Cod: 4.3016

NDL-PCB LEVELS IN MOLLUSCS FROM MIDDLE ADRIATIC SEA (ITALY) AND TIME TREND IN MYTILUS GALLOPROVINCIALIS

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Introduction

Molluscs harvesting is a relevant commercial activity along the Adriatic Sea coasts. Mussels (Mytilus galloprovincialis), clams (Chamelea gallina), mutable nassa (Nassa mutabilis) and spiny dye-murex (Bolinus brandaris) are among the most consumed species. Because of their filter feeding and slow rate of detoxification, molluscs can accumulate many toxic contaminants. Laboratories in charged of official control are requested to monitor the presence of contaminants in molluscs and check the compliance to regulatory limits (1,2). Since 2011, when maximum limits were set for the sum of the six non-dioxin like Polychlorinated Biphenyls (ΣPCBs) identified as contamination indicators (PCB-28, -52, -101, -138, -153, -180) (3), these contaminants are regularly monitored in molluscs collected along the Marche coast in the frame of official sampling plans. This study reports the analytical results obtained in 2013-2016.
Since Mytilus Galloprovincialis is a sessile specie, they are often used as sentinel organisms for environmental pollution monitoring, therefore the investigation of PCBs in these organisms provides information on both the consumers' exposure and the contamination of the marine environment. Monitoring of mussels along the Marche coast was already undergone more than ten yers ago in the frame of a research project funded by the Italian Ministry of Health (4). The comparison of these data with the levels measured in 2013-2016 enables temporal trends evaluations on ndl-PCBs contamination. The present study reports the mean values measured for the sum of the six ndl-PCBs in different molluscs species collected along Marche seacoast and the comparison between the contamination figures measured in 2003-2004 and the ones determinated more than ten years later (2013-2016) in Mytilus galloprovincialis.

Materials and methods

Sampling
Fiftyeight samples of molluscs were harvested between november 2013 and may 2016 as follow: 16 clams, 8 mutable nassa, 8 spiny dye-murex and 26 mussels. All the samples were collected in classified areas or breeding plants along the Marche coast (Central Adriatic Sea, Figure 1) .
The bivalve molluscs were opened, the sand and solid residues removed under running water, the gastropods were rinsed with water and the the molluscs removed from the shells (200 g), drained, homogenized and stocked at–20°C.

Analytical method
The analytical method was already described elsewhere (5). Briefly, after the addition to the six $^{13}C_{12}$-ndl-PCBs analogues, 25g of sample were freeze-dryed, extracted in ASE (Accelerated Solvent Extractor) and cleaned-up on an Extrelut NT3 column acidified with sulfuric acid and sequentially connected on top of a 1g/6mL silica SPE column. The chromatographic separation was achieved on a SGE-HT8 PCB capillary column (60m x 0.25mm x 0.25μm) by a GC-MS/MS Triple Quad (7890A and 7000 Triple Quad, Agilent Technologies).

Statistical analysis.
The ΣPCBs results were analyzed using one-way ANOVA. Since the data obtained in 2003-2004 are not normally distributed, to study the difference in contamination levels between the mussels (Mytilus galloprovincialis) sampled in 2003-2004 and in 2013-2016, the non-parametric Wilcoxon rank-sum statistical method was used. The softwares employed were Stata® 11.1 and Arcgis® 10.3.

Results and discussion

Table 1 shows the levels measured for the upper-bound sums of the six indicator PCBs for all the species considered in the two time periods 2003-2004 and 2013-2016. The ΣPCB means measured in 2013-2016 in the four species and compared by one-way ANOVA shows a significant difference among species ($F(3,104)=13.85$, $p=0.000$) (Figure 2). The mussels are the most contaminated (3.12 ng/g) while the clams the lowest (1.01 ng/g).
In Chamelea gallina the levels range from 0.22 to 2.24 ng/g. The results are comparable with the ones obtained from Visciano et al. (6) in the Abruzzi Region (Italy): they measure a mean value of 2.10 ng/g for the sum of the six ndl-PCB in all the samples analysed. The gastropods mean concentration found is 1.44 (min 0.66 – max 2.38 ng/g) and 2.33 ng/g (min 1.04 – max 3.49 ng/g) for Nassa mutabilis and Bolinus brandaris respectively. Giandomenico et al. in 2015 (7) reported PCB levels two order of magnitude higher in banded dye-murex (Hexaples trunculus) collected in Mar Piccolo (Taranto, Italy), with a mean of 247.7 ng/g (min 181.4 – max 304.6 ng/g). The higher ΣPCB levels were measured in mussels (Mytilus galloprovincialis) with concentrations from 1.61 to 5.56 (mean 3.12) ng/g. A study on #ΣPCB contamination in mussels bred on the Croatian coast of the Adriatic sea reported values between 0.88 and 17.54 ng/g (8); the maximum level reported is surely significantly higher than the level measured in the present study. The Giandomenico et al. paper (7) reports also the #ΣPCB levels in mussels collected from Mar Piccolo (Taranto, Italy); they report a mean of 89.3 ng/g (min. 61.5 and max. 138.7 ng/g). The #ΣPCB found in the present study in mussels and gastropods seem well below the concentration measured in the area of Taranto (Italy) which is, thereafter, well known as a specific contaminated zone because of the presence of an important industrial district. The levels in mussels are also lower than the ones measured in some breeding areas located on the Croatian Adriatic coast.
The box and whisker plot in Figure 3 represents the mean #ΣPCB in mussels (Mytilus galloprovincialis), for the two time periods and it shows a significant higher level in 2003-2004 respect to 2013-2016 (Wilcoxon rank-sum: $z = 4.993$ $p= 0.0000$). Therefore a contamination decreasing time trend is highlighted comparing the two group of results. Trying to understand if there are coastal areas more contaminated than others, all the Mytilus galloprovincialis sampling areas were put together in three geographical groups (North, Centre and South) and compared. In the box and whisker plot in Figure 4 the different contamination levels in North Center and South mussels production areas are shown; testing the data by one-way ANOVA, no statistical differences were found between the three groups ($F(2.23)=0.76$, $p=0.47$), therefore the PCB contamination is homogeneously distributed along the coast

Conclusions

More data on the PCB contamination of molluscs other than mussels should be produced to have a better description of the levels. Also in litterature very few are the information on PCBs in gastropods. Surely the decreasing mussels contamination time trend is an interesting issue but it needs further investigation also in other species.

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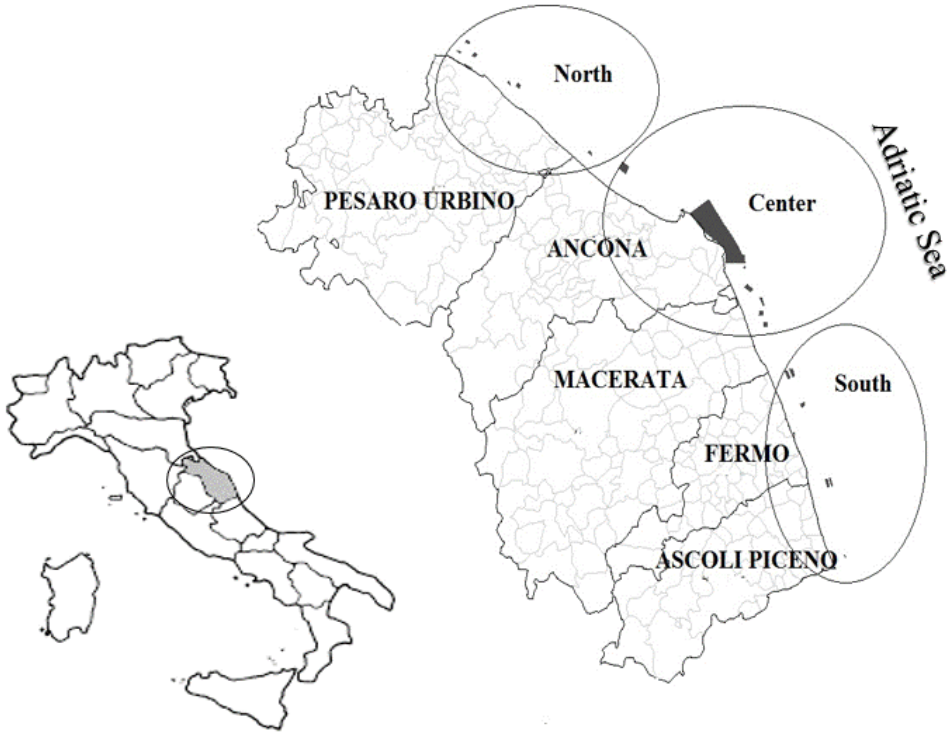

Figure 1: Sampling sites

Table 1: Σ6-ndl-PCB levels in all the species in the two time periods (2003-2004 and 2013-2016)

Species	N°	ng/g wet/weight							
		mean	sd	median	min	max	p25	p75	iqr
2003-2004									
Mytilus galloprovincialis	50	5.12	2.93	4.91	1.25	23.5	4.18	5.51	1.33
2013-2016									
Mytilus galloprovincialis	26	3.12	0.99	3.05	1.61	5.56	2.51	3.61	1.10
Chamelea gallina	16	1.01	0.54	0.86	0.22	2.24	0.64	1.39	0.75
Bolinus brandaris	8	2.33	0.87	2.38	1.04	3.49	1.66	3.01	1.35
Nassa mutabilis	8	1.44	0.66	1.40	0.66	2.38	0.89	1.97	1.08

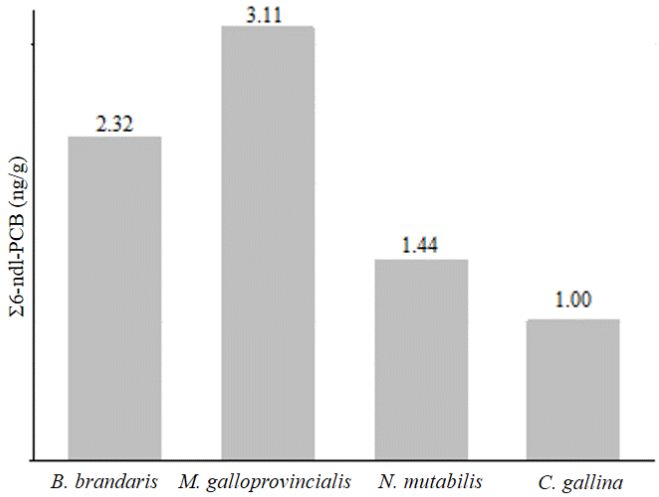

Figure 2: Σ6-ndl-PCB mean concentration bar graph for the four species (2013-2014)

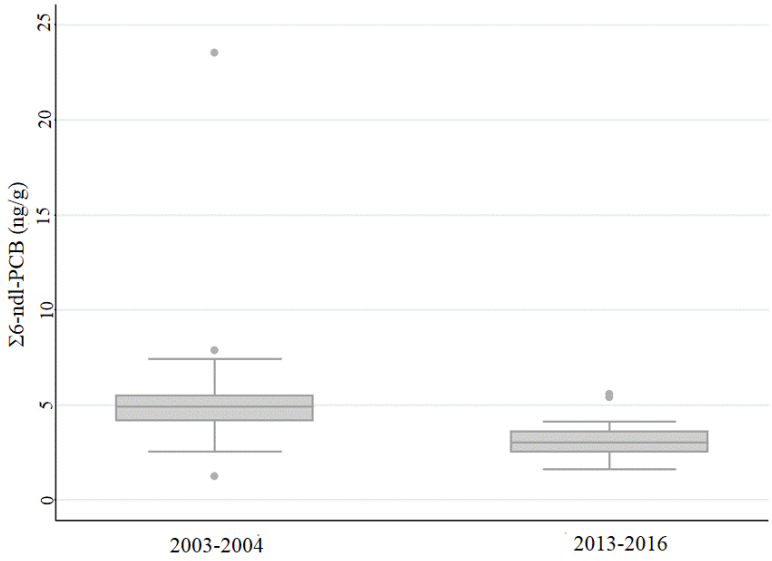

Figure 3- Box and whisker plot describing the PCB concentrations in *M. galloprovincialis* from Marche region: comparison of the two time periods 2003-2004 and 2013-2016.

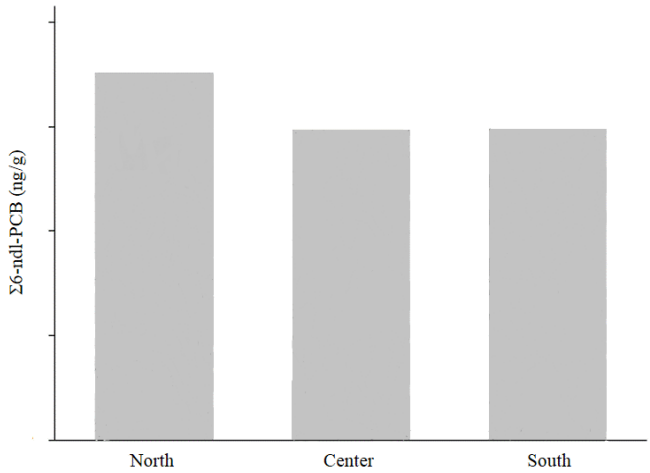

Figure 4: Bar graph describing the PCB concentrations in *M. galloprovincialis* from North, Centre and South coastal areas