The effect of short-term Cu exposure on the oxygen consumption and Cu accumulation of mudfish (*Labeo capensis*) and the largemouth bass (*Micropteris salmoides*) in hard water

WJ van Aardt* and M Hough

School of Environmental Sciences and Development, N.W.U, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2025, South Africa

Abstract

Sediment samples from 3 dams near the gold-mining area in the Mooi River catchment, South Africa, and fish tissue from the mudfish (*Labeo capensis)* and largemouth bass *(Micropteris salmoides*) were analysed for Cu to assess environmental pollution. Copper concentrations of sediment samples in 50 mm deep profiles at Klerkskraal Dam (22.2 mg Cu·kg^{-1}), Boskop Dam (14.1 mg Cu·kg^{-1}) and Potchefstroom Dam (21.7 mg Cu·kg^{-1}) and profiles 100 mm and 150 mm deep were above the risk assessment values for Cu, as implemented by the US EPA. Lowest Cu concentrations were found in gonads and blood samples in fish from both species in the 3 dams, but accumulated 3 to 5 times more, to 110.1±17.8 mg Cu·kg^{-1} dry mass in the liver. After 120 min Cu exposure at 20ºC to 10 mg Cu·ℓ$^{-1}$ (157.3 mmol Cu·ℓ$^{-1}$) and a 96 h Cu exposure to 1 mg Cu·ℓ$^{-1}$ (15.73 mmol Cu·ℓ$^{-1}$) Cu accumulated mainly in liver tissue and gills. For the mudfish, upon exposure to 10 mg Cu·ℓ$^{-1}$ (157.3 mmol Cu·ℓ$^{-1}$), the opercular frequency increased significantly from 80 (± 5.7) cycles·min^{-1} to above 100 (± 5.8) cycles·min^{-1} after 90 min, but thereafter decreased to zero cycles·min^{-1}. For largemouth bass the same increase in opercular frequency was found during 10 mg Cu·ℓ$^{-1}$ exposure, but this Cu level did not stop opercular frequency. For *L. capensis* the oxygen consumption rate $\dot{M}O_2$ for the two hour exposure period at 10 mg Cu·ℓ$^{-1}$ decrease significantly from 5.17 (± 0.32) mmol O_2·ℓ$^{-1}$·kg^{-1}· h^{-1} for the controls to 4.5 (± 0.37) mmol O_2 ℓ$^{-1}$·kg^{-1}·h^{-1} and for experimental *M. salmoides* from 4.91(± 0.45) mmol O_2·ℓ$^{-1}$·kg^{-1}·h^{-1} to 3.13 (±0.74) mmol O_2·ℓ$^{-1}$·g^{-1}·h^{-1}. For the exposure period of 96 h at 1 mg Cu·ℓ$^{-1}$, $\dot{M}O_2$ for both fish species, decreased to 2.9 (± 0.3) mmol O_2·ℓ$^{-1}$·kg^{-1}·h^{-1}. It is concluded that:

- The imported *M. salmoides* from the USA is biologically more tolerant to acute Cu exposure compared to the endemic mudfish, *Labeo capensis*
- For the 2 fish species Cu accumulates mainly in the liver, followed by the gills and kidney
- [Cu] above 20 mg Cu·kg^{-1} dry sediment may be released in the water column if the pH value decreases below 5 and, together with the physical disturbance of the sediment layer, acute Cu pollution will be the result
- Copper is about 10 times more toxic for the 2 fish species studied compared to Pb and Cd in hard water as found in previous studies.

Keywords: Cu, sediment, fish, $\dot{M}O_2$, opercular frequency, hard water

Introduction

Effluents from eroded or disused slimes dams at the Anglo-Gold Mine at Carletonville, previously known as the West Wits Goldfields, contain 84.97 µmol Cu·ℓ$^{-1}$ (5.4 mg·ℓ$^{-1}$) and 3.97 mmol Zn·ℓ$^{-1}$ (26.0 mg Zn·ℓ$^{-1}$) while the dry pelitic sediments contain 484 mg Cu·kg^{-1} (7616.2 µmol Cu·kg^{-1}) and 6440 mg Zn·kg^{-1} (98501 µmol Zn·kg^{-1}). The slimes dams receive discharges of high acidity (pH 1.7) containing large amounts of soluble ions (Wittmann and Förstner, 1977). About 139 Mℓ·d^{-1} (Winde, 2007) of underground water from these mines, mainly dolomitic in origin (Midgley et al., 1990), are pumped into the Wonderfonteinspruit, part of the upper Mooi River system. Since that study the polluted status of the slimes dams and the mining activities has not changed appreciably (Wade et al., 2002; Coetzee et al., 2006). Van Aardt and Erdman (2004) found a mean value of 35 (± 7.5) mg Cu·kg^{-1} and 60 (±10.4) mg Zn·kg^{-1} in the dried clay fraction of sediment samples from the Boskop Dam about 38 km downstream from the slimes dams. The [Cu] in the water of the Wonderfonteinspruit is between 2 and 6 µg Cu·ℓ$^{-1}$ but in sediment from a dam located in the Wonderfonteinspruit 305 mg Cu·kg^{-1} was found (Coetzee et al., 2006). The average [Cu] in soils world-wide is 20 mg·kg^{-1} (Nriagu, 1979). In *Labeo capensis,* a mudfish from the Mooi River Dams, Van Aardt and Erdmann (2004) found 150 (±17.8) mg Cu·kg^{-1} and 130 (± 23.1) mg Zn·kg^{-1} in dried liver. Copper concentrations for relatively unpolluted water include a mean of 5 µg Cu·ℓ$^{-1}$ for 'average river water' while the Amazon and its tributaries range from 0.3 µg·Cu·ℓ$^{-1}$ to 2.3 µg Cu·ℓ$^{-1}$ (Boyle, 1976; Kelly, 1988). According to the South African Water Quality Guidelines (DWAF, 1996) for aquatic ecosystems the acute effect value for Cu is 12 µg Cu·ℓ$^{-1}$ in very hard water.

Generally for aquatic animals such as tubificids, amphipods, crayfish, mud snails and mussels, (Hodson et al., 1979) and in tilapia fish (Van Aardt and Hough, 2006) Cu decreases the oxygen consumption rates. Water hardness affects acute Cu toxicity because Cu-complexes, especially from carbonates such as $CuCO_3^0$ and $Cu(CO3)_2^{2-}$, are less toxic than cupric or

* To whom all correspondence should be addressed.
☎ +27 18 299-2503; fax: +27 18 299-2511;
e-mail: willie.vanaardt@nwu.ac.za
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Cu hydroxyl ions (Andrew et al., 1977). In hard water systems Cu tends to accumulate in sediments but, if dissolved in water, can still be as high as 25 µg Cu·ℓ$^{-1}$ (Smith et al., 1996). Toxic Cu levels found in water systems originate mainly from mining processes, the leaching of Cu from minerals due to acid mine drainage and, thirdly, effluents from heavily urbanised and industrialised areas. Generally Cu in aquatic systems is 10 times more toxic compared to Pb, Ni and Zn (Kelly, 1988).

Rainbow trout, *Oncorhynchus mykiss*, at a 96 h exposure to 311 µg Cu·ℓ$^{-1}$ in pH 7.9 water with only dissolved calcium, undergoes severe iono-regulatory failure combined with a progressive systemic hypoxia and massive haemoglobin concentration (Wilson and Taylor, 1993). This is usually accompanied by excessive production of mucus by the mucus cells on the gill epithelium. The mucus largely stays on the gill surface probably as a protective layer (Tkatcheva et al., 2004). The 96 h LC_{50} Cu concentrations for fish start from 10 µg Cu·ℓ$^{-1}$ and end at 10.0 mg Cu·ℓ$^{-1}$ (McKim et al., 1978). Water hardness, high pH values, or water alkalinity reduce the lethality of Cu to fish (Zitko and Carson, 1976; Howarth and Sprague, 1978; Erickson et al., 1996) but at a pH of lower than 6.5 toxic Cu hydrides are formed (Stouthart et al., 1996). Compared to blue gill and fathead minnow, LC_{50} for Cu is about 10 times lower in the salmon family (Hodson et al., 1979).

A change in respiration rate ($\dot{M}O_2$) is one of the common physiological responses to metal toxicants (Connell et al., 1999) and is easily detectable through changes in the oxygen consumption rates. Generally the $\dot{M}O_2$ in fish is reduced when exposed to sub-lethal levels of Cu (Beaumont et al., 2003). However, at lethal Cu concentrations for blue gill (O'Hara, 1971) and *O. mykiss* (Wilson and Taylor, 1993) $\dot{M}O_2$ first increases above normal values before it declines before death. At a water pH of lower than 4.0, standard oxygen consumption in fish is reduced and the values of critical oxygen tension (Herried, 1980) increase, with the result that fish are less able to handle environmental hypoxia at low pH values (Ultsch et al., 1978; Van Dijk et al., 1993).

This paper reports the acute effects of Cu on the $\dot{M}O_2$ and gill frequency in hard water of an indigenous mudfish, *L. capensis* and the largemouth bass, *Micropteris salmoides,* imported from the USA. Measurements were also made of the Cu concentrations in the water and sediments of the dams in the Mooi River that harbours these fish and also receives water from the Carletonville mining area. Copper levels in the fish tissue for the 2 fish species, before and after exposure to Cu in the laboratory, were also analysed.

Material and methods

Collection and preparation of sediment samples

Six core samples were collected during the summer and winter season of 1998 from each of the 3 dams (Klerkskraal Dam: 26.3.745/27.09.063; Boskop Dam: 26.31.531/27.07.348; Potchefstroom Dam: 16.39.714/27.05.328) in the Mooi River catchment. Sampling localities were situated in the water near the dam inflow and were between 50 and 100 m apart. The core samples were collected using a specially made stainless steel core sampler with a mechanical valve (to be able to salvage the sediment sample), a cutting face and an integral hammer device to be able to drive the core sampler deeply into the dam sediment. The sampler was operated from a boat and took sediment samples from about 2 m below the water surface. Each sample, with an average mass of 900 g, was taken from the core sampler, enclosed in its polyvinyl jacket, and air dried in a vertical position for 24 h (Van Aardt and Erdmann, 2004).

The top 50 mm sample layer was removed and dried for 12 h at 80ºC. With the aid of a Wolfram-ring swing mill (Siebtechnik, Mulheim, Germany) the samples were pulverised for 15 s. One gram from each sediment sample was weighed, 1mℓ de-ionised water, 2 mℓ HNO_3 (70% *pro analisi*) and 1mℓ $HClO_4$ (65% *pro analisi*) from Merck were added and digested at 80˚C for 12 h. The digested samples were filled up with de-ionised water to a total volume of 10 mℓ.

Copper analysis of the sediment and Mooi River water was performed by flame atomic absorption spectrometry at 324.8 nm using standard reference materials (MESS-2) from The National Research Council, Canada, in quality assurance protocols. The calibration graph, with an accuracy of 10 µg Cu·ℓ$^{-1}$ was linear between 0.01 mg Cu·ℓ$^{-1}$ to 8.88 mg Cu·ℓ$^{-1}$. One gram samples from the pulverised cores were also used for energy dispersive X-ray spectrometry analysis (EDAX), using a Phillips EDAX-analyser (EDAX, CDU LEAP Detector) and an electron microscope (XL 30 Phillips Dzi). The sample buttons for each sample were prepared using 'sticking film'. The sample was slightly pressed onto the film and covered with a layer of carbon (Emscope TB 500).

Fish collection and the fish-keeping plant

Labeo capensis were collected by gill-netting from the 3 dams in the Mooi River during the summer and winter of 1998. During the same period *Micropteris salmoides* were caught by line fishing. Klerkskraal Dam was used as a control dam compared to Boskop and Potchefstroom Dams (Fig. 1a). The latter 2 dams receive water from mining effluents via the Mooi River (Wade et al., 2002). The fish were transported in a 400 ℓ container filled with aerated dam water and spiked with 12 g NaCl·ℓ$^{-1}$ water (Walsh, 1984). At the fish plant the fish were treated with 2% formaldehyde and 33 mg malachite green·ℓ$^{-1}$ for 10 s and transferred to the 5 000 ℓ fish-holding tanks (Van Aardt and Booysen, 2004). *M. salmoides* were fed earthworms or fish fry (*Tilapia sparrmanii)* from Boskop Dam. All fish were kept for at least 3 weeks in the fish-holding tanks at 20˚C ±0.5˚C before experiments were undertaken at this temperature range.

Chemical analysis of Mooi River water

Water samples were analysed for hardness and macro-elements (Midvaal Water Co., accredited laboratory number T0132).

Copper levels in fish before experimental Cu exposure

From 20 fish caught per dam a 1 mℓ blood sample was immediately taken by heart puncture whereupon the fish were transported to the laboratory. One gram fish tissue (gonads, the jejunum part of the intestines, gills, liver, kidney and muscle) was dissected out. In a glass vial 2 mℓ HNO_3 (70% *pro analisi*, Merck) and 1 mℓ $HClO_4$ 65% *pro analisi* (Merck) were added and each type of tissue was processed separately and digested as described above for sediment samples. The analysis protocols for Cu were the same as for the sediments except that the standard reference material used was (Dorm-2) from the National Research Council, Canada. To express the metal concentrations obtained from *L. capensis* per gram dried tissue, the percentage of water for each of the seven tissue types was determined as described by van Aardt and Erdmann (2004).

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Because of the low numbers of *S. micropteris* caught in Boskop and Klerkskraal Dams, only the tissues from *L. capensis* were analysed for Cu (Fig. 5).

Copper levels in fish after copper exposure

After the Cu exposure experiments, and $\dot{M}O_2$ determinations for both *S. micropteris* and *L. capensis* to different Cu concentrations, the 7 types of fish tissue were also harvested from the experimental fish and digested and analysed as described above for control fish.

Oxygen consumption rate measurements ($\dot{M}O_2$)

Measurements were done on individual fish after exposure for 120 min to 10 mg Cu·ℓ$^{-1}$ (157.3 µmol Cu·ℓ$^{-1}$) as Cu$(NO_3)_2.3H_2O$. Individual fish were also exposed for 96 h to 1 mg Cu·ℓ$^{-1}$ (15.7 µmol Cu·ℓ$^{-1}$) as Cu$(NO_3)_2$ $3H_2O$). Control fish, without Cu exposure, were measured for $\dot{M}O_2$ using the same batch caught during the same season as for the experimental fish. All $\dot{M}O_2$ measurements for Cu exposed fish were made 2 h after handling of the fish and 24 h after feeding (Jobling and Davies, 1980; Jobling, 1981). To determine the effects of handling on the $\dot{M}O_2$, oxygen consumption measurements were also made 0, 2, 4, 6, and 12 h after handling of the fish (Fig. 1a). The mean live fish mass for both control and experimental fish was 864.1 (± 85.1) g for *S. micropteris* and 992 (±112.0) g for *L. capensis.*

$\dot{M}O_2$ and opercular frequency was determined after 10 mg Cu·ℓ$^{-1}$ exposure using individual fish in a large 23.03 ℓ Perspex respirometer (Fig. 2) set up as a closed system respirometer (Bridges and Butler, 1989) with continuous PO_2 monitoring of the water. $\dot{M}O_2$ calculations and $\dot{M}O_2$ corrections for the different fish masses were done according to Van Aardt (1990) and Van Aardt and Booysen (2004). The PO_2 values to calculate $\dot{M}O_2$ for each fish were taken at a starting PO_2 value of 120 mm Hg and, after about a 20 min measuring time, again at a PO_2 of 80 mm Hg. In another experiment the progressive decrease of the PO_2 in the respiration water was determined for both fish species at 10 min intervals for 120 min (Fig. 3 a, b). At the same 10 min intervals the opercular frequency was also visually counted (Fig. 4 a, b). The $\dot{M}O_2$ determined in 10 control fish (no Cu) and 10 experimental fish exposed to 1 mg Cu·ℓ$^{-1}$ for 96 h at 20ºC (±0.5ºC), was done in a smaller volume (5.15 ℓ) respirometer without PO_2 and opercular monitoring. This was done by exposing the free swimming fish for 84 h in a 1 000 ℓ exposure tank. The fish were then caught and placed individually in open respirometers (Van Aardt and Booysen, 2004) in the same exposure tank for a further 12 h in order to eliminate handling stress on $\dot{M}O_2$. After this period the water-tight lids were screwed on for 20 min. The ΔPO_2 was then determined to enable the calculation of $\dot{M}O_2$ (Van Aardt, 1990; Van Aardt and Booysen, 2004) (Fig. 8a, b).

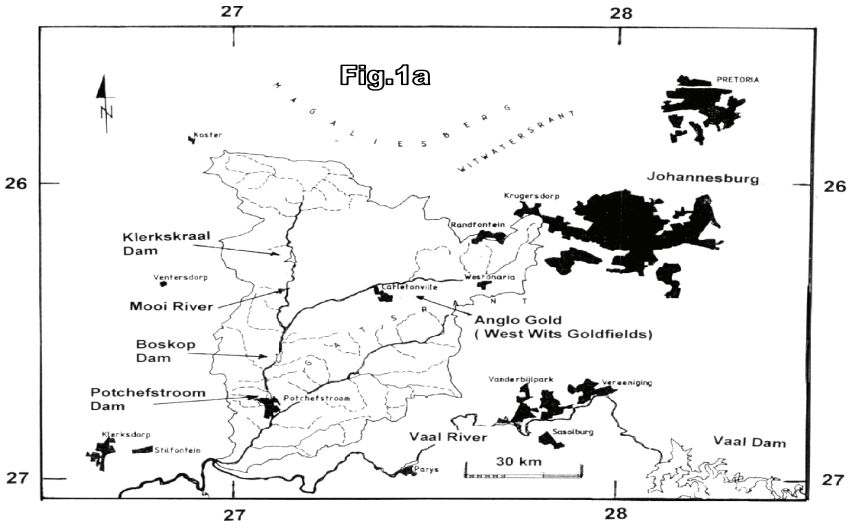

Figure 1a
Map of the Mooi River catchment indicating the three dams and the associated towns and cities

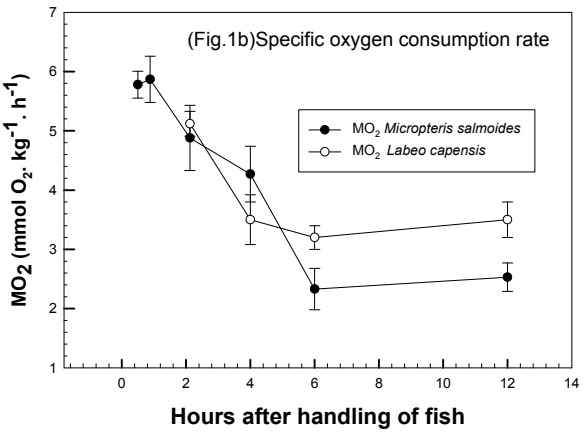

Figure 1b
*The effects of fish handling on the oxygen consumption rate of the mudfish (*Labeo capensis*, n =10) and bass (*Micropteris salmoides*, n =10). Vertical bars denote the standard deviation from the mean.*

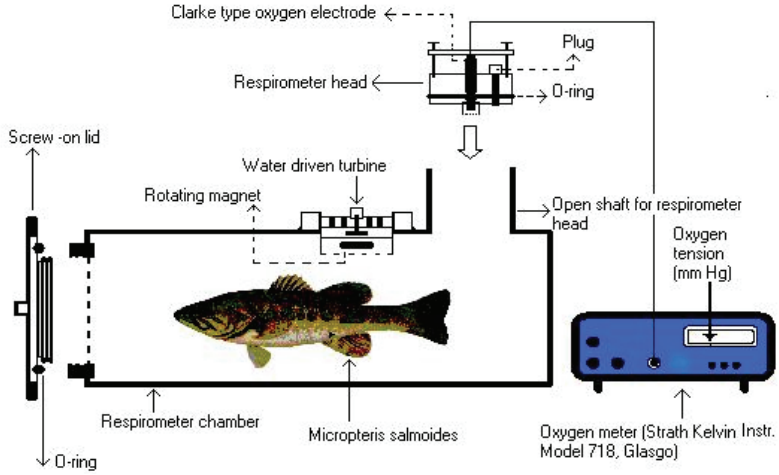

Figure 2
A diagrammatic representation (not to scale) of the large size closed system respirometer. During measurements the respirometer chamber was submerged in a thermostatically controlled water bath. The water turbine prevent the development of partial oxygen tension gradients between the fish and the oxygen electrode.

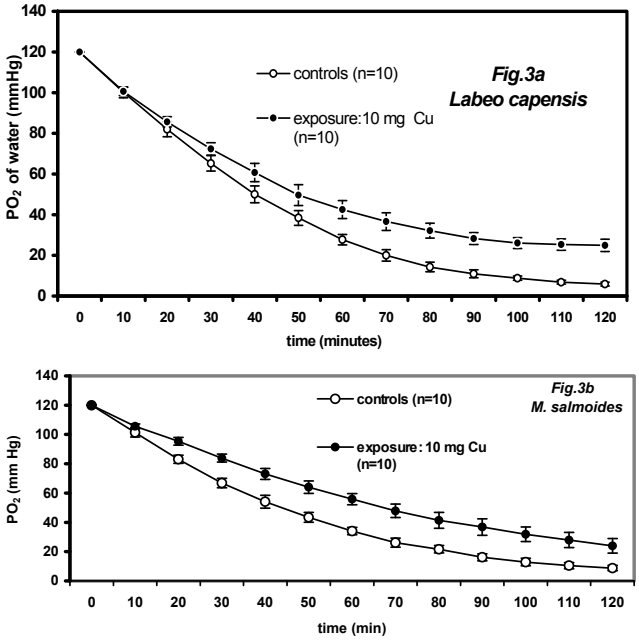

Figure 3

The progressive decrease of the mean PO_2 in the water measured for (a) L. capensis *and (b)* M. salmoides *in the large respirometer. Each fish was monitored for 2 h, exposed to 10 mg Cu·ℓ$^{-1}$(n=10). Vertical bars denote the standard deviation from the mean*

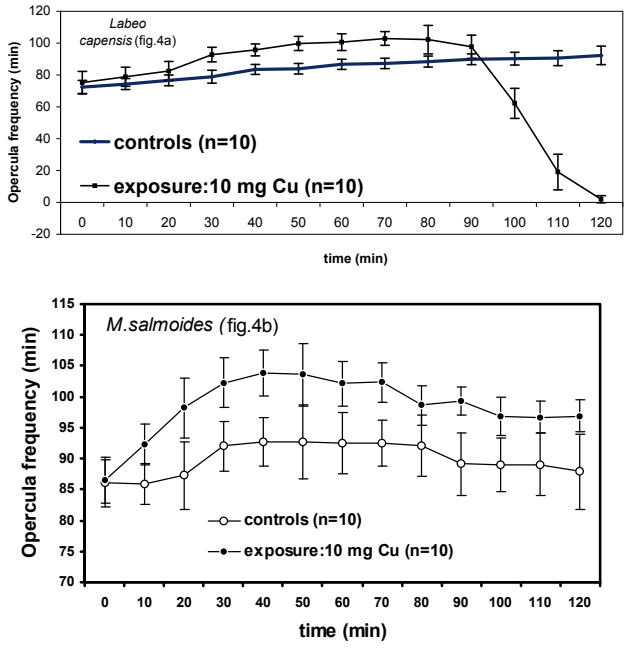

Figure 4

The mean opercular frequency during a 2 h exposure period of (a) L. capensis *and (b)* M. salmoides *to 10 mg Cu·ℓ$^{-1}$. (n = 10). Vertical bars denote the standard deviation from the mean.*

Results

The water in the 3 dams of the Mooi River can be classified as very hard (Cooney, 1995) with total alkalinity (as $CaCO_3$) of 252 mg·ℓ$^{-1}$; Ca: 67 mg·ℓ$^{-1}$; Cl: 36 mg·ℓ$^{-1}$; Mg: 50 mg·ℓ$^{-1}$; K: 4 mg·ℓ$^{-1}$; Na: 28 mg·ℓ$^{-1}$; SO_4: 115 mg·ℓ$^{-1}$; pH 8.2. Copper levels in dam sediment (Fig. 5) were 21.1 ± 6.1 µg Cu·g^{-1}, 14.0 ± 3.3 µg Cu·g^{-1} and

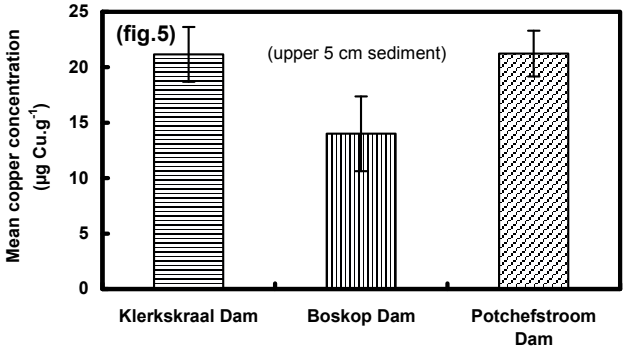

Figure 5

The mean Cu concentration in the upper 50 mm sediment fractions (n = 6 per dam) in the three dams situated in the Mooi River system. Vertical bars denote the standard deviation from the mean,

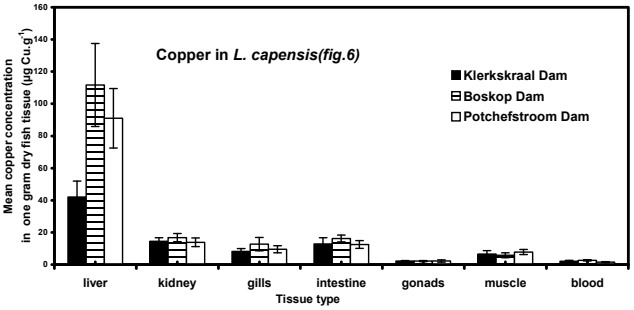

Figure 6

The mean Cu concentration (µg Cu·g^{-1}) in 1 g dried tissue from L. capensis *collected from the three dams during 1998. Twenty fish per dam were analysed. Vertical bars denote the standard deviation from the mean.*

21.2 ± 4.7 µg Cu·g^{-1} respectively for Klerkskraal-, Boskop- and Potchefstroom Dam. The percentage Cu in sediment samples from Boskop Dam, analysed by energy dispersive X-ray analysis, (EDAX) was 0.55 ± 0.06%. For Potchefstroom Dam it was 0.54 ± 0.05% and for Klerkskraal Dam it was 0.51± 0.1%. This value represents the percentage occurrence of Cu on the surface of the scanned film compared to the other metal elements.

For the 3 dams Cu in mudfish accumulates 3 to 5 times more in the liver compared to other tissues (Fig. 6). Lowest Cu concentrations were found in gonads and blood samples. The Cu concentration for mudfish liver in the Klerkskraal Dam was 42.10 ±9.8 mg Cu·kg^{-1} dry tissue. This was less than half the value found for liver tissue in Boskop Dam (110.1±17.8 mg Cu·kg^{-1}) or Potchefstroom Dam (90.5 ±12.2 mg Cu·kg^{-1}) dried liver.

Copious secretion and production of mucus on the gill lamellae were observed during the 10 mg Cu·ℓ$^{-1}$ exposure for both *L. capensis* and *M. salmoides* but not for the 1 mg Cu·ℓ$^{-1}$ exposure experiment. In both fish species the cough-reflex was observed when exposed to 10 mg Cu·ℓ$^{-1}$. Presumably the inside surface of the oral and opercular cavity was irritated or blocked with mucus (Heath, 1995). For both fish species, Cu accumulated mainly in the liver during the exposure of 1 mg Cu·ℓ$^{-1}$ for 96 h or 10 mg Cu·ℓ$^{-1}$ for 2 h (Figs. 6 and 7). Copper concentrations in fish tissue, after a 96 h exposure to 1 mg Cu·ℓ$^{-1}$ or after 2 h to 10 mg Cu·ℓ$^{-1}$, increased more than twofold in liver, kidney and gills for both species. The data were almost the same obtained for *M. salmoides* exposed to 1.0 mg Cu·ℓ.$^{-1}$ or 10 mg Cu·ℓ$^{-1}$ (Fig. 6).

The PO_2 depletion capacity by both *M. salmoides* and *L. capensis* of the water medium was significantly reduced when

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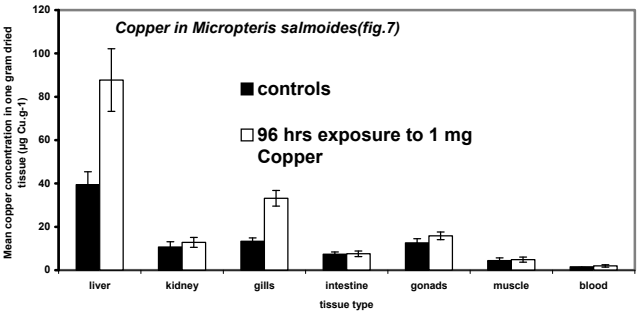

Figure 7

The mean Cu concentration (µg Cu·g^{-1}) in 1g dried tissue from M. salmoides *collected after exposure in the laboratory to 10 mg Cu·ℓ$^{-1}$ for 2h. The results for 1 mg Cu exposure and the 10 mg Cu exposure for both species were the same when compared with data expressed in Fig. 7. Vertical bars denote the standard deviation from the mean.*

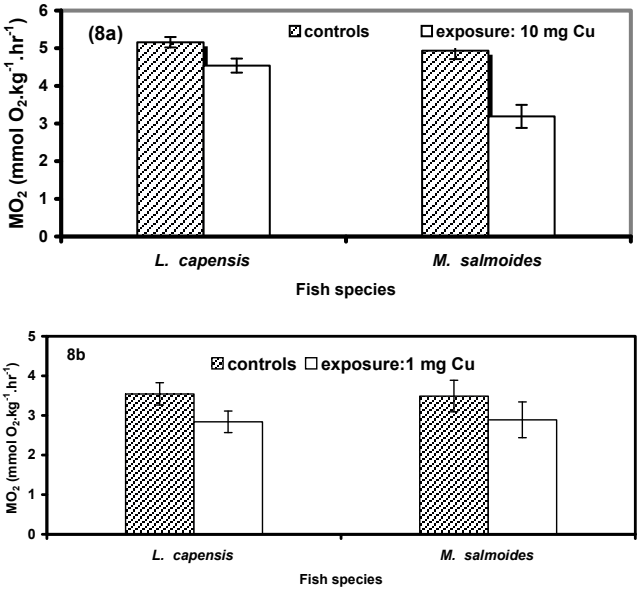

Figure 8

The oxygen consumption rate from control (n=10) and experimental fish (n=10) for both species exposed to (a) 10 mg Cu·ℓ$^{-1}$ and (b) to 1 mg Cu·ℓ$^{-1}$.Vertical bars denote the standard deviation from the mean.

fish were exposed to 10 mg Cu·ℓ$^{-1}$ compared to the controls (Fig. 3a, b). However, when the opercular frequencies between the two fish species were compared, *L. capensis* showed an increase in both control and experimental fish before a traumatic collapse in the opercular frequency for experimental mudfish occurred (Fig. 4 a, b). This happened at PO_2 values of the water below 30 mm Hg and after about 80 min exposure to Cu. All *L. capensis* specimens died before the end of the 120 min exposure time to 10 mg Cu·ℓ$^{-1}$. For the largemouth bass an initial increase in opercular frequency was found in acute Cu exposure, but it levelled off after about 60 min exposure time (Fig. 4). No deaths or collapse of the opercular frequency were found for largemouth bass. During progressive hypoxia (Fig. 3) it was visually observed that the opercular stroke volume for bass was increased by lowering the lower jaw and the floor of the oral cavity during each opercular opening. This reaction of the gill ventilation to Cu was not observed for mudfish. The $\dot{M}O_2$ values obtained for *M. salmoides* using the closed respirometry system compares favourably with the open system operated by Beamish (1970) and Beamish (1974) on the same fish species.

The $\dot{M}O_2$ of largemouth bass and mudfish decreased nearly twofold from 5.9 mmol O_2·kg^{-1}·h^{-1} to 2.12 mmol O_2·kg^{-1}·h^{-1}, 6 h after handling (Fig. 1). After this period the oxygen consumption rate stabilised, most probably as resting (standard) $\dot{M}O_2$. Beamish (1970) found for *M. salmoides* at 20ºC a standard $\dot{M}O_2$ of 3.43 mmol O_2·kg^{-1}·h^{-1} for a 150 g fish. In our experiments a statistically significant decrease in the $\dot{M}O_2$ between control fish and fish exposed for 2 h to 10 mg Cu·ℓ$^{-1}$ was found. For the 96 h exposure period to 1 mg Cu.ℓ$^{-1}$ for both the mudfish and largemouth bass the $\dot{M}O_2$ for experimental fish was significantly lower compared with the controls.

Discussion

The hard water in the Mooi River system probably played an important role in the precipitation of Cu compounds before the water from the mines reached the Potchefstroom and Boskop dams. The Cu concentration in the water decreased by more than half, 2h after the pH of the water was increased from a value of below 7 to above 8, in agreement with the findings reported by Shaw and Brown (1974). The result of this pH effect is that a relatively low Cu level is found in Mooi River water. Furthermore the high pH values of the water in the 3 dams increased the adsorption of Cu onto the small clay particles (Kishk and Hassan, 1973; Farrah and Pickering 1977; Daoust et al. 2006). An average of 18.7 (±7.5) mg Cu·kg^{-1} dried sediment was found in sediment samples collected for the 3 dams in the Mooi River. Most of the Cu is contained in the clay fractions (Van Aardt and Erdmann, 2004).

The results of the closed system respirometry used in this study compare favourably with the elaborate open system respirometry used by other workers (Beamish, 1970; Ultsch et al., 1980; Van Aardt and Frey, 1985; De Boeck et al., 1995). To circumvent the possible effects of accumulated excretory products in the closed system respirometry that may influence the respiration rate, the volume of the respiration water should be at least 100 times larger than the volume of an individual fish.

From the opercular frequency results it is clear that the largemouth bass (*Micropteris salmoides*) is physiologically better adapted to acute exposure to Cu in hard water compared to the indigenous *L. capensis*. Why the opercular frequency and $\dot{M}O_2$ in *M. salmoides* does not collapse during acute Cu exposure, compared to *L. capensis*, is difficult to explain. It is expected that the much larger gill surface area of largemouth bass, compared to that of the mudfish, should result in a higher Cu exposure per unit surface of the gills resulting in an increased uptake of this metal. McKim et al. (1978) found for *M. salmoides* hatchlings a LC_{50} of 6.97 mg Cu·ℓ$^{-1}$. This is the most tolerant fish species to Cu toxicity when compared with 7 other freshwater species (McKim et al., 1978). Furthermore the Salmonidae are more sensitive to Cu exposure compared to the Percidae (Spear and Pierce, 1979; Taylor et al., 2003). In this study, opercular frequency measurements indicated that, apart from electrolyte loss, *Labeo capensis*, as a member of the Cyprinidae, is much more sensitive to Cu than largemouth bass, belonging to the Centrarchidae. However, if the oxygen consumption rates of mudfish and largemouth bass are compared on a mass-specific basis, the metabolic rates are nearly the same. Furthermore, the amount of Cu entering the liver, gills and kidney at a 96 h and 2 h exposure periods, respectively, is the same in both fish species. From these data we suggest that *M. salmoides*, being a highly active predator (Rice, 1990), may have better developed neuro-muscular

functions which enables it to operate its opercular muscles during acute Cu exposure, than the relatively sedentary mud fish.

Generally the oxygen consumption rate in fish exposed to Cu decreases. However, an initial stimulation of $\dot{M}O_2$ was found for blue gill (O'Hara,1971), although this was not found by De Boeck et al.(1995) who found an immediate $\dot{M}O_2$ decrease in carp. Higher than normal $\dot{M}O_2$ values were also noted in our experiments but it could be ascribed to irritation of the gill membranes as well as the epithelium of the oral cavity causing the fish to move around more frequently in the large volume (23.03 ℓ) respirometer compared to the small volume (5.15 ℓ) respirometers. The standard $\dot{M}O_2$ of 1.8 mmol $O_2 \cdot kg^{-1} \cdot h^{-1}$ in acidified water at 15ºC was found for 1.960 kg carp, *Cyprinus carpio*, (Ultsch et al., 1980) while De Boeck et al.(1995) measured standard $\dot{M}O_2$ as 5.3 mmol $O_2 \cdot kg^{-1} \cdot h^{-1}$ in their small 25 g carp. However, for 25 g carp exposed to 53.37 µg $Cu \cdot \ell^{-1}$ (0.84 µmol $Cu \cdot \ell^{-1}$) the $\dot{M}O_2$ decreased substantially in hard water at 20ºC to well below 3 mmol $O_2 \cdot kg^{-1} \cdot h^{-1}$ (De Boeck et al., 1995).

In general, the ability of fish, exposed to Cu, to deplete the oxygen content in the water is impaired, compared with controls. This points to a disruption of the branchial structure in fish, probably due to apoptotic death (Wendelaar-Bonga and Lock, 1992; Dang et al., 2000) and not due to damage of the oxygen receptors in the gill arteries that govern gill frequency and ventilation volume (Hughes and Shelton, 1958; Hughes, 1966). According to Heath (1995) metabolic depression observed in fish during Cu exposure could be explained as inhibition of enzymatic reactions related to respiration, and also a decrease in muscle tone resulting in a decrease in spontaneous swimming activity.

The high Ca^{2+} levels found in Mooi River hard water may reduce the permeability of cell membranes including the gill epithelium to water and ions as well as ionic Cu species. High levels of calcium in the water modify the impact of most pollutants by changing membrane permeability to substances such as Cu (Wendelaar-Bonga, 1997). In diluted seawater it was found that ionoregulatory disturbances are caused by increased gill permeability to water and ions by Cu that displaces gill surface bound calcium (Wilson and Taylor, 1993).

Liver Cu concentrations in *M. salmoides* living in Mooi River water are 41.4 ±3.7 µg$Cu \cdot g^{-1}$. This is nearly the same concentration (47.7 µg $Cu \cdot g^{-1}$) found for *M. salmoides* living in a reactor cooling reservoir (Pinder and Giesy, 1981). These values are not above normal values found in fish (Pelgrom et al., 1995). However, the 10 mg $Cu \cdot \ell^{-1}$ and 1 mg $Cu \cdot \ell^{-1}$ exposed fish in our study had Cu levels of above 75.0 µg$\cdot Cu^{-1}$ in liver tissue and gills, indicating that the liver (Felts and Heath, 1984) and the branchial epithelium (Van Heerden et al., 2004) are actively sequestering Cu through metallothionein synthesis (Dang et al., 1999). According to Collvin (1985) the liver in fish implies an increased maintenance cost to detoxify excess Cu. Contrary to other heavy metals and organic pollutants such as organophosphates, Cu exposure of fish does not seem to cause liver histopathology (Baker, 1969). Fish species differences in the pathology to Cu have been recorded (Leland, 1983).

Although chronic and acute stress are mostly associated with increased metabolic rate in fish (Wendelaar-Bonga, 1997) the metabolic rates, as reflected by $\dot{M}O_2$ in *M. salmoides* and *L. capensis*, were decreased during short-term exposure to Cu. This suggests a direct toxic effect of Cu on the gills because in both species examined Cu accumulation was significantly higher in gill tissue and also to a degree in kidneys.

The dolomitic origin (Midgley et al., 1990) of the hard water in the Mooi River system in South Africa, with a hardness of 252 mg$\cdot \ell^{-1}$ (as CO_3), should result in the speciation of cupric Cu to $Cu(CO_3)_2^{-2}$; $CuCO_3$; or $Cu(OH)_2$ in a ratio, respectively, of 0.5; 0.8 and 1.0 (Leckie and Davis,1979; Tao et al., 2002). It was found (Wagemann and Barica, 1979; Stouthart et al., 1996) that Cu $(OH)_2$ is the most toxic Cu species.

It is known that acute as well as prolonged Cu exposure to freshwater fish disrupts the osmoregulatory mechanisms in fish with wide differences in sensitivity and tolerance between species to Cu exposure (Taylor et al., 2003). Generally a decrease in K, Na and Cl uptake was found as well as a substantial loss of these electrolytes from the blood plasma, causing massive haemoglobin concentration, increases in the arterial blood pressure and heart failure (Lewis and Lewis, 1971; Courtois and Meyerhoff, 1975; Heath, 1984; Lauren and McDonald, 1985; Wilson and Taylor, 1993).The mechanism of the effect of Cu toxicity on osmoregulation is an inhibition of Na and Cl uptake by the gills caused by Cu that suppresses the activity of Na^+/ K^+ ATP-ase in gill tissue (Heath, 1995; Li et al., 1996; Dang et al., 2000).

Pioneering experiments by Lauren and McDonald (1985) showed that differences in Cu toxicity between hard water and soft water are related to the affinity for complex carbonate, and not to water hardness *per se*. Thus water hardness (e.g. calcium) does not affect Cu speciation but may influence or modify the effects of Cu on physiological functions such as oxygen consumption rate and bio-membrane function in fish. Pagenkopf (1983) suggested that calcium in the water medium may compete with metals for binding sites on fish gill epithelium. Lauren and McDonald (1985) proved that the physiological basis for the ameliorative effects of alkalinity lies in the reduction of toxic effects of Cu on the fish gill capacity to regulate ions. Several physiologists (Potts and Fleming, 1971; Cuthbert and Maetz, 1972) have shown that calcium reduces gill permeability in fish and thus, in freshwater, reduces the diffusion loss of ions across the epithelial layer.

Acute exposure to Cu causes swelling and curling of the gill lamellae and tends to increase the blood/water distance for diffusion, resulting in internal hypoxia in fish (Hughes and Perry, 1976; Satchell, 1984; Mallat,1985; Poleksić and Mittrivić-Tutundžić,1994) and in a reduction of the oxygen consumption rate.

Because of its flexible redox state, Cu in fish plays a vital role in cellular respiration, with cytochrome-c oxidase as the most important Cu protein (Bury et al., 2003). Although Cu is an essential element, with a daily requirement of between 15to 60µ mol Cu $\ell^{-1} \cdot kg^{-1}$dry mass (Lanno et al., 1985), copper is toxic to salmonid fish exposed at concentrations as low as 11.95 µmol$\cdot \ell^{-1}$ (0.76 mg$\cdot \ell^{-1}$) at pH 8.6 in hard water (De et al., 1976) and 0.157 µmol$\cdot \ell^{-1}$ (0.1 mg$\cdot \ell^{-1}$) in soft water (Anderson et al., 1989). This metal also causes the immuno suppression of antibody-producing cells (Anderson et al., 1989), and an increase in the number of leucocytes in tilapia (Nussey et al., 1995). Mature, fully functional chloride cells decrease substantially and necrotic apoptotic chloride cell numbers increase in tilapia exposed to copper (*Oreochromis mossambicus*) fish (Dang et al., 2000).

In Mooi River hard water, copper is about 10 times more toxic for tilapia fish compared to cadmium (Van Aardt and Booysen, 2004) or lead (Van Aardt and Venter, 2004). These metals are known for their high toxicity to fish in soft water but in high alkalinity hard water, Cd and Pb completely precipitate from the water column compared to Cu.

Conclusions

It is concluded that:

- The imported *M. salmoides* is physiologically more tolerant to acute Cu exposure compared to the endemic mudfish.

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- The high [Cu] found in the sediments in the Mooi River catchment, if released in the water through pH and physical changes would result in acute Cu environmental pollution.
- Copper is about 10 times more toxic for the 2 fish species studied compared to Pb and Cd in hard water.
- The PO_2 depletion capacity for *M. salmoides* exposed to Cu decreases significantly after 15 min compared with the controls. For *L. capensis* PO_2 depletion capacity decreases significantly after 45 min.
- Copper toxicity decreases in hard water but is still toxic if tested at between 1 mg and 10 mg $Cu \cdot \ell^{-1}$.
- Copper as toxicant accumulates mainly in fish liver, gills and kidney. This metal can be evaluated, in short-term exposure regimes, using oxygen consumption rate and opercular frequency as physiological parameters.
- Experimental data from the literature (Wendelaar Bonga and Lock, 1992; Tkatcheva et al., 2004) advocate that electrolyte concentrations of Cl, Na and K and the osmotic value of blood plasma should also be measured to evaluate Cu-uptake during Cu exposure studies.

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