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METAL DETOXIFICATION AND SPILLOVER IN SCORPIONFISH

In the previous two papers it was shown that organisms collected directly from the sea can detoxify inorganic metals by sequestering them in their metallothionein pools. The objective of this study is to determine how much more metal could be accumulated in organisms before the loading capacity of their metallothionein is exceeded. Determining this factor allows us to calculate how much of the metal detoxification capacity is currently being utilized in organisms exposed to present levels of metals in the sea, thus, providing a preliminary indication as to how much more metal could be taken up by organisms before the additions cause toxic effects.

In this preliminary study, scorpionfish were exposed for four days to ionic Cd concentrations several orders of magnitude higher than those which fish could encounter in even the most contaminated marine environments. The results demonstrated that greater than fifty-fold increases of Cd above normal could be accommodated in the metallothionein pools of livers, kidneys, and gills of exposed fish. The very high exposure levels used here caused spillover into the enzyme pool in gill and kidney tissue; this coincided with histopathological effects in these tissues. Although these results show that spillover coincides with damage to tissues, the large increases of Cd in the metallothionein pools suggest that spillover probably would not occur at more environmentally realistic Cd exposure levels.

METHODS

Scorpionfish (*Scorpaena guttata*) were captured by otter trawl from near Dana Point in March 1982. Tissues, including liver, kidney and gills, were removed from ten scorpionfish and processed as described previously in this report (Brown *et al.*) to determine the natural range of variability of Cd, Cu, and Zn in the metallothionein-containing (MT), enzyme-containing (ENZ), and glutathione-containing (GSH) pools. In addition, tissues were examined for any histopathologic conditions. A number of fish captured at Dana Point were returned alive to the laboratory and acclimated for two weeks before commencement of Cd exposures. During this acclimation period, fish were held in aquaria containing seawater which was maintained at 11°C and received biological and charcoal filtration. Nonionized ammonia concentrations were monitored and usually found to be below the recommended safe concentration of 10 ug/liter (Spotte 1973).

After the acclimation period, groups of three scorpionfish were exposed to a range of Cd (as $CdCl_2$) concentrations for 96-hrs (or until death if less than 96-hr) to determine the 96-hr LC50. These exposures were conducted in aquaria which received aeration, but no filtration.

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Exposure solutions were changed at 48-hrs to replenish Cd concentrations and reduce the build-up of waste products. Cadmium, dissolved oxygen, salinity, pH, and ammonia concentrations were monitored daily. Except for ammonia, which increased up to 7 ug/liter (nonionized), water quality parameters remained stable throughout the exposure period. Survival data indicated that the 96-hr. LC50 was 62 mg Cd/liter. At the termination of exposure, the aforementioned tissues were removed from scorpionfish exposed to 0 (Control), 25, and 50 mg Cd/liter, for determination of cytosolic distribution of metals and histopathological changes. The degree of loading of the MT pool with Cd in Cd-exposed scorpionfish was compared with the degree of loading in scorpionfish from unexposed controls.

RESULTS AND DISCUSSION

The natural ranges of variability of metals in each of the ENZ, MT and GSH pools in livers of scorpionfish sampled directly from the sea were measured so that we could determine minimal sample sizes needed to obtain adequate precision for statistical tests to be employed in our laboratory experiments (Table 1). Co-efficients of variation ranged from 14.2% for Zn in the ENZ pool to 100% for Cd and Zn in the GSH pool. These data show that an acceptable degree of precision (20%) can be obtained for metals in the MT pool with a sample size of only three (Table 2). Therefore, this sample size was used in the laboratory exposures.

Table 1. Concentrations (mean ± standard deviation) of Cd, Cu, and Zn in each of the ENZ, MT, and GSH pool in liver of scorpionfish sampled directly from the sea at Dana Point. The coefficients of variation ($CV = \frac{\text{standard deviation}}{\text{mean}} \times 100$) of metals in the ENZ and MT pools are less than 50% indicating that the natural range of variability is moderate. Metal concentrations are given in mg/wet kg.

	ENZ	MT	GSH
Cd (X + SD)	0.067 ± 0.031	1.25 ± 0.46	0.0045 ± 0.0045
(CV)	46.3%	36.8%	100%
Cu (X + SD)	0.83 ± 0.035	17.4 ± 3.9	0.064 ± 0.045
(CV)	42.2%	22.4%	70.3%
Zn (X + SD)	28.8 ± 4.1	60.3 ± 17.3	0.20 ± 0.20
(CV)	14.2%	28.7%	100%

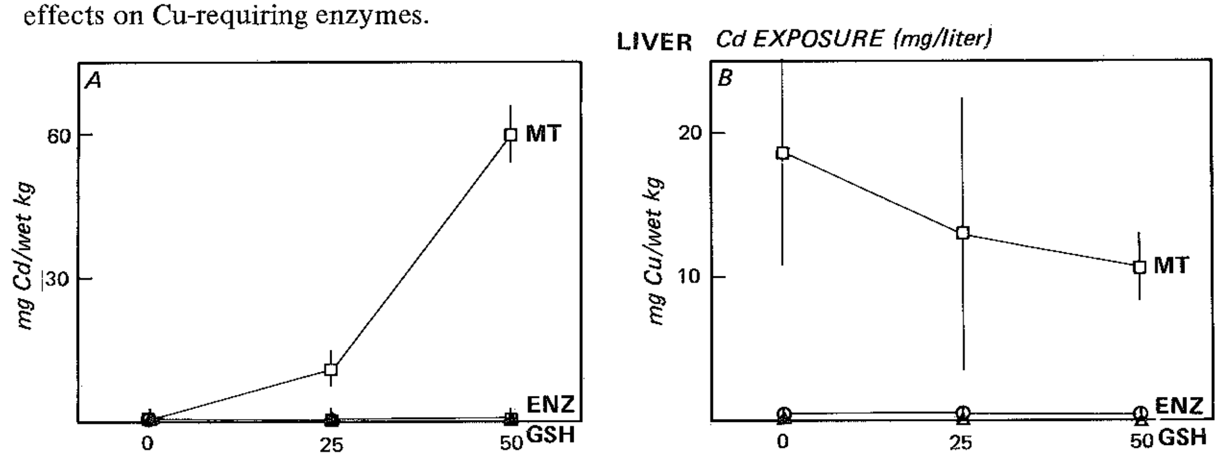
The results obtained demonstrate that the liver has a remarkable capacity to detoxify high levels of cadmium in a short period of time. Over 98% of Cd which accumulated in the liver cytosol at 25 and 50 mg Cd/liter exposure levels was accumulated in the MT pool (Figure 1). There was a 60-fold increase in concentration of Cd in the MT pool relative to laboratory controls after only 96 hrs of exposure to 50 mg Cd/liter. This means that less than 2% of the detoxification capacity of MT for Cd is being utilized in livers of scorpionfish in southern California coastal waters. This determination of available detoxification capacity is a preliminary estimate based on an acute exposure and may be modified by chronic exposure to other contaminants.

There was no spillover of Cd into the ENZ pool of the liver, indicating the Cd was effectively kept away from this site of toxic action (Figure 1). There were no occurrences of histopathological effects (Figures 1 and 4) demonstrating that when Cd is partitioned into the MT pool it is effectively detoxified. There was a slight reduction of Cu in the MT pool, but this should have little effect on the normal functioning of the liver because MT is a storage depot for excess Cu over the requirements of the ENZ pool (Brown and Chatel 1978).

Table 2. Sample sizes needed to obtain varying degrees of precision of metals in the pools. Values are calculated using the formula $n = \frac{(\text{standard deviation})^2}{(\text{precision})^2(\text{mean})^2}$ and data shown in Table 1.

Degree of Precision	ENZ	Cytosolic Pool MT	GSH
Cd 10%	21	14	100
20%	5	3	25
30%	2	2	11
Cu 10%	18	5	49
20%	4	1	12
30%	1	1	6
Zn 10%	2	8	100
20%	1	2	25
30%	1	1	11

Whereas most Cd was detoxified in liver tissue, this was not the case in gill tissue (Figure 2). At exposure levels of both 25 and 50 mg Cd/liter, more than half of the Cd in gill cytosol was present in the ENZ pool. This represents 40- and 90-fold increases of Cd in the ENZ pools relative to controls at 25 and 50 mg Cd/liter exposures, respectively. This degree of spillover was accompanied by a trend (not statistically significant) towards increases of histopathological effects at the 50 mg Cd/liter exposure, but not at 25 mg Cd/liter (Figures 2 and 4). Fish exposed to 50 mg Cd/liter had severe hyperplasia (increase in cell numbers) and hemorrhaging of gill lamellae. Gill lamellae hyperplasia was also present in laboratory controls, but not in fish sampled directly from the sea, indicating that laboratory conditions used here may be stressful to these fish. Copper decreased in the ENZ pool with both Cd exposures; this could have adverse effects on Cu-requiring enzymes.



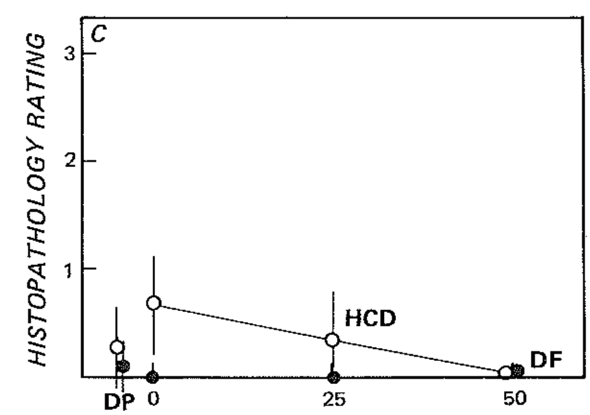


Figure 1A. This shows the distribution of Cd between the ENZ, MT and, GSH pools in livers of scorpionfish at the Cd exposures of 0,.25, and 50 mg Cd/liter water for 96 hrs. Cadmium was partitioned almost exclusively onto the MT pool, indicating that it was effectively detoxified.

Figure 1B. Copper decreased in the MT pool with increasing Cd exposure.

Figure 1C. Histological examination indicate no increases of hepatic cord disarray (HCD) or degenerative foci (DF) with increasing exposure. Livers of scorpionfish sampled directly from the sea at Dana Point (DP) were in similar condition to laboratory exposed fish. Ratings: 0 = none, 1 = mild, 2 = moderate, 3 = high.

Values are mean + standard deviation; n = 3 except DP histopathology where n = 10.

*p 0.05; Analysis of Variance and Multiple Range Test.

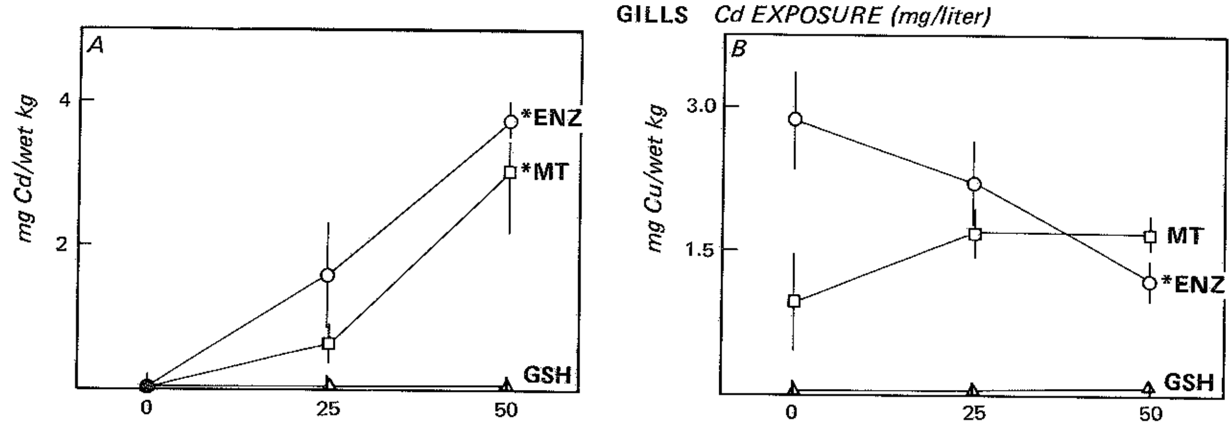


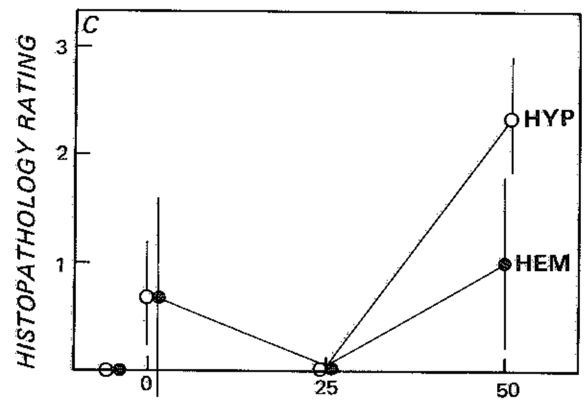
Figure 2A. Cadmium increased in the MT and ENZ pools of gills with increasing exposure, indicating that the detoxification capacity of the MT pool has been exceeded.

Figure 2B. Copper decreased in the ENZ pool and increased in the MT pool with Cd exposure.

Figure 2C. Gill epithelial hyperplasia (HYP) was higher in fish exposed to 50 mg Cd/liter relative to controls. Hyperplasia was present in laboratory controls, but not in those from Dana Point. Hemorrhaging (HEM) of gill lamellae was present in laboratory controls, as well as 50 mg Cd/liter exposed fish. Ratings: 0 = none, 1 = mild, 2 = moderate, 3 = high.

Values are mean + standard deviation; n = 3 except DP histopathology where n = 10.

*$p < 0.05$; Analyses of Variance and Multiple Range Test.

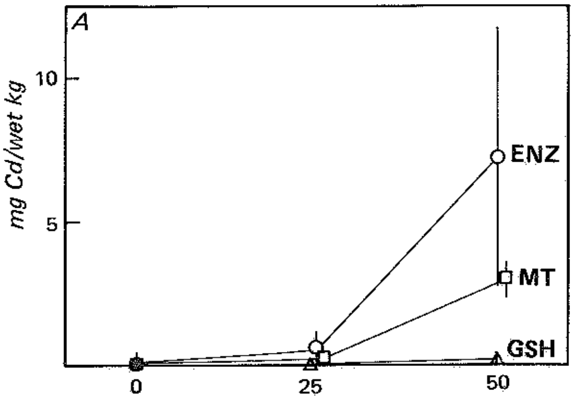


Even though spillover did occur in gill tissue there was a 250-fold increase of Cd in the gill MT pool relative to controls at the higher exposure. This means that less than 1% of the binding capacity of MT for Cd is being utilized in gills of scorpionfish from southern California coastal waters. Therefore, it seems likely that at environmentally realistic exposure levels, most Cd reaching gill tissues would be effectively detoxified.

There was a 65-fold increase of Cd in the MT pool of kidneys relative to controls at the 50 mg Cd/liter exposure level (Figure 3). This indicates that less than 2% of the binding capacity of kidney MT for Cd is being utilized in scorpionfish from southern California coastal waters. However, there was a significant spillover of Cd into the ENZ pool at this exposure level indicating that not all Cd was effectively detoxified in kidneys during this acute exposure. This spillover coincided with a trend towards increases of histopathological conditions in kidneys (Figures 3 and 4). However, as in gills, histopathological effects were present in the kidneys (in the form of granular casts in kidney tubule epithelia) of laboratory control animals, but not in fish sampled directly from the sea. This again suggests that laboratory conditions used in this experiment were stressful to these fish. No clear trends were shown for Cu values in any of the kidney pools.

When tissues are compared for susceptibility to Cd toxicity it can be seen that the gills appear to be most sensitive. Spillover was linear with exposure concentration in gills, and significant at

KIDNEY Cd EXPOSURE (mg/liter)



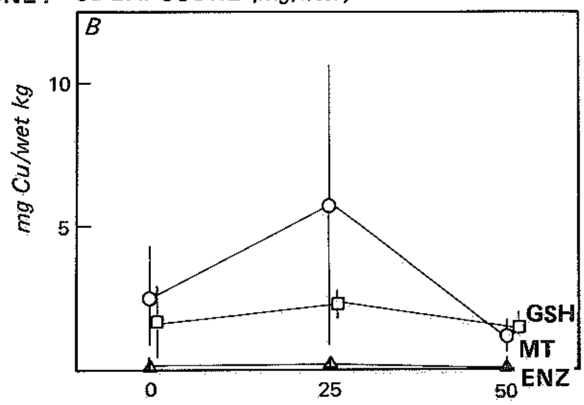


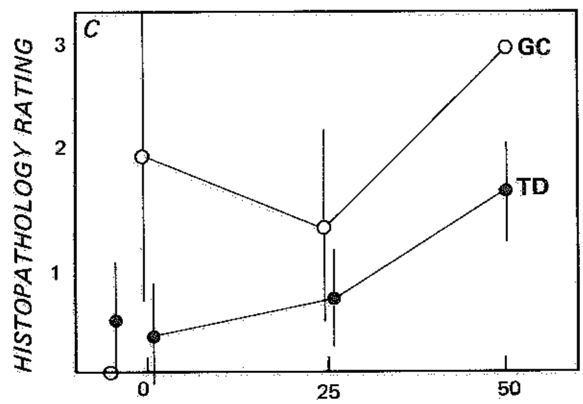
Figure 3A. There was a significant spillover of Cd into the ENZ pool of kidneys at 50 mg Cd/liter exposure.

Figure 3B. Copper showed no clear trends.

Figure 3C. Granular casts (GC) in kidney tubular epithelial cells and kidney tubule dilation (TD) were increased over laboratory controls at 50 mg Cd/liter exposure. Granular casts were increased in a laboratory fish relative to those sampled directly from the sea at Dana Point. Ratings: 0 = none, 1 = mile, 2 = moderate, 3 = high.

Values are mean + standard deviation; n = 3 except DP histopathology where n = 10.

*p 0.05; Analysis of Variance and Multiple Range Test.



both exposures. The kidney appeared to be the second most susceptible tissue since spillover was significant at the higher exposure level. The liver appeared to be least susceptible since no spillover occurred. Since gills and kidney are most susceptible to acute metal toxicity, these tissues should be considered when evaluating the biological impact of metals.

Histopathological effects tended to increase where there was spillover. Histopathological conditions, such as gill hyperplasia and kidney tubule granular casts, were similar to those previously reported to result from Cd exposure (Gardner and Yevich 1970). However, some histopathological conditions were also found in laboratory control fish. This indicates, first, that histopathological changes are not contaminant specific, a fact supported by other research (Patton 1982); and second, that our laboratory conditions were stressful to these fish. There was a buildup of nonionized ammonia to concentrations of approximately 4 to 7 ug/liter in our test aquaria; these concentrations are near those shown by others to cause pathology in sensitive fish (Spotte 1973). These unfavorable conditions will be alleviated in future studies.

CONCLUSIONS

Less than 2% of the detoxification capacity of MT for Cd is presently being utilized in scorpionfish from southern California coastal waters. The Cd exposure concentration at which spillover from MT to the ENZ pool occurs indicates that gills are most susceptible to Cd toxicity, followed next by kidneys, and then liver. Histopathological effects tend to coincide with spill-over, but these can also result from unfavorable laboratory conditions.

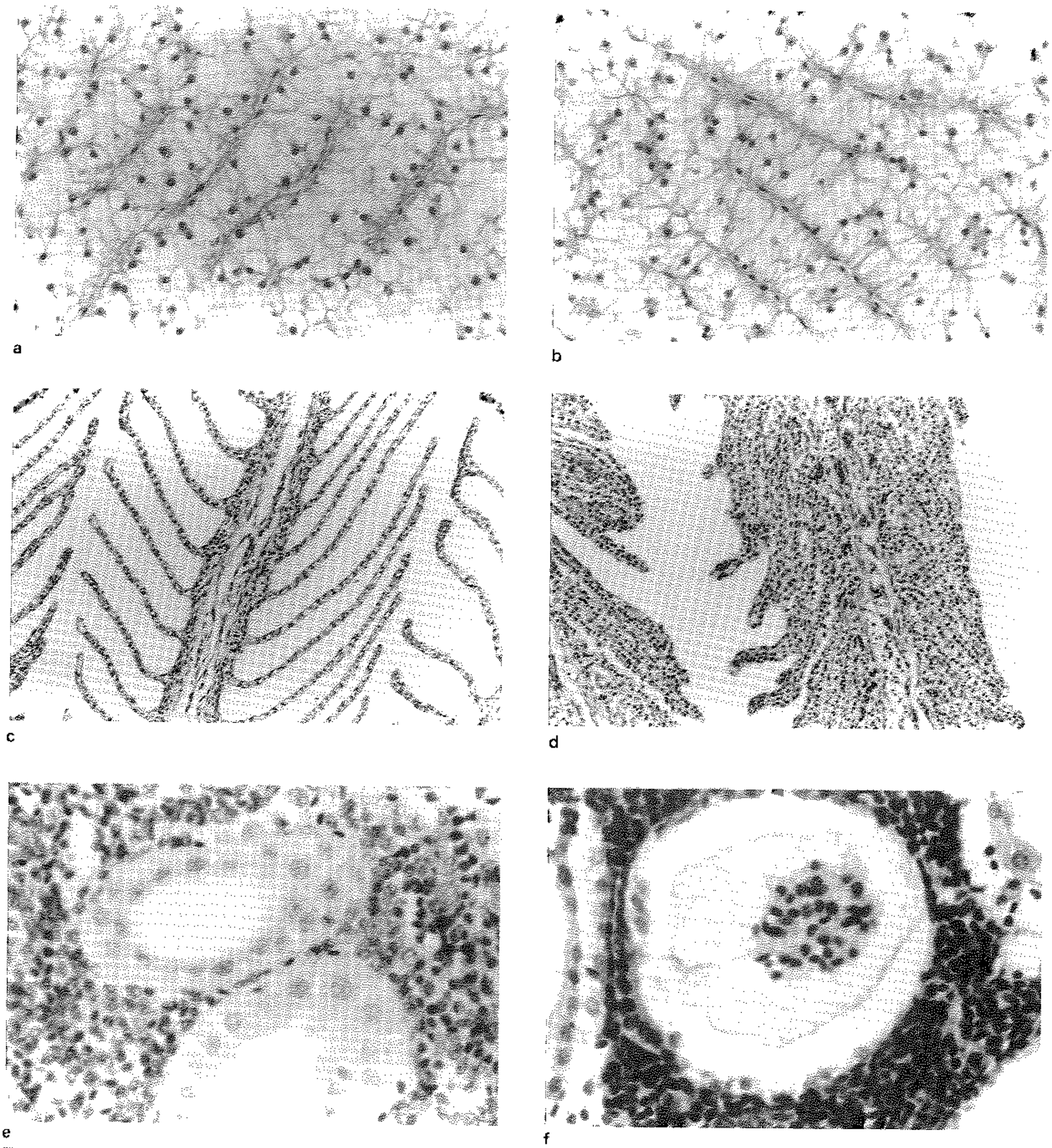


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Figure 4. These photos show the condition of liver, gills, and kidney of scorpionfish sampled directly from the sea at Dana Point and those exposed to 0, 25, and 50 mg Cd/liter for 96-hr in the laboratory.

A. Liver of fish from Dana Point showing normal cord structure. 200X
B. Normal liver of fish exposed to 50 mg Cd/liter. 200X
C. Normal gill lamellae from a Dana Point fish. 100X
D. Gills of fish exposed to 50 mg Cd/liter with severe hyperplasia. 100X
E. Kidney of fish from Dana Point showing normal tubules. 400X
F. Kidney of fish exposed to 50 mg Cd/liter showing dilation, degeneration, and congestion of tubules and granular casts in tubule epithelia. 400X

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Jeffrey Cross for guidance on statistical analysis.

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