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Biochemical Biomarkers in Fish from Different River Systems Reflect Exposure to a Variety of Anthropogenic Stressors

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Evaluating environmental quality in freshwater ecosystems requires reliable methods for toxicological risk assessment in natural populations. Because distinctive biota inhabit central Chilean river mouths that flow into the Pacific Ocean, these ecosystems are of particular interest. For thirty years, these areas have been heavily polluted by a number of industrial and urban effluents (Focardi et al. 1996; Sanchez –Hernández et al. 1998). Field studies by means of bioindicator species and biomarkers of pollution are critical in assessing damage that natural populations may suffer.

Comparing biomarker responses between indicator organisms sampled in presumably polluted areas and those sampled in more pristine environments can be especially useful in assessing environmental quality (McCarthy et al. 1989; McCarthy and Shugart 1990; Fossi et al. 1991; Fossi and Leonzio 1994). High levels of chlorinated organic pollutants in muscle tissues of several feral fish and bird species of the Biobio River mouth area were detected in previous studies (Fossi et al. 1995; Focardi et al. 1996).

This paper presents biomarker data obtained during two sampling campaigns in 1995 and 1996, aimed at comparing biomarker responses in two key fish species resident at the mouths of two major fluvial systems in Chile: the Itata and Biobio Rivers. Agriculture and forestry activities are carried out in the Itata river basin, while the Biobio River basin is surrounded by industrial plants (petrochemical, urban and paper mills) located mostly near the mouth.

Two typical fish species of central Chilean estuaries, *Mugil cephalus* and *Eleginops maclovinus,* were used as bioindicator organisms. The induction of liver CYP P450 1A1 measured as 7-ethoxresorufin-O-deethylase dealkylation (EROD) activity and brain acetylcholinesterase inhibition (AChE) were the biomarkers of exposure to pollutants. These species were chosen because of their commercial importance and for their suitability in biomarker studies. They are easy to catch, do well in laboratory settings and feature high body lipid contents in which lipophilic pollutants are incorporated and stored (Focardi et al. 1996).

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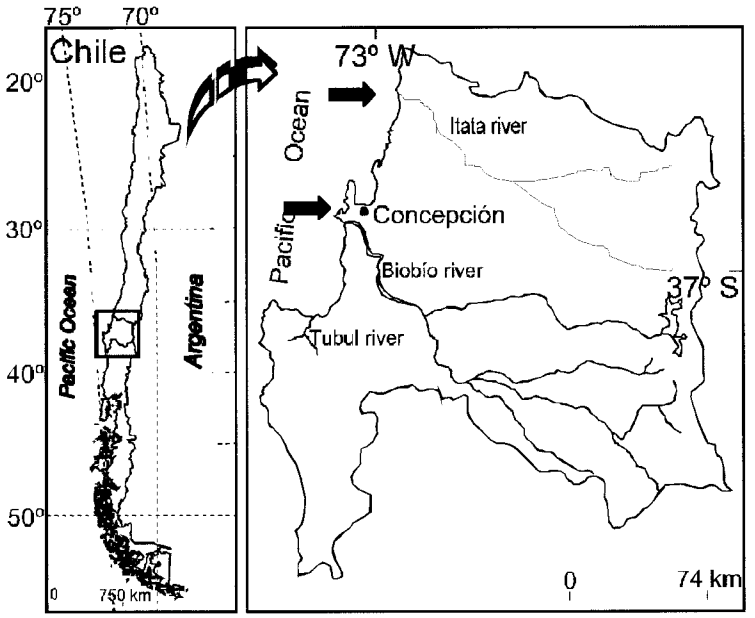

Figure 1. Study area and sampling sites in Central Chile.

MATERIALS AND METHODS

Twenty Juvenile male specimens of *Mugil cephalus* and of *Eleginops maclovinus* were netted at the Biobio, Itata, and Tubul River mouths (VIII Region Chile, as illustrated in Figure 1). The unpolluted Tubul River was selected as the reference environment (Stuardo et al. 1993). The fish species were chosen on the basis of their distribution in the study areas. *M. cephalus* is a omnivorous species, feeding on detritus as well as benthic organisms associated with the sediment, while *E. maclovinus* is a carnivorous predator of the water column. Mean (± SD) weight and length of fish collected from all three rivers 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*. Fish were sacrificed in the field and livers and brains were immediately removed, flash frozen and stored in liquid nitrogen until analysis.

Biomarker analyses were performed at the Biomarkers Laboratory of the EULA-Chile center in Concepción, Chile. In preparing microsomal fraction, the frozen livers were weighed and the subsequent procedures were carried out at 4° C. Tissues were homogenized at a ratio of 1:5 W/V (tissue weight/buffer volume) in 0.25 M sucrose buffer pH 7.5 in a potter Teflon homogenizer at 4°C. The resultant homogenate was centrifuged at 9,000 x g for 20 min. The supernatant was ultracentrifuged at 100,000 x g for 60 min and the resulting microsomal pellet was resuspended in 1.15% KCl solution at pH 7.5. These fractions were immediately used for determining enzyme activity. All enzyme assays were carried out at 25°C. MFO activity was measured in the liver microsomal fraction

by ethoxyresorufin dealkylation (EROD) (Lubet et al, 1985). Briefly, a final volume of 2.5 mL containing buffer Tris-HCl 50 mM, 25 mM MgCl2 at pH 7.5 was used, and the ethoxyresorufin was incubated at 20°C. The reaction began with the addition of NADPH 10 mM, and the activity was followed by fluorescence measurements in an spectrofluorimeter model LS50 B (Perkin-Elmer) with an Excitation wavelength of 522 nm and an Emission wavelength of 586 nm. EROD activity was expressed in pmoL of substrate/min/mg microsomal protein. Microsomal proteins were quantified by the Bio-Rad protein assay.

Esterase activities were measured as follows: whole fish brains were homogenized in 0.1% Triton X-100 in 25 mM Tris-HCl (pH 7.6) and assayed immediately for AChE activity. Brain AChE was tested by the method of Ellman et al. (1961). Acetylthiocholine iodide was used as substrate and the subsequent detection of released thiocholine by reaction with 5,5-dithiobis (2-nitrobenzoic acid) DTNB was monitored over a 5 min period with the recording spectrophotometer set at 410 nm. Activity was expressed as μmol/min/mg protein. Data were analyzed using statistical software STATISTICA. For not normal distributed data, the Mann-Whitney U test was used to detect significant differences between sites in each sampling period.

RESULTS AND DISCUSSION

The induction of CYP1A1 activity in bioindicator fish is a tool for evaluating exposure to organochlorines and other compounds such as planar PCBs, PAHs, dioxins, etc. Different levels of CYPP450 1A1 induction detected in specimens from different aquatic ecosystems are used to discriminate between exposure levels from different systems.

EROD activity was statistically higher (81.5%) in *M. cephalus* collected at the Biobio River mouth in spring 1996 (Mann-Whitney U test $p<0.01$) compared to activity of specimens from the control area (Figure 2). Because other factors can influence this activity (i.e., reproductive status, temperature, etc.), juvenile males were used in this study to minimize the effect of variables contributing to induction that might be related to reproductive status, etc. Likewise, EROD activities were higher (92.4%) in organisms from the Itata River as compared to activities of the control site organisms. Such induction levels indicate that chlorinated compounds and PAHs are significant pollution types to which this fish species is exposed in both rivers. In fact, recent results show detectable levels of lindane, PCP and other pollutants at the mouth of the Itata River, as well (Parra and Habit 1998).

EROD activity in *M. cephalus* (Figure 2) were similar to those reported by Fossi et al. (1995), revealing that pollution in the Biobio mouth generally not changed in the last few years. *M. cephalus* sampled in summer 1996 continued to show similar statistically significant differences with values 89.9% greater than EROD activities in fish from the reference site (Figure 2, Mann-Whitney U test $p< 0.05$). Samples from the Itata River were not available in Summer of 1996.

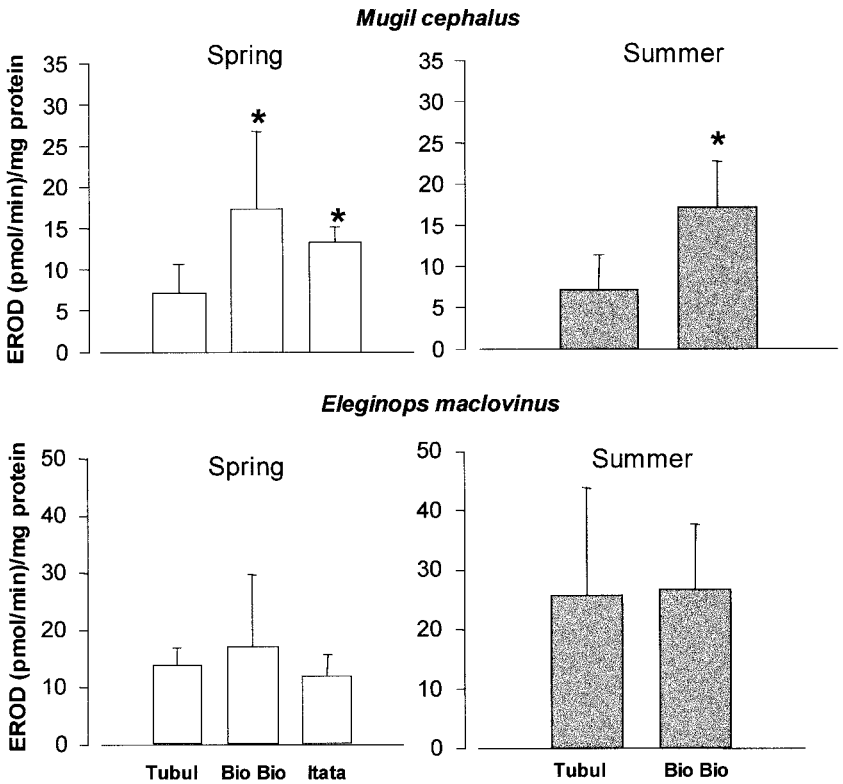

Figure 2. Mean (± SD) cytochrome P4501A1 (based on 7-ethoxresorufin-O-deethylase dealkylation, EROD) activity in *M. cephalus* and *E. maclovinus* caught in the spring (white bars) and summer (gray bars) at the Tubul, Biobio and Itata River mouths. Asterisks indicate EROD activities significantly ($p < 0.01$, Mann-Whitney U test) different from the reference group (Tubul River).

In *E. maclovinus,* no statistical differences in EROD induction were found between sites in the two sampling periods (Figure 2). Differences in EROD induction between the two species may be explained by their different sensitivity and/or feeding behaviors, i.e. *Mugil cephalus* is primarily a bottom feeder being more readily exposed to levels of chlorinated compounds and PAHs associated with sediments, whereas *E. maclovinus* resides in the water column where its exposure to PCBs and pesticides in sediments is minimal. In addition, *M. cephalus* has more body lipids than does *E. maclovinus*, making this species even more susceptible to lipophilic compounds via bioaccumulation, resulting in a higher EROD induction. Previous results show that levels of potential CYPP450 1A1 inducers (mostly PCBs) in fish captured at the Biobio River mouth were higher in *M. cephalus* (1,842 and 2,012 ng g^{-1} on lipid basis, in muscle and liver, respectively) than in *E. maclovinus* (424 and 515 ng g^{-1} on lipid basis, in muscle and liver, respectively) (Focardi et al. 1996).

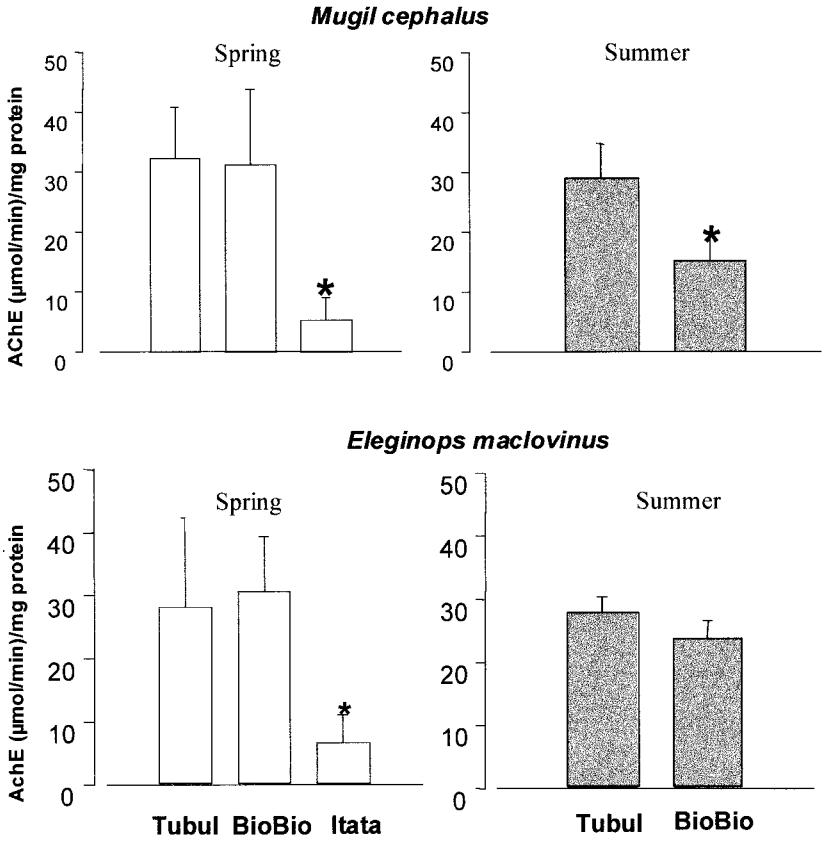

Figure 3. Mean (± SD) brain acetylcholinesterase activity in *M. cephalus* and *E. maclovinus* caught in spring (white bars) and summer (gray bars) at the Tubul, Biobio and Itata river mouths. Asterisks indicate AChE activities significantly ($p < 0.01$, Mann Whitney U) different from the control group Tubul River.

Acetylcholinesterase activities in *M. cephalus* from the Itata River showed a marked reduction, particularly in spring, when compared to activities in fish from the reference site (Figure 3). The Itata River basin has a greater agricultural and forestry land use than does the reference area (Parra and Habit, 1998) potentially explaining this difference. *M.cephalus* collected at the mouth of the Biobio River did not show statistically significant differences when compared to the reference site in the Spring, although significant reduction ($p<0.05$) was observed in the Summer. These results seem to be related to the short-lived presence of organophosphates (OPs) and carbamate compounds in the study area during the Spring and Summer periods, according to the traditional agricultural practices of that area (Barra et al. 1996). It was recorded a marked inhibition of the AChE in fish from the Itata River related to the same activity recorded in the fish from the Biobio River, in both fish species AChE activity inhibition was detected only in *E. maclovinus* of the Itata River during the spring. *In vitro* inhibition experiments

showed higher sensitivity of *M. cephalus* AChE towards carbaryl and other OPs (data not shown).

Though CYPP4501A1 induction in fish from the Itata mouth is lower that of fish from the Biobio River sediments, concentrations of xenobiotics are still considerable. In fact, recent results (Orrego 1998) show the presence of some PAHs in Itata River sediments. Moreover, even though chlorinated compounds have only been detected sporadically, some concentrations reported in water samples from the Itata mouth are even higher than those from the Biobio River mouth (Parra and Habit 1998). All these findings indicate that both ecosystems, are subject to chemical stressors at different degrees, even though pulp mill effluents are discharged into the Biobio river. Table 1 lists pollutant levels within the study area in different environmental compartments. It is likely that the CYPP4501A1 induction observed in the fish inhabiting these ecosystems may also be due to a mixture of pollutants (Payne et al. 1987).

Table 1. Levels of pollutants detected in the Biobio and Itata river mouths in fish, water and sediments.

		Contaminants			
Site	Compartment	PCBs	PAHs	DDTs	PCP
Biobio	Water[1] (ng l^{-1})	22,18	-	-	157
	Biota[2] (ng g^{-1})	1842	-	1429	-
	Sediments[3] (ng g^{-1})	2,93	5800	-	-
Itata	Water[4] (ng l^{-1})	-	-	-	63
	Sediments[5] (ng g^{-1})	-	178	-	-

[1]Parra et al. (1998),[2]Focardi et al. (1996),[3]Sánchez-Hernández et al. (1998),[4]Parra and Habit (1998),[5]Orrego (1998)

The fact that *M. cephalus* is a feeder of soft bottom sediments rich in organic matter (Carevic et al. 1982), as well as the fact that it absorbs lipophilic contaminants both suggests that this species is exposed to particularly high levels of sediment pollutants reported to be toxic for teleost fish (O'Hare et al. 1995). Recent results (Sanchez-Hernández et al. 1998) show that induction of CYPP4501A1 in fish from the Biobio River mouth is linked to PAHs and PCBs in these sediments.

The different exposure levels can also be attributed to the different feeding behavior between the two species, i.e. benthic detritivore versus limnetic predator. In fact greater mobility of *E.maclovinus* (Pequeño 1978) may reflect exposure to pollutants mostly through the water column and the food web.

Biomarker analysis and chemical monitoring of fish species with different characteristics and feeding habits, as in the species studied, are central in understanding which species are more susceptible to pollutant exposure, for identifying pollutant sources and mantaining environmental health of such

particular ecosystems, as well. In particular, analysis of reproductive status and growth bioindicators can provide a more detailed view of chemical stressor effects at more ecologically relevant perspectives.

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