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CYP1A Expression in *Mullus barbatus* and *Merluccius merluccius* from the Adriatic Sea in Serbia and Montenegro

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants of mostly anthropogenic origin. Due to their mutagenic and carcinogenic properties, measurements of PAH concentrations are included in most environmental monitoring programs (Meador et al. 1995). Polychlorinated biphenyls (PCBs) were produced for diverse industrial applications and although their production and use has been banned since the late 1970s, they continue to cycle through the environment due to their resistance to breakdown and tendency for bioaccumulation (Van der Oost et al. 2003). CYP1A is an established biomarker of exposure in response to environmental contaminants (Stegeman and Hahn 1994). PAHs and PCBs in the aquatic environment lead to a dose-dependent induction of transcription of CYP1A genes and resulting increased concentrations of proteins. CYP1A induction can be assessed by an enzymatic assay or by measuring the increase of its concentration in the hepatopancreas of fish by ELISA, immunoblot analysis or histochemical techniques (Bucheil and Fent 1995).

The aim of this study was to examine the relative changes of CYP1A concentrations in the hepatopancreas of the red mullet *Mullus barbatus* and European hake *Merluccius merluccius* in winter and spring from different localities of the Adriatic Sea in Serbia and Montenegro. Both species are of considerable commercial importance (Lionetto et al. 2003). The locations were chosen in view of their distinct characteristics: Platamuni is an open sea locality, Valdanos, a locality of low urban and industrial influence and the area near Bar is a locality of intense anthropogenic and industrial activity.

MATERIALS AND METHODS

Specimens of *Mullus barbatus* and *Merluccius merluccius* were collected by trawling in the area of Platamuni (UTM BN 9.9), Valdanos (UTM CM 4.5) and near Bar (UTM CM 3.6) in the Adriatic Sea in Serbia and Montenegro as shown in Fig. 1. The investigations covered two seasons, winter (25th February) and spring (25th May). At least seven (and up to nine) individual fish of one species from one locality during one season were pooled. The fish were killed

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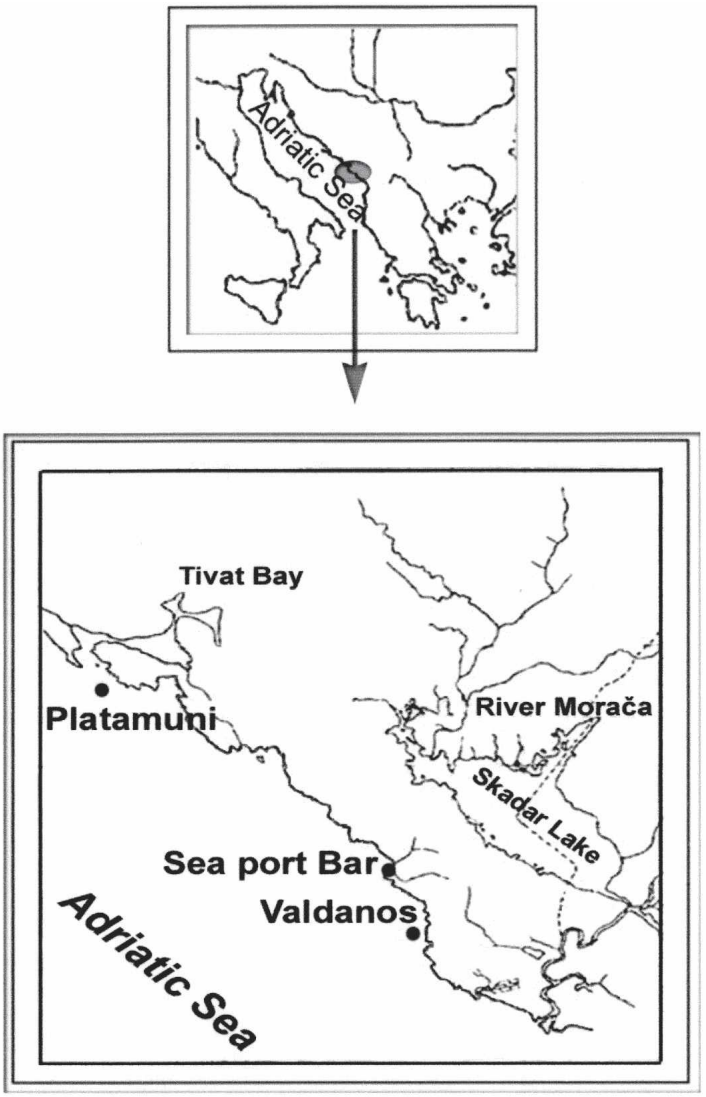

Figure 1. A map of the Adriatic coast of Serbia and Montenegro. The locations where seawater samples were collected and the fish caught are indicated.

immediately by spinosectomy according to standard animal care regulations. *Mullus barbatus* from locality near Bar was not caught in spring.

PCB concentrations were determined by gas chromatography (GC) with an ECD detector and linear programmable temperature vaporizer (PTV) injector. PAH concentrations were determined by gas chromatography (GC) with a FID detector

and a linear programmable temperature vaporizer (PTV) injector. The absence of individual peaks was not reported as zero but as less than the detection limit.The seawater was always sampled from the greatest possible depths, i. e. from 80 m in Platamuni, 20 m in Valdanos and 60 m near Bar.

The microsomal fraction of the hepatopancreas was prepared following the procedure of Krauss et al. (1983), SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970) and immunoblot analysis was performed according to Towbin et al. (1979), using a polyclonal antibody to fish CYP1A (CP226, Biosense Laboratories, Norway). Antigen-antibody complexes (changes of the relative concentrations of CYP1A), were analyzed by densitometry using Total Lab (Phoretix) electrophoresis software.

RESULTS AND DISCUSSION

The examination of CYP1A expression described here is the first of its kind to be carried out on the Adriatic coast of Serbia and Montenegro. As the Adriatic Sea is relatively shallow and virtually land-locked it differs from other seas in its basic physico-chemical and biological characteristics. It is the final recipient for terrestrial wastewaters that contain chemical pollutants. Thus, its pollution state depends largely on the anthropogenic input (Perugini et al. 2004).

The chemical analyses of the PAH and PCB contents in seawater at the examined localities during winter and spring are shown in Table 1. The presence of PAHs (Table 1A) was observed only in spring. The concentration of fluorene was 0.18 μg/l in Valdanos and 0.554 μg/l near Bar. In Valdanos the measured concentration of anthracene was 4.217 μg/l. The concentration of phenantrene in Platamuni was 0.373 μg/l. In all of the examined locations in winter, the concentrations of PAHs were below the limit of detection as established using a GC column ($\leq$0.01 μg/l). Compared to the values reported in the Environmental Quality Standards for the Mediterranean Sea in Israel (2002) the concentration of fluorene in Valdanos and near Bar was below the recommended maximum concentration in both locations. The measured concentration of anthracene (4.217 μg/l) in Valdanos was slightly above the recommended value of 4 μg/l (Nagpal 1993). A slight increase of the concentration of phenantrene (0.373 μg/l) above the recommended maximum concentration of Nagpal (1993) (0.3 μg/l) was measured in Platamuni. Increased concentrations of PCBs were observed only in winter and only near Bar where the concentrations of pcb28, pcb52 and pcb153 were 20, 15 and 15 ng/l respectively (Table 1B). In light of the recommended maximal total PCB concentration of 0.1 ng/l according to Nagpal (1992), the concentrations of PCBs measured near Bar could be taken as a significant contamination of the seawater. However, compared to the minimal concentration of PCBs of about 42 ng/l that exert a negative impact on marine organisms, as reported in the Environmental Quality Standards for the Mediterranean Sea in Israel (2002), the concentrations detected near Bar are at an acceptable level. As the limit of detection of PCBs using a GC column is 10 ng/l (which is above the recommended maximum concentration described by Nagpal

Table 1. Concentrations of PAHs (A) and PCBs (B) in seawater collected from Platamuni, Valdanos and near Bar.

A) PAH concentrations (ng/l)

	Platamuni (winter)	Platamuni (spring)	Valdanos (winter)	Valdanos (spring)	near Bar (winter)	near Bar (spring)
Acenaphthylene	<10	<10	<10	<10	<10	<10
Fluorene	<10	<10	<10	**180**	<10	**554**
Phenantrene	<10	**373**	<10	<10	<10	<10
Anthracene	<10	<10	<10	**4217**	<10	<10
Pyrene	<10	<10	<10	<10	<10	<10
Benz(A)anthracene	<10	<10	<10	<10	<10	<10
Chrysene	<10	<10	<10	<10	<10	<10
Benzo(B)fluoranthene	<10	<10	<10	<10	<10	<10
Benzo(K)fluoranthene	<10	<10	<10	<10	<10	<10
Benzo(A)pyrene	<10	<10	<10	<10	<10	<10
Benzoperylene	<10	<10	<10	<10	<10	<10
Indeno(1.2.3.cd)pyrene	<10	<10	<10	<10	<10	<10
Dibenzo(A)anthracene	<10	<10	<10	<10	<10	<10

B) PCB concentrations (ng/l)

	Platamuni (winter)	Platamuni (spring)	Valdanos (winter)	Valdanos (spring)	near Bar (winter)	near Bar (spring)
2,4,4'-trichlorobiphenyl (pcb28)	<10	<10	<10	<10	**20**	<10
2,2',5,5'-tetrachlorobiphenyl (pcb52)	<10	<10	<10	<10	**15**	<10
2,2',4,5,5'-pentachlorobiphenyl (pcb101)	<10	<10	<10	<10	<10	<10
2,2',3,4,4',5'-heksachlorobiphenyl (pcb138)	<10	<10	<10	<10	<10	<10
2,2',4,4',5,5'-heksachlorobiphenyl (pcb153)	<10	<10	<10	<10	**15**	<10
2,2',3,4,4',5,5'-heptachlorobiphenyl (pcb180)	<10	<10	<10	<10	<10	<10

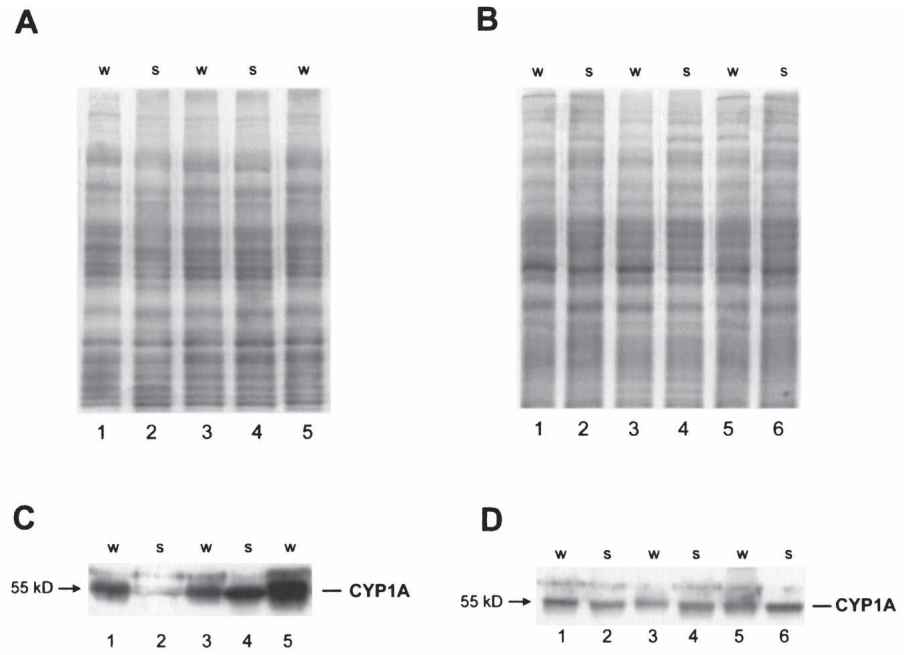

Figure 2. SDS-PAGE profiles (A and B) and immunoblot analysis (C and D) of the microsomal fraction proteins prepared from the hepatopancreas of *Mullus barbatus* (A and C) and *Merluccius merluccius* (B and D) with anti-CYP1A antibody.
Lanes 1 and 2 – samples from the Platamuni locality; lanes 3 and 4 – samples from the Valdanos locality; 5 and 6 – samples from near Bar locality; w – winter; s – spring. Twenty μg of proteins were subjected to 12% SDS-PAGE. The gels were stained with Coomassie Brilliant Blue R-250. Immunoblotting was performed with a polyclonal antibody for CYP1A. Antigen-antibody complex formation was detected using an ECL detection system.

(1992)), the presence of other PCBs on other localities cannot be ruled out.

The observed presence of PAHs and PCBs in seawater at the indicated localities led us to examine the induction of CYP1A, a well-established biomarker of exposure of fish to aromatic hydrocarbons. To that end, equal quantities of microsomal proteins prepared from *Mullus barbatus* and *Merluccius merluccius* were separated by SDS-PAGE (Fig. 2A and B respectively), and subjected to immunoblot analysis using polyclonal antibodies to CYP1A protein (Fig. 2C and D respectively). Within the same species, as judged by Coomassie-staining (Fig. 2A and B), neither qualitative or quantitative differences in the protein profiles were observed regardless of whether comparisons were made between samples that were obtained from different localities or seasons of the year. However, interspecies differences in the protein profiles were evident. In addition, species-

specific differences in CYP1A expression were observed. Assuming that the cross-reactivity of CYP1A was similar in different species, the results shown in Fig. 2 suggest that in the heapatopancreas of *Mullus barbatus* (Fig. 2C) the protein concentration of CYP1A was relatively higher than in *Merluccius merluccius* (Fig. 2D). In both species of fish that were caught in Platamuni the relative concentrations of CYP1A were decreased during spring as compared to the winter period (Fig. 2C and D, lanes 1 and 2). In *Mullus barbatus* the relative concentration decrease 4.5-fold and in *Merluccius merluccius* for about 14%. In both species of fish that were caught in Valdanos the relative concentrations of CYP1A were increased during spring as compared to the winter period (Fig. 2C and D, lanes 3 and 4). In *Mullus barbatus* the relative concentration increased for about 67% and in *Merluccius merluccius* 20%. The relative concentration of CYP1A was highest in both fish species that were caught near Bar (Fig. 2C and D, lanes 5 and 6). As was observed in fish that were caught in Valdanos, the relative concentration of CYP1A in the hepatopancreas of *Merluccius merluccius* was also increased in spring, in this case for about 35% (Fig. 2D, lanes 5 and 6). In *Mullus barbatus* a considerable increase of CYP1A was detected in winter. The relative concentration of CYP1A in fish caught near Bar locality was 50% and 100% higher than in fish caught in Platamuni and Valdanos in winter (Fig. 2C, lanes 1, 3 and 5).

In light of the observed presence of fluorene and anthracene in seawater specimens from Valdanos in spring (Table 1A), and of increased concentrations of fluorene in spring (Table 1A) and pcb28, pcb52 and pcb153 in winter from the locality near Bar (Table 1B), it was assumed that the relatively increased concentrations of CYP1A were the result of CYP1A induction in response to the presence of organic pollutants in seawater. However, CYP1A was observed in fish caught in all of the examined locations (Fig. 2C and D).

The potential toxicity of PAHs and PCBs in seawater on the examined organisms was provided by the observed upregulation of CYP1A. CYP1A is a sensitive indicator of the entry of pollutants into an organisms and their distribution in tissues. However, this biochemical response is only the first signal of exposure to contaminants; it is usually reversible, contrary to the changes manifested at higher levels of organisation of an organism, the population, community and ecosystem (Bayne et al. 1985). Whether the observed exposure resulted in other toxicity was not examined in this work.

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