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METAL AND ORGANIC DETOXIFICATION/TOXIFICATION IN FISH LIVERS AND GONADS

Contaminant bioaccumulation and biological effects have been observed in organisms from coastal southern California, but there is no direct evidence to show which, if any, of the contaminants are causative for effects. The objective of this study was to use a contaminant-specific assay to determine which contaminants are likely to be causative. The assay is based upon a direct measurement of the portions of contaminants which are internally detoxified and the portions which are present at sites of toxic action. Results showed that oxygenated metabolites of both DDTs and PCBs are likely to be responsible for reduced catalase activity and reduced metallothionein- and enzyme-metal binding in Palos Verdes scorpionfish in relation to those from Cortes Bank. However, normal seasonal differences, which have not been thoroughly investigated, may also play a role in observed differences.

Previous studies have shown that trace metals can be detoxified within organisms by being bound to a protein called metallothionein (Kägi and Nordberg 1979). Most organic contaminants can be metabolized by the mixed function oxygenases to highly reactive epoxides which may then be enzymatically conjugated to glutathione, glucuronic acid, or sulfates (Jerina and Daley 1974). In most cases this conjugation has been shown to render organics detoxified (Gillette et al. 1974), although exceptions have been observed for some compounds (Boyd et al. 1984). Metals and oxygenated metabolites which exceed the capacity of detoxification systems, or otherwise escape detoxification, can impact a number of sites of toxic action including enzymes, genetic materials, and biomembranes (Brodie et al. 1971; Bremner 1974). The appearance of contaminants at sites of toxic action appears to coincide with the onset of molecular effects including enzyme activity alterations (Pruell and Engelhardt 1980), and the onset of tissue-level pathology (Reid and Krishna 1973). Although most studies of detoxification have been done on mammals, similar biochemical processes appear to occur in most marine organisms (Lee 1975; Malins 1977; Bend et al. 1977; James et al. 1979; Roesijadi 1980; 1982; Brown et al. 1984a, b, c).

This study is a continuation of our examination of the relationship between the cytosolic distribution of metal and organic contaminants and the occurrence of toxic effects (Brown et al. 1982c). Here, however, individuals rather than composites are analyzed, more species are examined, and more detailed biological effects indices are employed. A simple gel filtration column chromatography technique was used to separate tissue extract into the following fractions: 1) a high molecular weight (>20,000 daltons) enzyme-containing (ENZ) pool which is a site of toxic action for both oxygenated metabolites and metals in excess of normal metalloenzyme requirements; 2) a medium molecular weight (3,000-20,000 daltons) metallothionein-containing (MT) pool which is a site of detoxification for metals; and 3) a low molecular weight (<3,000 daltons) glutathione-containing (GSH) pool which is a site of detoxification of oxygenated metabolites by glutathione, glucuronic acid, and sulfates (Brown et al. 1984b). Toxic effects examined in this and concomitant studies included metal depletions, enzyme activity changes, histological differences (Rosenthal et al., this volume), and reproductive success (Cross et al., this volume).

MATERIALS AND METHODS

Sampling

Four species of fish including scorpionfish *(Scorpaena guttata)*, yellowchin sculpin *(Icelinus quadriseriatus)*, longspine combfish *(Zaniolepis latipinnis)*, and California tonguefish *(Symphurus atricauda)* were captured at Palos Verdes (PV) Station 7-3 (Brown et al., this volume) near the Los Angeles County municipal wastewater outfall in June 1983, using standard otter trawls. Scorpionfish were also captured at Cortes Bank (CB), 90 miles offshore of the municipal outfall, in Janurary 1984, using hook and line. Cortes Bank fish were dissected onboard ship, while Palos Verdes fish were brought back to the laboratory alive and promptly dissected using sterilized carbon-steel scalpels. Livers and gonads were excised; one portion was placed in 10% buffered formalin for histological examination (Cross et al., this volume; Rosenthal et al., this volume) and another in kilned (2,000°F) and acid-washed glass jars with teflon lid liners for chemical (Brown et al., this volume) and biochemical analysis.

Analysis

Samples for biochemical analysis were thawed; diluted between 3:1 and 25:1, depending on the sample size available, with 0.05 M Tris-HCl, pH 8.1; homogenized using a glass mortar and teflon pestle; and centrifuged at 100,000 x g for 60 min. The supernatant (cytosol) was collected and subsampled for enzyme analysis, glutathione, and gel chromatography. Gel chromatography was done by applying 1 to 7 ml of cytosol to Amicon (0.9 x 60 cm) or Pharmacia (2.6 x 60 cm) columns, for metals and metabolites, respectively, packed with Sephadex G-75 gel. Fractions of 1.5 or 3.0 ml were eluted at 0.5 ml/min or 1 ml/min. Forty fractions of 1.5 ml or 3.0 ml were collected per column run. The locations of the ENZ, MT, and GSH pools were determined by Zn and Na scans using flame atomic absorption spectrophotometry (Brown et al. 1984b). Fractions comprising each of these pools were combined and analyzed for metals and oxygenated metabolites (Brown et al. 1982a), enzyme activities (Bay et al., this volume), and unbound glutathione (Szalay 1982).

Of 25 scorpionfish collected from Cortes Bank, the six with the lowest oxygenated metabolite values were selected for biochemical analysis. This was done because, although no single sample of adequately uncontaminated controls could be obtained in coastal California (Brown et al., this volume), the degree of biological effects from chlorinated hydrocarbons should have been minimal in those fish with lowest oxygenated metabolite values (Brown et al. 1984c; Rosenthal et al., this volume; Gossett et al., this volume).

RESULTS AND DISCUSSION

Cytosolic Trace Metal Distribution

Results from this study indicate that the largest portion of metals is successfully detoxified in fish livers from southern California coastal waters, but a small portion escapes detoxification. As cytosolic metal concentrations increased, the largest increases of metals occurred in the MT pool rather than the ENZ pool (Figures 1 and 2). This finding is in accordance with the notion that nonessential metals (e.g., Cd) and increases of essential metals (e.g., Cu and Zn) above metalloenzyme requirements are stored and detoxified on metallothionein (Brown and Chatel 1978). However, in almost all cases the ENZ-metal content showed a small positive slope with increasing cytosolic metal concentration (Table 1), which indicates that a small portion of metals always escaped detoxification by MT. The distribution of metals between the ENZ and MT pools was significantly correlated in many cases (Table 2), suggesting that some exchange of metals between these pools occurred due to equilibrium dynamics.

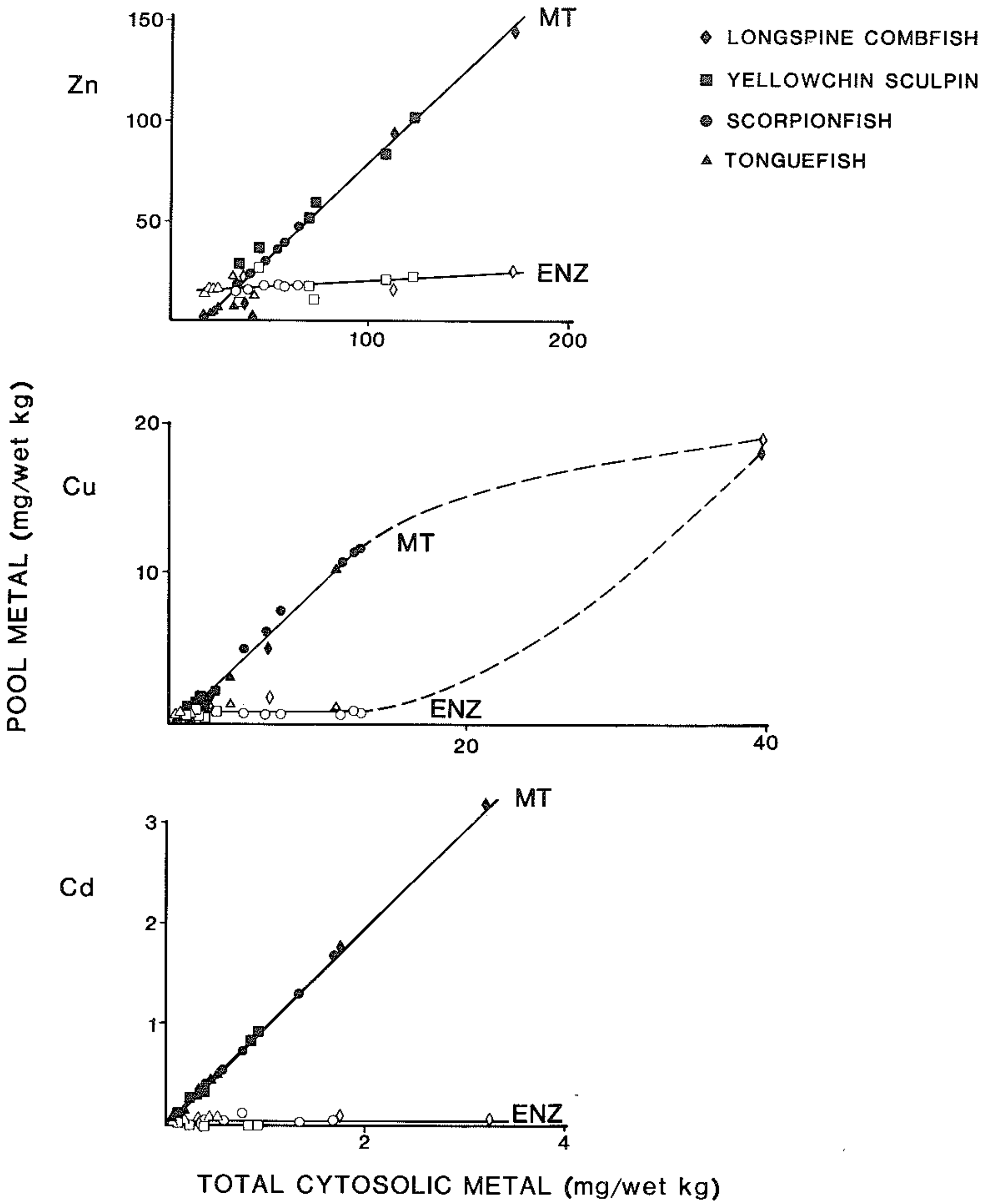

Figure 1. The concentration of Cd, Cu, and Zn increased predominantly in the MT pool in liver cytosols of scorpionfish, yellowchin sculpin, California tonguefish, and longspine combfish from Palos Verdes. In the longspine combfish with the highest cytosolic Cu concentration, there was an apparent saturation of the MT-pool Cu-binding capacity and a concurrent increase of Cu in the ENZ pool (solid lines are drawn from linear regressions). The highest Cu point was not included in the regression for reasons discussed in the text.

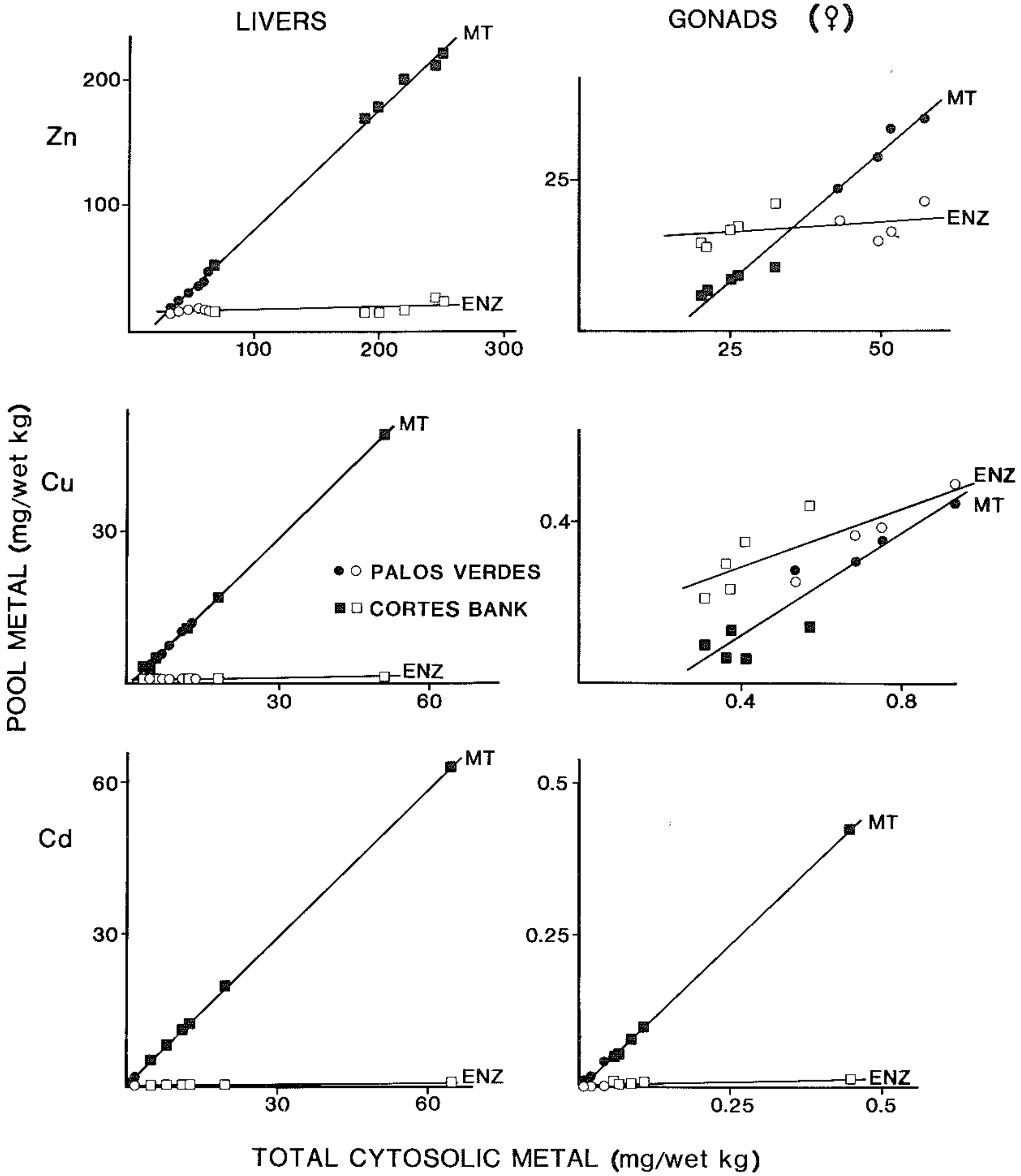

Figure 2. Comparison of cytosolic metal distributions in scorpionfish livers and gonads from Palos Verdes and Cortes Bank show that, with the exception of Cu in gonads, these appeared to follow the same linear regression patterns, with most metal partitioned into the MT pool. Cortes Bank fish comprised the higher range of metal values in liver, while, with the exception of Cd, Palos Verdes fish comprised the higher range of metal in gonads.

Table 1. Slopes for regressions of ENZ- and MT-pool metals versus total cytosolic metals in livers and gonads of fish from Palos Verdes and Cortes Bank.

		Four Species Combined from Palos Verdes[a] (n)	Scorpionfish from Palos Verdes and Cortes Bank[b] (n)
Liver			
Zinc	ENZ vs Total	0.056 (21)	0.025 (12)
	MT vs Total	0.947 (21)	0.951 (12)
Copper	ENZ vs Total	0.0094 (20)	0.016 (12)
	MT vs Total	0.964 (20)	0.973 (12)
Cadmium	ENZ vs Total	-0.0004 (21)	0.0062 (12)
	MT vs Total	0.986 (21)	0.978 (12)
Gonad ♀			
Zinc	ENZ vs Total	NA[c]	0.066 (9)
	MT vs Total	NA	0.846 (9)
Copper	ENZ vs Total	NA	0.364 (9)
	MT vs Total	NA	0.637 (9)
Cadmium	ENZ vs Total	NA	0.027 (9)
	MT vs Total	NA	0.964 (9)

[a] Regressions shown in Figure 1.
[b] Regressions shown in Figure 2.
[c] Not analyzed.

The cytosolic distribution of trace metals appears to be similar among species. When data for individual livers of scorpionfish, yellowchin sculpin, California tonguefish and longspine combfish from Palos Verdes were combined, increases of ENZ-and MT-metal were linear and significantly correlated (Figure 1 and Table 2). In only one fish of the 21 examined was the detoxification capacity of the MT pool greatly exceeded, as indicated by a plateau of MT-Cu and a large abrupt increase of ENZ-Cu in relation to the other fish examined (Figure 1). This one observation was consistent with the model of "spillover" (Brown et al. 1977). The amounts of metal in the ENZ pool for all four fish species combined, but excluding the one Cu spillover point, were Zn: 17.0 ± 1.0, Cu: 0.71 ± 0.08, and Cd: 0.034 ± 0.007 mg/wet kg (mean ± 1 standard error; n = 20-21). These amounts of ENZ-Zn and

Table 2. Correlation coefficients (r) and statistical significance (1-tailed test) for regressions of ENZ- versus MT-pool metals in livers and gonads of fish from Palos Verdes and Cortes Bank.

	Four Species Combined from Palos Verdes[a] (n)	Scorpionfish from Palos Verdes and Cortes Bank[b] (n)
Liver		
Zinc	$r = 0.879$; $p<0.05$ (21)	$r = 0.526$; $p<0.05$ (12)
Copper	$r = 0.309$; $0.10>p>0.05$ (20)	$r = 0.750$; $p<0.05$ (12)
Cadmium	$r = 0.399$; $p<0.05$ (21)	$r = 0.889$; $p<0.05$ (12)
Gonad ♀		
Zinc	NA[c]	$r = 0.334$; $0.25>p>0.10$ (9)
Copper	NA	$r = 0.558$; $0.10>p>0.05$ (9)
Cadmium	NA	$r = 0.748$; $p<0.05$ (9)

[a] Regressions shown in Figure 1.
[b] Regressions shown in Figure 2.
[c] Not analyzed.

-Cu most likely represent the requirements of metalloenzymes and, as such, would be subject to regulation. The function of the much smaller amount of ENZ-Cd is unknown, but it may represent the threshold amount of Cd that must be free from MT before MT synthesis is induced.

The fact that the amounts of ENZ-metals are similar among species means that these amounts could be used to determine if spillover from MT has occurred in future fish liver samples. With the exception of the one longspine combfish discussed above, excesses of cytosolic metal over the normal ENZ complement were partitioned almost exclusively into the MT pool. These plots of ENZ- and MT-metals could serve as the basis for standard curves of normal metal distribution in Palos Verdes fish liver (Figure 1). However, these "standard curve" plots should be used with caution since cytosolic metal distribution has been shown to vary seasonally (Brown et al. 1982b).

Although no fish were obtained which satisfied the criteria for "normal" controls (Brown et al., this volume), a comparison was done of highly contaminated Palos Verdes scorpionfish and less contaminated Cortes Bank scorpionfish. The purpose of this comparison was to determine if a gradient of effects could be observed along a contamination gradient, even though no samples of fish were free of contaminants (Brown et al., this volume) and few fish were free of pathology (Rosenthal et al., this volume). Scorpionfish were chosen for this initial comparison because their livers are large enough to allow a multitude of analyses with no problems of contaminant detectability due to small sample size.

Results of scorpionfish cytosolic metal distribution showed ENZ-Zn and -Cu values to be similar between stations and between livers and gonads (Figure 2; Table 3). Most cytosolic Zn and Cu fluctuations were restricted to the MT pool, indicating their relatively successful detoxification in scorpionfish. However, there were large differences in MT-Zn and -Cu between samples. Cortes Bank scorpionfish liver MT-Zn and -Cu values were 5.4- and 1.7-fold higher, respectively, than those from Palos Verdes; but Palos Verdes gonadal MT-Zn and -Cu were 3.8- and 3.7-fold higher, respectively, than those from Cortes Bank. Previous studies have related reduced MT metal values in Palos Verdes fish livers to the occurrence of oxygenated metabolites in the MT pool (Brown et al. 1982a; 1983; 1984b, c). As will be discussed later, the presence of oxygenated metabolites may be a factor here, but the apparent inverse relationship between liver and gonadal values indicates that seasonal factors must also be considered; in the present study, Palos Verdes fish were sampled in June when they were reproductively active, while Cortes Bank fish were sampled in January when they were inactive. It is possible that, in reproductively active Palos Verdes fish, a portion of essential metals was transferred from the liver to the gonad and held as a reservoir of MT-bound essential metals for the developing embryo.

Cytosolic Cd was much higher in both livers and gonads of Cortes Bank scorpionfish. This is again in accordance with previous studies showing Palos Verdes fish to be metal depleted in relation to reference fish (Brown et al. 1982a; 1983; 1984b,c). It appears that, unlike ENZ-Zn and -Cu, ENZ-Cd was significantly reduced in Palos Verdes fish compared to those from Cortes Bank. Although this reduction seems to indicate that fish from Palos Verdes are less subject to Cd toxicity than those from Cortes Bank, there is no evidence at the present time to show that ENZ-metal values within the range of control values represent a toxic state. In fact, trace metal reductions may be indicative of toxicity, but from organic contaminants (Brown et al. 1982a).

Cytosolic Oxygenated Metabolite Distribution

Analysis of the cytosolic distribution of oxygenated metabolites of DDTs

Table 3. Concentrations (mg/wet kg) of trace metals found in cytosolic pools of scorpionfish from Cortes Bank and Palos Verdes (mean ± 1 standard error; n = 6). Cortes Bank scorpionfish livers had higher MT-Cd, -Cu, and -Zn and ENZ-Cu and -Cd concentrations than those from Palos Verdes; Palos Verdes scorpionfish gonads had higher MT-Zn and -Cu, lower MT-Cd, and similar amounts of ENZ-metals.

	ENZ	MT	GSH
Liver			
Zinc			
Cortes Bank	18.9 ± 2.3	172 ± 26	4.29 ± 0.57
Palos Verdes	16.4 ± 0.5	31.9 ± 4.4[a]	0.63 ± 0.41[a]
Copper			
Cortes Bank	0.785 ± 0.131	14.7 ± 7.3	0.208 ± 0.085
Palos Verdes	0.497 ± 0.047	8.59 ± 1.21	0.334 ± 0.096
Cadmium			
Cortes Bank	0.202 ± 0.054	19.7 ± 8.9	0.321 ± 0.154
Palos Verdes	0.036 ± 0.015[a]	0.807 ± 0.213	0.044 ± 0.009[a]
Gonad ♀			
Zinc			
Cortes Bank	16.8 ± 0.9	7.9 ± 0.7	ND[b]
Palos Verdes	17.8 ± 1.3	30.2 ± 2.6[a]	2.6 ± 1.2[a]
Copper			
Cortes Bank	0.311 ± 0.036	0.095 ± 0.014	ND
Palos Verdes	0.377 ± 0.049	0.351 ± 0.039[a]	ND
Cadmium			
Cortes Bank	0.008 ± 0.002	0.139 ± 0.066	0.003 ± 0.002
Palos Verdes	ND	0.021 ± 0.007[a]	0.007 ± 0.007

[a] $p<0.05$; 2-tailed Student's t-test.
[b] Not detected.

(DDTols) and PCBs (PCBols) showed the following: 1) Palos Verdes scorpionfish liver cytosol contained higher metabolite levels than those from Cortes Bank; 2) these metabolites had apparently exceeded the capacity of or otherwise escaped detoxification by substances in the GSH pool of both Palos Verdes and Cortes Bank fish and had spilled over into the MT and ENZ pools (Figure 3); and 3) spillover was greater in Palos Verdes fish than in Cortes Bank fish. As in previous studies on croakers and Dover sole, when oxygenated metabolites were in excess of the binding capacity of the GSH pool, spillover into the MT pool was greater than into the ENZ pool (Brown et al. 1982a; 1983; 1984b, c).

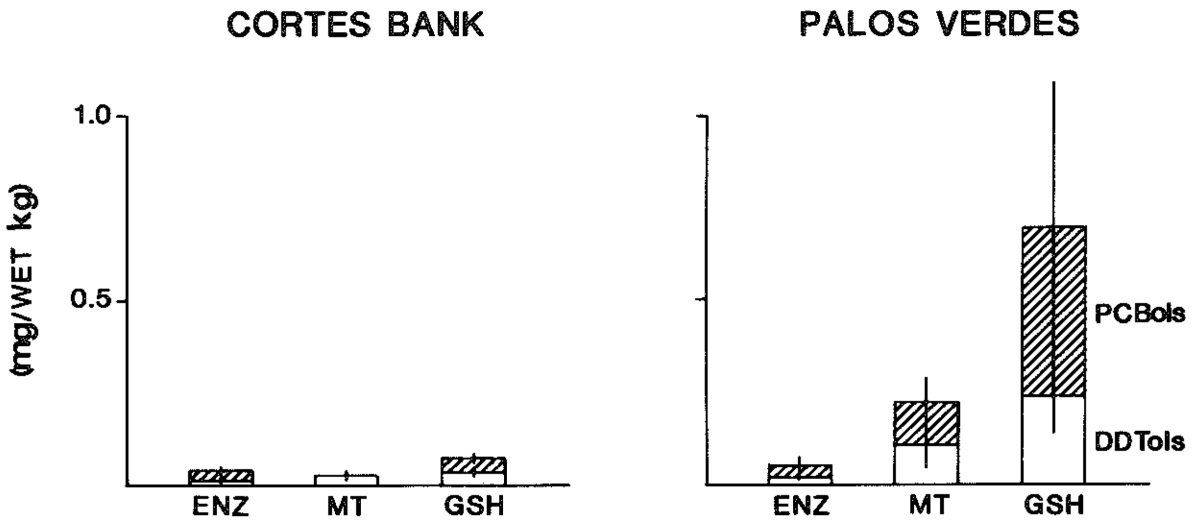

Figure 3. Comparison of cytosolic oxygenated metabolite distributions in scorpionfish livers from Palos Verdes and Cortes Bank shows that both had the majority of metabolites in the GSH pool, but Palos Verdes fish contained more metabolites in each of the GSH, MT, and ENZ pools. Differences not significant; Student's t-test (mean ± 1 standard error; n = 4).

Biological Effects Indices

It would appear, on the basis of the cytosolic distribution of metabolites, that the detoxification capacity of the GSH pool had been exceeded in scorpionfish livers from both Palos Verdes and Cortes Bank. However, measurement of unbound glutathione levels in Cortes Bank and Palos Verdes scorpionfish liver cytosol revealed no differences (Figure 4). Thus, it appears that oxygenated metabolites spilled over into the MT and ENZ pools before the detoxification capacity of glutathione had been exceeded or even depleted. There are at least two possible explanations for this premature spillover. One, spillover of chlorinated hydrocarbon oxygenated metabolites in scorpionfish livers may not be related to a saturation-dependent threshold value, but rather may be a function of simple equilibrium dynamics between the GSH, MT, and ENZ pools. This would be unlikely if conjugation of oxygenated metabolites was catalyzed by glutathione transferase, as in other species (Jerina and Daley 1974; James et al. 1979). However, it is possible that the catalytic capacity of the glutathione transferases rather than gluthathione was exceeded, or that the glutathione transferase was not specific for the substrates encountered here. Second, glutathione may not be the predominant detoxification substance in scorpionfish. Both glucuronic acid and sulfate, which would occur in the low molecular weight GSH pool, have been shown to function as detoxification substances in fish (Malins et al. 1979). Elucidation of predominant detoxification substances in scorpionfish will require utilization of enzymatic deconjugation procedures.

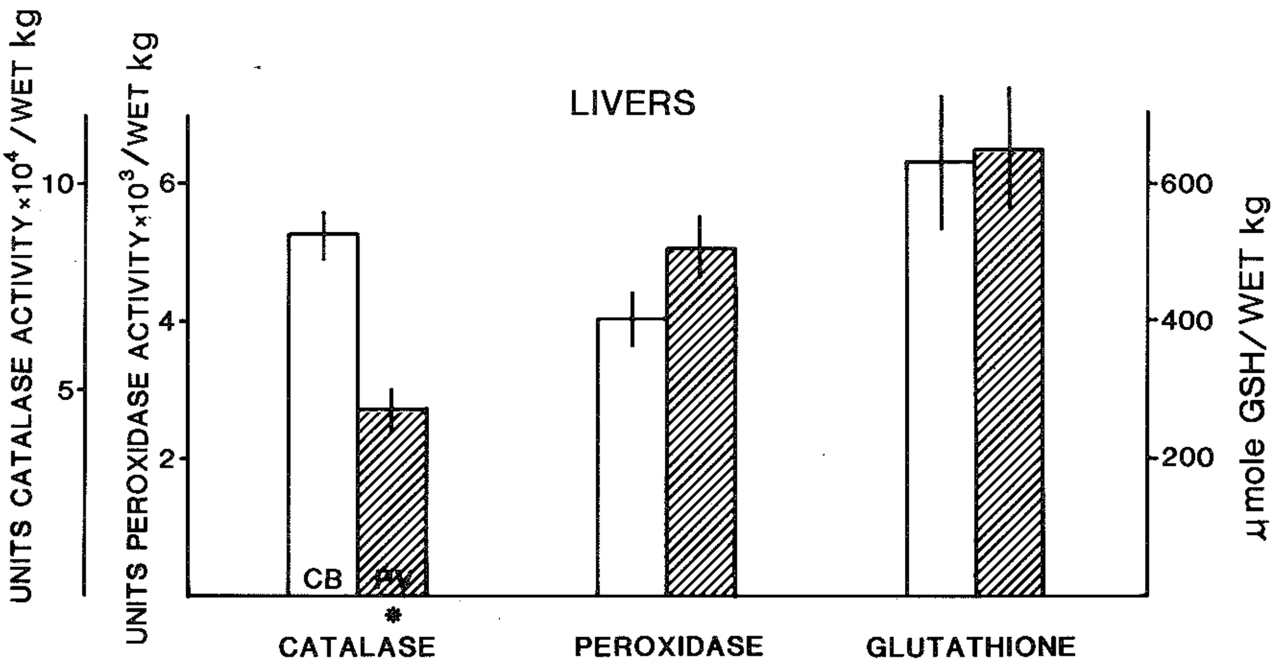

Figure 4. Activity of the enzyme catalase was reduced in Palos Verdes (PV) scorpionfish liver cytosols compared to Cortes Bank (CB). Selenium-dependent glutathione peroxidase activity and unbound glutathione reserves were similar. Asterisk indicates $p<0.05$; Student's t-test (mean ± 1 standard error; n = 6).

As in previous studies (Brown et al. 1982a; 1983; 1984b, c), the occurrence of oxygenated metabolites in the MT pool of Palos Verdes fish corresponded with a reduction of metal-binding in this pool (Figure 2 and 3; Table 1). It has been suggested that oxygenated metabolites interfere with MT-metal binding, resulting in reduced uptake and retention of metals. This interference could be caused directly by competition for metal binding, resulting in reduced uptake and retention of metals, or indirectly through inhibition of synthetic mechanisms. Trace metal reductions could be harmful, since many metals are essential for normal metalloenzyme functioning (Friedberg 1974). All Zn reductions occurred in the MT pool and should have produced no effect on metalloenzyme function, but because Cu reductions (not significant) occurred in both the MT and ENZ pools, their consequences could be detrimental. Trace metal depletions have been shown to be associated with diseases in mammals, including cancer (Brown et al. 1980) and diabetes (Lau and Failla 1984).

The occurrence of oxygenated metabolites in the ENZ pool of Palos Verdes scorpionfish coincided with reductions in the activity of catalase but not selenium-dependent glutathione peroxidase (Figure 4). This could indicate that catalase is a more sensitive site of toxic action than peroxidase. However, since Palos Verdes and Cortes Bank fish were sampled at different times of the year, the effects of seasonality on enzyme activities must be considered.

The occurrence of severe hepatocellular hypertrophy and vacuolation in Palos Verdes and Cortes Bank scorpionfish (Rosenthal et al., this volume) may result from the presence of oxygenated metabolites in excess of amounts which can be successfully detoxified. One of the mechanisms responsible for the development of fatty livers is the inhibition of lipoprotein synthesis by oxygenated metabolites, once the detoxification capacities of low molecular weight substances including glutathione have been exceeded (Dianzani 1979). This mechanism prevents the normal transport of lipids synthesized in liver tissue to peripheral storage depots and results in fatty accumulation in the liver.

CONCLUSIONS

This study indicates that chlorinated hydrocarbons, and not trace metals, are likely to be responsible for some of the observed biological effects in southern California coastal fish. With one exception, trace metals were successfully detoxified over the range of concentrations encountered in southern California fish livers. However, a portion of oxygenated metabolites of DDTs and PCBs were not detoxified. The presence of a large portion of DDTols and PCBols in the MT and ENZ pools of Palos Verdes fish relative to those from Cortes Bank may be responsible for the observed reduced metal binding, reduced catalase activity, and increased hepatocellular hypertrophy and vacuolation. However, confirmation of these relationships will require comprehensive seasonal and laboratory exposure studies.

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