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Fish Cytochrome P4501A1 Activity Induced by Biobio River Sediments, South Central Chile

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The toxic potential of sediments is commonly assessed by Microtox® or Mutatox® methods as well as by standardized bioassays using amphipods or algae as test organisms (Ingersoll et al. 1998). Induction of the cytochrome P4501A1 (CYP1A1) isoenzyme in fish livers has been recognized as a useful biomarker of exposure to man-made organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), several polychlorinated biphenyl (PCBs) congeners and dioxins (Schoor et al. 1991; Andersson and Förlin 1992; van der Weiden et al. 1993). CYP1A1 induction has been generally employed to assess the field exposure to these organic pollutants by comparison of enzyme activity in fish caught in polluted and unpolluted environments or by deploying caged fish in polluted areas (Soimasuo et al. 1995). In the laboratory, this biomarker has been used to assess sediment toxicity by exposing fish to organic-solvent extracts of sediments collected from polluted areas (Collier et al. 1986; Ueng et al. 1995). This approach however does not take into account site-specific pollutant bioavailability because contaminants are chemically extracted from sediment, which do not reflect biological availability in natural ecosystems.

In this study, CYP1A1 isoenzyme induction was studied in relation to the pollutant burden of the sediment from the Biobio river mouth. This river supports various human activities such as forestry, industry, farming and tourism along its banks and supplies drinking water to Concepción, the second largest Chilean city (population 320,000). Field and laboratory experiments were performed in order to: (i) determine CYP1A1 activity in several fish species that inhabit the Biobio river mouth in comparison to that of the same fish species from an unpolluted area (Tubul river), (ii) assess the potential capacity of Biobio river sediments in inducing CYP1A1 activity, and (iii) detect concentrations of major pollutants in the Biobio river sediments responsible for the adverse responses that can be measured in biota.

MATERIALS AND METHODS

Juvenile male specimens (n = 10) each of *Mugil cephalus* and *Eleginops maclovinus* were netted at the Biobio and Tubul river mouths (VIII Region, Central Chile) in Spring 1995 and Summer 1996. The unpolluted Tubul river was

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selected as the reference environment. The fish species were chosen based on their distribution and accessibility in the study areas. *M. cephalus* is an omnivorous species associated with bottom sediments which feed on detritus as well as benthic organisms, while *E. maclovinus* is a pelagic predator of the water column. Mean (± Standard Deviation, SD) weight and length of fish were 10.9 ± 2.8 g and 9.7 ± 0.9 cm for *M. cephalus*, and 12.8 ± 2.1 g and 10.2 ± 0.6 cm for *E. maclovinus*, regardness the river. Fish were sacrificed *in situ* and livers were immediately removed and stored in liquid nitrogen.

Sediment samples from the Biobio river were used for field exposure simulation carried out in the laboratory using juvenile specimens of rainbow trout (*Oncorhynchus mykiss*, mean length and weight (± SD) were 80 ± 10 mm and 4.7 ± 2.2 g, respectively). Acclimatization of *M. cephalus* and *E. maclovinus* under laboratory conditions was difficult. For this reason, *O. mykiss* was selected to test sediment toxicity; a species commonly used for toxicological assays. Groups of 12 fish were placed in three different 60-L glass tanks. One tank contained sediment and water from the Tubul river (Group A, control group), another contained sediment and water from the Biobio river (Group B) and a third contained sediment from the Biobio river and water from Tubul river (Group C). Sediment was collected at the beginning of the experiment and stored at –20°C for analysis; water was periodically replaced (every 3-4 days) with fresh water from the corresponding sampling sites. Water temperature and pH were serially measured throughout the experiment, ranging from 10-12°C and 7.8-8.0, respectively. After 15 days of exposure, fish were sacrificed and liver samples were immediately removed and stored at –80°C.
Liver samples were homogenized in a 0.1 M sucrose buffer (pH 7.5) and centrifuged at 12,000 g for 20 min at 4°C. The supernatant was centrifuged at 100,000 g for 60 min at 4°C to obtain a microsomal pellet, which was resuspended and homogenized in a volume of 0.15% KCl solution (pH 7.5). The homogenate was centrifuged again under the same conditions to purify the microsomal fraction. In juvenile rainbow trouts, the post mitochondrial supernatant (PMS) obtained after the first centrifugation was used as a source of enzymes for monooxygenase assays (O'Hare et al. 1995) due to the small volume of the liver homogenate. CYP1A1 activity was assayed by 7-ethoxyresorufin-*O*-deethylase dealkylation (EROD) and benzo(a)pyrene hydroxylation (BPMO). EROD measurements were based on the method by Lubet et al. (1985) and expressed as pmol resorufin/min per mg of microsomal protein. BPMO activity was tested according to Kurelec et al. (1977) and the enzyme activity was expressed as fluorescence units (F.U.)/min per mg of microsomal protein. Microsomal proteins were measured by the Bio-Rad Protein Assay using BSA as a reference standard.

Sediment samples were analyzed for PAHs and PCBs because of their known induction of CYP1A1 activity. Wet sediment samples were prepared in three 5-gram replicates and PAHs were extracted following the Eschenbach et al. (1994) method. Briefly, samples were sonicated with acetone, centrifuged, evaporated,

and redissolved in n-hexane. Acetone-extracted sediment was then subjected to an alkaline hydrolysis with 2N KOH:MeOH (1:4 v/v) and extracted twice by liquid/liquid n-hexane partitioning. The hexane extracts from both extraction steps (sonication and hydrolysis) were combined and evaporated to near dryness. Samples were subjected to clean up in a Florisil (1.5 g) glass column with 1 g of anhydrous Na_2SO_4 at the top. The PAH fraction was eluted with n-hexane:DCM (1:1, v/v), evaporated under a N_2 stream and redissolved with 1 ml n-hexane. Wet/dry weight ratio was obtained by drying 3 sediment subsamples at 105°C until a constant weight (wt) was obtained. PCBs were extracted from wet sediment (about 10 g) samples according to Fuoco et al. (1993) with some modifications. Samples were sonicated with n-hexane:acetone (1:1, v/v), centrifuged, and then cleaned up in a Florisil (2 g) glass column with about 1 g of anhydrous Na_2SO_4 at the top. PCBs were selectively eluted with n-hexane and then treated with Cu powder. The eluate was concentrated and run on a glass column (5 mm i.d.) packed with 250 mg of activated carbon to separate non-*ortho* PCB congeners (elution with benzene/ethylacetate, 1:1 v/v) from the bulk of PCBs (elution with hexane/CH_2Cl_2 1:4 v/v).
PAHs were analyzed by GC/MS and quantification was performed in the selected ion monitoring (SIM) mode. PCBs were analyzed by GC-ECD and external standards (PAH mix, Arochlor 1260 and individual PCB congeners) were used for identification/quantification procedures. Spiked sediments and blanks were analyzed for each batch of samples. Mean recovery rates of the PAH- and PCB-spiked sediment samples of 68 % was obtained for naphthalene, over 82 % for individual PAHs and over 89 % for PCBs. Data are expressed without correction by recovery rates.

RESULTS AND DISCUSSION

Mean (± SD) values of CYP1A1 activity in *M. cephalus* and *E. maclovinus* from the Biobio and Tubul river mouths are shown in Figure 1. *E. maclovinus* did not show any significant difference in enzyme activity, between sample site or either season . However, a strong ($p < 0.01$, Kolmogorov-Smirnov test) induction of CYP1A1 activity, by a factor of 3, was found in *M. cephalus* from the Biobio river in both sampling periods compared to specimens from the Tubul river. BPMO activity was not measured in these samples because of small volume of microsomes. Moreover EROD activity generally is the most sensitive catalytic probe to assess the induction of CYP1A1 (Goksøyr and Förlin 1992). The differences between the CYP1A1 activity in the two fish species could be attributed to their different feeding habits. *M. cephalus* is an omnivorous species that feeds on benthic invertebrates and detritus, disturbing the sediment which in turn could result in exposure to CYP1A1 inducers that settle out in bottom sediments. The other way of exposure to pollutants from the sediments may occur by direct ingestion of polluted sediments. The relative importance of each route of exposure was difficult to assess because of the experimental design and sampling. The induction of this enzymatic activity is generally explained as a

response to contaminant exposure, such as to certain PAHs and molecules with a basic planar structure (e.g., coplanar PCBs or dioxins).

The PMS was used as source of monooxygenases in juvenile trouts because microsomal pellets obtained by ultracentrifugation were too small to test both enzyme activities (EROD and BPMO). O'Hare et al. (1995) demonstrated that the PMS is sufficiently sensitive to evaluate monooxygenase activity in roaches (*Rutilus rutilus*) exposed to β-naphthoflavone. Results of the two-week exposure period in the simulated field study showed a highly significant ($p < 0.01$, Kolmogorov-Smirnov test) induction of CYP1A1 assayed in terms of EROD and BPMO activities in fish exposed to Biobio sediment (groups B and C) respect to that of group A (Figure 2). Several specimens from group A showed no CYP1A1 activity. Low enzyme activity, at times even undetectable, has also been reported in untreated individuals or those from uncontaminated areas (Goksøyr and Förlin 1992, Andersson and Förlin 1992).

Because of the enzyme induction observed in both field and laboratory experiments, guided our attention to determine CYP1A1 inducers such as PAHs and coplanar PCBs, in the Biobio river sediment. A mean (± SD) PAH concentration of 5.8 ± 2.7 μg g^{-1} dry wt was found in Biobio river sediment samples (Figure 3). All 16 PAHs screened were detected, and most of the residue included concentrations of three- and four-fused ring compounds over 400 ng g^{-1} dry wt. In comparing induction levels in *O. mykiss* with the results obtained from sediment analyses, the mean concentration of total PCBs (2.93 ± 0.5 μg g^{-1} dry wt) was apparently too low to account for such high induction of CYP1A1 activity alone (Brumley et al. 1995).

On the other hand, the mean concentration of total PAHs fell within ranges of magnitude similar to those found in river sediments of industrialized countries (Johnson and Larsen 1985), which are thought to trigger inductive responses of EROD activity in fish. Focardi et al. (1993) have also reported high induction of the CYP1A1 in other fish species and seabirds living in Biobio river mouth. Several studies have demonstrated that the EROD induction is linked to high PAH concentrations in sediments. Machala et al. (1997) reported inductive responses of EROD activity in carps that inhabit ponds with differing PAH levels in sediments. Van Veld et al. (1990) also found that sediments containing elevated PAH concentrations, in the order of μg per gram, induced EROD activity in *Leiostomus xanthurus*. Although the biochemical marker used in the current study is not totally specific for a particular group of organic pollutants, the high PAH concentrations measured in the sediment samples from the Biobio river mouth appears to be the main contaminant responsible for the high induction observed in *M. cephalus.*

In conclusion, high induction of CYP1A1 isoenzyme observed in *M. cephalus* (field experiment) and juvenile rainbow trouts (simulated field experiment) are likely to be caused by pollutants released into the Biobio river by industrial

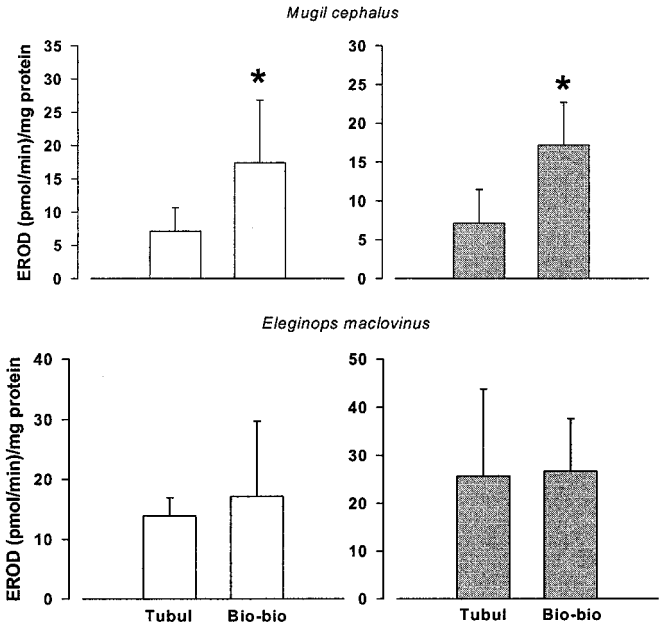

Figure 1. Mean (± SD) EROD activity in *M. cephalus* and *E. maclovinus* caught i the spring (white bars) and in the summer (gray bars) at the Tubul and Biobio rive mouths. EROD activities significantly ($p < 0.01$, Kolmogorov-Smirnov test different from the control group (Tubul river) are indicated by an asterisk.

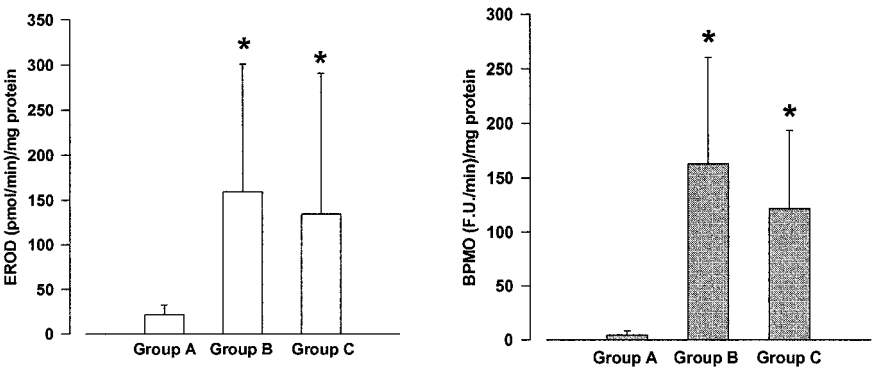

Figure 2. Mean (± SD) cytochrome P4501A1 activities in *Oncorhynchus mykiss* experimentally exposed to sediment from the Biobio river mouth. EROD and BPMO activities significantly ($p < 0.01$, Kolmogorov-Smirnov test) different from the control group (Group A) are indicated by an asterisk. Group A, sediment and water from Tubul river; group B, sediment and water from Biobio river; group C, sediment from Biobio and water from Tubul river.

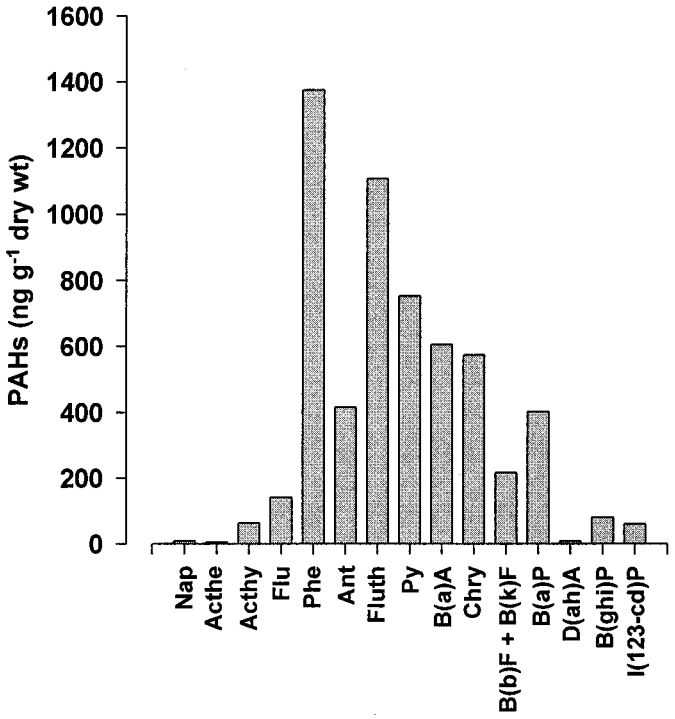

Figure 3. Mean PAH concentrations in sediment collected at the Biobio river mouth. Nap, napththalene; Acthe, acenaphthene; Acthy, acenaphthylene; Flu, fluorene, Phe, phenanthrene, Ant, anthracene; Fluth, fluoranthene; Py, pyrene; B(a)A, benzo(a)anthracene; Chry, chrysene; B(b)F, benzo(b)fluoranthene; B(k)F, benzo(k)fluoranthene; B(a)P, benzo(a)pyrene; D(ah)A, dibenzo(a,h)-anthracene; B(ghi)P, benzo(g,h,i)perylene; I(123-cd)P, indeno(123-cd)pyrene.

activities located near to the mouth. PAHs concentrations of the order of μg per gram in the sediment suggested that these pollutants are probably main responsible for induction of CYP1A1. Furthermore, the experiments prove this isoenzyme to be a suitable biochemical marker for primary assessment of sediment toxicity and to address the chemical analysis of the abiotic matrix.

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