al., 2011), we believe that the age as well as the wide range of benthic organisms devoured by moray eels show the accumulation of significant traces of OC residues. A significant decrease in DDT and OC levels from the 1970s to the present was noted in the Croatian waters of the Adriatic Sea (Picer and Picer, 2003), with the lowest concentrations of OC residues reported at locations in the vicinity of Ploče to Dubrovnik (Čalić et al., 2007). We therefore conducted a site-specific seasonal study with the hypothesis that if we collected seasonally-distinct moray samples from the same unpolluted locality and got similar readings, the species could be considered as a potential candidate for the bioindicator model. By comparing winter and summer captures in an uncontaminated area, we wanted to test such a hypothesis to see if there were consistencies in accumulation of individual OC in the examined species.

Materials and methods

Collecting of fish

Morays were collected in the same location in summer (August 2009) and winter (January 2010) in the Adriatic Sea, near the Elaphite Islands, Dubrovnik, Croatia (lat. 42°45′ 38.8″; long. 17°45′53.6″). Nine fish were caught per each field trip at 5–10 m depths by 200 m longlines for a total of 18 fish (total in summer and winter, N = 18). Fish were caught on the same day in each season to ensure that they were analyzed under approximately the same environmental conditions and that the sample would be uniformly representative. Taking into account the nocturnal habits of the species, the longline hooks were set at 03 : 00 in the morning and collected 2 h later. All fish appeared healthy and very agile (active-aggressive). Each fish was sedated individually for 15 min with MS222 (Sigma) in a separate 100-L plastic barrel in oxygenated seawater (MS222 dose = 250 mg L^{-1}). After sedation, morphometric parameters (BL = body length, BM = body mass, VG = ventral girth, CG = caudal girth) were measured and the moray sacrificed by instant decapitation. Age was estimated by analyzing otholiths of each individual, as described by Matić-Skoko et al. (2011), determined by counting growth rings on the posterior otolith region. Three experienced researchers independently read the otoliths. The index of average percent error (IAPE) (Beamish and Fournier, 1981) was calculated as well as the mean coefficient of variation (CV) (Chang, 1982) to estimate the relative precision between readings. A ring mark was considered to be the outer edge of the opaque zone. Opaque and translucent zones exhibited an alternating pattern. The total number of translucent zones was recorded in order to assign an estimated age to the Mediterranean moray eel specimens. The periodicity of opaque zone formation was examined by the edge-type analysis (Palazón-Fernandez et al., 2010). The marginal increment was measured along the long axis of each otolith (0.01 mm) and used to validate the ageing. Morphometric features and ages of the fish are given in Table 1.

Each fish was measured 10 cm caudally from the anus, and muscle tissue and skin were taken for pesticide detection. Samples were weighed immediately thereafter and frozen at −18°C until processed further in the laboratory.

Pesticide analyses

Each moray eel was examined individually for a total of 18 individual analyses. Requiring 10 g of fish, each individual sample extract was measured, then homogenized; 25 ml of n-Hexane was added to each sample and shaken vigorously. Sodium sulfate (5 g) was added to each sample and centrifuged for 5 min. The extracts were then transferred in flasks and the entire procedure repeated. Both extracts were collected and evaporated by rotary evaporator. A maximum 0.5 g of evaporated samples was transferred in a PTFE test tube to which 3 ml acetronitril : dichlormethan in a 75 : 25 ratio was added. Prepared samples were frozen at −15°C for 1 h. The extracts were centrifuged for 20 min and dried in a stream of nitrogen. The levels of 16 organochlorines were determined using an external standard method on a Shimadzu GC-2010 gas chromatograph with an electron capture detection GC-ECD (Ni^{63}). The separation was performed on a RTX-CL pesticides capillary column (30 m × 0.32 mm ID × 0.5 μm). Nitrogen was used as a carrier gas (minimum purity 99.999%) with a flow rate of 1.4 ml min^{-1}, using a 150°C temp. oven for 1 min and 250°C oven temp. for 15 min at a rate of 10°C min^{-1}. The injection inlet was set at 250°C, ECD detector at 300°C and makeup 30 ml min^{-1}. The volume of the 1 μl of sample was injected by the autosampler AOC-20i in the splitless mode (Stoichev et al., 2005).

Table 1
Biometric parameters and age of moray eels in bioaccumulation study of individual organochlorine residues in summer and winter (2010/2011), Adriatic Sea (Elaphite Islands, near Dubrovnik, Croatia)

Trait		All morays (N = 18)	Summer (N = 9)	Winter (N = 9)
Total length (cm)	Mean	77.1	72.3	82.5
	SD	18.8	13.4	23.2
	Median	70.5	64.7	74.5
	Minimum	53	60.2	53
	Maximum	127.5	93	127.5
Body weight (g)	Mean	1282.7	1296.3	1267.5
	SD	877.4	804.4	1009.7
	Median	927	1043	852.5
	Minimum	264	751	264
	Maximum	3322	3321	3302

	Age distribution					
Number of fish	5 years	6 years	7 years	8 years	9 years	10 years
Summer	3	2	1	0	2	1
Winter	2	4	0		1	2
Total	5	6	1	0	3	3

SD = standard deviation.

Statistical analysis

The computational program STATISTICA 9.1 (Statistica Software, Tulsa, OK) was used to determine descriptive statistics and data analysis. Statistical differences between measurements of various cell sizes were compared by Student's *t*-test, with the statistical significance level set at P ≤ 0.05.

Results

Morphometric data of Muraena helena

A total of 18 *Muraena helena* were captured and analyzed for the presence of organochlorine pesticides. Measured and calculated morphometric parameters are shown in Table 1. There was a significant correlation between body length (BL) and body weight (BW) of the fish (R^2 = 0.796, P = 0.0102). There were no statistically significant differences in morphometric parameters (Table 1) such as in body length and weight between fish caught in the summer (N = 9) or winter (N = 9).

Organochlorine pesticides (OC)

Organochlorine pesticides were detected in all sampled fish, but not all pesticides were present in every fish. Total average concentration of organochlorine pesticides in jointly analyzed seasonal samples was 5.406 ± 4.538 $\mu g\ kg^{-1}$ (range 1.425–18.220 $\mu g\ kg^{-1}$). Total average concentration of organochlorine pesticides in all summer samples was 6.036 ± 5.990 $\mu g\ kg^{-1}$ (range 1.425–18.220 $\mu g\ kg^{-1}$) and in all winter samples 4.776 ± 2.643 $\mu g\ kg^{-1}$ (1.800–9.645 $\mu g\ kg^{-1}$). There was no significant difference (P ≤ 0.05) between the average seasonal concentrations of OC in morays, although the maximal total OC concentrations in a few individuals were higher in summer than in winter (as shown by range), which affected the average seasonal concentration. Figure 1 presents the average concentration percentages of each individual pesticide within these total concentrations in jointly analyzed (Fig. 1a) seasonal samples, in summer (Fig. 1b), as well as in winter samples (Fig. 1c). Generally, the array of detected concentration levels was Endrin > ppDDE > Heptachlor epoxide > Heptachlor > Aldrine > Lindane > *β*HCH > *δ*HCH > Endrin aldehyde. Lindane and pp-DDE were detected in all 18 analyzed fish, followed by Endrin, which was absent in only one fish. Heptachlor epoxide and Aldrine were present in half or more of sampled fish. Endrin with an average concentration of 4 $\mu g\ kg^{-1}$ and approx. 63% proportion (Fig. 1) was the significantly highest (P ≤ 0.05) detected concentration of all analyzed pesticides. All other pesticides ranged from 0.1 to 0.7 $\mu g\ kg^{-1}$. In summer samples of Endrin, maximum concentrations of 14.75 and 10.97 $\mu g\ kg^{-1}$ were recorded in two individuals, which were also the highest detected concentrations of individual pesticides. This was approximately 10- to 14-fold higher than other detected pesticide concentrations; the values in the two samples showed that Endrin was the only pesticide nearing, but not exceeding, the maximal concentrations proposed by the European Union and Republic of Croatia. Endrin did not reach such high concentrations in the winter samples, and did not exceed 8.35 $\mu g\ kg^{-1}$ followed

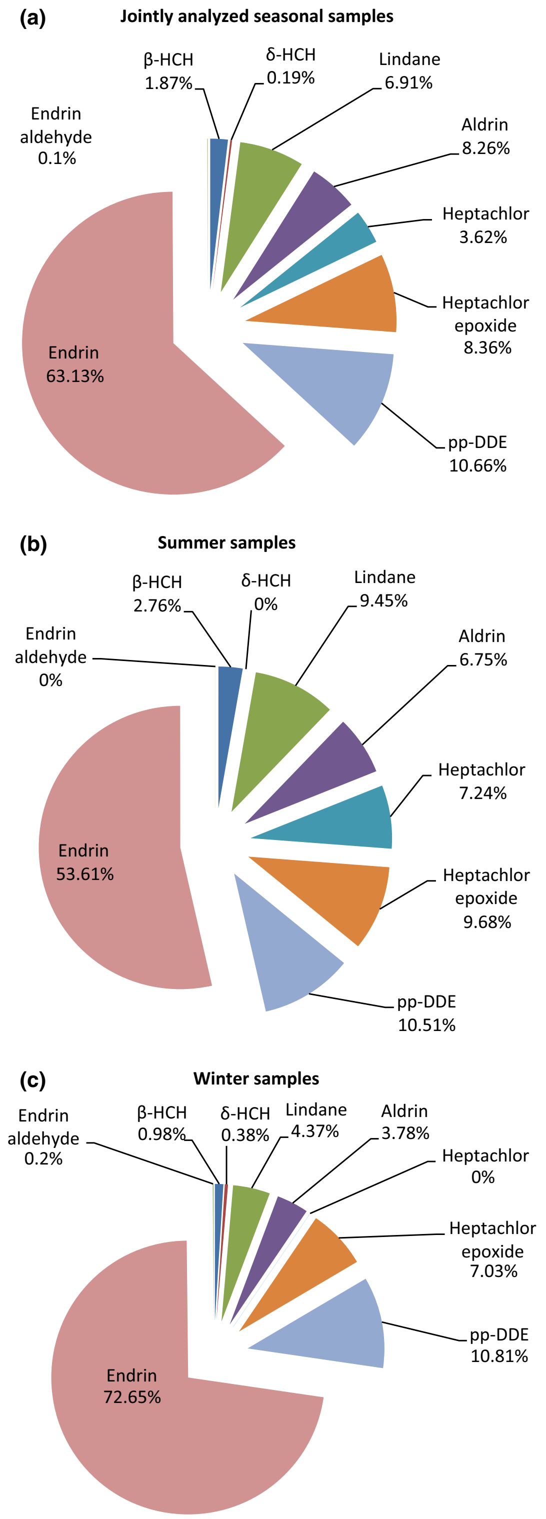

Fig. 1. Average percentage proportion of individual organochlorine residues within total detected concentrations in moray eels in bioaccumulation study of individual organochlorine residues, summer and winter, 2010–2011, Adriatic Sea (Elaphite islands, near Dubrovnik, Croatia). (a) Average percentage of jointly analyzed seasonal samples (N = 18 fish); (b) Average percentage proportion of organochlorine residues, summer (N = 9); (c) Average percentage of organochlorine residues, winter (N = 9)

by 4.65 and 4.78 μg kg^{-1} in three separate individuals, respectively. Regardless of slight differences in maximal concentrations between the summer and winter groups, on average the Endrin concentrations were not significantly different ($P \leq 0.05$) between the two seasons. Although the average concentrations did not differ, it was noted that there was an almost 20% higher proportion of Endrin in winter due to the changes in total OC concentrations and, consequently, the percentages of other pesticides. Similarly, the average level of pp-DDE was different between the two seasons but the percentage proportions were not different (Fig. 1). In third place was Heptachlor epoxide, which was found in higher concentrations and in more analyzed fish than was Heptachlor, which was not detected whatsoever in winter but was significantly higher ($P \leq 0.05$) in summer with an almost double percentage proportion. δ-HCH and Endrin aldehyde were the only pesticides not detected in summer-caught fish. In winter each pesticide was found only individually in two separate fish.

Discussion

The biological life history of moray eels is still relatively unknown and remains to be understood (Đikić et al., 2011; Đikić et al., 2013). To the best of our efforts even the basic data on ecotoxicology of *M. helena* or any reports on this fish species being used as a bioindicator organism could not been found. Since there was no significant statistical difference between morphometric parameters in fish collected during different field trips at the same location (Table 1), a comparison between pesticide concentrations in summer- and winter-caught animals was made. The weight/length range of the sampled fish was in accordance with reports of average weight/length range recorded in the Adriatic Sea; the collected fishes shared morphometric features representative of the Adriatic population (Matić-Skoko et al., 2011). Previous studies on biological indices showed that *M. helena* is a carnivore with a trophic level of 4 and more, which confirms the proposed idea of an appropriate benthic bioindicator model organism. Diet composition includes at least 14 different prey taxa. A benthic life style has an advantage compared to other species such as tuna or swordfish that may be similarly positioned on a high trophic level but are more agile and which tend to bioaccumulate pollutants from larger, mainly pelagic, areas (De Metrio et al., 2003; Stefanelli et al., 2004). Amongst pray taxa of moray, various shellfish – and amongst shellfish the genera *Mytillus* – were broadly represented, which are important ecotoxicology model organisms (Kožul et al., 2009). This allows the study of food web accumulation and comparisons with previous data.

However, the moray is difficult to investigate for it is a species of low population density in a restricted area. A critical point of weakness here is the sample of N = 18 fish total, since we persisted in collecting each sample group under the same conditions. The numbers of fish investigated and the numbers of fish representing distinct age classes (5–10 years) seem not always to be sufficient for statistics. The sample size is small, but knowing the difficulty of collecting this species it is understandable that we had to rely on a relatively small sample size, justified by pesticide analyses where the samples were not pooled and where each moray eel was examined individually, with a total of 18 individual pesticide analyses performed. Until studies are further expanded, the data are of interest as background information for international scientists working in a coastal habitat environmental field.

In a small closed sea such as the Adriatic, many studies have been conducted in near shore and pelagic waters, on sediment and biota, including mollusks and fish from Adriatic coastal areas (Solé et al., 2000; Bayarri et al., 2001; Frignani et al., 2001, 2004; Sekulić et al., 2004; Kljaković-Gašpić et al., 2006; Storelli et al., 2006b; Borrell et al., 2007; Ferrante et al., 2010). Bioaccumulation of organochlorine compounds is governed by the physico-chemical properties of these compounds as well as ecological and biological factors such as trophic position, feeding behaviors, habitat, age, sex, and health as well as the lipid composition of the animal tissue or organ (Gray, 2002; Greco et al., 2010).

The source of residues detected within this work might be explained considering the Adriatic current flow (Artegiani et al., 1997) as the main source of detected xenobiotics pending from the Otranto inflow, the Albanian river systems, the Neretva River and Bosnian and Montenegro tributaries, or remobilization from the north (mainly the River Po) or from the river systems of central Italy. The recorded pollutants have been banned for decades, and bearing in mind the early ban of the pesticides DDT (1980) and Aldrin (1991) in the EU, their concentrations may well be related to a remobilization of the Adriatic sediments (Eggleton and Thomas, 2004).

From the Albanian reports on OC compounds from the years 2002–2005, it seems that this location was partially similar to the composition and pollutant levels measured in morays. The report of Koci et al. (2012) showed detection of hexachlorobenzene (HCB), dieldrin, endrin, hexachlorocyclohexane (HCH and isomers), heptachlor, heptachlor epoxide, and the DDT-related chemicals (o,p-DDE, p,p-DDE, p,p-DDD, p,p-DDT), which were found in our samples, but with no methoxychlor or mirex found in the Albanian studies and none detected in the moray.

Similarly, studies from the North African and Turkish coasts recorded that OC in the biota are of a somewhat different composition, thus this excludes the presumption that the pollutant levels are the result of the Otranto currents or a remobilization of Mediterranean concentrations (Barakat et al., 2002; Kucuksezgin et al., 2006; Fouial-Djebbar et al., 2010).

A comparison of analyzed biota records from the Italian side of the Adriatic (De Metrio et al., 2003; Perugini et al., 2004; Stefanelli et al., 2004; Storelli et al., 2004, 2005, 2006a, b; Storelli et al., 2009 Borrell et al., 2007) showed similar compositions of detected pollutants as in our samples where the most dominant residues in various fish and other biota were pp-DDE (a metabolite of DDT) and HCH; both were found in similar or higher concentrations than those recorded in the analyzed moray eels. Interestingly, Aldrin, Endrin, Heptachlor epoxide and Heptachlor were not detected in fish tissues from the Italian side of the Adriatic, whereas they were found in the moray eels in low, but detectable, concentrations. Most probably the source of pollutants recorded in the moray is the surrounding inland of Bosnia and the Neretva River or the industry in Kaštela Bay near Split. In addition, Picer and Picer (2003), Milun et al. (2007), and Milun and Zvonarić (2008) reported that during recent warfare, the karstic area of Croatia, Bosnia and Herzegovina, and Kosovo was jeopardized by hazardous waste. In the Neretva River delta, situated at the shore part of Croatia, there is intensive agricultural production, possibly explaining the offshore source and transport of contaminants from the past presumably when OC pesticides were still in use. Similarly, the Kaštela Bay (Split) in the middle Adriatic included a chlor-alkali industrial unit up until the 1990s Picer (2000). Thus this might be responsible for the HCB, heptachlor and heptachlor epoxide, detected in industrial OC concentrations. We can conclusively say that our analyses showed not one single fish free of OC, regardless of the season. The detection levels were lower than in most reports on Mediterranean or Adriatic fish. Some of the detected compounds were endocrine and reproductive disruptors but their influence on reproductive physiology of the Adriatic moray population remains to be determined in future studies. We can conclude that moray eels as a bioindicator organism are positive in that they are large enough for extensive sample preparation and analysis; however, researchers should anticipate problems with acquiring enough samplings. Researchers should account for the high interspecies variability in the bioaccumulation potential of different compounds and slight seasonal differences, a result of diverse food consumption between and among individuals and seasons (Endrin, Aldrin, δ-HCH and Endrin aldehyde in this work changed with the season). This work adjoins the systematic and ongoing data collection and analyses of OC substances in the Croatian part of the Adriatic Sea (Croatia, 2005; Second, Third and Fourth National Communication of the Republic of Croatia under the United Nations Framework Convention on Climate Change, 2006; Republic of Croatia Ministry of environmental protection, physical planning and construction, 2009) and global monitoring of biogeochemical cycles of examined pollutants prescribed by Stockholm convention. It is estimated that in the next decades, the marine pollutant loads of organochlorine compounds will continue to increase in many areas, even if no new input occurs; closed seas such as the Adriatic are especially threatened (Tateya et al., 1988; Borrell et al., 2007). Biomonitoring of deposited pollutants in the Adriatic from both Italian and Croatian sides must be continuous and more frequent. This work is the first to draw attention to moray eels as being possible excellent bioindicator organisms in the studies of bioaccumulation trophic levels.

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