



Baseline

Levels of chemicals in two fish species from four Italian fishing areas

Cristiana Guerranti^{a,*}, Eleonora Grazioli^a, Silvano Focardi^b, Monia Renzi^{a,c}, Guido Perra^b^a Bioscience Research Center, Via Aurelia Vecchia, 32, 58015 Orbetello, GR, Italy^b Department of Physical, Earth and Environmental Sciences, University of Siena, Via Mattioli, 4, 53100 Siena, Italy^c CoNISMa, Consorzio Nazionale Interuniversitario delle Scienze per il Mare, Piazzale Flaminio, 9, 00192 Rome, Italy

ARTICLE INFO

Article history:

Received 29 April 2016

Received in revised form 1 July 2016

Accepted 2 July 2016

Available online 14 July 2016

Keywords:

Red mullet

Anchovy

Protected area

PCBs

PAHs

Oil extraction areas

ABSTRACT

This paper reports the results of an assessment of contamination by polychlorinated biphenyls and polycyclic aromatic hydrocarbons in two demersal species of commercial importance. The organisms considered were *Mullus barbatus* and *Engraulis encrasicolus* from three marine areas off the Italian coasts: North and South Adriatic Sea and North Tyrrhenian Sea. The instrumental analyzes have revealed, in many samples examined, the presence of most of PCB congeners and 5 of the 16 PAHs considered. Organisms caught in the waters of Apulia have greater contamination values, while the samples from Tuscany showed the lowest ones, results probably referable to the environmental quality differences between the areas of origin, at least for PAHs, since the sampling areas are represented respectively by areas with presence of oil extraction plant (Adriatic), and by an area subject to environmental protection.

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Pollution by certain classes of toxic, lipophilic and persistent molecules is widely recognized as one of the main factors that impact on environmental quality and on the health of fish stocks, also affecting consumers' health. The most commonly caught species in Mediterranean Sea are Osteichthyes, which accounted for 40 species of which >50% is commercially important, including *Mullus barbatus* (Linnaeus, 1758) and *Engraulis encrasicolus* (Linnaeus, 1758) (Osio et al., 2015). The economic performance of these activities is threatened since European fisheries have the 88% of the stocks declared overfished (Osio et al., 2015) furthermore the marine pollution due to strong human activities contributes to worsen the overall condition. Persistent organic pollutants (POPs) have physic-chemical proprieties that may favour atmospheric transport to other areas and may participate in exchange or deposition mechanisms for the inputs to open waters and coastal areas of the sea (Baek et al., 1991). For what concerns polycyclic aromatic hydrocarbons (PAHs), local sources of emission can be represented by refineries and fossil fuels extraction plants (Yassaa and Cecinato, 2005).

In this general condition, our study aims to investigate the state of contamination by POPs, considering polychlorinated biphenyls (PCBs) PAHs of *M. barbatus* and *E. encrasicolus* from the northern to the southern Adriatic Sea and in North Tyrrhenian Sea, both to assess the health status of the fish resource and considering such organisms as bioindicators.

Two sampling campaigns were carried out, one in late spring and one in autumn in four distinct areas from the northern to the southern

Adriatic Sea and in North Tyrrhenian Sea (Fig. 1). From an environmental point of view, there are differences between the Tuscany area and the Adriatic areas, especially with regard to potential sources of pollution by hydrocarbons: in the Adriatic, in fact, extraction plants of fossil fuels are present, while the sea facing the Tuscan coast includes protected areas Tuscan Archipelago National Park and Sanctuary of Cetaceans.

The organisms were caught, through nets, from professional fishing boats. *E. encrasicolus* sampled specimens ranged 2.10–24.10 g in weight and 7–16 cm in length, while *M. barbatus* 55.03–74.02 g and 7–19 cm. The tissue chosen was liver, the main site of accumulation of the contaminants of interest. The minimum quantity of tissue needed for chemical analysis has been a constraint, since 10–15 specimens of *E. encrasicolus* and 6–8 *M. barbatus* specimens were necessary to constitute an analyzable pool (3 g).

A total of 77 pools were analyzed. PAHs considered were the 16 compounds indicated by the US-EPA ClearWater Act, while PCB congeners considered were the following IUPAC numbers: 70-76-95-60-56-101-99-110-151-123-144-135-118-149-114-146-153-105-141-176-137-138-158-178-187-183-167-128-185-174-177-156-100-71-200-257-172-180-199-170-196-201-189-195-207-194-205-206.

Purification and separation of PCBs and PAHs was performed according to the procedure described by Cousins and Jones (1998). PCB analyses followed the procedures and details reported by Guerranti and Focardi (2011). PCB congeners were then confirmed using a GC/MS (ion trap mass detector: ThermoFinnigan, Trace™ GC 2000/GCQ Plus) with a RTX-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm; Resteck) using splitless injection mode and helium as carrier gas. The

* Corresponding author.

E-mail address: cristiana.guerranti@bsrc.it (C. Guerranti).



Fig. 1. Sampling sites (Google Maps).

limits of detection (LOD) of the compounds were estimated as 3σ (IUPAC criterion) and was 0.01 ng/g for each congener. PAHs were quantified as in a previous study (De Sanctis et al., 2013). Quantitative analysis was done against a three-point linear calibration of PAH solution, obtained by dilution of the certified standard mixture TLC 16-PAH mix (Supelco). Satisfactory linearity was obtained, with values of the correlation coefficient R above 0.99. Detection limit, estimated as 3σ (IUPAC criterion) for each PAH compound was 0.5 ng/g.

Procedural blanks, prepared by the same procedure used for the samples, was included every five samples and results were blank corrected. Blanks and replicate samples were used as quality control procedures, and their reproducibility and recovery were high (>85%) for both classes of analytes. The accuracy of the analytical procedure was determined by analysis of certified materials from the National Institute of Standards and Technology, US Department of Commerce (Gaithersburg, MD, USA), the National Council of Canada, Institute for National Measurement Standards, and the Community Bureau of Reference. The following compounds were determined in the following materials: PCBs in cod liver oil (NIST), PCBs in carp muscle (CARP-1, NRC), PCBs in pig fat (ERM-IRMM) and PAHs in harbour sediments (HS-6 NRC). Recovery was >85% in all cases. Recoveries were also evaluated by the method of additions at the time of extraction, using homogeneous replicates and were always >90%.

Statistical analysis was performed using Kruskal-Wallis one-way ANOVA by ranks, Wilcoxon-Mann-Whitney test, and Spearman's correlation coefficient (r_s). A probability level of <0.05 was considered significant. Averages and standard deviations were calculated assuming values of zero for the compounds below the LOD (lower bound).

The analyses have revealed, in many samples examined, the presence of most of PCB congeners and 5 of the 16 PAHs considered. Data obtained from tissue analysis are summarized in Table 1. The results for *Engraulis encrasicolus* samples represent the first data on contamination by PCBs and PAHs measured in the liver of this organism. The results for North Tyrrhenian are the first data on the concentrations of PCBs in red mullet, as well as for the anchovies. Organisms caught in the waters of Apulia reported the greatest contamination values, while these from Tuscany showed the lowest ones. The results of PCBs showed an upward trend from north to south Adriatic for both species, while the lowest recorded are those of samples from North Tyrrhenian. This trend is valid for both species and in both samplings carried out, although in the latter the recorded values are much lower than in the first. PAH values obtained are similar from North to South although in the first sampling recording a peak for anchovies from Apulia's coasts. Organisms caught in the period May–June showed higher liver contaminant concentrations of those caught in October: this could be due to the incidence of reproductive stage in the spring, resulting in an increase of food

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