BIOACCUMULATION AND BIOMARKER RESPONSES TO ORGANOCHLORINES, POLYCYCLIC AROMATIC HYDROCARBONS AND TRACE METALS IN ADRIATIC SEA FISH FAUNA

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Abstract

The primary aim of our study was set up the current knowledge of the environmental quality and the state of health of a great impacted basin, such as the Adriatic Sea, by an evaluation of the "state of chemical stress" of its fish communities. Bioaccumulation of trace metals (Hg, Pb, Cd, Zn, Cu, Fe) and of persistent contaminants such as polychlorobyphenyls (PCBs), dichlorodiphenyltrichloro-ethan (DDT), hexachlorobenzene (HCB), polycyclic aromatic hydrocarbons (PAHs) were investigated in liver samples of three fish species : *Merluccius merluccius, Sprattus sprattus* and *Sardina pilchardus* were collected in three different sites of north Adriatic Sea. A suite of biochemical biomarkers such as the induction of 7-ethoxyresorufin-O-deethylase (EROD), benzo(a)pyrene monooxygenase (BPMO) activities and metabolic intermediates (porphyrins associated with haem synthesis) were measured in the liver of fish samples in order to determine their environmental exposure to xenobiotic compounds. Preliminary results are : trace metal levels were higher in sprat than in whiting; levels of organochlorines and polycyclic aromatic hydrocarbons compounds were higher in whiting than in sprat and pilchard. These results were compared with biomarker responses on CYT. P-4501A activities were detected both in whiting and pilchard but absent in sprat. The highest EROD activity was recorded in whiting and BPMO activity in pilchard.

Key-words: Adriatic Sea, bio-indicators, chlorinated hydrocarbons, metals, PAH.

Introduction

The specific aim of this research was to investigate environmental quality and the health status of this basin by evaluating the state of chemical stress of its fish communities. The Adriatic Sea has been the subject of attention because of the periodic occurrence of degenerative phenomena, due partly to its structural and oceanographic characteristics and to human impact. Evaluation of the effects of pollution on marine communities is essential to safeguard the marine environment.

The objective of the present study was to assess the presence of contaminants of different types (trace metals, PCBs, DDT, HCB and PAHs) and their impact and/or toxic effects on specimens of certain fish communities regarded as being a target. This evaluation was done by a biomonitoring programme based on residue analysis of these contaminants and on the assessment of CYT. P4501A activity, determined by 7-ethoxyresorufin-O-deethylase (EROD) and benzo(a)pyrene monooxygenase (BPMO) assays, which is commonly used as a biochemical biomarker of exposure. A biomarker is "a change induced by a contaminant in a biochemical or cellular component of a process, structure or function that can be measured in a biological system" (1). The specificity and sensitivity of biomarker responses combined with data on accumulation levels could make it possible to determine dose-effect relationships for compounds present in the environment, in this case the Adriatic Sea. These contaminants are known to induce detoxification systems and may therefore be responsible for a toxic effect in organisms (2). Comparison of tissue concentrations of these compounds, levels of enzyme induction of CYT P-4501A activity and accumulation of metabolites in these fish could confirm that given environmental concentrations of these contaminants cause changes in biological functions and, in severe cases, damage to organisms and communities (3).

Materials and Methods

The first stage of our biomonitoring programme was carried out at three sites in the northern Adriatic Sea : 51 near the Lagoon of Venice, 50 further north at the limits of territorial waters, and 156 off the coast of Venetum (Fig. 1). Specimens of *Merluccius merluccius* and *Sprattus sprattus* were captured in December 1996 in site 50 and site 51 and stored on ice and frozen at -20°C on reaching the laboratory. Some specimens of both species were dissected on the spot and their livers placed in liquid nitrogen and stored at -80°C in the laboratory. Specimens of *Sardina pilchardus* were captured in March 1997 in site 156 and placed whole in liquid nitrogen. The following analyses were performed on individual liver samples of all species: trace elements (Hg, Cd, Pb, Zn, Fe, Cu) by plasma emission spectrometry according to the method of Broekaert (4) and mercury by flow injection mercury system by the method of Stoeppler and Backhaus (5). Blanks and standard reference materials (SRM n°1566a "oyster tissue" supply by U.S. Department of Commerce National Bureau of Standards Gaithersburg, USA) were analysed in each batch of samples.

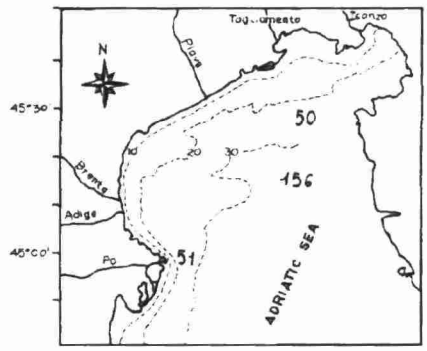

Fig. 1. Map of North Adriatic Sea with the locations of the sampling sites.

Total and coplanar PCBs, HCB, DDT were extracted and cleaned up from individual liver samples by the method of alkaline-alcohol digestion (6). Analysis was performed with a Perkin-Elmer Autosystem model gas chromatography equipped with Ni^{63} electron capture detector and type SBP-5 (Supelco) bonded-phase, fused silica capillary columns. Pure reference standard solution (Aroclor 1260 for total PCBs supply by Supelco Inc., PCB coplanars non-*ortho* substituted as PCB-77, PCB-126, PCB-169 supply by Dr. Ehrenstorfer GmbH, DDT and HCB supply by Supelco Inc.) were used for determination, quantification and evaluation of total PCBs, coplanars non-*ortho* substituted PCBs, DDT and HCB. Mixtures of specific isomers (PCB-77; PCB-126; PCB-169) were used for calibration, recovery evaluation and confirmation of non-*ortho* coplanar PCB congeners.

Polycyclic aromatic hydrocarbons (PAHs) were extracted and separated by the method of alkaline-alcohol digestion with KOH/MeOH (1:4 v/v) for the extraction and n-hexane for separation. Toluene eluates were reserved for PAHs determination using an HPLC/fluorescence and GC/MS. Pure reference standard solution for total PAHs were supplied by Supelco.

Biochemical analysis on CYT. P4501A activity (induction of benzo-(a)pyrene monooxygenase and 7-ethoxyresorufin O-deethylase activities) were performed previous isolation of microsomal fraction. Microsomal EROD activity was measured by spectrofluorimetric assay using the original method of Burke and Mayer (7) by kinetic measurement at 30°C using a Perkin Elmer LS50B spectrofluorimeter (Ex 544 nm, Em 584 nm). The amount of resorufin produced was calculated from a calibration curve of standard resorufin (Pierce) in the range 0.01-5μM range. BPMO activity was measured by the method of Kurelec *et al.* (8). The protein content of microsomal samples was evaluated following the Bio-Rad assay, using serum albumin for the standard calibration curve.

Metabolic intermediates (liver porphyrins) were extracted by the method of the Matteis and Lim (9). The method of Grandchamp *et al.* (10) was used for quantitative determination of porphyrins with reference standard of porphyrin products (supply by Logan, Utah, USA). This fluorimetric procedure was used to determine the percentages and concentrations of uroporphyrin, coproporphyrin and protoporphyrin in a mixture of porphyrins in the nanomolar range. The porphyrin were measured using a Perkin Elmer LS 50B spectrofluorimeter.

Results and Discussion

Hg, Cd, Zn, Cu and Fe levels detected in liver samples of *M. merluccius* and *S. sprattus* are summarised in Figure 2 (also see Table 1). Lead levels in both species were below the instrumental detection limit and therefore

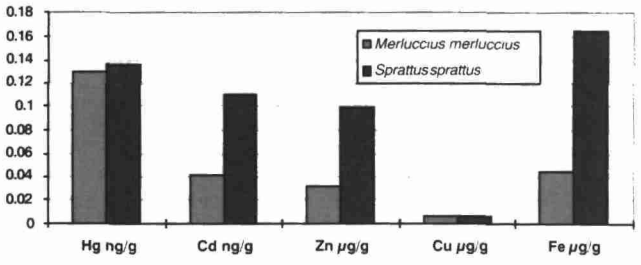

Fig. 2. Mean concentrations (ng/g and μg/g f.w.) of trace metals in the liver of *M. merluccius* and *S. sprattus*.