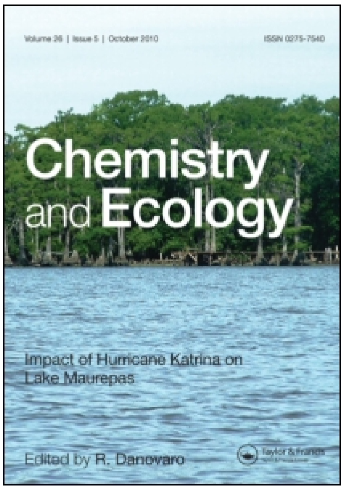

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LIVER METALLOTHIONEIN LEVEL AND METAL CONTENT IN FISH OF CHUNG-KUNG STREAM, TAIWAN-TILAPIA AND *LIZA MACROLEPSIS*

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A survey of Chung-Kung sttream fish was undertaken on six occasions from March 1994 to August 1995. Tilapia (*Oreochromis* hybrid) and *Liza macrolepsis* were the major species found downstream and were chosen as the target species for metallothionein (MT) analysis of liver tissue. In total MT of 77 tilapia and 48 *Liza macrolepsis* liver samples were analyzed. Significantly higher values ($p < 0.05$) were found for the August 1995 samples than for samples taken earlier at site B (6 km from the sea) for tilapia and at site A (estuary) for *Liza macrolepsis*. Liver zinc, copper and cadmium were analyzed for the 1995 samples. For tilapia, liver MT concentration was found to correlate well with zinc concentration ($r = 0.84$, $p < 0.001$). For *Liza macrolepsis*, good correlation was found for MT with both zinc ($r = 0.89$, $p < 0.001$) and copper ($r = 0.90$, $p < 0.001$). These results indicate that in the Chung-Kung stream, zinc and copper may be the major inducers of fish liver MT. Since these two species are ubiquitous in tropical areas, they would provide a good biomarker for evaluation of integrated metal exposure in the tropical aquatic environment.

KEY WORDS: Fish, metallothionein, zinc, copper, cadmium, biomarker.

INTRODUCTION

The metallothionein was purified from the equine kidney in 1957 (Margoshes and Vallee, 1957); thereafter, it was found throughout living organisms (Schaffer and Kagi, 1991). No clear physiological function has been assigned to MT, but some think that it may have to do with the homeostasis of copper and zinc (Whanger and Ridlington, 1982; Vallee, 1991). The MT will be induced when the animal is exposed to heavy metals of cadmium, copper, zinc and mercury. Metal will bind with MT instead of functional groups of enzymes and membrane epithelial organelles. This detoxication method of MT makes this protein important in toxicology (Schaffer and Kagi, 1991).

The induction of tissue MT when fish are exposed to heavy metals in the environment has been shown (Hamilton and Merhle, 1986). Neff (1985) reviewed biomarkers useful in pollution studies. He listed a dozen species of freshwater and marine fish for which metal binding MT as a useful indicator; he suggested that MT was a useful indicator of metal exposure. Olsson and Haux (1986) studied perch in the river Eman in Sweden polluted by a cadmium discharge. Higher cadmium level was here found in nearby river water, sediment and plants; the perch liver MT level was to be correlated with the cadmium counts. Hogstrand *et al.* (1991) studied effect of copper and zinc discharge from the cadmium refinery plant. Higher liver MT levels were found in fish caught at a site with higher metal content in water and closer to the plant. Roch *et al.* (1986) studied the effect of mining wastes which might induced MT through zinc, copper and cadmium; the fish liver metallothionein was to be correlated to the zinc concentration in water. Other studies included rainbow trout in pulp mill effluent (Gagne and Blaise, 1993), and fathead minnow at flyash deposition (Benson and Birge, 1985); all exposures showing a positive effect on MT induction in fish tissues. The studies showed that many successful applications of fish MT as a heavy metal exposure indicator. According to Roch *et al.* (1986), the metal concentrations needed for the induction of rainbow trout liver MT are comparable with levels causing toxicity. Using a mixture of zinc, copper, and cadmium at a ratio at 400:20:1, the lowest observable level for MT induction was found to be 40 ppb zinc. This induction of fish liver MT was thus indicated at the level that metal toxicity occurred.

There are MT induction studies have been done with tilapia in the laboratory (Fu *et al.*, 1990). However, there has been study on the MT inductor of warm waters or indigenous fish in the field taken in Taiwan. What is the level of fish liver in MT Taiwan? Is there correlation and metals in MT and with what metals? How appropriate is for fish tissue MT as a biomarker of metal pollution in the aquatic environment in Taiwan.

Chung-Kung stream in northern Taiwan was studied as the study area. The river is heavily polluted downstream from municipal, industrial and agricultural discharges. The middle section and the upstream is unpolluted (DOEP, 1984; Wang *et al.*, 1996). This correlation with liver MT level and metal concentrations of the fish sample for the Chung-Kung stream was analysed. The suitability of the fish liver MT as a metal exposure indicator is discussed.

MATERIALS AND METHODS

Sampling Sites and Species

The Chung-Kung stream runs from east to west into Taiwan Strait in the northern part of Taiwan. Figure 1 shows a map of Chung-Kung stream and sampling sites. The total length of the stream is 54 km. The sources of pollution for Chung-Kung were municipal, industrial, and agricultural and the stream is considered heavily polluted (at sites A and B) and mildly polluted at the middle section (as sites C and D). Fish were caught using gill net, cast net or electric shock apparatus. Samples were collected in March, June and September, 1994 and in February, May and August, 1995. Two major fish species were found downstream. Tilapia was the most abundant at sites B and C, and it was also found at site D. Most of the tilapia in Chung-Kung are hybrids of the *Oreochromis nitolicus* and *Oreochromis mossambius*. The other tilapia caught were of the species *T. zilli*, but these were few and were not intended for analysis. The size of the tilapia caught ranged from 8–26 cm in standard length and 20–590 g in body weight. *Liza macrolepsis* is a peripheral

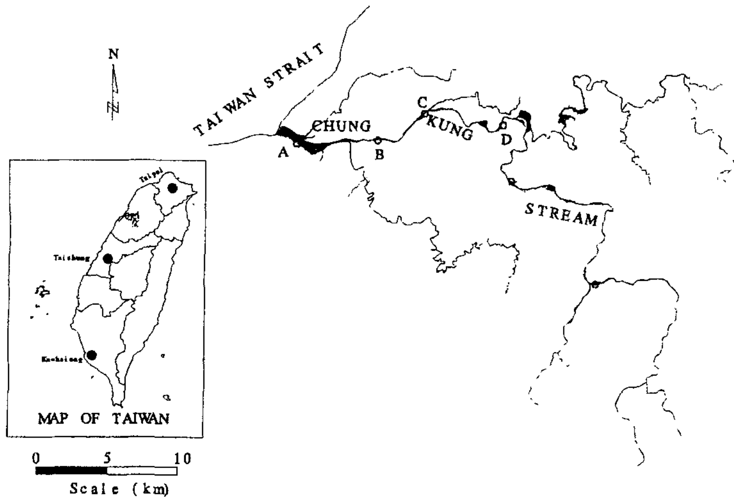

Figure 1 Map of Chung-Kung stream in Taiwan and sampling sites indicated by capital letters.

(Miyadi *et al.*, 1996) freshwater fish found commonly at the junction of the river and sea. It was the major fish species found at site A. The size of *Liza macroleplsis* caught ranged from 14–21 cm at a standard length and 60–170 g in body weight. On site, the liver of each fish was dissected and some pooled for the sampling site if the sample size is small. Liver samples were put in small vials and frozen in liquid nitrogen immediately and left frozen at −80°C until analysis.

Methods of Analysis

The silver saturation method of Scheuhammer and Cherian (1986) was used for MT analysis using a Hitachi z-6100 atomic absorption spectrometer. About 0.4 g of liver sample was used; the detection limit is about 5 μg g^{-1}. Recovery of standard pure rabbit liver MT (Sigma) was 80–100% for a concentration of 0.15–3.0 ppm. For metal analysis, about 0.8 g of liver sample was digested with about 5 ml HNO_3 and 2 ml H_2O_2 in a pyrex beaker on a hot plate. Residue grade of HNO_3 was used. Samples were diluted with double distilled water to a fixed volume after digestion was complete. Copper and zinc were analysed using a GPC908 and cadmium with a Varian 300 atomic absorption spectrophotomoter, with Zeeman background correction. Average recovery of the three metals was 93% for zinc, 98% for copper, and 105% for cadmium. Student's t test and Fisher's F test were used to compare statistical significance.

RESULTS

Liver MT values of tilapia caught at each sample season is shown in Table I. For site B, fish caught in August 1995 had higher liver MT values ($p < 0.05$) compared to

Table I Liver metallothionein content of tilapia from Chung-Kung stream (μg g^{-1}).

Site/Time		*Mar 94*	*Jun 94*	*Sept 94*	*Feb 95*	*May 95*	*Aug 95*
B	Mean	16.7	40.8	28.4	19.3	15.0	76.3*
	SE	3.8	8.5	4.6	3.3	0.9	10.1
	No.	3	6	10	10	12	12
C,D	Mean	29.0	38.3	13.0	24.2	–	14.8
	SE	11.0	13.4	14.6	8.4	–	2.2
	No.	3	2	5	7	0	7

* significantly ($p < 0.05$) higher than all previous groups at site B.

Table II Liver metallothionein content of *Liza macrolepsis* from Chung-Kung stream (μg g^{-1}).

Time	*Mar 94*	*Jun 94*	*Sept 94*	*Feb 95*	*May 95*	*Aug 95*
Mean	173.3	84.1	194.2	225.4	90.9	256.7*
SE	44.6	27.8	53.6	50.9	14.0	25.0
No.	6	8	6	3	10	15

48 of 50 *Liza macrolepsis* was caught at the mouth of Chung-Kung stream (site A) and MT content analyzed.
*MT content of the group caught in August 1995 was significantly ($p < 0.05$) higher than group caught at the previous season.

the previous five seasons. For site C and D, there was no difference between different sampling times.

For *Liza macrolepsis*, forty eight of a total fifty samples were caught at site A; only two were caught at site B. When the liver MT values for each sampling time was compared for site A, the result were shown in Table II; the average MT value in August 1995 was higher ($p < 0.05$) than in the previous sampling time. Also, it was higher ($p < 0.05$) than in June, 1994. These results would indicate that at higher metal exposure and induction of MT occurred. The results of liver zinc, copper and cadmium concentration for the three sampling data in 1995 for tilapia is shown in Figure 2. Liver zinc concentration was significantly higher ($p < 0.05$) for the group caught in August 1995, but there was no difference for copper and cadmium concentration at the different sampling times. This would indicate that zinc is the major metal the fish has been exposed for the induction of liver MT. Figure 3 shows tilapia liver contents of metals and MT for sites B and C. There was significant difference ($p < 0.05$) for liver cadmium, copper and MT level for tilapia caught at the two sites, which would indicate cadmium and copper may have a contribution for liver MT induction for tilapia further downstream. However, when correlation was analyzed between metals and MT level in tilapia liver as in Figure 4, good correlation between zinc and MT level ($r = 0.84$) was found for the fortyfive liver samples analyzed. Correlations for copper and cadmium were 0.07 and 0.03 respectively. These results would suggest although cadmium and copper did accumulate in the liver of tilapia further downstream, it was zinc, but not copper or cadmium, that might have caused MT induction in tilapia.

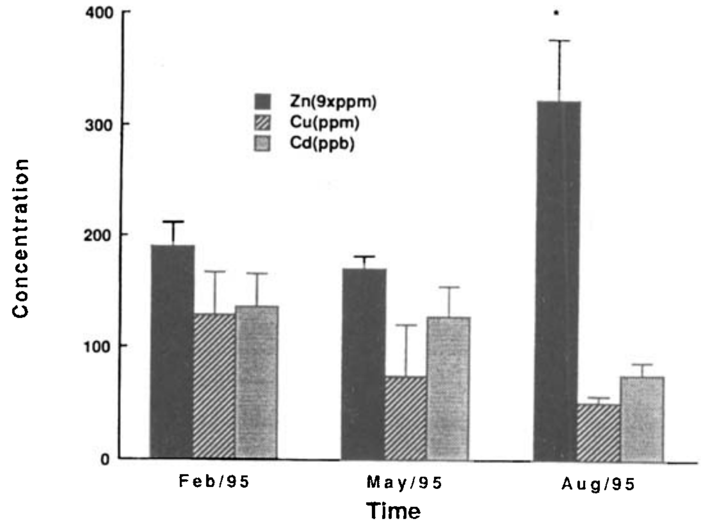

Figure 2 Liver metal concentrations of tilapia caught at site B of Chung-Kung stream. *Zinc concentration was significantly ($p < 0.05$) higher in August 1995. A total of 9, 12 and 10 samples were analyzed for August, May and February groups respectively.

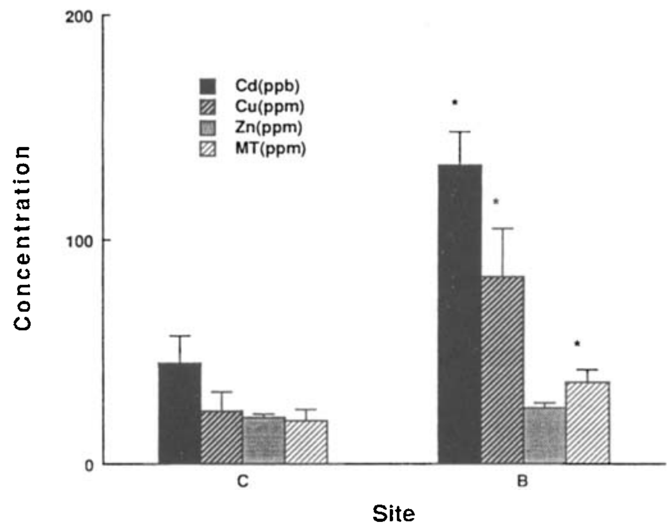

Figure 3 Liver concentrations of cadmium, copper, zinc and MT of tilapia caught at site B and C of Chung-Kung stream. *Mean value at site B significantly ($p < 0.05$) higher than the other site.

Fifteen liver samples of the species *Liza macrolepsis* caught at site A in August 1995 were analyzed for metals and the results shown along with MT concentration in Figure 5. *Liza macrolepsis* is the major species for site A and tilapia the major species for site B since both of these groups of fish caught in August 1995 showed

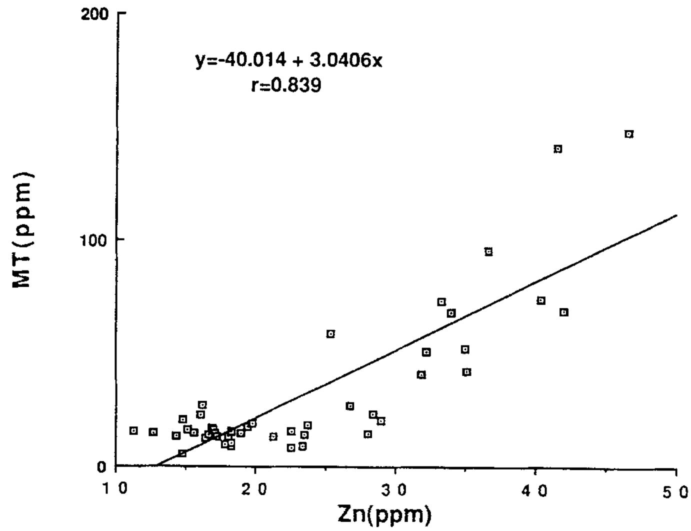

Figure 4 Correlation of tilapia liver MT with liver zinc concentration. Data included 45 liver samples of tilapia caught at Chung-Kung stream for the period of March 1994–August 1995.

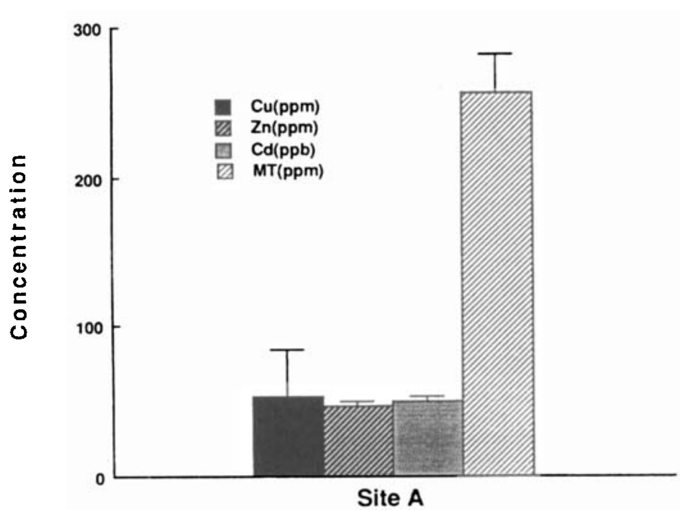

Figure 5 Liver copper, zinc, cadmium and MT concentration of *Liza macrolepsis* caught at site A of Chung-Kung stream August 1995.

higher liver MT values compared to fish of the same species caught in the previous season. Metal content of these two species caught at the same season was compared. *Liza macrolepsis* had in average about the same level of copper, 1.3 fold higher in zinc level, and about two-third this amount in cadmium compared to tilapia. Average MT level was 257 μg g^{-1} liver, which was 3.4 times compared to tilapia. Clearly there is species difference of MT in fish liver.

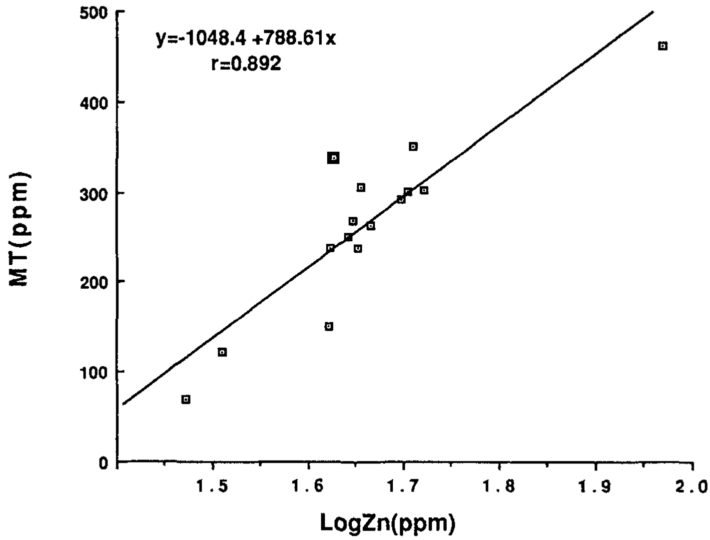

Figure 6 Correlation of liver MT with liver zinc level of *Liza macrolepsis* caught at site A of Chung-Kung stream.

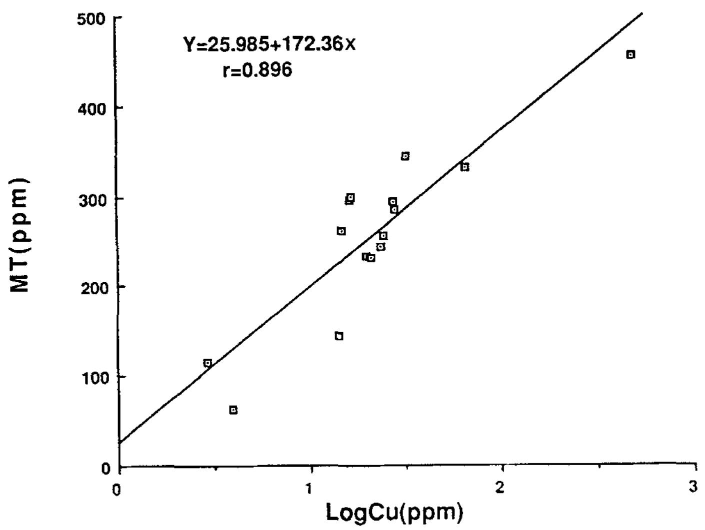

Figure 7 Correlation of liver MT with liver copper level of *Liza macrolepsis* caught at site A of Chung-Kung stream.

Since most of the *Liza macrolepsis* were caught at site A, very few fish were caught further upstream. Analysis was done on the correlation between liver metals and MT concentration for fish caught at site A in August 1995. The results are in Figure 6 and Figure 7. Good correlation was found for MT with zinc ($r = 0.89$) and copper ($r = 0.90$). For cadmium and MT, the correlation was poor ($r = 0.11$). These results indicate zinc and copper were the major metals that caused liver MT induction in the fish, *Liza macrolepsis*, of Chung-Kung stream.

DISCUSSION

Chung-Kung stream is not known to be heavily polluted by metals. However, significantly higher values of MT in fish liver were detected in a few sampling visits, indicating metal exposure. There is a bridge under construction near site B during the year 1995; in the August visit at site B, bulldozer and other heavy machineries were in the middle of stream and hauling the bottom gravel and sediment of the stream. This would presumably mobilize metals in the sediment and increase exposure for fishes in the stream. This can be used to explain the highest average value (76.3 $\mu g\,g^{-1}$) of MT found in tilapia liver. This value is close to that found for tilapia in Erh-jen stream (81 $\mu g\,g^{-1}$, unpublished data), which is known to be heavily polluted by metals. Also high value (256.7 $\mu g\,g^{-1}$) was found in *Liza macrolepsis* caught at site A for the same season. This could be an indication that the metals had been carried further downstream and caused the exposure.

Roch *et al.* (1982) demonstrated MT in liver of rainbow trout was induced by water contaminated by zinc, copper and cadmium. Olsson and Haux (1986) found close correlation between liver MT and cadmium in perch from a cadmium polluted river. Hogstrand *et al.* (1991) found correlation of liver MT with copper and zinc of perch influenced by brasswork discharge, a lower but significant correlation has been found for cadmium. Our study adds two more tropical fish species to the list of demonstrated induction of MT in the field. Tilapia is a fresh water fish, *Liza macrolepsis* is a marine fish and liver MT of both these species of fish downstream Chung-Kung stream was higher in the August 1995 sampling season. It is reasonable to think environmentally exposed fish should be responding to whichever metal(s) that reached high enough level to cause MT induction. Thus correlation between fish liver MT and various metals may be different for each specific environment. In our study, zinc showed the greatest increase in tilapia liver from May 1995 to August 1995, while copper and cadmium decreased in the same time (Fig. 2). Thus a good correlation between liver zinc level and MT is demonstrated. As there should be a basal MT level in fish for the normal physiological function, and also exponentially fitted line would have better correlation for liver zinc and MT. Correlation between metals and MT level was calculated excluding samples with lower MT values; however, there is only a slight increase of correlation (from $r = 0.851$ to 0.854) when five samples with MT values lower than 10 $\mu g\,g^{-1}$ were excluded. Correlation would decrease when further exclusion of sample was performed. This would be a preliminary indication that tilapia basal liver MT value may be around 10 $\mu g\,g^{-1}$.

Factors like developmental stage, weight and season may influence MT and metal levels (Waalkes and Goering, 1990; Olson and Hogstrand, 1987; Shears and Fletcher, 1985). However, since there may be complex environmental variations in different sampling seasons, size was the only factor analyzed here. Tilapia caught in February, May and August 1995 was analyzed for correlation of body weight and length with MT level separately. No correlation was found for each of the three groups of fish. However, this is different with *Liza macrolepsis*; correlation between liver MT and body weight was low ($r = 0.43$) but significant ($p < 0.01$) for the fifteen *Liza macrolepsis* caught in August 1995. Further study on physiological regulation of MT in these fish is needed to understand these differences and its significance.

For *Liza macrolepsis*, which appear almost exclusively at the mouth of Chung-Kung stream (site A) and presumably are exposed to higher concentrations of various metals from various effluent sources along their migratory trip toward the mouth of the stream, since cadmium concentration in fish liver was about one thousandth of zinc or copper, in spite of the stronger inducing effect known of cadmium, good correlation of MT level was found with copper and zinc level but not cadmium. Metals were analyzed for 20 samples of *Liza macrolepsis*, including 3 in February, 2 in May and 15 in August of 1995. The correlation between liver copper and zinc with MT level tends to decrease when samples of different seasons were calculated together. Possibilities exist that there were different polluting metal sources in different seasons, or seasonal variation of different interaction among pollutants, temperature and rate of metabolism could also have influence. Further study is needed to reveal the factors of importance.

The gene of MT in several species of fish have been sequenced and its expression studied (Kay *et al.*, 1992; Norey *et al.*, 1990). Olsson (1993) reviewed MT gene expression and regulation in fish. There is strong molecular basis of this metal inducing protein to be used as biomarker of metal exposure. However, it is still unclear what are the processes, control and metabolism of this protein. For example, although there are inducers other than metals like hormones, pressure factors found in mammals (Waalkes and Goering, 1990), little is known if and how they work in fish. This protein would be even more useful when more is known of its control mechanism. At present, Livingstone (1993) reviewed and suggested MT along with cytochrome P4501A and DNA-adducts would be good biomarkers and useful in pollution monitoring.

Tilapia originated from Africa but are now common over the warmer parts of the world. *Liza macrolepsis* is commonly found in the indo-west Pacific area (Yoshino and Senou, 1984). Since tilapia are commonly found downstream in rivers and streams in Taiwan and *Liza macrolepsis* is a migratory coastal species commonly found at the river mouth, these places are usually more polluted with a mixture of complex pollutants often including many metals compared to upstream of the river. Also these fish are resistant to pollution and can live even in very polluted waters in Taiwan. As the good correlations shown in this field study indicate, the specificity and sensitivy of this protein to metal induction, liver MT of both tilapia and *Liza macrolepsis* would be a good biomarker of metal exposure in the aquatic environment in Taiwan. These fishes would also be promising biomarker species in the warmer part of the world.

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