



Biliary PAHs metabolites in red mullet (*Mullus barbatus*) from Spanish Mediterranean coast

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants present in marine environment as a consequence of their continuous input from either land- or marine-based sources. Fishes have a higher capacity to metabolize and excrete PAHs than invertebrates such as mollusks, therefore the accumulation of PAHs metabolites in their bile fluid is used to assess the environmental fish exposure to the parent compounds (Porte et al., 2002; Martínez-Gómez et al., 2013). In this study the bile concentration of PAHs metabolites in red mullet (*Mullus barbatus*) from 10 areas from the Spanish Mediterranean coast were characterized (Barcelona, Ebro Delta, Tarragona, Mallorca, Valencia, Santa Pola, Cartagena, Almería, Castell de Ferro and Málaga).

MATERIAL AND METHODS

STUDY AREAS AND SAMPLING PROCEDURE

SAMPLING:

Red mullets from 12 to 18 cm were sampled in autumn 2012, 2013 and 2014 (12 specimens per area, except in Valencia).

2012: Barcelona, Delta del Ebro, Palma de Mallorca, Santa Pola and Valencia

2013: Cabo de Palos, Cartagena, Almería, Málaga and Castell de Ferro

2014: Barcelona and Tarragona

Bile samples were treated individually and maintained at -20°C until analysis.



PAHs METABOLITES ANALYSIS

10 µL of red mullet bile was diluted with bidistilled water, incubated with β-glucuronidase/arylsulfatase for 2 hours at 37°C, then the reaction was stopped with cold methanol and the sample centrifuged at 10000 rpm for 10 minutes (Beyer et al., 2010).

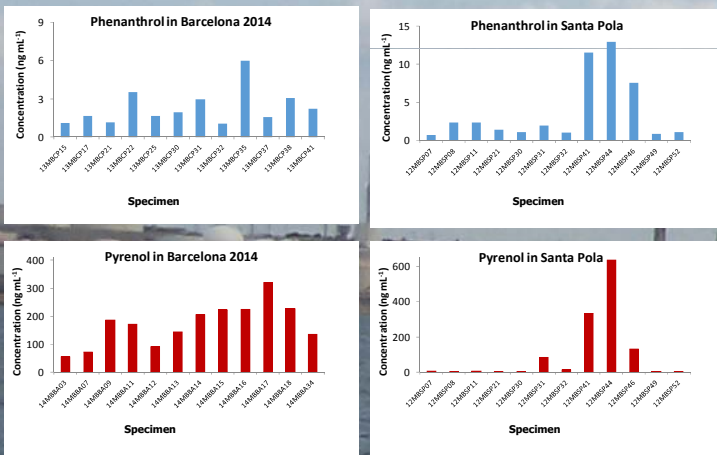
Phenanthrol and pyrenol were determined by HPLC with fluorescence detection using specific λ_{excitation} and λ_{emission} for each PAH metabolite.

m-Chryseno was used as internal standard.

Limits of quantification (LOQ) were 5.4 ng g⁻¹ and 0.1 ng g⁻¹ for phenanthrol and pyrenol respectively.

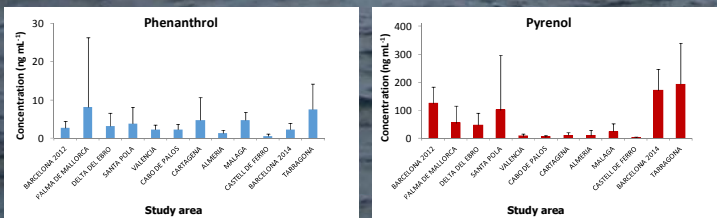
RESULTS AND DISCUSSION

PAHs METABOLITE CONCENTRATIONS



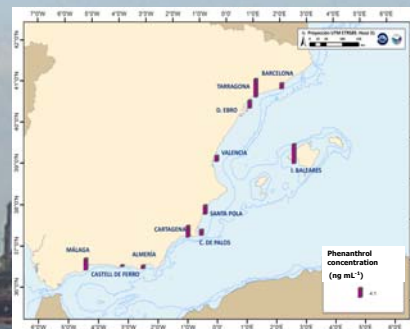
Phenanthrol and pyrenol were found in all bile samples and showed a high variability between specimens and areas.

Phenanthrol and pyrenol concentrations ranged between non detected and 65 ng/mL bile (Palma de Mallorca) and LOQ and 635 ng/mL bile (Santa Pola), respectively.



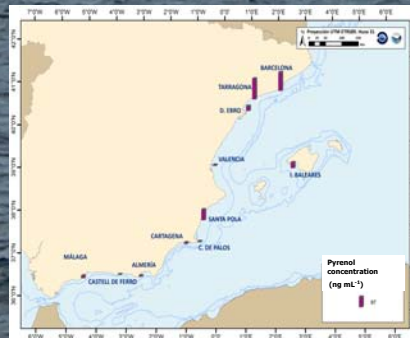
The concentration variability between different specimens of one area (and between areas were high (high standard deviations).

SPATIAL DISTRIBUTION IN MEDITERRANEAN COAST



Pyrenol was the predominant metabolite and showed the highest concentration in all specimens and areas.

The highest mean concentrations of pyrenol were found in Barcelona and Tarragona, being significantly higher (p < 0.05) than in the rest of study areas. Those results agree with the previous ones obtained by Martínez-Gómez et al., (2013), which detected maximum values in Barcelona too. On the other hand the lowest pyrenol concentrations were found in Castell de Ferro (Granada).



The pyrenol concentrations were similar to the detected ones in red mullet from other European areas (Porte et al., 2002).

PAHs concentrations in muscle showed low variation between areas (León et al., 2014), therefore PAHs metabolites in bile discriminate better the exposure and effects of the PAH sources than PAHs in muscle.

CONCLUSIONS

Phenanthrol and pyrenol were the major PAH metabolites detected in red mullet bile, and their concentrations were analyzed by liquid chromatography with fluorescence detection using a standard solution for external calibration. Phenanthrol and pyrenol were found in all samples, with pyrenol reaching the highest concentrations. The metabolite concentrations were higher in specimens sampled close to Barcelona than in the rest areas. In contrast, the minimum values were found in Castell de Ferro. These results showed that accumulation of PAHs metabolites was related with the human pressure. Phenanthrol and pyrenol concentrations in red mullet bile clearly discriminated environmental fish exposition to PAHs between areas. Results obtained in the reference site Castell de Ferro can be used for Background concentrations in this species for the Mediterranean Region, although additional data will be required to get that reference values.

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