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# Zinc Effects on the Embryos and Larvae of the Sharptooth Catfish, *Clarias gariepinus* (Burchell, 1822)

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The acute toxicity of zinc has long been known and is well documented. Target water quality guidelines for zinc range from 0.002 to 1.2mg/L, (Department of Water Affairs & Forestry 1996; United States Environmental Protection Agency 1999; Australian & New Zealand Environment & Conservation Council 2000). Fingerlings of the channel catfish, *Ictalurus punctatus* (Rafinesque) died within 40hr after exposure to 12mg/L zinc (Lewis and Lewis 1971). Studies concerned with the survival of adult fish indicated that lethal concentrations ranged roughly from 0.01mg/L (Affleck 1952) to 330mg/L (Carpenter 1927).

Sublethal zinc levels have been shown to reduce the growth rate and fecundity of various fish species (Lloyd 1992). Most results concerning the effects on growth have however been inconsistent. At 0.5 to 2.0mg/L growth of Atlantic herring larvae improved, whilst growth declined from six to 12mg/L (Somasundaram et al. 1984). Several authors (Rosenthal and Alderdice 1976; Somasundaram et al. 1984; Heath 1987) reported on reductions in incubation times. Zinc exposure resulted in decreased embryonic activity causing hatching enzymes to be poorly distributed throughout the embryo. It accumulated in the head region and induced a partial hatch (Rosenthal and Alderdice 1976). Zinc has also been shown to reduce the tensile strength of eggs, resulting in fragile eggs prone to premature hatching (Brungs 1969; McFarlane and Franzin 1978; Holcombe et al. 1979; Somasundaram et al. 1984). Spawning of white sucker, *Catostomus commersoni* (Lacepède) and fathead minnows, *Pimephales promelas* (Rafinesque) were impaired and there were also reductions in the number of eggs laid per female (Brungs 1969, McFarlane and Franzin 1978). In salmonids zinc has been linked with coagulated yolk disease (Affleck 1952). Zinc has been shown to penetrate cell membranes and accumulated in fish eggs as follows; 70.8% in the chorion, 26.1% in the perivitelline fluid, 2.3% in the embryo and 0.8% in the yolk (Wedemeyer 1968, Somasundaram et al. 1984) although zinc can accumulate up to 41 fold in the embryo itself (Van Coille et al. 1975).

The aim of this study was to assess the sensitivity of the early life stages of the sharptooth catfish, *Clarias gariepinus* (Burchell) in order to determine which stage to use during rapid effluent assessments.

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## MATERIALS AND METHODS

Hatching and survival experiments were conducted in a flow through system consisting of 10 reservoir tanks connected to 10 exposure tanks. Zinc was added in the form of zinc sulphate and zinc chloride. The amounts added were calculated to achieve zinc concentrations ranging from 0 to 9mg/L. The software package JESS (Murray and Wade 1996) was used to model the chemical speciation of zinc in the water. This was done to check whether the calculated zinc concentrations would be available in the borehole water and in what form it would be. The available zinc was identified as $Zn^{2+}$.

Each batch of fertilised ova was separated into 10 alienates of 5mL, each containing ±10 000 ova. Inseminated ova were incubated at 27±1°C for 24hr. Thereafter the free and dead embryos were counted and the hatching percentage calculated for each of the exposure solutions. Hatching percentages in the controls were calibrated as a 100% hatch. To test the sensitivity of the free embryos 50 free embryos incubated under control conditions were transferred to the exposure tanks immediately after hatch and exposed for 48hr after which they were removed and their survival noted. Thereafter 50 larvae raised under control conditions were transferred to each of the exposure tanks and their survival noted after 48hr.

After each series of exposure experiments, 10 live individuals (if available) were fixed in a 10% phosphate buffered formaldehyde solution. Yolk diameter (YD) and yolk width (YW) were measured where after yolk volumes (YV) were calculated according to the equation $YV=YD^2 \times YW^2 \times \pi/6$ (Hüppop and Wilkens 1991). Total body lengths (FTL) were also measured.

Results from the three replicates were pooled. The correlation between hatching percentage and survival against exposure concentration were calculated and tested using Pearson's simple linear correlation (r). $EC_{50}$ and $LC_{50}$ values were calculated with a Multi Probit Analyses for the statistical approach for predicting chronic toxicity of chemicals to fishes. The significance of the values were described by a Chi-square value with its corresponding

## RESULTS AND DISCUSSION

Hatching success decreased significantly ($p \leq 0.05$) in both zinc sulphate and zinc chloride solutions. $EC_{50}$ values were 6.01 and 3.87mg/L for zinc sulphate and zinc chloride respectively ($p \leq 0.05$). Previous studies have shown that from 0.5mg/L, hatching in zebrafish, *Danio rerio* (Hamilton), eggs were negatively affected (Dave et al. 1987). The eggs of the fathead minnow hatched in 1.1mg/L zinc but the fry died after three days (Pickering and Vigor 1965) whilst no eggs hatched at 2.8mg/L [$CaCO_3$ 200mg/L] (Brungs 1969). Zinc at 1.4mg/L ($CaCO_3$ 43-48mg/L) reduced the survival of brook trout embryos (Holcombe et al. 1979). As a result of the fast embryonic development of *C. gariepinus* the fertilised eggs of

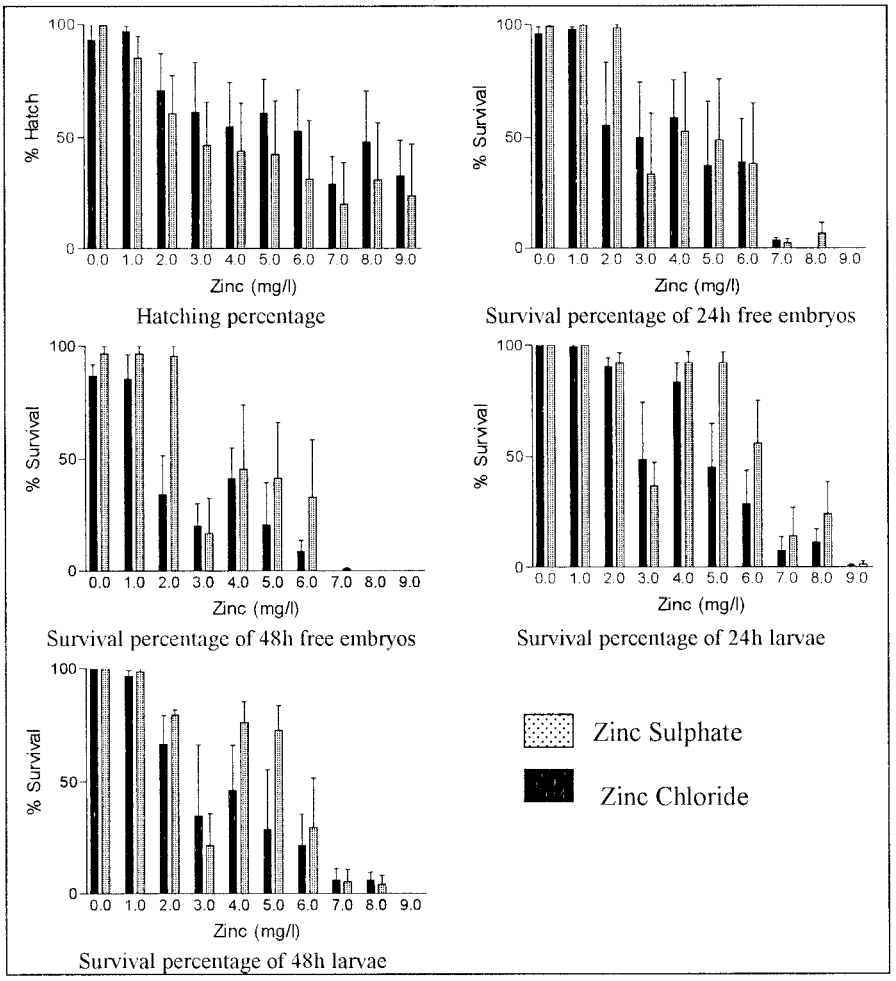


**Figure 1.** Hatching and survival percentages (Standard errors added) of newly hatched free embryos (after 24hr), free embryos and larvae (after 24 and 48hr) in zinc solutions.

*C. gariepinus* are probably more likely to hatch in a zinc polluted environment than those of many other species, specifically the salmonids, whose embryonic stages last longer.

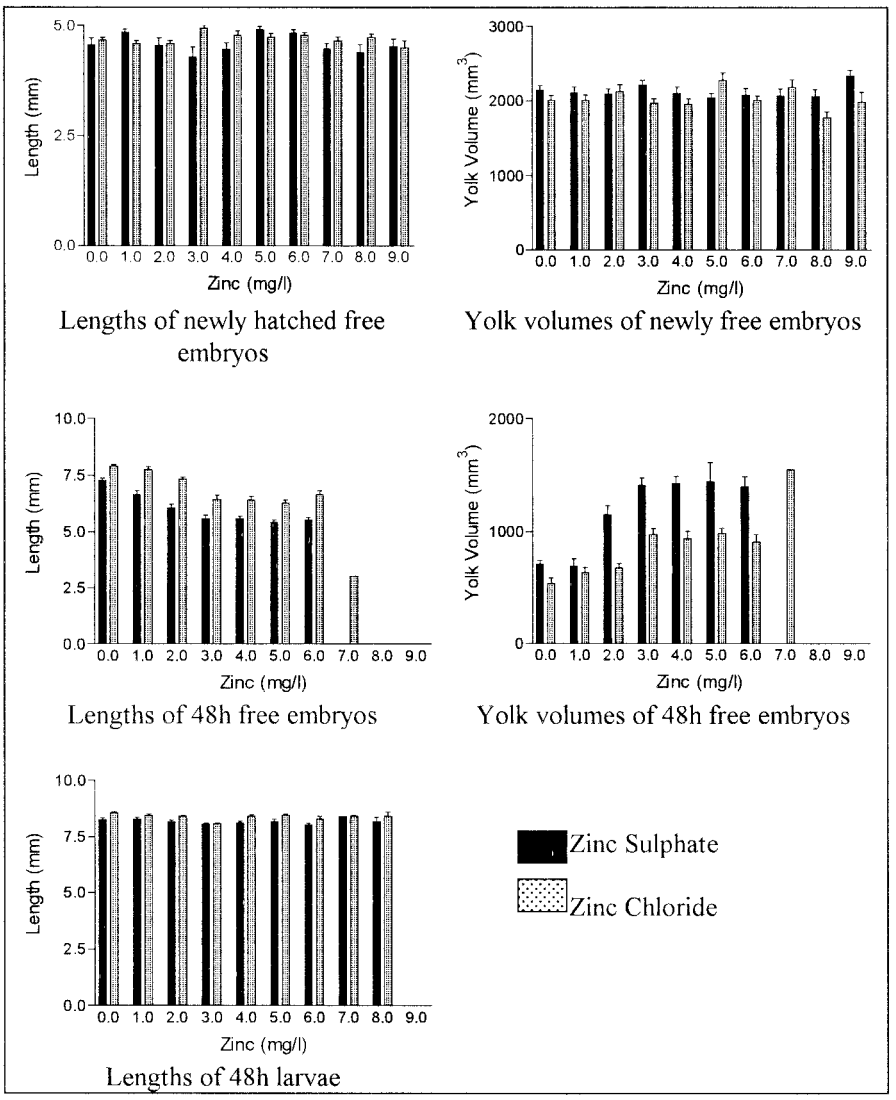


**Figure 2.** Lengths and yolk volumes (Standard errors added) of newly hatched free embryos, 48hr free embryos and 48hr larvae in zinc solutions.

Free embryo and larval survival generally decreased as zinc concentrations increased. This pattern was clear after 24hr and persisted for the full 48hr exposure. Since the reduction in survival of the free embryos was most marked during these two stages (Figure 1) it is clear that the larvae of *C. gariepinus* are vulnerable towards zinc pollution. Free embryos displayed 24hr $LC_{50}$ values of 3.86 and 4.04mg/L for zinc sulphate and zinc chloride respectively ($p \leq 0.05$) and

respective 48hr $LC_{50}$ values of 2.21 and 3.61mg/L ($p \leq 0.05$). The larvae were only slightly more resistant with 24hr $LC_{50}$ values of 4.62 and 5.61mg/L for zinc sulphate and zinc chloride respectively ($p \leq 0.05$) and respective 48hr $LC_{50}$ values of 3.45 and 4.40mg/L ($p \leq 0.05$). Results from this study indicated that $Zn^{2+}$ has the ability to destroy 80% of the larval population within 48hr of exposure at concentrations from 4.56mg/L.

No marked effects were evident on either the embryonic length or yolk volumes of newly hatched free embryos in either zinc solution ($p > 0.05$; $r(ZnSO_4)=-0.1649$ and $r(ZnCl_2)=-0.1243$). Growth in 48hr free embryos was significantly reduced. This was characterised by reduced lengths ($p \leq 0.05$; $r(ZnSO_4)=-0.8998$ and $r(ZnCl_2)=-0.8187$) and increased yolk volumes as zinc concentrations increased ($p \leq 0.05$; $r(ZnSO_4)=0.8730$ and $r(ZnCl_2)=0.8703$). Although there was also a general decrease in the lengths of the larvae this decrease was not significant ($p > 0.05$; $r(ZnSO_4)=-0.0770$ and $r(ZnCl_2)=-0.1243$). The growth reduction, manifested itself during only a short time of the development. This together with the fact that growth is interdependent on various other factors (Sprague 1971) provide some understanding as to why Woltering (1984) stated that growth is a poor indicator of stress in fish. However, during this study growth was useful in emphasising the sensitivity of the free embryo stage.

Results from this study have indicated that the free embryo stage from hatching up unto 48h thereafter, of *C. gariepinus* is probably the most sensitive stage and that this stage can be used successfully as suitable test subject for the monitoring of chemicals and effluents.

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