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Heavy metal content and biotransformation enzymes in two fish species from the NW Mediterranean

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ABSTRACT: Trace metal concentrations (Cd, Cu, Fe, Zn) and total calcium content were measured in the gills of 2 marine fish (painted comber *Serranus cabrilla* and striped mullet *Mullus barbatus*) sampled in different stations in the cove of Cortiou, where the outlet collecting the waste waters of Marseille, France, is situated (NW Mediterranean). In parallel, the activity rates of some phase I enzymes (ethoxyresorufin-0-dealkylase, EROD; pentoxyresorufin-0-dealkylase, PROD) and phase II enzymes (glutathione-S-transferase, GST) were determined in the livers of both species of fish. Cadmium and copper concentrations were highly variable for both species. Two sampling stations situated in the dispersion area of waste waters seemed affected by chemical pollution. At these stations, iron concentrations appeared to be higher in gills of *S. cabrilla* and *M. barbatus*. Elevated iron concentrations were thought to be due to the water treatment plant, which uses iron chloride as a flocculant. Likewise, at both stations calcium and cadmium concentrations were higher in *M. barbatus*. EROD and PROD activities appeared to be generally lower in the livers of *S. cabrilla* collected at both 'polluted' stations; GST activities did not differ according to the sampling stations. Enzyme activities (PROD and GST) in *M. barbatus* were lower at the stations in the dispersion area of waste waters. The results imply that heavy metals, which can also be accumulated in some target organs of fish, may lower biotransformation enzyme activities which are induced in the livers of fish exposed to organics.

KEY WORDS: Heavy metal · Biotransformation enzymes · Fish

INTRODUCTION

The purpose of this study was to measure trace metal concentrations (Cd, Cu, Fe, Zn) and total calcium content in the gills of 2 marine fish (painted comber *Serranus cabrilla* and striped mullet *Mullus barbatus*) sampled in the cove of Cortiou, France, where the outlet collecting the waste waters of Marseille is situated. The activity rates of some phase I enzymes (ethoxyresorufin-0-dealkylase, EROD; pentoxyresorufin-0-dealkylase, PROD) and phase II enzymes (glutathione-S-transferase, GST) in the livers of the 2 fish species were also studied. The waste waters contain both types of chemical pollutants: heavy metals, and organics such as PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated biphenyls) and pesticides. In this area of mixed pollution, we evaluated whether the biotransformation enzymatic activities, induced in fish exposed to organics, could be modified by the presence of heavy metals.

Trace metal accumulation in the tissues of fish is a general phenomenon. The gills are the first target in metal accumulation because they are directly in contact with seawater. But some correlations between tissue concentrations of metals and the development of hepatic perturbations have been reported (Establier et al. 1978a, b, Gutierrez et al. 1978, Dubale & Shah 1979, Sinovcic et al. 1980, Sørensen et al. 1980, Gony et al. 1988).

Heavy metals are known to interfere with calcium homeostasis in mammals and fish (Shephard & Simkiss 1978, Bansal et al. 1985, Reddy et al. 1988, Zhang et al. 1990). Viarengo et al. (1988) showed that calcium concentrations significantly increased in the digestive gland of mussels exposed to pollutants in the field, as well as in contaminated (copper and diesel oil mixture) mesocosm basins. Regoli et al. (1991) observed a net increase of total calcium concentration in the body of mussels during metal accumulation.

Fish, as well as mammals, possess the ability to perform a wide variety of detoxication reactions. The first metabolic step is mediated by mono-oxygenase or mixed-function oxygenases (MFO), which introduce functional groups into the substrates (reviewed by Buhler & Williams 1988). Primary oxidation products arising from the so-called phase I reactions are then excreted or further transformed into products of greater water solubility. This second step (phase II) is mediated by a series of conjugating enzymes (reviewed by Foureman 1989). The major organ involved in xenobiotic metabolism in fish is the liver. Hepatic MFO induction in fish has been used on many occasions as a monitoring tool (Payne et al. 1987).

MATERIALS AND METHODS

The area of study is shown in Fig. 1. As there are no tidal currents, circulation of waters in the Cortiou area is mostly wind-induced (Castelbon 1972). Charmasson (1982) showed that sediments alone in this area can account for the variability of the aquatic system on a long-term basis. Significant dilution gradients of Cortiou effluent were found by Arnoux (1988) as a function of depth and along 2 axes, one heading to the west and the other to the southeast. We elected to sample 2 transects along this latter axis (Stn TR1, along the island of Calesraigne, and Stn TR2, along the mainland coast near the waste water outlet of Cortiou) by obliquely towing the trawl from 80 to 25 m. Additional samples were collected by trawling for 10 min at different depths following the 30, 40, 50, 60 and 70 m isobaths. All sampling was performed from the RV 'Roselys' (IFREMER).

On board, 5 specimens of each species (*Serranus cabrilla* and *Mullus barbatus*) at each station were measured, weighed and identified as to sex. Fish were then dissected and the organs separated. Gills were carefully rinsed with a solution of 1 M glycine and 10 mM Tris(hydroxymethyl-aminomethane)-HCl. Rinsing gills with this solution, which has the same osmolarity as seawater, allows the elimination of seawater, which contains 11 mM of calcium. Whole livers and gills were kept in liquid nitrogen until analysis. Metal determinations were carried out on gills, whereas the activities of biotransformation enzymes were determined in the livers of fish.

Metal determinations in fish gills. Gills were first thawed and dried at 60 °C to a constant weight. Digestion of gills was performed in a microwave oven (CEM-MDS81D) as follows. First, samples were placed in high-pressure vessels and concentrated nitric acid (65 %)

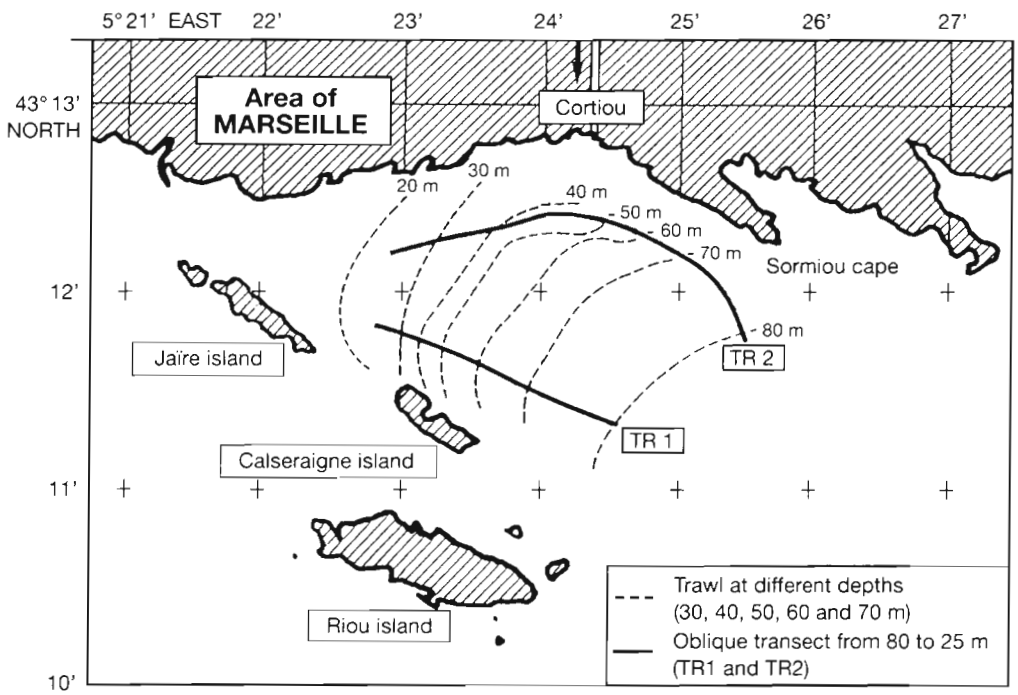

Fig. 1. Sampling locations in the cove of Cortiou, Marseille, France

(Merck Suprapur) was added. The digestion procedure then followed 3 steps: microwaving at 30 W for 15 min; 60 W for 15 min; and finally 90 W for 5 min. Trace metal concentrations were determined by atomic absorption spectrophotometry (Philips Pye Unicam SP9) with flame for copper, iron and zinc and with a graphite furnace for cadmium (Philips PU 9095 video furnace). Deuterium background correction was used when necessary. Calcium was determined on the digested solution by flame emission spectrophotometry. The analytical procedure was checked regularly using standard reference material (lobster hepatopancreas TORT-1) provided by the National Research Council of Canada.

Table 1. *Serranus cabrilla*. Mean values ± 1 SD of dry weight and calcium and trace metal concentrations in the gills of painted comber. Median values are also given under the means, due to the high standard deviations

Stn	Dry wt (g)	Ca (mg g^{-1})	Cd (ng g^{-1})	Cu (µg g^{-1})	Fe (µg g^{-1})	Zn (µg g^{-1})
30 m	0.45 ± 0.08	65 ± 8	19 ± 10	9.4 ± 5.6	190 ± 25	101 ± 63
n = 5	0.45	64	18	7.6	192	93
40 m	0.52 ± 0.08	70 ± 8	57 ± 49	19.9 ± 13.9	161 ± 41	123 ± 59
n = 5	0.51	72	35	27.2	145	93
50 m	0.55 ± 0.07	64 ± 6	110 ± 172	25.7 ± 34.9	144 ± 14	103 ± 59
n = 5	0.55	63	34	10.3	140	79
70 m	0.57 ± 0.10	70 ± 7	62 ± 55	21.9 ± 16.1	207 ± 37	82 ± 19
n = 5	0.59	71	20	21.3	220	79
TR1	0.41 ± 0.09	76 ± 8	63 ± 46	23.6 ± 27.4	246 ± 82	102 ± 24
n = 5	0.40	79	47	5.3	229	86
TR2	0.66 ± 0.09	73 ± 13	20 ± 11	11.9 ± 5.4	327 ± 150	87 ± 37
n = 5	0.65	74	20	11.0	333	76

Hepatic biochemical measurements. The hepatic microsomal fractions were prepared as follows. Upon return to laboratory, livers were thawed and rapidly homogenized in a cold volume of 10 mM Tris - 250 mM sucrose buffer (pH 7.4), containing protease inhibitors (phenylmethylsulfonylfluoride, PMSF) and 20% (v/v) glycerol. The homogenates were centrifuged at 105 000 × *g* for 15 min and the supernatant collected and centrifuged again at 105 000 × *g* for 60 min. The microsomal pellets were then suspended in a 10 mM Tris / 250 mM sucrose buffer (pH 7.4). EROD and PROD were determined according to Burke & Mayer (1974) and Lubet et al. (1985) respectively. GST was determined with the substrate 1-chloro-2,4-dinitrobenzene (Habig et al. 1974). Proteins were analyzed by the method of Lowry et al. (1951).

RESULTS

Calcium and trace metal concentrations of the gills of both fish are shown in Table 1 (*Serranus cabrilla*) and Table 2 (*Mullus barbatus*). Mean values (5 samples) ± 1 SD and median values of all metal concentrations are given in Tables 1 & 2. Cadmium and copper concentrations were highly variable for both species. Iron concentrations appeared to be higher at Stns TR1 and TR2 for *S. cabrilla* and *M. barbatus*. At both stations, calcium and cadmium concentrations were higher in *M. barbatus*.

The activities of enzymes EROD, PROD (pmol min^{-1} mg^{-1}) and GST (nmol min^{-1} mg^{-1}) found in the livers of *Serranus cabrilla* are presented in Fig. 2 (mean values of 5 samples ± 1 SD). EROD values were lower at Stns TR1 (12 pmol min^{-1} mg^{-1}) and TR2 (9 pmol min^{-1} mg^{-1}) than those reported for the different depths (e.g. 28 pmol min^{-1} mg^{-1} at 50 m). The same tendency was observed for PROD activities. GST values did not differ among sampling stations.

Hepatic enzyme activities in *Mullus barbatus* are shown in Fig. 3. Lower activities of PROD were observed at Stn TR2 and the same phenomenon occurred at both Stns TR1 and TR2 for GST. Generally, the values were ca 80 pmol min^{-1} mg^{-1} for EROD, ca 4 pmol min^{-1} mg^{-1} for PROD and ca 200 nmol min^{-1} mg^{-1} for GST.

Tables 3 & 4 give the correlation matrix between the different variables analysed for *Serranus cabrilla* and

Table 2. *Mullus barbatus*. Mean values ± 1 SD of dry weight and calcium and trace metal concentrations in the gills of striped mullet. Median values are also given under the means due to the high standard deviations

Stn	Dry wt (g)	Ca (mg g^{-1})	Cd (ng g^{-1})	Cu (µg g^{-1})	Fe (µg g^{-1})	Zn (µg g^{-1})
50 m	0.49 ± 0.06	37 ± 2	12 ± 9	44.5 ± 18.1	172 ± 14	94 ± 11
n = 5	0.48	38	12	42.8	172	95
60 m	0.43 ± 0.06	32 ± 2	6 ± 3	37.6 ± 21.1	207 ± 29	83 ± 15
n = 5	0.43	31	7	40.6	209	88
70 m	0.39 ± 0.09	36 ± 4	129 ± 148	60.3 ± 59.8	195 ± 31	100 ± 26
n = 5	0.40	36	31	48.1	187	98
TR1	0.50 ± 0.09	55 ± 12	60 ± 37	38.4 ± 41.1	397 ± 174	95 ± 17
n = 5	0.49	55	56	17.6	332	92
TR2	0.42 ± 0.06	51 ± 12	230 ± 263	32.6 ± 3.4	442 ± 176	96 ± 16
n = 5	0.42	49	86	33.9	390	96

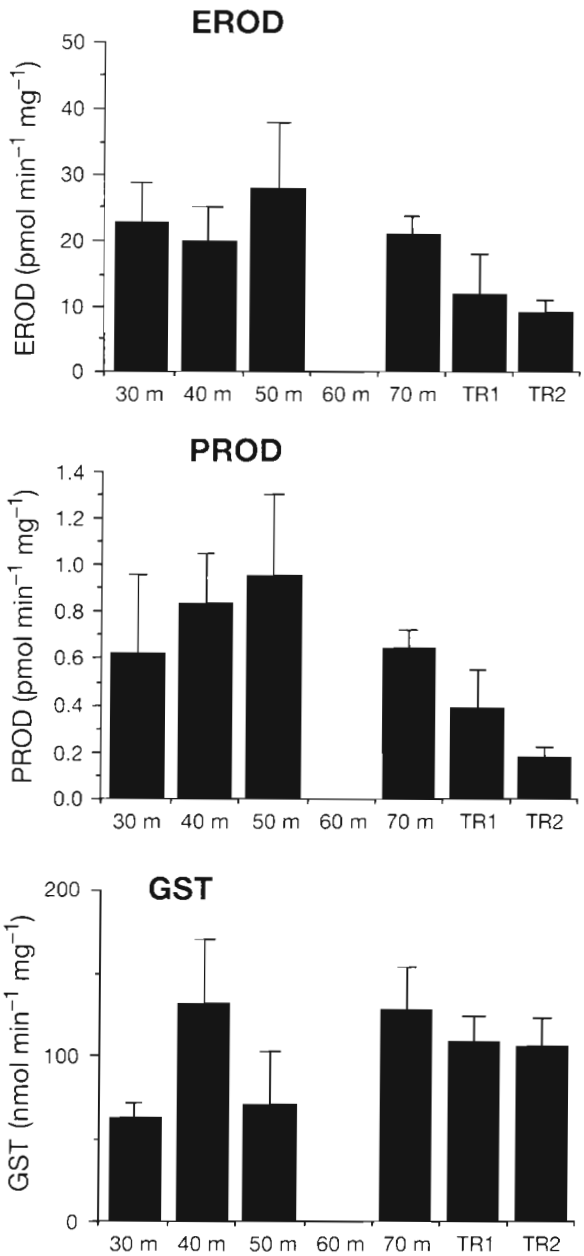

Fig. 2. *Serranus cabrilla*. Activity of ethoxyresorufin-0-dealkylase (EROD), pentoxyresorufin-0-dealkylase (PROD) and glutathione-S-transferase (GST) measured in livers of painted comber (n = 5 samples at each station)

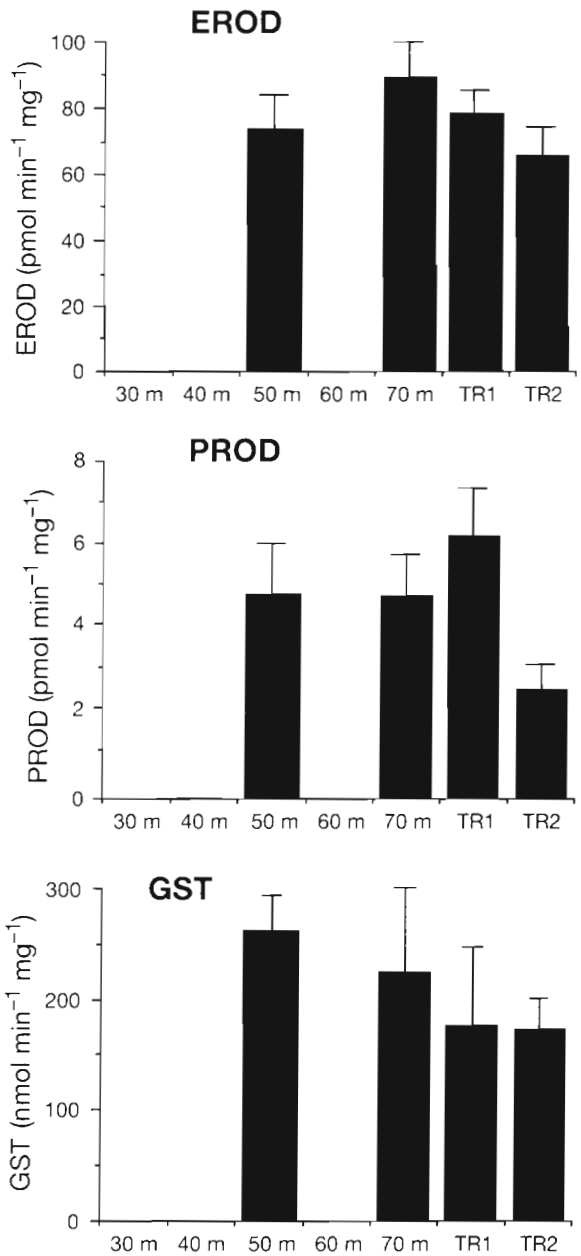

Fig. 3. *Mullus barbatus*. Activity of ethoxyresorufin-0-dealkylase (EROD), pentoxyresorufin-0-dealkylase (PROD) and glutathione-S-transferase (GST) measured in livers of striped mullet (n = 5 samples at each station)

Mullus barbatus respectively. As data display a wide range of variation, a log transformation was done. The sampling stations studied were those with complete data for biotransformation enzyme activities and metal concentrations. Eight variables were taken into consideration (Ca, Cd, Cu, Fe and Zn concentrations, and EROD, PROD and GST activities). This corresponds to 25 samples for *S. cabrilla* and 20 samples for *M. barbatus*.

With regard to *Serranus cabrilla* (Table 3), there was a positive correlation between EROD and PROD ($r = 0.709$), as well as between copper and zinc ($r = 0.492$). Both enzyme activities were negatively correlated with iron (EROD/Fe, $r = -0.626$; PROD/Fe, $r = -0.546$) and calcium (EROD/Ca, $r = -0.404$; PROD/Ca, $r = -0.419$).

With regard to *Mullus barbatus*, calcium and iron were positively correlated ($r = 0.715$), as were copper

Table 3. *Serranus cabrilla*. Correlation coefficients between the 8 variables analysed in painted comber (n = 25). *Significant at $p < 0.05$

	Ca	Cd	Cu	Fe	Zn	EROD	PROD	GST
Ca	1.000							
Cd	0.092	1.000						
Cu	−0.354	0.002	1.000					
Fe	0.198	−0.148	0.098	1.000				
Zn	0.178	−0.173	0.492*	−0.122	1.000			
EROD	−0.404*	−0.085	0.175	−0.626*	−0.018	1.000		
PROD	−0.419*	0.122	0.257	−0.546*	0.161	0.709*	1.000	
GST	0.128	0.073	0.279	0.242	0.132	−0.298	−0.092	1.000

Table 4. *Mullus barbatus*. Correlation coefficients between the 8 variables analysed in striped mullet (n = 20). *Significant at $p < 0.05$

	Ca	Cd	Cu	Fe	Zn	EROD	PROD	GST
Ca	1.000							
Cd	0.286	1.000						
Cu	−0.196	−0.094	1.000					
Fe	0.715*	0.434	−0.212	1.000				
Zn	0.097	0.132	0.720*	−0.119	1.000			
EROD	−0.466*	0.284	−0.246	−0.535*	−0.123	1.000		
PROD	−0.147	−0.305	−0.202	−0.331	0.200	0.465*	1.000	
GST	−0.123	−0.305	−0.340	−0.171	−0.481*	−0.266	−0.212	1.000

and zinc (r = 0.720) and EROD and PROD (r = 0.465), whereas EROD was negatively correlated with calcium (r = −0.466) and iron (r = −0.535), and GST with zinc (r = −0.481).

DISCUSSION

Generally, results were highly variable for metal concentrations in gills, as well as for hepatic enzyme activities, as shown by the elevated standard deviations. It must be emphasized that fish are not exactly in the same metabolic state and, due to their movement, they do not necessarily integrate the same pollution gradient. This renders interpretation difficult. Nevertheless, the fish which were analysed were of the same length (20 to 25 cm) and weight class (60 to 80 g), with a mean length and weight 25 cm and 73 g for *Serranus cabrilla* and of 20 cm and 67 g for *Mullus barbatus*. Fish had not begun their reproductive cycle since it occurs from April to July for *S. cabrilla* and from April to August in the case of *M. barbatus* (Fischer et al. 1987). Moreover, since *S. cabrilla* is a hermaphroditic species, this eliminates possible variations due to sex in measuring hepatic enzymatic activities.

The results on metal concentrations were compared to previous results (Gnassia-Barelli et al. 1992) obtained on *Serranus cabrilla* in different parts of the NW Mediterranean Sea (n = 47) at different periods of the year. Gills were found to have calcium (76 ± 28 mg g^{-1}), cadmium (28 ± 41 ng g^{-1}), iron (179 ± 71 µg g^{-1}) and zinc (91 ± 20 µg g^{-1}) concentrations which were not significantly different from those reported here, whereas copper concentrations in the gills were much higher in the samples collected from Cortiou than in those from other parts of the Mediterranean (5.3 ± 2.3 µg Cu g^{-1}). For the gills of *Mullus barbatus*, comparison was possible because of results obtained on samples (n = 7) collected in a Mediterranean coastal zone (Banyuls-sur-Mer). Mean calcium, iron, zinc and copper concentrations were 43 ± 8 mg g^{-1} (dry wt), 295 ± 123 µg g^{-1}, 53 ± 7 µg g^{-1} and 3.6 ± 0.7 µg g^{-1} respectively. Copper and zinc concentrations were lower than those reported for the gills of *M. barbatus* collected from Cortiou.

Arnoux (1988) reported high levels of total hydrocarbons (18000 µg g^{-1}), zinc (2550 µg g^{-1}) and copper (680 µg g^{-1}) in sediments collected in the dispersion area of waste waters from Cortiou; cadmium levels (9 µg g^{-1}) seemed slightly higher than values reported for coastal sediments. Chabert & Vicente (1981) found a mean maximal value of 5.5 µg Cd g^{-1} in sediments sampled in a Mediterranean bay not far from Cortiou. High levels of metals (particularly copper, zinc and lead) were found in the gonads and in the digestive content of the sea urchin *Paracentrotus lividus* collected in the Cortiou area (Delmas 1986/1987). Pergent-Martini (1992) also reported high levels of metals (particularly copper and iron) in the marine phanerogam *Posidonia oceanica* from this area as compared to levels found in unpolluted areas.

At both Stns TR1 and TR2 (which correspond to a trawling from 80 to 25 m in the area of the effluent dispersion, TR2 being the transect closest to the outlet), high levels of iron were noted for *Serranus cabrilla*; these levels were even more significant for *Mullus barbatus*. It must be emphasized that the water treatment plant at Cortiou uses iron salts (254.4 kg of iron chloride per day since 1987) as a flocculant. Delmas (1990) found, among populations of *Paracentrotus lividus* living near the outlet of Cortiou, individuals with brown to black gonads. An ultrastructural study demonstrated the presence of extracellular and intracellular

crystals and an X-ray microanalysis revealed the presence of iron, chlorine and sulphur in these crystals. The author suggested that this bioaccumulation of iron is linked to the release into coastal water of iron chloride from the treatment plant of Cortiou.

At Stns TR1 and TR2, calcium concentrations were also higher in the gills of both fish species compared to calcium concentrations in the gills of fish from the other stations. This holds true especially in the case of *Mullus barbatus*, and the differences found in calcium concentrations cannot be attributed to a difference in the gill weights since fish analysed were of the same weight and size class and did not have different gill weights. Allemand et al. (1989) measured the gonad calcium content of *Paracentrotus lividus* collected near the sewage outfall of Cortiou and found that this content was 2.6-fold higher than that measured in sea urchins from an unpolluted zone. A similar increase of cell calcium content was measured by Viarengo et al. (1988) in the digestive gland of mussels exposed to high levels of copper and diesel oil. Thus, increase in calcium concentrations detected in some organs of marine animals may be considered as an index of general toxicity.

Very few data on PROD activities in fish are found in the literature. The majority of studies report a general lack of response to phenobarbitone induction in fish (Kleinow et al. 1987). Although no correlations were found between organic micropollutants and PROD activity (Van der Oost et al. 1991), these authors reported indications for the existence of a phenobarbitone-type inducible enzyme system for 2 fish species: pike (PROD activity ranging from 0.6 to 4.9 pmol min^{-1} mg^{-1}) and eel (from 2.7 to 6.6 pmol min^{-1} mg^{-1}). Addison et al. (1991) found that, for the winter flounder *Pseudopleuronectes americanus* treated with a PCB-substitute, Ugilec-141®, the significant increase in PROD:EROD ratios reflected the absence of measurable PROD activity in control fish and the authors noted a slightly induced PROD activity (41.8 pmol min^{-1} mg^{-1}) in treated dab *Limanda limanda* compared to controls (22.9 pmol min^{-1} mg^{-1}).

The correlation matrices demonstrate that, in general, the variations of enzyme activities EROD and PROD in the livers of *Serranus cabrilla* and *Mullus barbatus* are negatively correlated with metal concentrations in the gills of these fish. Pollutant trace metals may inhibit induction of biotransformation enzyme activity. Addison & Edwards (1988) reported that exposure of flounder to diesel oil and copper in mesocosms over several months did not induce EROD or benzo[a]pyrene hydroxylase (B[a]PH). In addition, in the same mesocosm system Suteau et al. (1988) found that mussels, which accumulated significant amounts of copper, showed a drop in the membrane-bound enzyme activity of B[a]PH and epoxide hydrolase (EH). Fair (1986) noted that administration of cadmium alone to the black sea bass *Centropristis striata* had no effect on B[a]PH and GSH-S-transferase. In plaice *Pleuronectes platessa*, George & Young (1986) found that the cotreatment with both PAH and heavy metal caused an apparent synergistic effect which led to an inhibition of the conjugating enzymes.

The EROD, PROD and GST activities in the liver of *Serranus cabrilla* and *Mullus barbatus* were significantly lower than those previously reported (Mathieu et al. 1991, Narbonne et al. 1991). The presence of high metal concentrations (iron and copper) reported by different authors for sediments and marine organisms collected in this area (Delmas 1986/1987, Arnoux 1988, Pergent-Martini 1992) and also found in this study for the gills of *S. cabrilla* and *M. barbatus* may explain the low hepatic biotransformation enzyme activities.

In conclusion, in samples collected in the area of dispersion of waste waters from the outlet of Cortiou, low biotransformation enzyme activities in the liver of 2 fish species appear to be associated with high levels of metals, particularly iron and copper, in the gills of the organisms. Goksøyr & Förlin (1992) reported that the mechanism of heavy metals, such as cadmium, which lower P-450 activities is not known and should be further studied to give a basis for evaluating the response of biotransformation enzymes in mixed contamination situations, i.e. with organic chemicals and heavy metals present in the environment at the same time. This work is not a general statement about metal accumulation and biotransformation; it reports some local conditions. Nevertheless, in many coves of the NW Mediterranean, treatment plants, using the same processes as in Cortiou, are now in operation and mixed pollution may occur.

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LITERATURE CITED

Addison, R. F., Edwards, A. J. (1988). Hepatic microsomal mono-oxygenase activity in flounder *Platichthys flesus* from polluted sites in Langesundfjord and from mesocosms experimentally dosed with diesel oil and copper. Mar. Ecol. Prog. Ser. 46: 51–54

Addison, R. F., Hansen, P.-D., Pluta, H.-J., Willis, D. E. (1991). Effects of Ugilec-141®, a PCB substitute based on tetrachlorobenzyltoluenes, on hepatic mono-oxygenase induction in estuarine fish. Mar. environ. Res. 31: 137–144

Allemand, D., Walter, P., Delmas, P., De Renzis, G. (1989). Alteration of calcium transport as a mechanism of cell injury induced by $HgCl_2$ in sea urchin eggs. Mar. environ. Res. 28: 227–230

Arnoux, A. (1988). Micropollution dans les sédiments et la

matière vivante (moules et oursins). Etat de référence 1987. In: La reconquête du milieu marin. Direction Générale des Services Techniques, Direction des Services Industriels, Service de l'Assainissement, Ville de Marseille, p. 1–32

Bansal, S. K., Murthy, R. C., Chandra, S. V. (1985). The effects of some divalent metals on cardiac and branchial Ca^{2+} - ATPase in a freshwater fish *Saccobranchus fossilis*. Ecotoxicol. environ. Safety 9: 373–377

Buhler, D. R., Williams, D. E. (1988). The role of biotransformation in the toxicity of chemicals. Aquat. Toxicol. 11(1–2): 19–28

Burke, M. D., Mayer, R. T (1974). Ethoxyresorufin: direct fluorimetric assay of a microsomal 0-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metabol. Dispos. 2: 583–588

Castelbon, C. (1972). Etude de la circulation des masses d'eau dans le golfe de Marseille. Téthys 4: 269–312

Chabert, D., Vicente, N. (1981). Pollution chimique par les métaux lourds et les composés organochlorés d'un milieu lagunaire (lagune du Brusc, Méditerranée, France). J. Etud. Pollut. CIESM 5(1980): 323–333

Charmasson, S. (1982). Etude d'un système marin perturbé par le rejet d'un effluent anthropique: modélisation des processus de dispersion et études courantologiques. Thèse 3ème cycle, Université d'Aix-Marseille II

Delmas, P. (1986/1987). Dynamique des concentrations en métaux lourds dans les gonades et les contenus digestifs de *Paracentrotus lividus* (Lam.) provenant d'une zone soumise à une pollution à dominante domestique et transplantés dans la réserve sous-marine de Monaco. Données préliminaires. In: Compte-rendu des activités 1986–1987. Association Monégasque pour la Protection de la Nature, Monaco, p. 29–31

Delmas, P. (1990). Etude structurale de bioaccumulations de cristaux de fer dans les gonades de l'échinoïde *Paracentrotus lividus* (Lam.) soumis à des rejets de chlorure ferrique. C.r. Acad. Sci., Paris 311 (Sér. III): 69–74

Dubale, M. S., Shah, P. (1979). Toxic effect of cadmium nitrate on the liver of *Channa punctatus*. Experientia 35(5): 643–644

Establier, R., Gutierrez, M., Arias, A. (1978a). Acumulacion y efectos histopatologicos del mercurio inorganico en la lisa (*Mugil auratus* Risso). Invest. Pesq. 42(1): 65–80

Establier, R., Gutierrez, M., Arias, A. (1978b). Acumulacion del mercurio inorganico a partir del agua de mar por el robalo, *Dicentrarchus labrax* L., y sus efectos histopatologicos. Invest. Pesq. 42(2): 471–483

Fair, P. H. (1986). Interaction of benzo(a)pyrene and cadmium on GSH-S-transferase and benzo(a)pyrene hydroxylase in the black sea bass *Centropristis striata*. Arch. environ. Contam. Toxicol. 15: 257–263

Fischer, W., Schneider, M., Bauchot, M.-L. (1987). Fiches FAO d'identification des espèces pour les besoins de la pêche, Méditerranée et Mer Noire, Vol. 2, Vertébrés. FAO, Rome

Foureman, G. L. (1989). Enzymes involved in metabolism of PAH by fishes and other aquatic animals: hydrolysis and conjugation enzymes (or phase II enzymes). In: Varanasi, U. (ed.) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, FL, p. 185–202

George, S. G., Young, P. (1986). The time course of effects of cadmium and 3-methylcholanthrene on activities of enzymes of xenobiotic metabolism and metallothionein levels in the plaice *Pleuronectes platessa*. Comp. Biochem. Physiol. 83C: 37–44

Gnassia-Barelli, M., Roméo, M., Mathieu, A., Romana, A., Lafaurie, M. (1992). Contenu en calcium comme indicateur de pollution chez des poissons de Méditerranée. Rapp. Comm. int. Mer Médit. 33: 174

Goksøyr, A., Förlin, L. (1992). The cytochrome P-450 system in fish, aquatic toxicology and environmental monitoring. Aquat. Toxicol. 22: 287–312

Gony, S., Lecomte-Finiger, R., Faguet, D., Biagianti, S., Bruslé, J. (1988). Etude expérimentale de l'action du cadmium sur les juvéniles d'anguille: biologie du développement et cytopathologie. Océanis 14(1): 141–148

Gutierrez, M., Establier, R., Arias, A. (1978). Acumulacion y efectos histopatologicos del cadmio y el mercurio en el sapo (*Halobatrachus didactylus*). Invest. Pesq. 42(1): 141–154

Habig, W. H., Pabst, M. J., Jakoby, W. B. (1974). Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130–7139

Kleinow, K. M., Melancon, M. J., Lech, J. J. (1987). Biotransformation and induction implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. Environ. Health Perspect. 71: 105–119

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Lubet, R. A., Mayer, R. T., Cameron, J. W., Nims, R. W., Burke, D., Wolff, T., Guengerich, P. (1985). Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. Arch. Biochem. Biophys. 238(1): 43–48

Mathieu, A., Lemaire, P., Carrière, S., Drai, P., Giudicelli, J., Lafaurie, M. (1991). Seasonal and sex-linked variations in hepatic and extrahepatic biotransformation activities in striped mullet (*Mullus barbatus*). Ecotoxicol. environ. Safety. 22: 45–57

Narbonne, J. F., Ribera, D., Michel, X., Raoux, C., Garrigues, P., Monod, J. L., Lemaire, P., Galgani, F., Roméo, M., Salaün, J. P., Lafaurie, M. (1991). Indicateurs biochimiques de contamination de l'environnement marin: étude comparative en mer Méditerranée. Océanis 17(3): 257–275

Payne, J. F., Francey, L. L., Rahimtula, D., Porter, E. L. (1987). Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp. Biochem. Physiol. 86C: 233–245

Pergent-Martini, C. (1992). Contribution à l'étude des stocks des flux d'éléments dans l'écosystème à *Posidonia oceanica*. 1. Etude expérimentale sur la croissance. 2. Mémorisation des teneurs en métaux traces. Diplôme de DESS 'Ecosystèmes méditerranéens', Université de Corse

Reddy, R. S., Jinna, R. R., Uzodinma, J. E., Desaiah, D. (1988). *In vitro* effect of mercury and cadmium on brain Ca^{2+} - ATPase of the catfish *Ictalurus punctatus*. Bull. environ. Contam. Toxicol. 41: 324–328

Regoli, F., Orlando, E., Mauri, M., Nigro, M., Cognetti Affiniti, G. (1991). Heavy metal accumulation and calcium content in the bivalve *Donacilla cornea*. Mar. Ecol. Prog. Ser. 74: 219–224

Shephard, K., Simkiss, K. (1978). The effect of heavy metal ions on Ca^{2+} - ATPase extracted from fish gills. Comp. Biochem. Physiol. 61B: 69–72

Sinovcic, G., Gutierrez, M., Establier, R. (1980). On the accumulation of mercury in the blood, liver, spleen and kidney of *Halobatrachus didactylus* Schneider and resulting haematologic, cytohaematologic and histopathologic alterations. Acta adriat. 21(1): 219–225

Sørensen, E. M. B., Ramirez-Mitchell, R., Harlan, C. W., Bell,

J. S. (1980). Cytological changes in the fish liver following chronic environmental arsenic exposure. Bull. environ. Contam. Toxicol. 25: 93–99

Suteau, P., Daubeze, M., Migaud, M. L., Narbonne, J. F. (1988). PAH-metabolizing enzymes in whole mussels as biochemical tests for chemical pollution monitoring. Mar. Ecol. Prog. Ser. 46: 45–49

Van der Oost, R., Heida, H., Opperhuizen, A., Vermeulen, N. P. E. (1991). Interrelationships between bioaccumulation of organic trace pollutants (PCBs, organochlorine pesticides and PAHs), and MFO-induction in fish. Comp. Biochem. Physiol. 100C(1/2): 43–47

Viarengo, A., Mancinelli, G., Martino, G., Pertica, M., Canesi, L., Mazzucotelli, A. (1988). Integrated cellular stress indices in trace metal contamination: critical evaluation in a field study. Mar. Ecol. Prog. Ser. 46: 65–70

Zhang, G. H., Yamaguchi, M., Kimura, S., Higham, S., Kraus-Friedmann, N. (1990). Effects of heavy metals on rat liver microsomal and sequestering. J. Biol. Chem. 265: 2184–2189

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