DIOXIN ACCUMULATION IN DEEP-SEA MEGAFAUNA (BLANES SUBMARINE CANYON, NW MEDITERRANEAN)

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Introduction

A preliminary study dealing about the accumulation of dioxins in the deep-sea rose shrimp *Aristeus antennatus* showed that this accumulation was observed either close to the coast line and as deep as 2500 m from the Western to the Eastern Mediterranean Sea[1]. Moreover, it is accepted that deep-sea canyons accumulate continental sediments and the anthropogenic contamination associated to them.
The objective of the present study was to evaluate the effect of canyon structure in dioxin accumulation in crustacean and fish species and relate them with the habits of these species (pelagic, nektobenthic and benthic).

Materials and methods

Three species of crustaceans (*Phasiphaea multidentata* – pelagic; *Aristeus antennatus* – nektobenthic, i.e. benthic species with swimming abilities; *Polycheles typhlops* – benthic) and three of fishes (*Lampanyctus crocodilus* – pelagic; *Phycis blennoides* – nektobenthic; and two flat fish species of the genus *Symphurus*, *S. nigrescens and S. ligulatus* – benthic) were caught with bottom trawl commercial vessels landing in Blanes harbour (NW Mediterranean Sea). Two fishing grounds were selected: the first one at the canyon head at 600 m deep (with geomorphological connection to the Tordera River), called Rocassa, and the second one, at the NE on the open slope (outside the canyon influence) at 900 m deep, called Barana.
Twenty individuals for each species were collected in order to have enough quantity of sample for analyzing dioxins and to minimize individual variability. Once at the laboratory, the non-edible parts of fish were removed and the muscle meat, skin excluded, was freeze-dried and re-homogenized as a pretreatment step to the extraction of the analytes[2]. On the contrary, crustaceans were kept entire to have enough quantity to analyze dioxins.
For PCDD/F analysis, samples were extracted in a Soxhlet for ~24h with toluene:cyclohexane (1:1) after being spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS, Wellington Lab., Guelp, Canada). Next, the extracts were rotary evaporated and kept in the oven overnight (105 ºC) in order to eliminate the solvents prior to gravimetrical fat determination. Afterwards, fat residues were dissolved again in *n*-hexane. Organic components, fat and other interfering substances were removed by treating the *n*-hexane extracts with silica gel modified with sulphuric acid (44%). The extracts were then rotary concentrated and filtered prior to the next clean-up step. Further sample purification and instrumental analysis by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) are described elsewhere[3]. All analyses were performed on a 6890N Network GC System Agilent gas chromatograph (Agilent Technologies Inc., Palo Alto, USA) fitted with a DB-5 ms fused silica column (J&W Scientific, Folsom, USA) and connected through a heated transfer line kept at 280 ºC to an Auto-Spec Ultima NT high resolution mass spectrometer with an EBE geometry (Waters, Manchester, UK).

Results and discussion

Concentrations of PCDD/Fs, in terms of sum of the individual concentrations of the 17 toxic congeners, as well as the total WHO-TEQs, are shown in Table 1. For PCDD/Fs, the highest levels were found in crustaceans with total values ranging from 3.5 to 23.05 pg/g fresh weight (fw), while fish species showed lower levels (values ranging from

1.15 to 3.39 pg/g fw). In addition, in terms of total WHO-TEQ, crustaceans also presented the highest values expressed in fresh weight.

No significant differences were observed on dioxin accumulation for a determined species between the two areas studied in the continental margin off Blanes. Only *P. multidentata* presented lower levels of dioxins, expressed as lipid weight (lw), in canyon head ground when compared to open slope ground, but this is related to the different percentage of fat found in each area for this particular crustacean species (i.e. 4% (Rocassa) vs 1.7% (Barana)). In addition, species habits do not seem to have a clear influence on dioxin accumulation, and opposite to what was expected, a benthic species such as *P. typhlops* presented lower dioxin accumulation, even though it has feeding habits close related in the sediment. On the other hand, the nektobenthic commercial crustacean species *A. antennatus* presented the highest level of dioxins among the studied species in the canyon ground, either referred as pg/g fw or pg/g lw.

Besides fishing ground and species habits, dioxin content might also be related to trophic level of each singular species. Table 2 shows the mean trophic level values calculated after their content on $\delta^{15}N$ (white muscle for fishes and whole organisms for crustacean) after Polunin *et al.* (2001)[4] and Stergiou and Karpouzi (2002)[5]. In this sense, the nektobenthic fish *P. blennoides,* that has the highest trophic level among the fish species, do not show a high dioxin accumulation, in terms of pg/g fw or pg WHO-TEQ/g fw. However, when the results are expressed in lipid basis, the accumulation rate increased up to levels equal or higher to those of the other fishes due to the lower percentage of fat in this species. In the case of crustacean, also the nektobenthic species *A. antennatus* has the highest trophic level among the three species and showed the highest dioxin accumulation independently of the way the results are expressed.

An interesting finding is that the three crustacean species considered in this study presented accumulation of toxic dioxins as well as non-toxic dioxins, whereas the fishes only presented toxic congeners. As an example, Figure 1 shows the HRGC-HRMS chromatograms of a crustacean sample (*P. multidentada*) and a fish sample (*L. crocodilus*). The particular dioxin fingerprint in crustacean is very unusual, since only 2,3,7,8-substituted PCDD/Fs are normally accumulated in biotic samples. However, the pattern is comparable to that found in the case of bivalve samples reported by Abad et al. (2003)[6]. For the case of the toxic congeners, the distribution profile in crustacean and fish samples was characterized by an important contribution from 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and OCDD. In all cases, OCDD is the main congener that contributes to the total profile in all crustaceans and fish samples (30-64%), followed by TCDF and PeCDF (Figure 2). When the data are expressed in WHO-TEQs, the major contribution comes from the 2,3,4,7,8-PeCDF and the 1,2,3,7,8-PeCDD, followed by 2,3,7,8-TCDF, with the rest of the isomers remaining in a minor proportion (Figure 3).

References

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