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Mechanism of Copper Tolerance in Black-Banded Rainbowfish *(Melanotaenia nigrans)* from the East Branch, Finniss River

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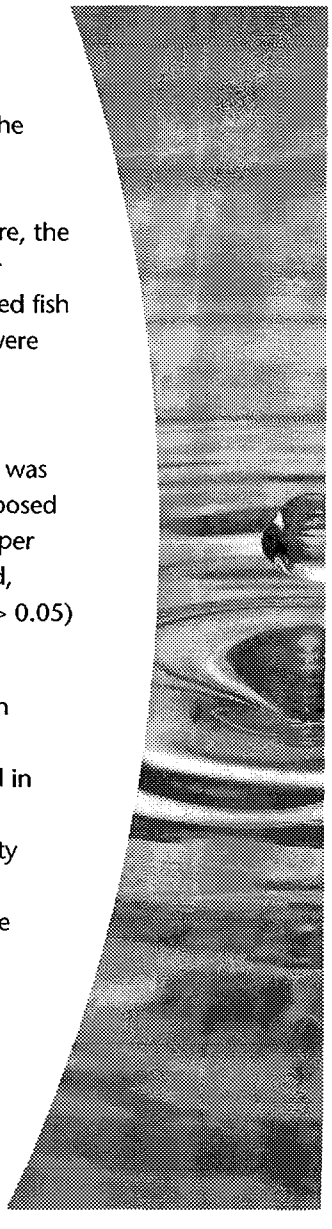
Mining is a common cause of copper pollution in the aquatic environment. Several studies have demonstrated the ability of fish to develop copper tolerance in the laboratory following sublethal exposure (Dixon and Sprague, 1981; Buckley et al., 1982; Taylor et al., 2000). Metal tolerance can be either the result of phenotypic plastic responses following exposure to sublethal concentrations (physiological acclimation), or it can be inherited (genetically-based tolerance) (Mulvey and Diamond, 1991). The mechanism of copper tolerance in fish is unresolved and has not been investigated in wild populations or in populations exposed to elevated copper concentrations for consecutive generations. Mulvey and Diamond (1991) identified three possible mechanisms of metal tolerance: changes to metal uptake or elimination rates, the ability to bind or sequester metals, and decreased enzyme sensitivity to inhibition by metals.

The population of black-banded rainbowfish (*Melanotaenia nigrans*) (Richardson) inhabiting the East Branch, Finniss River, Northern Territory, Australia, has been exposed to elevated copper concentrations for over 40 years, due to metal contaminated leachate from the Rum Jungle uranium-copper mine. It was hypothesised that due to the selective pressure of lethal exposure, the exposed fish may have developed copper tolerance. This study aimed to demonstrate copper tolerance in the exposed fish and determine the mechanism(s) involved. In May 2000, exposed fish were collected from the East Branch, 6 km downstream of Rum Jungle mine. Reference fish were collected from Coomalie Creek, an uncontaminated catchment nearby.

Fish imbalance was used as a sublethal measure of copper exposure. The 96 h EC_{50} (i.e. the concentration of copper that affects 50% of fish over 96 h) of the reference and exposed fish was used as an initial indicator of copper tolerance. At the time of collection, the 96 h EC_{50} of exposed fish was 8-fold higher than that of reference fish. Following two months exposure to low copper concentrations (24 mg Cu L^{-1}) during housing, the 96h EC_{50} of reference fish increased 5-fold, representing physiological acclimation. The 96h EC_{50} of exposed fish did not significantly ($P > 0.05$) change during this period, and remained 1.6-fold higher than that of the reference fish.

The bioconcentration of $^{64/67}Cu$ was used to investigate the mechanism of copper tolerance in exposed fish. Both exposed and reference fish were exposed to low (30 mg L^{-1}) and elevated (300 mg L^{-1}) copper concentrations for 24 and 48 h, respectively. Radioactivity was measured in four tissue sections: head region (gills, heart and brain), internal organs (including the gastrointestinal tract, liver, kidneys and gonads), muscle section, and whole body. Radioactivity correlated to the total copper concentration in the tissue section. One-compartment bioconcentration models were fitted to data for reference and exposed fish (**Figure 1**) and the regressions compared using an F-test.



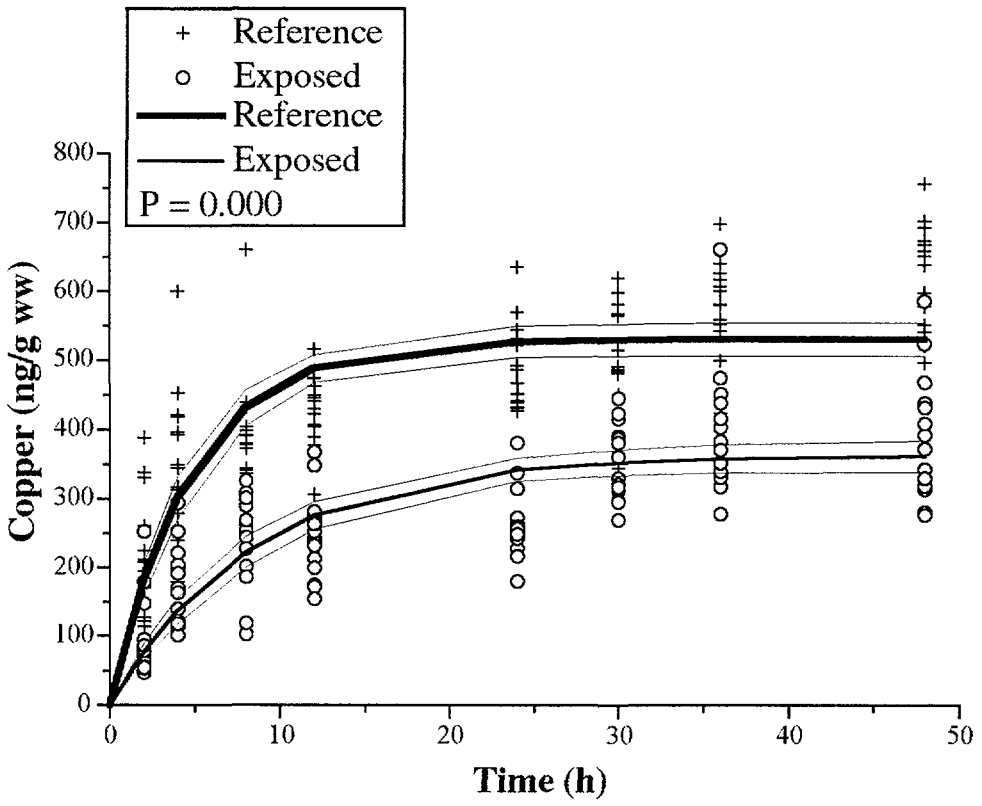


Figure 1. Accumulation of copper in the head region (gills, heart and brain) of reference and exposed fish during exposure to (a) low copper (30 mg Cu L^{-1}) and (b) elevated copper (300 mg L^{-1}) concentrations. Observed values, fitted models and 95% confidence intervals of the models are presented. Head Section included. ww; wet weight.

Copper accumulation in all tissue sections was significantly ($P \leq 0.05$) less (up to 50%) in exposed fish compared to the respective parts of the reference fish. However, copper accumulation in the internal organs was not significantly different ($P > 0.05$) between exposed and reference fish during exposure to elevated copper concentrations. Reduced copper accumulation by exposed fish indicated that binding or sequestration of copper was an unlikely mechanism of copper tolerance. The elimination rate constant was not consistently different ($P > 0.05$) between exposed and reference fish, indicating that increased elimination was also an unlikely mechanism of copper tolerance. Therefore, the reduced copper accumulation by exposed fish was due to reduced copper uptake, and as the gills are the primary site of copper uptake, the mechanism of reduced uptake is likely to occur there. Reduced gill uptake in exposed fish may have been due to changes in gill binding sites, gill surface area or gill mucus secretion rates, compared to reference fish.

Allozyme electrophoresis of seven enzymes was used to determine if genetic selection had occurred in the exposed fish population. Allozyme frequencies at the aspartate amino transferase-1 (AAT-1) and glucose-6-phosphate isomerase-1 (GPI-1) loci were significantly ($P \leq 0.05$) different between exposed and non-exposed fish (reference and captive-bred) (**Table 1**). Heterozygosity was reduced in exposed fish compared to that of unexposed fish. Collectively these results suggest that genetic selection may have occurred in the exposed fish population. Consequently, the selection of allozymes less sensitive to copper may be another mechanism of copper tolerance of exposed fish.

Table 1. Allozyme frequencies at two loci, AAT-1 and GPI-1, for fish exposed and not exposed (reference and captive-bred) to elevated copper concentrations

	Exposed	Reference	Captive-bred
AAT-1			
a	0.70	0.20	0.00
b	0.30	0.20	0.40
c	0.00	0.60	0.60
GPI-1			
a	0.00	0.40	0.05
b	0.10	0.35	0.45
c	0.80	0.10	0.30
d	0.10	0.15	0.20

This is the first study of the mechanism of copper tolerance in a wild fish population that has been exposed to elevated copper concentrations for consecutive generations. This study emphasises the importance of sample selection, and its implication for toxicity testing and risk assessment. Furthermore, as copper tolerance was due to exclusion rather than sequestration, the potential for toxic effects on higher levels of the food chain is less likely.

References

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