# FISH COLLECTED FROM OUTFALL-AFFECTED COASTAL WATERS SHOW SUBCELLULAR TOXIC EFFECTS

In laboratory exposures, scorpionfish and kelp bass respond to toxicants along a roughly similar pattern (reports in this volume, p. 36 and p. 38). A certain portion of the toxicant taken into body tissues is detoxicated within the cells by specific compounds, such as metallothionein for some trace metals and glutathione for organic contaminants such as DDT and PCBs. Another portion of the toxicant is not detoxicated but instead is associated with enzyme fractions of the cells where it potentially has toxic effects. In the case of scorpionfish, SCCWRP researchers (this volume p. 38) measured the activities of several enzymes in different tissues of scorpionfish exposed in the laboratory to cadmium and found evidence of toxic effects.

But how different are the biochemical responses of fish that have grown, swam, fed and spawned in contaminated environments? Does lifetime exposure to contaminants make them more or less capable of detoxifying these compounds?

To address these questions, in this study David A. Brown and his colleagues* demonstrate that fish collected near a major municipal outfall in coastal southern California exhibit biochemical effects indicative of exposure to organic contaminants.

Using the methods used to study effects in laboratory-exposed fish, they examined the partitioning of toxicants into enzyme-associated cell fractions and fractions containing detoxicating agents. The researchers tested liver tissue for the substances of interest. Sephadex G-75 chromatography was used to separate tissue homogenates into high, medium and low molecular weight fractions. The cytosolic enzyme pool is found in the high molecular weight fraction, metallothionein in the medium molecular weight fraction, and glutathione in the low molecular weight fraction.

They found some major differences between fish from a contaminated environment (Palos Verdes station PV 7-3, adjacent to the Los Angeles County JWPCP municipal outfall) and those from less contaminated reference stations (Santa Monica Bay station SMB 2-3 near Malibu Beach, and Cortes Bank station CB located 90 miles offshore).

Among their findings:

1. Despite higher metal levels in the sediments at the contaminated site, fish from this site had lower cytosolic metal levels than did fish from the reference site (Figure 1). Such lowered metal levels have been shown to result from the presence of oxygenated metabolites of PCBs and DDT which may interfere with metal binding. However, other explanations are possible — for example, metals at outfall sites may be less bioavailable due to binding by sewage particulates.

2. The largest differences in metal-partitioning responses were not between fish from different sites, nor between species, but between metals. For example, in three fish species examined — longspine combfish, yellowchin sculpin and California tonguefish — the cell fractions containing enzymes showed increasing copper concentrations as the amount of total tissue copper increased (Figure 1). In contrast, enzyme-associated cadmium showed no significant change in concentration as total cadmium increased. This suggests that cadmium is being successfully detoxicated by these species but copper is not.

3. Most of the copper, zinc and cadmium in the three fish species' cell fractions occurred in metallothionein pools, except for zinc in the California tonguefish which was largely in the enzyme pool (Figure 1).

As for the capability of fish from the three sites to handle oygenated metabolites of PCBs and DDT, the researchers made these observations:

1. Fish from Palos Verdes had higher levels of glutathione (considered the agent of detoxication of PCB and DDT metabolites) than did fish from the Santa Monica Bay site (Table 1).

2. Fish from both Cortes Bank and Palos Verdes showed higher levels of oxygenated metabolites in the glutathione cell fraction than in enzyme cell fractions (Table 1). This suggests that detoxication is going on. However, in contrast to Palos Verdes scorpionfish, those from Cortes Bank showed increases of oxygenated metabolites in the enzyme pool indicating a potential for toxic effects (Figure 2).

The investigation produced two further observations. The researchers measured activity of the enzyme catalase and found that its activity was higher in fish from the contaminated Palos Verdes site than in fish from Santa Monica Bay (Table 1). Increased catalase activity may be indicative of a compensatory response which increases resistance to lipid peroxidation induced by exposure to DDTs or PCBs (reviewed in Brown et al, 1987).

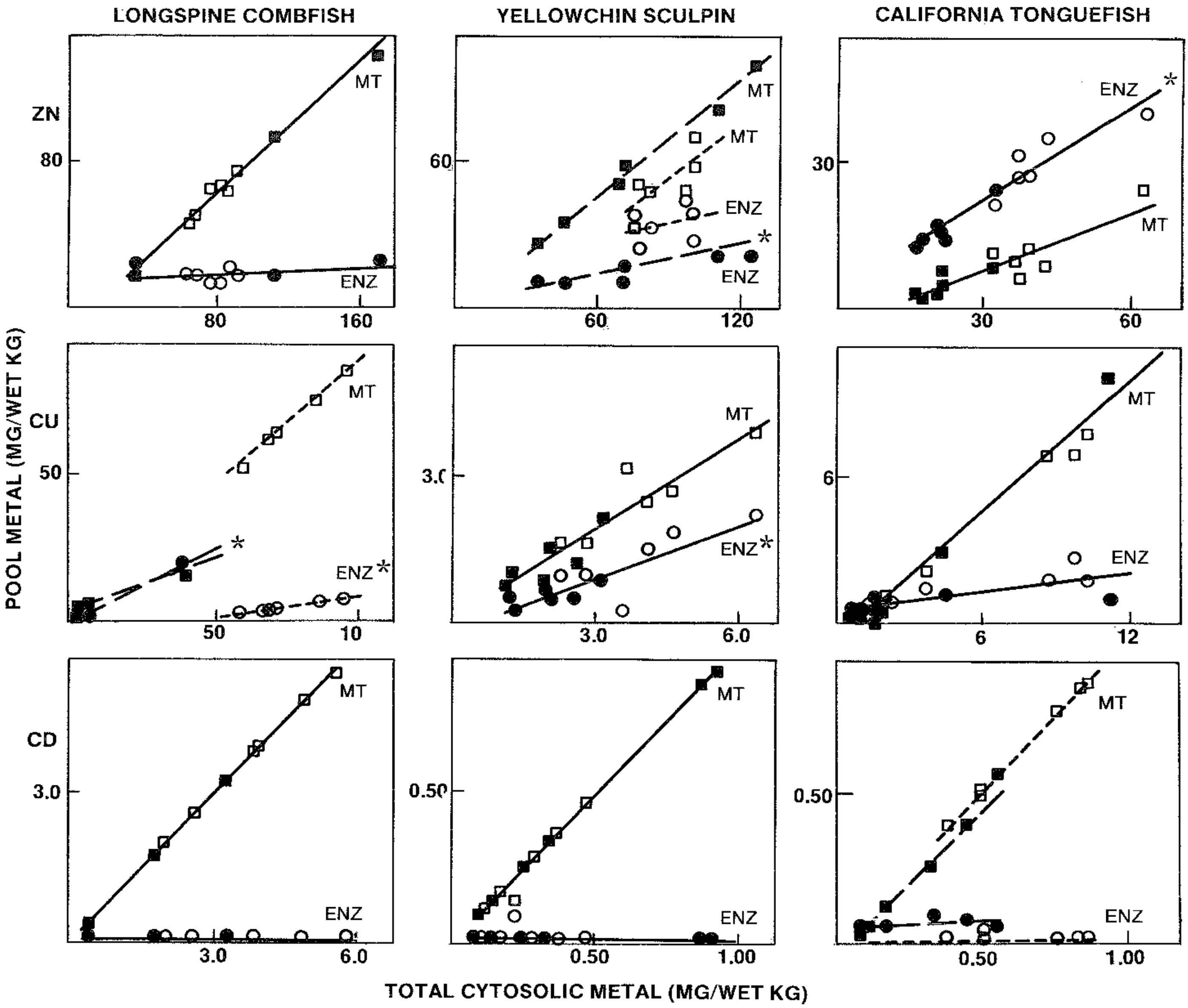


**Figure 1.** Distribution of Zn, Cu and Cd between the ENZ (circles) and MT (squares) pools in fish liver cytosols from SMB 2-3 (open symbols) and PV 7-3 data were combined. Dashed lines were used when SMB 2-3 (small dashes) and PV 7-3 (large dashes) data were plotted separately due to statistically significant ($P < 0.05$; Student's t-test) differences in slopes or intercepts of regressions. Asterisks indicate that regressions of ENZ-metals against MT- or total cytosolic-metals were significantly different from zero ($P < 0.05$; ANOVA).

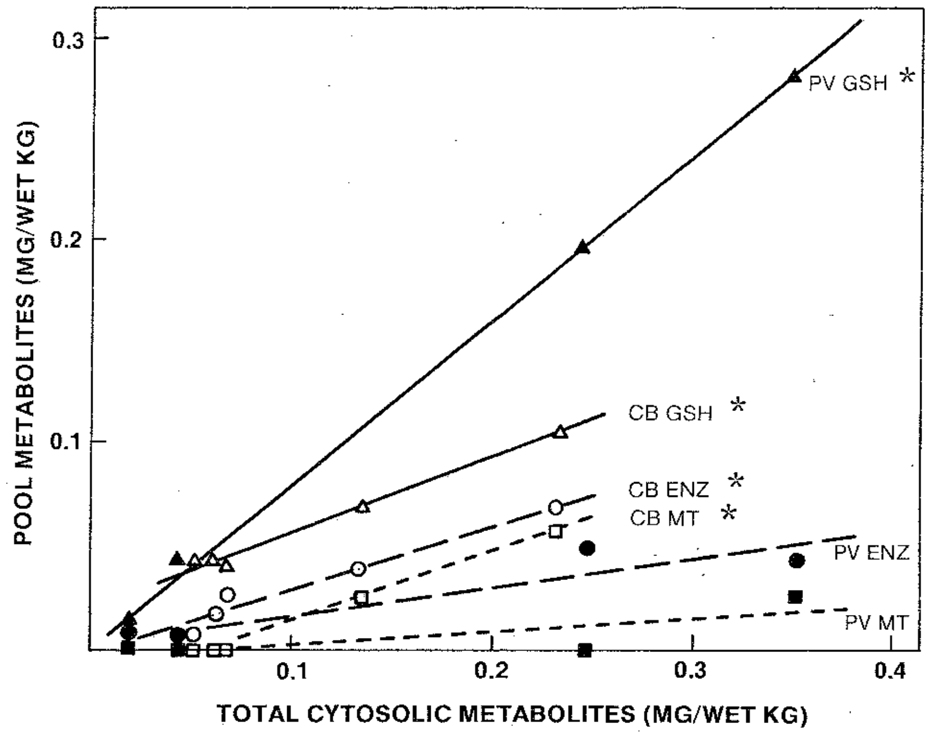


**Figure 2. Distribution of oxygenated metabolites of DDTs and PCBs between GSH (triangles, solid lines), ENZ (circles, large dashes) and MT (squares, small dashes) pools in scorpionfish liver cytosols from CB (open symbols) and PV 7-3 (closed symbols). Data for CB and PV 7-3 were plotted separately due to statistically significant ($P < 0.05$; Student's t-test) differences in slopes or intercepts of regressions. Asterisks indicate that regressions of ENZ- or MT-MTBs against GSH- or total cytosolic-MTBs were significantly different from zero ($P < 0.05$; ANOVA).**

| | | Protein (ug/0.1 ml cytosol) | Glutathione (umole/wet kg) | Catalase ($K \times 10^3$ /wet kg) | Catalase (K/g protein) |
|---|---|---|---|---|---|
| Longspine Combfish | SMB | 1413 ± 101 (6) | 443 ± 76 (6) | 335 ± 27 (6) | 7020 ± 180 (6) |
| | PV | 1547 ± 44 (3) | 1247 ± 566 (3) | 415 ± 35 (2) | 8130 ± 490 (2)* |
| Yellowchin Sculpin | SMB | 1372 ± 52 (5) | 129 ± 47 (6) | 110 ± 12 (4) | 2310 ± 160 (4) |
| | PV | 1245 ± 170 (6) | 643 ± 154(6)* | 172 ± 15 (6)* | 4330 ± 400 (6)* |
| California Tonguefish | SMB | 1588 ± 113 (6) | 413 ± 89 (6) | 198 ± 4 (4) | 4160 ± 360 (4) |
| | PV | 1652 ± 62 (6) | 637 ± 75 (6) | 257 ± 18 (6)* | 4570 ± 350 (6) |

* Significantly different from SMB station ($p<0.05$; 2-tailed Student's t-test)

**Table 1. Concentrations of protein and glutathione and activity of catalase in 3 species of fish sampled from Santa Monica Bay (SMB) and Palos Verdes (PV) in summer 1983 ($\bar{X} \pm$ SE (n) ).**

As Brown and his associates considered the subcellular partitioning of metals and organic contaminants seen in these fish, they found support for the idea that chronic exposures produce different responses to toxicants than do acute exposures. Both enzyme-associated levels of copper, zinc and oygenated metabolites rose as total cytosolic concentrations of these substances increased (Figures 1 and 2). This is suggestive of an equilibrium-dependent exchange of toxicants among the various cytosolic pools. This contrasts with the results of acute laboratory exposures to toxicants, which have provided evidence that detoxication systems can saturate and "spill over" into sites of toxic action. Since metal partitioning in the field-collected fish appears to follow different rules, it is evident that chronic effects cannot be predicted from acute bioassays (Brown, 1986).

To follow up this study, the researchers will look at the activities of two other enzymes involved in lipid peroxidation. And they will measure lipid peroxidation directly. Both measurements will be made as part of SCCWRP's recently-begun investigation of the effects of seasonal change on fish biochemistry and reproduction. They are particularly interested in investigating the relative sensitivity of various tissues to toxicants. Of special concern will be gonad tissue, since effects here should have consequences for fish populations.

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**Reference**

Brown, D. A., S. M. Bay, D. J. Greenstein, P. Szalay, G. P. Hershelman, C. F. Ward, A. M. Westcott, and J. N. Cross. 1987. Municipal wastewater contamination in the Southern California Bight: Part 2 - Cytosolic distribution of contaminants and biochemical effects in fish livers. Mar. Environ. Res. 21:2: 135-161.