

Abstract

The need to monitor fresh water ecosystems for pollution is increasing, as is the need to develop a biomarker sensitive to a range of environmental insults. Recently, heat shock proteins have been identified as possible biomarkers of environmental contamination. However, evaluation as to their use as a biomarker of metal contamination in fish species endemic to Southern Africa is limited.

The purpose of this study was to identify what members of the 70 kDa family of heat shock proteins (Hsp70) were present in the liver of *Oreochromis mossambicus*, and if their accumulation was altered after short-term (96 hour) exposure to aqueous copper. In addition to copper exposure, the effect of acclimation media was also examined. Tissue-level analysis was done by means of histological examination so as to determine if alterations in the accumulation of the Hsp70 family had a marked effect on the structural integrity of the liver.

Specimens of *Oreochromis mossambicus* acclimated in either aged tap water or borehole water were placed in flow-through systems and exposed to either 10% or 20% of the LC₅₀ value of cupric chloride for a duration of 96 hours. Control groups were run in conjunction with the exposure groups so as to set control values by which to compare. Heat shock protein analysis was done by Western blotting after separation of hepatic proteins by SDS-PAGE. For the purpose of histological analysis, representative samples were randomly selected.

Analysis of the hepatic heat shock protein 70 family identified the presence of three (3) members, each of a different molecular weight. These included members of 70 kDa (Hsp70), 74 kDa (Hsp74) and 76 kDa (Hsp76). In addition

to these findings, it was found that *Oreochromis mossambicus* accumulated high levels of particular members of the heat shock protein 70 family under unstressed conditions, affording the fish adaptability to environmental extremes. Furthermore, individuals acclimated in aged tap water showed decreased Hsp76 accumulation after exposure to sub-lethal copper concentrations, whereas those individuals acclimated in borehole water retained relatively high levels of Hsp76.

Additionally, it was shown that the hepatic structure deteriorated in those individuals acclimated to the aged tap water after copper exposure, with observed increases in vacuolation, number of macrophage centres present and the occurrence of intracellular golden-brown granules. However, there was little change from the already-altered hepatic structure of those individuals acclimated in borehole water, with conspicuous golden-brown granules the most obvious histopathological condition present. Histological examination therefore proved to supplement the heat shock protein results obtained.

This study thus concluded that a decrease in the accumulation of the Hsp70 family resulted in a negative organismal response, initiating deleterious alterations in the hepatic structure. Additionally, this study concluded that past water quality has a marked effect on a given biomarker response, and should be taken into careful consideration when conducting biomarker studies.