Technical Memorandum 8

# Acute Toxicity of Copper and Zinc to Three Fish Species from the Alligator Rivers Region

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Supervising Scientist for the Alligator Rivers Region

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Australian Government Publishing Service
Canberra 1984

Office of the Supervising Scientist
P.O. Box 387
Bondi Junction, N.S.W.
Australia, 2022

August 1984



ISSN 0810-9532
ISBN 0 644 01262 5

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Printed by Canberra Publishing and Printing Co.

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# SUMMARY

Baker, L. and Walden, D. (1984). Acute toxicity of copper and zinc to three fish species from the Alligator Rivers Region. Supervising Scientist for the Alligator Rivers Region, Tech. Mem. 8.

The ninety-six hour median lethal concentrations (96-h $LC_{50}$) of copper and zinc (in terms of nominal concentrations) for three species of fish were determined by static bioassay using water from Buffalo Billabong (Magela Creek).

The 95% confidence limits of these values for copper (mg Cu/L) were: Chequered Rainbow-fish 0.04-0.26; Black-striped Rainbow-fish 0.10-0.16; and Fly-specked Hardyhead 0.016-0.027. The 95% confidence limits of the 96-h $LC_{50}$ for zinc (mg Zn/L) were: Chequered Rainbow-fish 4.00-7.40; Black-striped Rainbow-fish 6.3-15.9; and Fly-specked Hardyhead 0.34-1.07. Two experiments were completed with each metal and each species, except for zinc and the Fly-specked Hardyhead where only one experiment was completed. Not all of the copper was present in the ionic form so that the true 96-h $LC_{50}$ values (in terms of 'available' copper) will be less than that defined. In the case of zinc, where all or the greater part was present in a biologically available form, this problem did not arise. All species tested were an order of magnitude more sensitive to copper than to zinc.

# 1 INTRODUCTION

The Alligator Rivers Region (ARR) in the tropical zone of the Northern Territory of Australia (Fig. 1) is located approximately 220 km east of Darwin (12°20'S 131°00'E - Fig. 1). Magela Creek is a north-flowing tributary of the East Alligator River, draining an area of 1720 $km^2$. Two major uranium deposits are located on the Magela Creek system (Fig. 2) and two others are located within the ARR.

A recommendation of the Ranger Uranium Environmental Inquiry (Fox et al. 1977, p. 326) was that initial quality standards for any waters which were to be released should be based on acute bioassay data and the use of application factors. The Inquiry identified several heavy metals as possible contaminants, of which copper and zinc have been well documented as being toxic to biota. (Chapman 1973; Chapman and Stevens 1978; Lewis 1978).

The objective of this investigation was to establish the acute lethal levels of copper and zinc to species of freshwater fish common in the ARR. The test program was designed to conform to the standardised methodology of toxicity bioassays suggested by the American Public Health Association (APHA) (1975) and the US Environmental Protection Agency (EPA) (1975).

Several criteria for selecting test organisms are listed by APHA and EPA, including sensitivity to the test materials or environmental factors under consideration, geographical distribution, seasonal availability, ecological importance, and reliability as testing organisms.

Six species of fish were investigated as possible test species: Chequered Rainbow-fish (*Melanotaenia splendida inornata*) (Allen and Cross 1982), Black-striped Rainbow-fish (*Melanotaenia nigrans*) (Allen and Cross 1982), Sail-fin Perchlet (*Ambassis* spp.), Bony Bream (*Nematolosa erebi*), Fly-specked Hardyhead (*Craterocephalus stercusmuscarum*) (Lake 1978) and Marjorie's Hardyhead (*C. marjoriae*). The bony bream were deemed unsuitable as they were extremely sensitive to handling and died after being kept in aquaria for short periods. The sensitivity of the various perchlet species could not be tested as the taxonomy of this group is under review. The Fly-specked Hardyhead was chosen in preference to Marjorie's because of its ready availability throughout the year.

The three species selected, Chequered Rainbow-fish, Black-striped Rainbow-fish and the Fly-specked Hardyhead, are found near the base of the food chain, feeding on phytoplankton, zooplankton and macrophytes (Bishop et al. in press). Should toxic materials be released into the Magela Creek system, two problems could occur: either larger fish could be deprived of these three species as a source of food, or the species could add to the biological uptake of heavy metals by larger predator fish and thus, inevitably, by man.

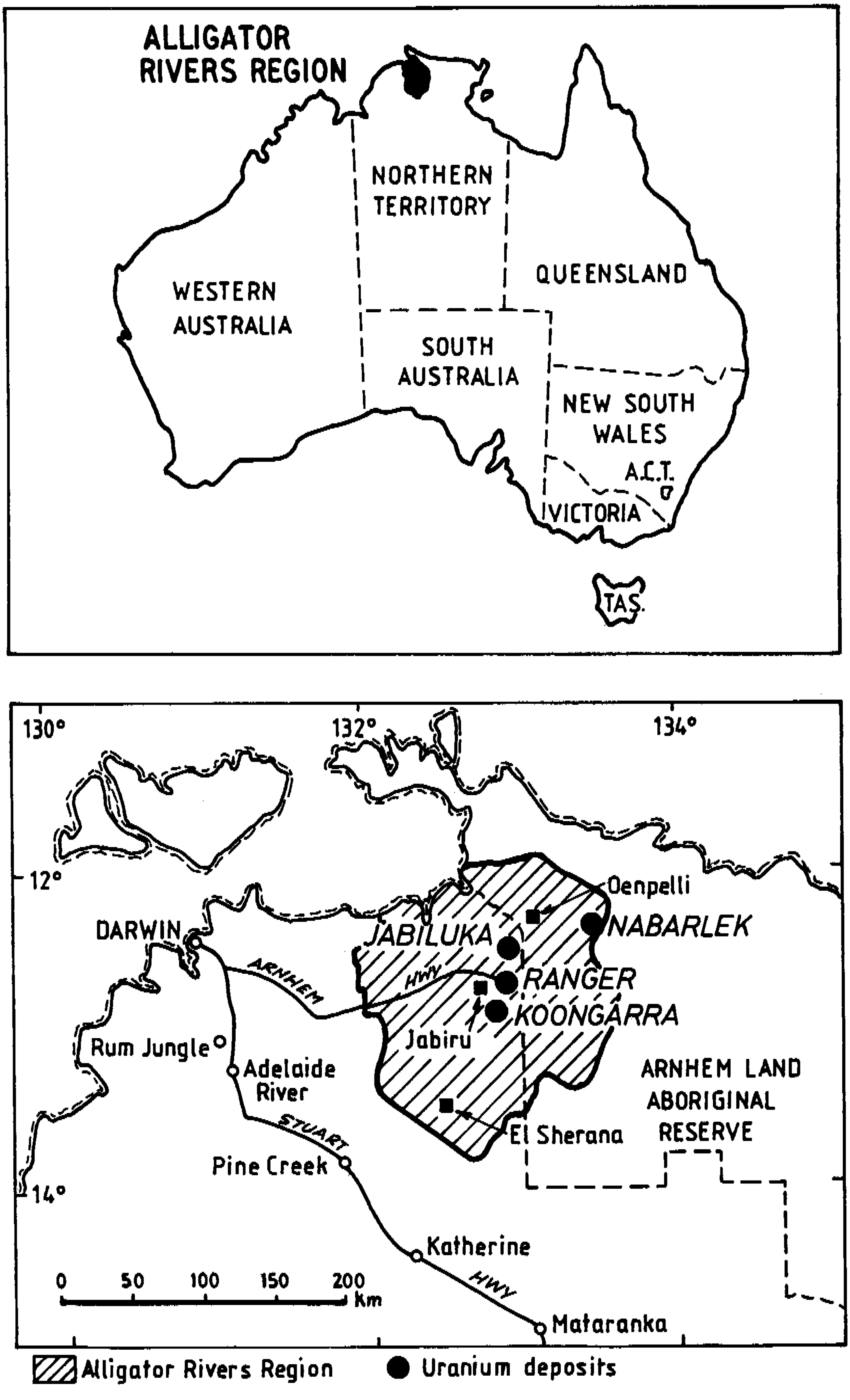


FIG. 1 LOCATION OF ALLIGATOR RIVERS REGION.

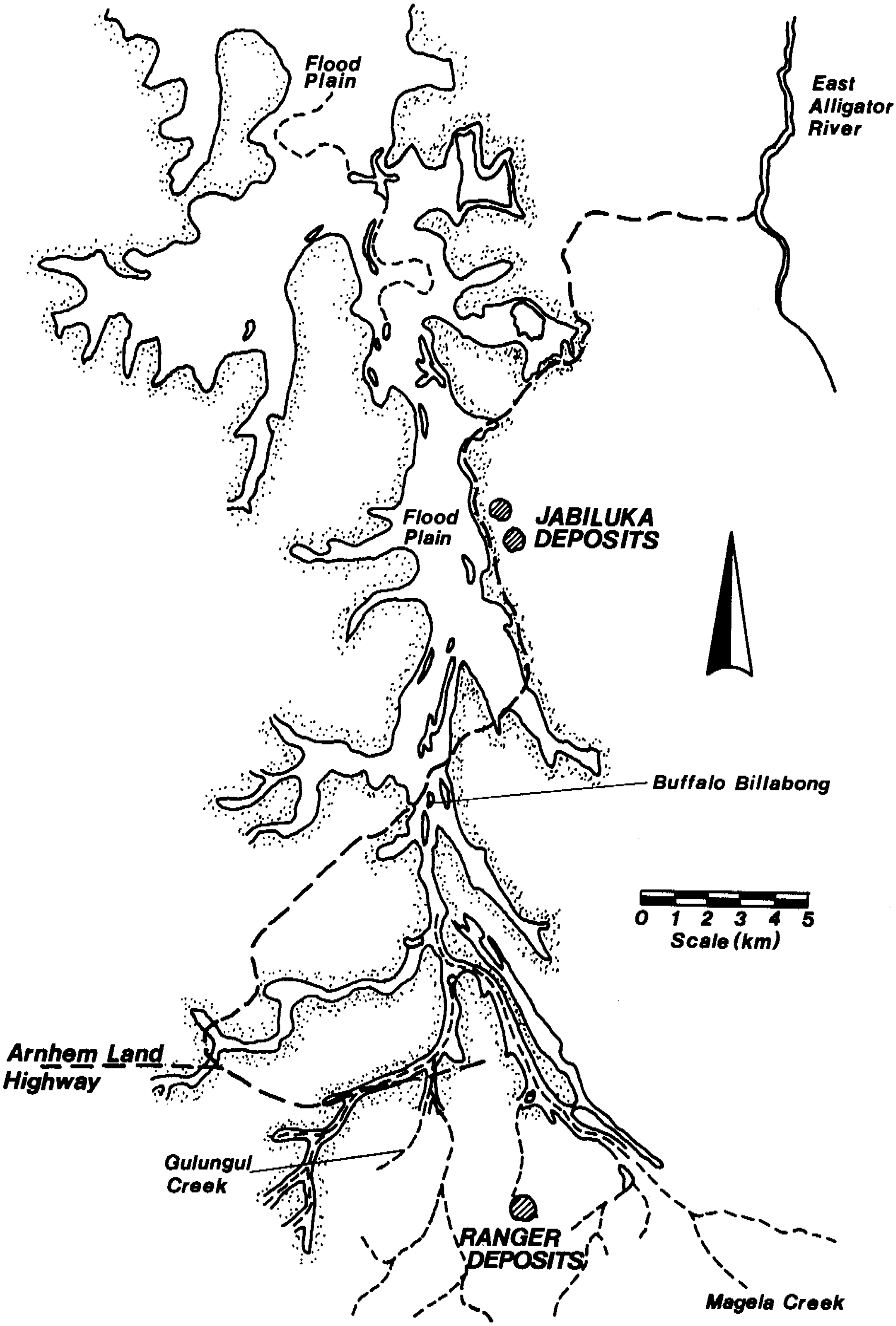


FIG. 2 MAGELA CREEK SYSTEM.

## 2 METHODS

The fish were collected by seine netting in billabongs and creeks of the Magela Creek and Nourlangie Creek systems. Both species of rainbow-fish were kept for approximately two weeks in local groundwater at ambient air temperatures, in perspex aquaria in a covered outdoor holding facility. Because of their sensitivity to handling and changes in water quality, the hardyheads were transferred directly from the field to the testing laboratory and kept in billabong water. (Facilities were not available to hold all of the fish in the testing laboratory.)

All tests were made in billabong water and because groundwater used in the stock tanks was an order of magnitude harder than local billabong water, the rainbow-fish were acclimatised by transferring them to tanks of billabong water five days before testing. The fish were fed a commercial tropical fish food (Tetramin) three times per day, and all test fish appeared to be in a healthy condition. The rainbow-fish were starved for 24 hours prior to and during testing (APHA 1975; Peltier 1978). The hardyheads, however, could not survive starvation, and were fed daily until testing commenced, and thereafter given small amounts of Tetramin on alternate days. The size classifications (fork lengths) of the fish were: Chequered Rainbow-fish 28-50 mm; Black-striped Rainbow-fish 36-42 mm and Fly-specked Hardyhead 22-30 mm.

Water for the tests was collected from Buffalo Billabong (Fig. 2) during the dry season, and from nearby Magela Creek during the wet season. The water was pumped by a 12-volt all-plastic submersible pump into a 500 L fibreglass tank for transport, and was transferred to two 1000 L covered black plastic holding tanks at the laboratory. The water was circulated through a 30 μm glass-fibre filter at a rate of 120 L/min for 36 hours to remove large suspended matter. The filter was then removed from the system and the water recirculated continuously until used for testing. The pump used had Teflon internal components and all piping was made of PVC. All apparatus was washed with 10% hydrochloric acid and rinsed three times with billabong water before use.

Before toxicity tests began, the billabong water was pumped into the test tanks and aerated for five hours. However, as recommended by APHA (1975) and Peltier (1978), aeration was not continued during the tests. There was no water renewal over the test period. Temperature in the test tanks was maintained at 27°C ± 1°C by the circulating ambient air. (Each test tank was fitted with a heater and a thermostat as a backup system to maintain water temperatures.)

Water quality was monitored throughout each trial with pH, dissolved oxygen (DO) and metal concentration being measured every 24 hours. Total hardness, alkalinity, conductivity and turbidity were analysed on the first and last days of each test. In later tests, analysis for calcium, magnesium, sodium and potassium by AAS was substituted for total hardness (sum of magnesium and calcium cations expressed as mg/L calcium carbonate equivalent) after the two techniques had been compared. All tests were performed according to methods of APHA.

Each experiment consisted of six test tanks and one control (i.e. without added metal) except where insufficient numbers of fish were available. Fifty litres of water was used per tank. Stock solutions of analytical grade copper sulphate and zinc sulphate were prepared using distilled water. An appropriate volume of one of these was added to each tank to give the required concentration. The solutions were stirred by a 12 volt pump for 5 minutes, metal concentrations determined and fish added randomly. Ten fish were placed in each tank, observing the limit of 1 gram of fish to 10 litres of water recommended for tropical waters (APHA 1975).

Whenever possible, fish mortality was observed hourly for the first 24 hours, after which only daylight observations were made. Weight (g), fork length (mm), and in some cases sex and maturity were recorded after death. Dead fish were removed immediately on discovery.

Two experiments were completed for each metal and with each fish species except for zinc where only one experiment was completed for the Fly-specked Hardyhead.

Metal concentration in the test tanks was determined every 24 hrs. Water samples were taken with either an acid-washed pipette (unfiltered sample), or in some instances, an acid-washed plastic syringe fitted with a filter holder and a 0.45 μm Sartorius membrane (filtered sample). Samples were preserved by the addition of 0.5 ml nitric acid (BDH Aristar) per 50 ml sample and stored in acid-washed polyethylene bottles. Total copper concentrations were measured by graphite furnace atomic absorption spectrophotometry (AAS) and total zinc concentrations by flame AAS, using the direct calibration technique. Background correction was used for both graphite furnace and flame AAS. No interferences were found when direct calibration results were compared with standard addition technique results (Noller pers. comm.).

It has been well documented that complexed metals are less toxic to fish than are their free ionic or labile forms (Pagenkopf et al. 1974; Brown et al. 1974; Hart and Davies, 1984). Further investigations into the ability of Buffalo Billabong water to complex free metal ion were carried out as separate experiments. Nominal concentrations of copper sulphate (0-400 μg/L) and zinc sulphate (0-15 mg/L) were added to Buffalo Billabong water (sampled in February 1982). Aliquots were then analysed as follows (Noller and Currey 1984):

(i) the sample was acidified and the total metal concentration determined by AAS.

(ii) the sample was filtered (0.45 μm Sartorius membrane filter), acidified, and the total metal concentration determined by AAS.

(iii) the sample was filtered and the concentration of acid-labile metal determined by chrono-potentiometric stripping analysis using 0.1M hydrochloric acid as the supporting electrolyte.

(iv) the sample was filtered and concentration of labile metal determined by chrono-potentiometric stripping analysis using acetate buffer (pH 4.8) as the supporting electrolyte.

(v) the sample was filtered and Chelex 100 resin (sodium form) added (for copper solution 1 g and for zinc solution 1-2 g per 50 ml sample). The solution was allowed to stand for 24 h, then filtered through a 0.45 μm membrane filter.

Measurement of the metal concentration in the filtrate by AAS gave the non-ion-exchangeable filtered metal concentration. The difference between this and the filtered total metal concentration (determined in (ii)) gave the ion-exchangeable filtered metal concentration.

To determine the 96-h $LC_{50}$ the data were analysed by the Litchfield-Wilcoxon Nomographic Method (Peltier 1978) and also a computer program, 'PRBT' (Bengston), based on probit analysis (Finney 1977).

## 3 RESULTS

The two 96-h $LC_{50}$ values of copper for the Chequered Rainbow-fish differed, as did the 96-h $LC_{50}$ values of zinc for the Black-striped Rainbow-fish. The results of repeat experiments for the other tests are in reasonable agreement. The three test species were all an order of magnitude more sensitive to copper than zinc. The Fly-specked Hardyhead appears to be an order of magnitude more sensitive to both metal species than either the Chequered or Black-striped Rainbow-fish. The Chequered Rainbow-fish was the next most sensitive and Black-striped Rainbow-fish the most resistant. The 96-h $LC_{50}$ values for copper and zinc and their 95% confidence limits are presented in Tables 1 and 2 respectively.

Water quality data collected throughout the experiments are in Tables 3 and 4. Tables 5 and 6 present the mean concentration of the metals per test tank and their standard deviations through the experiments, with the filtered metal concentrations where they were measured. The dosed metal concentration appeared to stay relatively constant throughout the experiments (Table 5). The groundwater analysis is given in Table 7 and Buffalo Billabong water quality data (April 1980-August 1981) are presented in Table 8.

Table 9 presents the results of the ion-exchange and chrono-potentiometric stripping analysis experiments. The acid-labile concentrations (determined by chrono-potentiometric stripping analysis) were approximately the same as the filtered metal levels measured by AAS techniques (Table 9). Differences between these two sets of data are attributable to the fact that chrono-potentiometric stripping analysis gave less precise results than did AAS (Noller and Currey 1984).

Difficulties were experienced in the measurement of labile copper (pH 4.8) by potentiometric stripping analysis and no values are reported. Complexing behaviour appears to influence the electrochemical response of copper at levels higher than those found in natural waters and for copper-complexing capacities above those now considered to be typical. This aspect is still under investigation. A study by Figura and McDuffie (1980) reported that the copper complexed with humic acids gave no 'ASV-labile' signal at pH 7.8 but 54% was found labile by the Chelex column technique.

All raw experimental data are given in Appendix I (Tables A1-A11).

# 4 DISCUSSION

The toxicity of heavy metals to fish is affected by a number of variables, including size of fish, water hardness, pH and dissolved oxygen levels. The latter two remained relatively constant throughout all experiments, but there was variation in water hardness and size of fish tested.

An increase in hardness of the test water causes a decrease in the toxicity of heavy metals to fish (Miller & Mackay 1980; Pagenkopf et al. 1974).

The data on the quality of water from Buffalo Billabong (Table 6), groundwater (Table 7) and the test waters (Tables 3 and 4) indicate that, in some cases, groundwater, used to prime the recirculation pumps, was present in the test tanks. Groundwater was an order of magnitude harder than billabong water and its presence as a contaminant in some of the test tanks led to a difference in the total hardness of water between replicate experiments. In such cases (Table 4) the tests run in water of lower hardness gave lower 96-h $LC_{50}$ values, in agreement with the findings of other workers (Zitko & Carson 1976).

The 96-h $LC_{50}$ can also be affected by biological factors, such as the size of the fish tested (Anderson and Spear 1980). The replicate experiments with copper and Chequered Rainbow-fish appear to confirm this, copper being more toxic to the smaller fish, although this result is confused by the lower hardness of the water in which the small fish were tested.

It has been well established that heavy metals are complexed by organic and inorganic compounds in the water which render a proportion of the metal unavailable as toxicant to the fish. The most toxic forms of metals are the free ionic or labile forms (Sylva 1976, Brown and Andrew 1976; Spear and Pierce 1979). The 96-h $LC_{50}$ values reported here were based on total metal concentrations as measured by AAS. Results show that added zinc was present predominantly as the labile metal ion and the $LC_{50}$ values therefore reflect the concentration of the labile zinc ion. A proportion of the added copper, on the other hand, was complexed (non-ion-exchangeable on Chelex 100) by the test waters as well as showing some degree of adsorption to particulate matter greater than 0.45 μm. As a result the 96-h $LC_{50}$ values reported for copper do not accurately reflect the concentration of labile copper in the test waters.

A combination of factors, both chemical and biological, contributed to the differences in the 96-h $LC_{50}$ figures observed in these experiments. The results give an indication of the acute toxicity of copper and zinc to the three fish species studied, but further better controlled investigations are needed to provide more accurate information.

## 5 ACKNOWLEDGMENTS

We would like to thank the following people for their assistance throughout the project: Mr Peter Body and Dr Barry Noller for the heavy metal sampling and analysis; Dr Walter Ivantsoff, Ms Helen Allison and Mr Chris Humphrey for help in setting up histological techniques and identifying gonad stages; Dr Neville Williams, Dr John Skidmore and Mr Ian Firth for information on toxicity testing techniques; Dr M. Bengston (CSIRO, Brisbane) for the computer program, based on Finney's (1977) probit analysis, and especially Dr Douglas Clay for his direction and assistance in the writing of this report.

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TABLE 1 96-h $LC_{50}$ VALUES FOR COPPER CALCULATED BY LITCHFIELD-WILCOXON, NOMOGRAPHIC METHOD OF ANALYSIS[a] AND BY COMPUTER PRBT ANALYSIS[b].

Dashes indicate insufficient data to calculate results using PRBT program.

| Fish Species | Date | Mean Weight (g) ± S.D. | Nomograph 96-h $LC_{50}$ (mg/L) | 95% Confidence Limits (mg/L) | PRBT 96-h $LC_{50}$ (mg/L) | 95% Confidence Limits (mg/L) |
|---|---|---|---|---|---|---|
| Chequered Rainbow-fish | 5.8.80 | 1.4 ± 0.5 (n=70) | 0.25 | 0.24 - 0.26 | 0.25 | 0.23 - 0.27 |
| | 2.11.81 | 0.32 ± 0.05 (n=50) | 0.06 | 0.04 - 0.09 | - | - |
| Black-striped Rainbow-fish | 9.6.80 | 0.41 ± 0.18 (n=70) | 0.12 | 0.10 - 0.14 | - | - |
| | 29.6.80 | 0.60 ± 0.24 (n=70) | 0.14 | 0.12 - 0.15 | 0.14 | 0.12 - 0.15 |
| Fly-specked Hardyhead | 28.9.81 | 0.16 ± 0.08 (n=60) | 0.017 | 0.016 - 0.027 | 0.019 | 0.008 - 0.028 |
| | 5.10.81 | 0.12 ± 0.07 (n=60) | 0.021 | 0.016 - 0.027 | - | - |

TABLE 2 96-h $LC_{50}$ VALUES FOR ZINC CALCULATED BY LITCHFIELD-WILCOXON NOMOGRAPHIC METHOD OF ANALYSIS[a] AND BY COMPUTER PRBT ANALYSIS[b].

| Fish Species | Date | Mean Weight (g) ± S.D. | Nomograph 96-h $LC_{50}$ (mg/L) | 95% Confidence Limits (mg/L) | PRBT 96-h $LC_{50}$ (mg/L) | 95% Confidence Limits (mg/L) |
|---|---|---|---|---|---|---|
| Chequered Rainbow-fish | 9.12.81 | 0.51 ± 0.09 (n=70) | 6.20 | 5.20 - 7.40 | 6.14 | 5.34 - 7.00 |
| | 8.4.81 | 1.70 ± 0.20 (n=70) | 4.80 | 4.00 - 5.70 | 4.73 | 3.65 - 5.51 |
| Black-striped Rainbow-fish | 10.11.80 | 0.56 ± 0.06 (n=70) | 13.90 | 10.00 - 15.90 | - | - |
| | 8.4.81 | 0.74 ± 0.14 (n=70) | 6.80 | 6.30 - 7.30 | 5.98 | 5.27 - 6.62 |
| Fly-specked Hardyhead | 27.4.81 | 0.07 ± 0.01 (n=70) | 0.60 | 0.34 - 1.07 | - | - |

[a] Peltier (1978). [b] Based on Finney's method (1977) - Dr. M. Bengston's (CSIRO Brisbane) PRBT program.

TABLE 3 QUALITY OF TEST WATER IN COPPER EXPERIMENTS.

Hardness (Mg + Ca) and alkalinity are expressed as mg/L $CaCO_3$.
n = 1 for conductivity, hardness, alkalinity, K, Ca, Na and Mg.
DO = dissolved oxygen; Conduct = conductivity.

| Fish Species | Date | pH[a] | DO[a] (mg/L) | Conduct[b] (µS/cm) | Hardness[b] | Alkalinity[b] | K[b] | Ca[b] (mg/L) | Na[b] | Mg[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| Chequered Rainbow-fish | 5.8.80 | 6.9±0.2 | 5.3±0.4 | 40.0 | 7.0 | 6.7 | – | – | – | |
| | 2.11.81 | 6.9±0.1 (n=25) | 6.7±0.3 (n=25) | 28.5 | 3.3 | – | 0.64 | 0.35 | 2.6 | 0.6 |
| Black-striped Rainbow-fish | 9.6.80 | 7.3±0.1 (n=35) | 5.4±0.1 (n=35) | 34.5 | 25.2 | 18 | – | – | – | – |
| | 29.6.81 | 7.2±0.3 (n=35) | 6.6±0.3 (n=35) | 57.5 | 25.5 | 17 | 0.99 | 3.5 | 3.3 | 4.0 |
| Fly-specked Hardyhead | 28.9.81 | 6.9±0.1 (n=30) | 6.9±0.4 (n=30) | – | 6.6 | – | 0.44 | 0.72 | 2.3 | 1.2 |
| | 5.10.81 | 7.3±0.5 (n=25) | 7.2±0.3 (n=25) | – | 6.6 | – | 0.48 | 0.73 | 2.5 | 1.2 |

[a] Mean sample values taken from all tanks over 96 hours.
[b] Sample values taken from control tanks at commencement of test.

TABLE 4 QUALITY OF TEST WATER IN ZINC EXPERIMENTS.

Hardness (Mg + Ca) and alkalinity are expressed as mg/L $CaCO_3$.
n = 1 for hardness, alkalinity, K, Ca, Na and Mg.
DO = dissolved oxygen; Conduct = conductivity.

| Fish Species | Date | pH[a] | DO[a] (mg/L) | Conduct[b] (μS/cm) | Hardness[b] | Alkalinity[b] | K (mg/L) | Ca (mg/L) | Na (mg/L) | Mg (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Chequered Rainbow-fish | 9.2.81 | 7.0±0.2 (n=35) | 5.5±0.4 (n=35) | 33±1.1 (n=2) | 27 | - | 1.16 | 3.7 | 3.0 | 4.4 |
| | 8.4.81 | 6.6±0.2 (n=35) | 4.8±0.6 (n=35) | 22 (n=1) | 7.3 | 7.0 | 0.38 | 0.91 | 1.8 | 1.2 |
| Black-striped Rainbow-fish | 10.11.80 | 6.8±0.5 (n=30) | 5.0±0.3 (n=30) | 40±3.9 (n=2) | 22 | 15 | - | - | - | - |
| | 8.4.81 | 6.7±0.2 (n=35) | 5.1±0.4 (n=35) | 33 (n=1) | 7.3 | 7.0 | 0.66 | 0.91 | 1.7 | 1.2 |
| Fly-specked Hardyhead | 27.4.81 | 7.2±0.1 (n=35) | 5.1±0.3 (n=35) | 42±3.5 (n=4) | 42 | - | 0.66 | 2.18 | 2.4 | 8.8 |

[a] Mean sample values taken from all tanks over 96 hours.
[b] Samples taken from control tanks at commencement of experiment (n=1), commencement and end (n=2), each day of test (n=4).

TABLE 5 MEAN CONCENTRATION OF COPPER IN TEST TANKS.

U = unfiltered, F = filtered, samples filtered through 0.45 µm (Sartorius) filter.

| Fish | Date | Concentration of Copper (mg/L)[a] ± SD | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | Control | |
| | | U | F | U | F | U | F | U | F | U | F | U | F | U | F |
| Chequered Rainbow-fish | 5.8.80 | 0.230 ± 0.01 | – | 0.243 ± 0.012 | – | 0.258 ± 0.016 | – | 0.265 ± 0.007 | – | 0.284 ± 0.008 | – | 0.299 ± 0.005 | – | 0.003 ± 0.001 | – |
| | 2.11.81 | 0.040 ± 0.002 | 0.035 | 0.085 ± 0.003 | 0.081 | 0.126 ± 0.001 | 0.110 | 0.172 ± 0.004 | 0.156 | – | | – | | 0.002 ± 0.001 | 0.0015 |
| Black-striped Rainbow-fish | 9.6.80 | 0.084 ± 0.008 | – | 0.099 ± 0.001 | – | 0.109 ± 0.005 | – | 0.132 ± 0.007 | – | 0.147 ± 0.011 | – | 0.195 ± 0.003 | – | 0.003 ± 0.001 | – |
| | 29.6.81 | 0.093 ± 0.006 | 0.087 | 0.114 ± 0.005 | 0.096 | 0.145 ± 0.008 | 0.131 | 0.149 ± 0.009 | 0.138 | 0.178 ± 0.009 | 0.158 | 0.214 ± 0.006 | 0.186 | <0.005 | – |
| Fly-specked Hardyhead | 28.9.81 | 0.010 ± 0.0004 | 0.010 | 0.015 ± 0.001 | 0.015 | 0.020 ± 0.001 | 0.019 | 0.023 ± 0.0001 | 0.023 | 0.028 ± 0.001 | 0.027 | – | – | 0.003 ± 0.0004 | 0.002 |
| | 5.10.81 | 0.011 ± 0.001 | 0.012 | 0.021 ± 0.001 | 0.022 | 0.035 ± 0.001 | 0.034 | 0.047 ± 0.003 | 0.045 | – | | – | | 0.002 ± 0.002 | 0.004 |

[a] Determined by graphite furnace AAS.

TABLE 6 MEAN CONCENTRATION OF ZINC IN TEST TANKS.

| Fish Species | Date | No. of Samples | Concentration of Zinc (mg/L)[a] ± SD | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | Control |
| Chequered Rainbow-fish | 9.2.80 | 6 | 1.4 ± 0.2 | 3.3 ± 0.3 | 4.9 ± 0.5 | 6.0 ± 0.5 | 7.0 ± 0.6 | 8.1 ± 0.6 | <0.1 |
| | 8.4.81 | 5 | 2.6 ± 0.1 | 4.9 ± 0.1 | 6.1 ± 0.2 | 6.8 ± 0.1 | 7.5 ± 0.1 | 9.6 ± 0.2 | <0.15 |
| Black-Striped Rainbow-fish | 10.11.80 | 5 | 7.1 ± 1.2 | 8.7 ± 2.0 | 11.2 ± 1.9 | 13.9 ± 2.3 | 21.4 ± 5.2 | 26.6 ± 2.9 | <0.4 |
| | 8.4.81 | 5 | 2.8 ± 0.1 | 5.2 ± 0.1 | 5.4 ± 0.1 | 6.9 ± 0.1 | 7.8 ± 0.2 | 9.9 ± 0.3 | <0.15 |
| Fly-specked Hardyhead | 27.4.81 | 5 | 0.088 ± 0.0004 | 0.31 ± 0.007 | 0.56 ± 0.009 | 0.75 ± 0.02 | 1.03 ± 0.02 | 1.59 ± 0.02 | <0.01 |

[a] Determined by flame AAS.

TABLE 7 QUALITY OF GROUNDWATER[a].

Hardness (Mg + Ca) and total alkalinity expressed as mg/L $CaCO_3$.

| Date | Number of Samples | Conductivity (µS/cm) | pH | Hardness | Total Alkalinity | COPPER (µg/L) | ZINC (µg/L) |
|---|---|---|---|---|---|---|---|
| 23 Sept. 1980 to 4 Nov.1981 | 13 | 209 ± 68 | 7.4 ± 0.3 | 109 ± 20 | 122 ± 26 | 450 ± 710 (<1-230) | 33 ± 72 (22-2000) |

[a] Ranger Uranium Mines Pty. Ltd., unpublished data. Note that water samples were taken some distance from the laboratory and therefore probably do not accurately reflect water conditions in the holding tanks.

TABLE 8 WATER QUALITY IN BUFFALO BILLABONG[a].

Hardness (Mg + Ca) and total alkalinity expressed as mg/L $CaCO_3$.

| Date | Number of Samples | Sample Time | Water Temperature (°C) | Conduct. (µS/cm) | pH | Hardness | Total Alkalinity | COPPER (µg/L) | ZINC (µg/L) |
|---|---|---|---|---|---|---|---|---|---|
| April 1980 to August 1981 | 20 | 0800-1530 | 29.2 ± 2.7 | 25.8 ± 7.8 | 5.8 ± 0.6 | 4.0 ± 1.2 | 4.2 ± 1.6 | <0.5-3.0 | <0.5-8.5 |

[a]Northern Territory Department of Transport and Works (1982)

TABLE 9 FRACTIONATION OF METAL WHEN (A) COPPER SULPHATE AND (B) ZINC SULPHATE SOLUTIONS WERE ADDED TO BUFFALO BILLABONG WATER.

(A) Concentration of Copper (μg/L)

| Nominal (μg/L) | Total[a] | Filtered[a,e] | Acid-labile Filtered[b,c,e] | Total[a] | Ion-exchangeable Filtered[a,e] | Non-ion-exchangeable Filtered[a,e] |
|---|---|---|---|---|---|---|
| 0 | <10 | <10 | <10 | <10 | <10 | <10 |
| 50 | 38 | 33 | 57 | 23 | 8 | 15 |
| 100 | 93 | 72 | 91 | 60 | 37 | 23 |
| 200 | 181 | 139 | 162 | 159 | 115 | 44 |
| 300 | 271 | 225 | 207 | 213 | 118 | 95 |
| 400 | 335 | 284 | 274 | 280 | 137 | 143 |

(B) Concentration of Zinc (mg/L)

| Nominal (mg/L) | Total[a] | Filtered[a,e] | Labile Filtered[b,d,e] | Acid-labile Filtered[b,c,e] | Filtered[a,e] | Ion-exchangeable Filtered[a,e] | Non-ion-exchangeable Filtered[a,e] |
|---|---|---|---|---|---|---|---|
| 0 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 0.5 | 0.43 | 0.42 | 0.4 | 0.36 | 0.4 | 0.4 | <0.1 |
| 1.0 | 0.98 | 0.98 | 0.93 | 0.91 | 1.0 | 0.9 | 0.1 |
| 5.0 | 4.7 | 4.6 | - | 4.3 | 4.9 | 4.4 | 0.5 |
| 10.0 | 9.3 | 9.2 | - | 8.3 | 10.2 | - | - |
| 15.0 | 14.6 | 14.3 | - | 12.6 | 16.0 | 15.9 | 0.1 |

[a] Measured by AAS. [b] Measured by chrono-potentiometric stripping analysis.
[c] 0.1M HCL used as supporting electrolyte. [d] Acetate buffer (pH4.8) used as supporting electrolyte.
[e] Solution was filtered through 0.45 μm Sartorius filter before analysis.