Vol. 192: 259–266, 2000 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published January 31

Xenobiotic metabolising enzymes and antioxidant defences in deep-sea fish: relationship with contaminant body burden

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ABSTRACT: Xenobiotic metabolising enzymes, namely the cytochrome P450 monooxygenase system and glutathione *S*-transferase, as well as antioxidant enzymes were determined in the liver of 3 deep-sea fish species (*Lepidion lepidion*, *Bathypterois mediterraneus* and *Coryphaenoides guentheri*). These organisms were collected from 3 stations along the NW Mediterranean, at depths ranging from 1500 to 1800 m. The enzymatic activities detected in deep-sea fish indicated the ability of these organisms to cope with pollutants and oxidative stress. Differences among the studied species may be related to habitat and diet, viz. the highest monooxygenase and antioxidant enzyme activities were observed in *L. lepidion*, a typical middle slope species, whereas the lowest activities were recorded for *B. mediterraneus*, an organism adapted to live at greater depths. Tissue concentration of persistent organochlorinated compounds, like polychlorinated biphenyls, DDTs and hexachlorobenzene, were in the lower range of those reported for coastal fish, and significant differences among sampling stations were not observed. Overall, the obtained results contribute to the knowledge of the NW Mediterranean deep-sea basin and reflect the chronic contamination of the area in terms of organochlorinated compounds, rather than the influence of coastal discharges.

KEY WORDS: Deep-sea fish · Cytochrome P450 · EROD · GST · Antioxidant enzymes · PCBs · PAHs in bile

INTRODUCTION

Deep-sea regions (depths >1000 m) encompass about 75% of the biosphere. Despite their remoteness, these regions are reached by man-made pollutants. Several models suggest that deep-sea sediments are the final accumulation site for organochlorinated compounds (Woodwell et al. 1971), and fish living in association with sediments are exposed to and accumulate these compounds (Hargrave et al. 1992, Berg et al. 1997). Likewise, polycyclic aromatic hydrocarbons (PAHs) occur in remote deep-sea areas (Karinen 1980, Lipiatou et al. 1997), and their bioaccumulation by deep-sea organisms has been documented (Steimle et al. 1990, Escartín & Porte 1999). Trace metals also occur in deep-sea fish at concentrations similar to those detected in coastal species (Steimle et al. 1990).

In contrast, very little work has focused upon the potential enzymatic systems that may be involved in xenobiotic biotransformation in deep-sea fish (Stegeman et al. 1986). Because deep-sea organisms have adapted to particular environmental conditions (high pressure, low temperature and absence of light), they may respond differently than coastal species to pollutants (Gross & Jaenicke 1994). Xenobiotics within the organism undergo a suit of reactions to facilitate their excretion. In all eukaryotes, the cytochrome P450 system and glutathione *S*-transferases (GSTs) play a key role in the biotransformation (monooxygenation and conjugation) of lipophilic foreign chemicals, such as PAHs and polychlorinated biphenyls (PCBs). Substantial differences in monooxygenase or transferase activities and number of isoenzymes have been reported in marine organisms, depending on habitat, pollutants load, etc. (Förlin et al. 1995, Beyer et al. 1996, Stegeman et al. 1997). Metabolic rates of deep-sea fish are known to decrease with depth as a result of several

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interacting factors, such as low temperatures, low food availability and poor locomotory capabilities (for a review see Gibbs 1997); thus strong differences from coastal species in terms of xenobiotic metabolising enzymes could be anticipated.

Many pollutants in aquatic systems exert their toxic effects due to oxidative stress (Winston & Di Giulio 1991, Thomas & Wofford 1993, van der Oost et al. 1996). Oxyradicals are continually produced in eukaryotes as unwanted biproducts of normal oxidative metabolism, and their production can be increased by conditions such as hypoxia/hyperoxia, redox cycling xenobiotics (e.g. metals, quinones, nitroaromatic compounds) and induction of enzymes, such as cytochrome P450 and P450 reductase (Premereur et al. 1986). Consequently, aerobic organisms have developed defence systems against oxidative damage (Di Giulio et al. 1989), consisting of antioxidant scavengers (glutathione, vitamin C, vitamin E, carotenoid pigments), and specific antioxidant enzymes: catalase (EC 1.11.1.6), superoxide dismutase (SOD; EC 1.15.1.1) and glutathione peroxidase (GPX; EC 1.11.1.9). These enzymes participate in the removal of reactive oxygen species and have been detected in a number of fish species (Lemaire & Livingstone 1993). However, no data are available for deep-sea fish.

This study will focus on 3 Mediterranean deep-sea fish species, *Lepidion lepidion*, *Coryphaenoides guentheri* and *Bathypterois mediterraneus*, sampled at depths of 1500 to 1800 m, and selected on the basis of their abundance and broad distribution in the area.

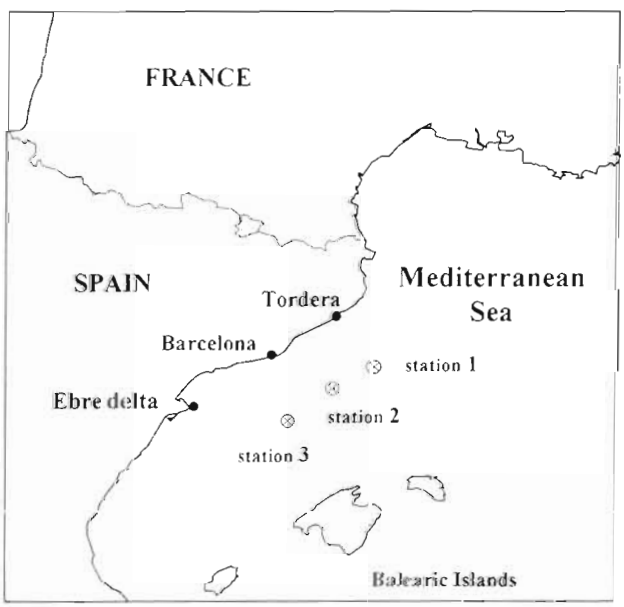

Fig. 1. Map of the western Mediterranean showing locations of the sampling sites. Stn 1: 41° 14' N, 3° 20' E (1812 m); Stn 2: 41° 01' N, 2° 34' E (1546 m); Stn 3: 40° 41' N, 1° 58' E (1601 m)

The western Mediterranean receives urban and industrial waste water discharges from bordering countries. Surveys have been carried out along the coast using fish as biomonitors (Porte & Albaigés 1993, Burgeot et al. 1996, Escartín 1999). Nothing is known, however, about the contamination of deep-sea fish or their ability to cope with pollutants. Hence, the purpose of this study was to identify and characterise xenobiotic metabolising enzymes and antioxidant defence systems in 3 deep-sea fish species from the area. In addition, the exposure of these organisms to persistent organic pollutants such as PCBs, DDTs and hexachlorobenzene (HCB) will be assessed.

MATERIALS AND METHODS

Sample collection and preparation. Fish samples were collected by trawling from 3 stations along the western Mediterranean at depths ranging from 1500 to 1800 m (Fig. 1). Once on board, individuals were immediately dissected, the liver frozen in liquid nitrogen and stored at −80°C for biochemical analysis. A piece of dorsal muscle was wrapped in clean aluminium foil and stored at −20°C for analysis of organochlorinated compounds.

Biochemical analysis. Cytosolic and microsomal fractions were prepared essentially as described in Förlin & Andersson (1985). After weighing, livers were flushed with ice-cold 1.15% KCl and homogenized in 4 vol. of cold 100 mM KH_2PO_4/K_2HPO_4 buffer pH 7.4, containing 0.15 M KCl, 1 mM dithiothreitol (DTT), 1 mM EDTA and 0.1 mM phenylmethylsulfonylfluoride (PMSF). Homogenates were centrifuged at 500 × *g* for 10 min, the fatty layer removed and the supernatant centrifuged at 10000 × *g* for 20 min. The 10000 × *g* supernatant was further centrifuged at 100000 × *g* for 60 min to obtain the cytosolic and microsomal fractions. Microsomal pellets were resuspended in a small volume of 100 mM Tris-HCl pH 7.4 containing 0.15 M KCl, 20% w/v glycerol, 1 mM DTT, 1 mM EDTA and 0.1 mM PMSF.

Cytosolic and microsomal protein content were measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Cytochrome P450 system. Cytochrome P450 system components were measured in the microsomal fraction. Cytochrome P450 was determined by the sodium dithionite-difference spectrum of carbon monoxide treated samples, assuming an extinction coefficient of 91 mM^{-1} cm^{-1} for P450 and 105 mM^{-1} cm^{-1} for the '420' peak (Estabrook & Werringloer 1978). NADPH-cytochrome *c* reductase activity was measured by the increase in absorbance at 550 nm (extinction coefficient 19.6 mM^{-1} cm^{-1}) after adding NADPH to the microsomal fraction (Shimakata et al. 1972). 7-ethoxyresorufin

O-deethylase activity (EROD) was determined at 30°C as described in Burke & Mayer (1974); 10 µl of microsomes were incubated for 10 min in a final volume of 1.0 ml containing 90 mM KH_2PO_4/K_2HPO_4 pH 7.4, 0.22 mM NADPH and 3.70 µM 7-ethoxyresorufin. The reaction was stopped by adding 2.0 ml of ice-cold acetone, samples were centrifuged at low speed, and 7-hydroxyresorufin fluorescence determined using a Perkin-Elmer LS-5 spectrofluorometer at 537/583 nm excitation/emission wavelengths.

Glutathione *S*-transferase. GST activity was measured in the cytosolic fraction using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, the final reaction mixture containing 1 mM CDNB and 1 mM reduced glutathione (Habig et al. 1974).

Antioxidant enzymes. Antioxidant enzymes were measured essentially as described in Livingstone et al. (1992). Catalase activity was determined in the cytosolic fraction (100000 × *g* supernatant) and in the 10000 × *g* pellet, after the latter was resuspended in a reduced volume of homogenisation buffer. The activity was measured by the decrease in absorbance at 240 nm (extiction coefficient 40 M^{-1} cm^{-1}) using 50 mM H_2O_2 as substrate, and expressed as the sum of both fractions' activities. SOD activity was determined by the degree of inhibition of cytochrome c reduction by superoxide anion radical, by recording the absorbance at 550 nm as described in McCord & Fridovich (1969). The activity of this enzyme is given in SOD units (1 unit = 50% of the inhibition of cytochrome c reduction); the assay conditions were 87 mM KH_2PO_4/K_2HPO_4 pH 7.8, 50 µM hypoxanthine, 10 µM cytochrome c and 1.8 mU ml^{-1} xanthine oxidase. GPX activity was measured by the NADPH consumption monitored at 340 nm (extiction coefficient 6.2 mM^{-1} cm^{-1}) during the formation of reduced glutathione by commercial glutathione reductase using 0.8 mM H_2O_2 as a substrate (Se-dependent GPX) or 3 mM cumene hydroperoxide (sum of Se-dependent and Se-independent activities, and referred as total-GPX) (Günzler & Flohé 1985).

Chemical analysis. Muscle samples of 4 to 6 individuals per location were pooled and subsamples of approximately 4 to 5 g were homogenised with anhydrous Na_2SO_4 and Soxhlet-extracted with *n*-hexane: dichloromethane (4:1) for 18 h. The solvent extract was evaporated near to dryness, the residue dissolved in 3 ml of *n*-hexane and cleaned-up by vigorous shaking with 1 to 2 ml of concentrated sulphuric acid. The extract was then injected onto a gas chromatograph (Hewlett Packard 5890) equipped with an ECD detector at 300°C. The column, a 50 m × 0.25 mm i.d. CP-Sil 5 CB fused silica (Chrompack, Middelburg, The Netherlands), was programmed from 80 to 180°C at 15°C min^{-1} and from 180 to 280°C at 3°C min^{-1}, keeping the final temperature for 15 min. The carrier gas was helium at a linear flow-rate of 50 cm s^{-1}. The injector temperature was 280°C. Quantification was performed using an external standard calibration mixture of selected congeners (IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180) supplied by Promochem (Wesel, Germany). These congeners were quantified separately and the PCB concentration defined as its sum. *p,p'*DDT and its metabolites *p,p'*DDE and *p,p'*DDD, and hexachlorobenzene (HCB) were also determined. Analyses were carried out in duplicate.

Statistics. Biochemical activities were determined individually in 4 to 12 organisms per station, assays run in duplicate, and the results showed as mean ± SEM. Chemical analyses of PCBs were run in duplicate from pooled tissue of 4 to 12 individuals, the results given as mean ± range. Statistical significant differences were assessed using a Student's *t*-test ($p \leq 0.05$).

RESULTS

Biological data of samples

Biological parameters of sampled individuals are listed in Table 1. Individuals were adults and samples

Table 1. Biological data of the sampled fish. Values are mean ± SEM; number of organisms analysed per station is given in parentheses. Condition factor: CF = [(body weight)/(body length)^3] × 100; Liver somatic index: LSI = [(liver weight/body weight) × 100]

	Stn 1	Stn 2	Stn 3
Weight (g)			
Lepidion lepidion	66.0 ± 9.9 (4)	92.7 ± 15.7 (6)	61.1 ± 4.3 (6)
Coryphaenoides guentheri	8.4 ± 0.6 (8)	7.1 ± 0.9 (10)	11.7 ± 1.6 (6)
Bathypterois mediterraneus	14.0 ± 0.4 (12)	16.0 ± 0.3 (12)	18.2 ± 0.6 (12)
Length (cm)			
L. lepidion	21.3 ± 0.7	23.9 ± 1.4	20.6 ± 0.2
C. guentheri	14.9 ± 0.3	12.5 ± 0.6	15.9 ± 0.6
B. mediterraneus	15.7 ± 0.4	15.1 ± 0.2	16.3 ± 0.2
CF (g cm^{-3})			
L. lepidion	0.68 ± 0.09	0.66 ± 0.03	0.70 ± 0.03
C. guentheri	0.25 ± 0.01	0.36 ± 0.02	0.28 ± 0.02
B. mediterraneus	0.37 ± 0.02	0.46 ± 0.01	0.42 ± 0.01
LSI			
L. lepidion	0.98 ± 0.25	2.00 ± 0.67	1.62 ± 0.34
C. guentheri	1.22 ± 0.10	2.36 ± 0.28	2.03 ± 0.16
B. mediterraneus	0.31 ± 0.05	0.58 ± 0.02	0.36 ± 0.08

homogeneous (size and weight). The condition factor (CF) evidenced strong differences among fish species, but no differences among sampling stations. Similarly, the liver somatic index (LSI) was not significantly different between sampling sites, though there was a trend towards larger livers in specimens sampled at Stn 2. Among species, the largest livers were observed in *Coryphaenoides guentheri* (1.2 to 2.4) followed by *Lepidion lepidion* and *Bathypterois mediterraneus* (0.3 to 0.4).

Cytochrome P450 system

The dithionite difference spectra of CO-treated microsomes showed a cytochrome P450 peak at 448 nm for all samples, and an additional peak at approximately 420 to 422 nm, which may correspond to cytochrome P420. Such apparent degradation varied among species and it could be due to the fact that the fish were dead on retrieval, given the time needed to collect the net from 1500 to 1800 m depth (approximately 45 min). Assuming an extinction coefficient of 105 mM^{-1} cm^{-1} for P420, all samples contained a greater amount of P420 than P450, and the average values for the P420/P450 ratio were 11.1 for *Coryphaenoides guentheri* and *Lepidion lepidion*, and 4.4 for *Bathypterois mediterraneus*. The presence of P420 suggests that the catalytic rates measured may not reflect the full catalytic capacity of the samples. Nonetheless, cytochrome P450 was detected in all 3 species, and was higher in *C. guentheri* (138 to 252 mol mg^{-1} protein) (Table 2). When combined with P420, differences among species were even more evident. The total P450 content approached 2.0 nmol mg^{-1} protein in *C. guentheri*, but was 0.5 nmol mg^{-1} protein in *B. mediterraneus*. No significant differences among sampling sites were detected.

The specific activity of NADPH cytochrome c (P450) reductase ranged from 13 to 28 nmol min^{-1} mg^{-1} protein, with the lowest activities in *Lepidion lepidion*, slightly higher activities for the other 2 species. Nevertheless, no statistically significant differences among species or sampling sites were observed (Table 2).

Strong differences among species were, however, seen in EROD activity, which was significantly elevated in *Lepidion lepidion* (111 to 192 pmol min^{-1} mg^{-1} protein) compared with *Coryphaenoides guentheri* (23 to 29 pmol min^{-1} mg^{-1} protein) and *Bathypterois mediterraneus* (2.6 to 7.2 pmol min^{-1} mg^{-1} protein). Differences were greater when EROD activity was expressed as an estimate of turnover number (pmol min^{-1} $pmol^{-1}$ P450), viz. *L. lepidion* showed a turnover number of 1.03 ± 0.19 pmol min^{-1} $pmol^{-1}$ P450, *C. guenteri* (0.16 ± 0.03) and *B. mediterraneus* (0.05 ± 0.02). These results suggest a higher catalytic efficiency of *L. lepidion*. No statistically significant differences among sampling sites were observed in terms of EROD activity, although *L. lepidion* and *B. mediterraneus* from Stn 2 showed a slightly higher EROD activity.

Table 2. Cytochrome P450 monooxygenase and glutathione *S*-transferase (GST) activities in deep-sea fish from the western Mediterranean. Values are mean ± SEM (n = 4 to 12 organisms analysed individually)

	Stn 1	Stn 2	Stn 3
Cytochrome P450[a]			
Lepidion lepidion	63.3 ± 31.5	128.2 ± 22.3	116.7 ± 18.5
Coryphaenoides. guentheri	252.3 ± 63.5	261.3 ± 79.3	137.6 ± 35.9
Bathypterois mediterraneus	157.1 ± 45.0	117.9 ± 46.3	98.0 ± 15.3
P450 + P420[a]			
L. lepidion	874 ± 223	1484 ± 418	1093 ± 137
C. guentheri	2054 ± 441	1944 ± 125	1820 ± 173
B. mediterraneus	480 ± 31	596 ± 100	545 ± 119
NADPH cyt. c reductase[b]			
L. lepidion	18.4 ± 4.5	16.6 ± 3.5	13.1 ± 2.5
C. guentheri	24.2 ± 6.2	19.2 ± 1.3	20.9 ± 3.2
B. mediterraneus	20.6 ± 2.8	19.5 ± 2.8	27.7 ± 14.2
EROD[c]			
L. lepidion	126.6 ± 56.3	192.0 ± 54.7	111.2 ± 28.0
C. guentheri	29.4 ± 9.1	26.8 ± 7.2	23.3 ± 6.8
B. mediterraneus	6.7 ± 1.0	7.2 ± 1.1	2.6 ± 1.2
GST[b]			
L. lepidion	958.3 ± 92.4	957.5 ± 123.6	983.9 ± 166.3
C. guentheri	1629.1 ± 177.9	1506.9 ± 212.3	1436.0 ± 427.3
B. mediterraneus	246.6 ± 128.6	206.9 ± 133.4	174.7 ± 102.1

[a]pmol mg^{-1} protein
[b]nmol min^{-1} mg^{-1} protein
[c]pmol min^{-1} mg^{-1} protein

Glutathione *S*-transferase

The activity of cytosolic GST measured with CDNB as substrate is given in Table 2. Strong differences among species were evident, the highest specific activity detected in *Coryphaenoides guentheri* (1.4 to 1.6 (mol min^{-1} mg^{-1} cytosolic protein). Lower activities were observed in *Lepidion lepidion* (0.9 µmol min^{-1} mg^{-1} protein) and *Bathypterois mediterraneus* (0.2 µmol min^{-1} mg^{-1} protein). Within species, no significant differences among sampling sites were observed.

Table 3. Antioxidant enzyme activities in deep-sea fish from the western Mediterranean. Values are mean ± SEM (n = 4 to 12 organisms analysed individually). *Significantly different from Stn 1 (Student's *t*-test, $p \leq 0.05$)

	Stn 1	Stn 2	Stn 3
Catalase[a]			
Lepidion lepidion	101.3 ± 16.4	82.9 ± 11.2	91.6 ± 9.8
Coryphaenoides guentheri	82.4 ± 11.7	78.3 ± 9.5	75.2 ± 7.4
Bathypterois mediterraneus	39.5 ± 4.9	18.6 ± 5.8	33.5 ± 6.4
SOD[b]			
L. lepidion	12.9 ± 1.9	16.9 ± 3.3	13.5 ± 1.8
C. guentheri	11.9 ± 1.3	10.2 ± 1.3	9.9 ± 1.2
B. mediterraneus	9.2 ± 4.5	7.2 ± 3.1*	8.9 ± 3.1
Se-dependent GPX[c]			
L. lepidion	40.0 ± 6.3	47.2 ± 7.7	33.3 ± 4.1
C. guentheri	38.6 ± 1.6	42.8 ± 5.1	37.0 ± 4.4
B. mediterraneus	18.7 ± 0.5	22.9 ± 1.5	25.0 ± 1.2*
Total-GPX[c]			
L. lepidion	62.3 ± 7.5	72.7 ± 8.8	78.3 ± 14.5
C. guentheri	98.3 ± 4.9	103.0 ± 7.7	98.9 ± 16.9
B. mediterraneus	18.2 ± 0.8	19.5 ± 0.5	23.8 ± 3.0

[a]mmol min^{-1} g^{-1} wet weight
[b]units min^{-1} mg^{-1} protein
[c]nmol min^{-1} mg^{-1} protein

Antioxidant enzymes

Antioxidant enzyme activities are shown in Table 3. As previously observed for cytochrome P450 system and GST activity, *Bathypterois mediterraneus* showed the lowest antioxidant enzyme activities. The highest activities were detected in *Lepidion lepidion*, with the exception of total GPX which was higher in *Coryphaenoides guentheri*. Differences among sampling sites were only observed for *B. mediterraneus*, which showed significantly depleted catalase activity at Stn 2, and elevated GPX(H_2O_2) activity at Stn 3. For the other species and activities, no significant differences among sampling sites were observed.

Organochlorinated compounds

Levels of organochlorinated compounds (PCBs, DDTs and HCB) were determined in fish muscle and the results are given in Table 4. While no geographical differences were observed, differences among species were evidenced. PCBs and DDTs were up to 5-fold higher in *Lepidion lepidion* and *Bathypterois mediterraneus* than in *Coryphaenoides guentheri*; conversely, HCB was highest in *C. guentheri*. We also found qualitative differences in PCB bioaccumulation profiles. Detailed examination of the GC-ECD chromatographic profiles enabled the identification of 30 individual congeners. The highly chlorinated PCB IUPAC Nos. 138, 153 and 180 were dominant in all 3 species, and accounted for 49 to 50% of the total detected PCBs in *L. lepidion* and *B. mediterraneus*, but only 38% in *C. guentheri*.

DISCUSSION

Hepatic microsomal preparations of the species studied showed active electron transport components and native cytochrome P450. Total cytochrome P450 content (P450+P420) in *Coryphaenoides guentheri* and *Lepidion lepidion* were in the higher range (0.9 to 2.0 mol mg^{-1} protein) reported for teleosts. The specific activity of NADPH-cytochrome c (P450) reductase, the microsomal flavoprotein that transfers electrons from NADPH to cytochrome P450, was also similar to that reported for other species (Mathieu et al. 1991, Haasch et al. 1993, Sleiderink et al. 1995). Therefore, a high monooxygenase capacity of deep-sea fish hepatic tissue could be anticipated. However, only *L. lepidion* exhibited an EROD activity comparable to coastal fish (Collier et al. 1995, Förlin et al. 1995, Escartín 1999). Similarly, when EROD activity is expressed as the turnover number (EROD/P450), *L. lepidion* demonstrated a high catalytic efficiency, similar to coastal fish from the

Table 4. Organochlorinated compounds in muscular tissue (mean ± range, n = 2) of different deep-sea fish collected from the western Mediterranean. Each sample is a pool of 4 to 12 organisms. Results are given in ng g^{-1} wet weight

	Stn 1	Stn 2	Stn 3
PCBs			
Lepidion lepidion	9.4 ± 3.1	8.3 ± 2.5	9.4 ± 0.5
Coryphaenoides guentheri	4.6 ± 2.3	3.0 ± 0.4	2.5 ± 0.8
Bathypterois mediterraneus	6.8 ± 2.4	6.0 ± 3.3	10.0 ± 4.2
DDTs			
L. lepidion	7.1 ± 2.0	6.0 ± 1.2	6.2 ± 0.1
C. guentheri	4.3 ± 1.8	2.5 ± 0.2	1.9 ± 0.5
B. mediterraneus	5.0 ± 1.3	5.9 ± 1.9	10.2 ± 4.9
HCB			
L. lepidion	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
C. guentheri	0.25 ± 0.08	0.67 ± 0.20	0.50 ± 0.36
B. mediterraneus	0.16 ± 0.04	0.12 ± 0.01	0.25 ± 0.03

region (Escartín 1999). To our knowledge, there are no data on EROD activity in deep-sea species apart from 2 macrourid species, *C. armatus* sampled in the western North Atlantic and *C. rupestris* caught in the North Sea (Stegeman et al. 1986, Förlin et al. 1995), which showed similar EROD activities to those we found. High pressure and cold temperatures may influence membrane functioning by reducing fluidity and affecting protein-protein interactions (Gibbs 1997). Thus, the interaction between cytochrome P450 and reductase in the membrane will be less effective in deep-sea fish than in shallow-water fish, which may lead to low cytochrome P450 catalytic activities. *L. lepidion*, a typical middle slope species (1000 to 1400 m), exhibited higher EROD activity (between 4- and 74-fold) than *Bathypterois mediterraneus* and *C. guentheri*; both organisms adapted to live at greater depths, with maximum abundance at 1600 to 2200 m (Stefanescu et al. 1992).

There were no differences between sampling sites in terms of cytochrome P450 system or associated activities (Table 4). Because this system is readily induced by PAHs, dioxins, PCBs and other planar halogenated hydrocarbons, our results suggest no significant differences in exposure.

In addition to characterising aspects of Phase I monooxygenation, GST—one of the major Phase II conjugation enzymes—was also examined. Cytosolic GST activity was in the range or even higher than the activities reported for coastal fish (Förlin et al. 1995, Escartín 1999). The high GST activity detected in *Coryphaenoides guentheri* is related to high P450 specific content, which is in accordance with the important role of GSTs in conjugation of electrophiles produced by P450 monooxygenation. Elevated GST activity may have been selected in these organisms as protection against toxic dietary chemicals. *C. guentheri* lives in direct contact with sediment, it feeds actively on small epibenthic and endobenthic invertebrates (Stefanescu et al. 1992), and it might therefore be exposed to a higher amount of sediment-trapped pollutants than the other 2 species. Although levels of PCBs in muscle tissue of *C. guentheri* were relatively low, previous work by Escartín & Porte (1999) found PAH metabolites in bile of this species up to 1 order of magnitude higher than in *Bathypterois mediterraneus* or *Lepidion lepidion*. Alternatively, low GST activities were observed in *B. mediterraneus*. This species also presented the lowest P450 content and has a diet restricted to zooplankton (Carrason & Matallanas 1990).

Interspecies variations were also observed in antioxidant enzyme activities. *Lepidion lepidion* showed higher catalase and SOD activities, higher catalytic efficiency (EROD/P450) and elevated cytochrome P450 content. A higher production of oxyradicals via reactions catalysed by cytochrome P450 enzymes can be anticipated, requiring mechanisms to remove those oxyradicals. Total GPX activity—due to some isozymes of GST (Se-independent GPX) plus Se-dependent GPX—was significantly increased in *Coryphaenoides guentheri*, which also showed the highest GST activity. Generally, the ratio total GPX activity to Se-GPX was rather low in *L. lepidion* and *C. guentheri*, and suggests that the major role of GSTs in these organisms is conjugation of xenobiotics rather than antioxidant function, particularly in *L. lepidion*. In contrast, Se-independent GPX was not observed in *Bathypterois mediterraneus*. This organism also exhibited low activities of catalase and Se-GPX (both detoxify H_2O_2) in comparison with the other species, and this may be related to the absence of a swim bladder. It is reported that with increasing depth and increasing hydrostatic pressure, most species maintain constant swim bladder volume by increasing mainly its oxygen content, that may make up to 90% of the gas mixture in deep-sea fish (Morris & Albright 1984). Thus, the gas gland tissue operates under conditions of hyperoxia, and this enhances oxyradical production (Jones 1985).

Coastal fish show great variations in antioxidant activities (Cassini et al. 1993, Filho et al. 1993, Förlin et al. 1995, Rudneva 1997), which are influenced by season, feeding habits, salinity and oxygen pressure (Desrochers & Hoffert 1983, Radi et al. 1985, Winston & Di Giulio 1991, Fitzgerald 1992). Pollutants also act as pro-oxidant chemicals through redox cycling and other processes. Taking into account all of these factors, and the fact that different workers use slightly different methods, SOD activities were in the range reported for coastal fish (Lemaire & Livingstone 1993, García de la Parra 1998), whereas catalase was generally higher, and total and Se-dependent GPX activities were lower in deep-sea fish. All these enzymes are inducible, they are of central importance for the antioxidant defence mechanisms of aquatic organisms (Winston & Di Giulio 1991), and were present at varying levels in the 3 deep-sea species we studied. Elevated antioxidant defences in deep-sea fish might reflect exposure to pollutants acting as pro-oxidant chemicals, but they may also represent an adaptative mechanism to low oxygen availability (Vig & Nemcsok 1989).

Concerning the exposure of these organisms to persistent organochlorinated compounds, we observed concentrations in muscle similar to those found in fish from relatively clean coastal areas of the region (Porte & Albaigés 1993, Pastor et al. 1996, García de la Parra 1998), which may be representative of basal or chronic pollution for the NW Mediterranean deep-sea basin. All stations and organisms indicated a prevalence of PCBs over DDTs, and a strong decrease of PCBs and DDTs from the corresponding coastal sites. No site

pollution gradient was observed and we did not detect any simple relationship between food preference and levels of organochlorinated compounds in deep-sea fish. On the contrary, accumulation of highly chlorinated congeners appeared to be a general rule for all the studied species; the hexa- to octochloro isomers constituted 80 to 92% of the total detected PCBs. This may be due to particle-bound transportation of PCBs from the surface to deep-sea environments, and the fact that highly chlorinated PCBs are relatively more adsorbed to suspended solids than low chlorinated ones. Nevertheless, other factors such as bio-degradability of the different congeners are also of importance. Muscle tissue of all 3 species was enriched in PCB congeners Nos. 153, 138, 187, 180 and 170. All these isomers have chlorine atoms in positions 2,4,5- in one (PCB-138, 187 and 170) or both rings (PCB-153 and 180) in common, a substitution particularly recalcitrant to degradation. In addition, a certain number of congeners were clearly depleted, namely those isomers containing vicinal H atoms at *meta* and *para* positions in at least 1 aromatic ring, irrespective of the *ortho* chlorine substitutions, viz. PCB-52, 101, 110, 151, 149 and 141. These congeners represented only 7 to 13% of total detected PCBs, against 15 to 19% in coastal fish or 35% in mussels from the area (Porte & Albaigés 1993). Thus, the detected PCB patterns indicate that deep-sea fish are exposed to highly degraded PCB patterns.

Overall, the obtained results describe hepatic metabolising enzymes and antioxidant defences in deep-sea fish and demonstrate the ability of these organisms to cope with xenobiotics and palliate oxidative damage. These enzymatic systems appear to be as catalytically efficient as those of shallow-water species, although one must be cautious in making generalisations, because of the great variations observed for different coastal fish species and sites.

Acknowledgements. This work was partially supported by the POLTOX Project (Generalitat de Catalunya) and the Spanish National Plan for Research (PLANYCIT) under Project Ref. AMB95-1092-CE. Dr Domingo Lloris and Dr Jaume Rucabado (ICM-CSIC, Barcelona- Spain) are gratefully acknowledged for their valuable experience in sampling, and help with species identification. This paper is dedicated to the memory of our friend and colleague Dr Jaume Rucabado, who generously introduced us to the world of deep-sea fish and, unfortunately, left us without seeing the results of his teaching.

LITERATURE CITED

Berg V, Ugland KI, Hareide NR, Aspholm PE, Polder A, Skaare JU (1997) Organochlorine contamination in deep-sea fish from the Davis Strait. Mar Environ Res 44:135–148

Beyer J, Sandvik M, Hylland K, Fjeld E, Egaas E, Aas E, Skare JU, Goksøyr A (1996) Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus* L.) and atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sørfjorden, Norway. Aquat Toxicol 36: 75–98

Burgeot T, Bocquéné G, Porte C, Dimeet J, Santella RM, García de la Parra LM, Pfhol-Leszkowicz A, Raoux C, Galgani F (1996) Bioindicators of pollutant exposure in the northwestern Mediterranean Sea. Mar Ecol Prog Ser 131: 125–141

Burke MD, Mayer RT (1974) Ethoxyresorufin: direct fluorimetric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-methylchlorantrene. Drug Metab Disp 2:583–588

Carrason M, Matallanas J (1990) Preliminary data about the feeding habits of some deep-sea Mediterranean fishes. J Fish Biol 36:461–463

Cassini A, Favero M, Albergoni V (1993) Comparative studies of antioxidant enzymes in red-blooded and white-blooded Antarctic teleost fish. *Pagothenia bernacchii* and *Chionodraco hamatus*. Comp Biochem Physiol 106C:333–336

Collier TK, Anulacion BF, Stein JE, Goksøyr A, Varanasi U (1995) A field evaluation of cytochrome P4501A as a biomarker of contamination exposure in three species of flatfish. Environ Toxicol Chem 14:143–152

Desrochers PE, Hoffert JR (1983) Superoxide dismutase provides protection against the hyperoxia in the retina of the rainbow trout (Salmo gairdneri). Comp Biochem Physiol 76B:241–247

Di Giulio RT, Washburn PC, Wenning RJ, Winston GW, Jewel CS (1989) Biochemical responses in aquatic animals: a review of determinants of oxidative stress. Environ Toxicol Chem 8:1103–1123

Escartín E (1999) Utilització de biomarcadors per a la vigilància de la contaminació ambiental del Mediterrani nord-occidental. PhD thesis, University of Barcelona

Escartín E, Porte C (1999) Hydroxylated PAHs in bile of deep-sea fish. Relationship with xenobiotic metabolizing enzymes. Environ Sci Technol 33:2710–2714

Estabrook RW, Werringloer J (1978) The measurement of difference spectra: Application to the cytochromes of microsomes. In: Fleischer S, Packer L (eds) Methods of enzymology, Vol 52. Biomembranes: Part C: Biological oxidations, microsomal, cytochrome P-450, and other hemoprotein systems. Academic Press, New York, p 212–220

Filho DW, Giulivi C, Boveris A (1993) Antioxidant defences in marine fish. I. Teleosts. Comp Biochem Physiol 106C: 409–413

Fitzgerald JP (1992) Comparative analysis of superoxide dismutase activities in a range of temperate and tropical teleost fish. Comp Biochem Physiol 101B:111–114

Förlin L, Andersson T (1985) Storage conditions of rainbow trout liver cytochrome P450 and conjugating enzymes. Comp Biochem Physiol 80B:569–572

Förlin L, Lemaire P, Livingstone DR (1995) Comparative studies of hepatic xenobiotic metabolizing and antioxidant enzymes in different fish species. Mar Environ Res 39: 201–204

García de la Parra LM (1998) Distribución de compuestos organoclorados en peces del Mediterráneo noroccidental. Metabolismo oxidativo y mecanismos de defensa. PhD thesis. University of Barcelona

Gibbs AG (1997) Biochemistry at depth. In: Randall DJ, Farrell AP (eds) Deep-sea fishes. Academic Press, New York, p 239–277

Gross M, Jaenicke R (1994) Proteins under pressure. The influence of high hydrostatic pressure on structure, func-

tion and assembly of proteins and protein complexes. Eur J Biochem 227:617–630
Günzler WA, Flohé L (1985) Glutathione peroxidase. In: Greenwald RA (ed) Handbook of methods for oxygen radical research. CRC Press, Boca Raton, FL, p 285–290
Haasch ML, Prince R, Wejksnora J, Cooper KR, Lech JJ (1993) Caged and wild fish: induction of hepatic cytochrome P-450 (CYP1A1) as an environmental biomonitor. Environ Toxicol Chem 12:885–895
Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione *S*-transferases. J Biol Chem 25:7130–7139
Hargrave BT, Harding GC, Vass WP, Erickson PE, Fowler BR, Scott V (1992) Organochlorine pesticides and polychlorinated biphenyls in the Arctic Ocean food web. Arch Environ Contam Toxicol 22:41–54
Jones DP (1985) The role of oxygen concentration in oxidative stress: hypoxic and hyperoxic stress. In: Sies H (ed) Oxidative stress. Academic Press, New York, p 151–195
Karinen JF (1980) Petroleum in the deep sea environment: potential for damage to biota. Environ Int 3:135–144
Lemaire P, Livingstone DR (1993) Pro-oxidant/antioxidant processes and organic xenobiotics interactions marine organisms, in particular the flounder *Platichthys flesus* and the mussel *Mytilus edulis*. Trends Comp Biochem Physiol 1:1119–1150
Lipiatou E, Tolosa I, Simó R, Bouloubassi I, Dachs J, Marti S, Sicre MA, Bayona JM, Grimalt JO, Saliot A, Albaigés J (1997) Mass budget and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean Sea. Deep-Sea Res II 44:881–905
Livingstone DR, Archibald S, Chipman JK, Marsh JW (1992) Antioxidant enzymes in liver of dab *Limanda limanda* from the North Sea. Mar Ecol Prog Ser 91:97–104
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193:265–275
Mathieu A, Lemaire P, Carriere S, Drai P, Giudicelli J, Lafaurie M (1991) Seasonal and sex-linked variations in hepatic and extrahepatic biotransformation activities in striped mullet (*Mullus barbatus*). Ecotox Environ Saf 22:45–57
McCord JM, Fridovich I (1969) Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 244:6049–6055
Morris SM, Albright JT (1984) Catalase, glutathione peroxidase, and superoxide dismutase in the rete mirabile and gas gland epithelium of six species of marine fishes. J Exp Zool 232:29–39
Pastor D, Boix J, Fernández V, Albaigés J (1996) Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus, Mugil cephalus* and *Dicentrarcus labrax*). Mar Pollut Bull 32:257–262
Porte C, Albaigés J (1993) Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustaceans, and fishes. Arch Environ Contam Toxicol 26: 273–281
Premereur N, van den Branden C, Roels F (1986) Cytochrome P450-dependent H_2O_2 production demonstrated in vivo. Influence of phenobarbital and allyl-isopropylacetamide. FEBS Lett 199:19–22
Radi AA, Hai DQ, Matkovics B, Gabrielak T (1985) Comparative antioxidant enzyme study in freshwater fish with different types of feeding behaviour. Comp Biochem Physiol 81C:395–399
Rudneva II (1997) Blood antioxidant system of Black Sea elasmobranch and teleosts. Comp Biochem Physiol 118C: 255–260
Shimakata T, Mihara V, Sato R (1972) Reconstitution of hepatic microsomal stearoyl-coenzyme A desaturase system from solubilized components. J Biochem 72: 1163–1174
Sleiderink HM, Oostingh I, Goksøyr A, Boon JP (1995) Sensitivity of cytochrome P450 1A induction in dab (*Limanda limanda*) of different age and sex as a biomarker for environmental contaminants in the Southern North Sea. Arch Environ Contam Toxicol 28:423–430
Stefanescu C, Rucabado J, Lloris D (1992) Depth-size trends in western Mediterranean demersal deep-sea fishes. Mar Ecol Prog Ser 81:205–213
Stegeman JJ, Kloepper-Sams PJ, Farrington JW (1986) Monooxygenase induction and chlorobiphenyls in the deep-sea fish *Coryphaenoides armatus*. Science 231. 1287–1289
Stegeman JJ, Woodin BR, Singh H, Oleksiak MF, Celander M (1997) Cytochromes P450 (CYP) in tropical fishes: catalytic activities, expression of multiple CYP proteins and high levels of microsomal P450 in liver of fishes from Bermuda. Comp Biochem Physiol 116C:61–75
Steimle FW, Zdanowicz VS, Gadbois DF (1990) Metals and organic contaminants in Northwest Atlantic deep-sea tilefish tissues. Mar Pollut Bull 21:530–535
Thomas P, Wofford HW (1993) Effects of cadmium and Aroclor 1254 on lipid peroxidation, glutathione peroxidase activity, and selected antioxidants in Atlantic croaker tissues. Aquat Toxicol 27:159–178
van der Oost R, Goksøyr A, Celander M, Heida H, Vermeulen NPE (1996) Biomonitoring of aquatic pollution with feral eel (*Anguilla anguilla*) II. Biomarkers: pollution-induced biochemical responses. Aquat Toxicol 36:189–222
Vig E, Nemcsok J (1989) The effects of hypoxia and parquat on the superoxide dismutase activity in different organs of the carp, *Cyprinus carpio* L. J Fish Biol 35:23–25
Winston GW, Di Giulio RT (1991) Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat Toxicol 19: 137–161
Woodwell GM, Craig PP, Johnson HJ (1971) DDT in the biosphere: where does it go? Science 174:1101–1107

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: April 29, 1999; Accepted: September 6, 1999
Proofs received from author(s): January 17, 2000