

# The effect of the heavy metals lead (Pb<sup>2+</sup>) and zinc (Zn<sup>2+</sup>) on the brood and larval development of the burrowing crustacean, *Callianassa kraussi*

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## Abstract

This investigation explored the effect of the heavy metals Pb (Pb<sup>2+</sup>) and Zn (Zn<sup>2+</sup>) individually and in combination (i.e. three metal solutions) on both the brood and larval development of the burrowing crustacean, *Callianassa kraussi*. Egg and larval mortalities at factorial (4x7) combinations of salinity (20, 24, 30, 35 mg/ℓ) and seven respective metal concentrations were examined. The results provided various statistically significant trends. Individual concentrations of Pb and Zn at various salinities impacted negatively upon the brood and larval development of *Callianassa kraussi*. Increasing the specific concentrations of both metals further demonstrated a negative influence, especially on brood development and to a lesser extent on larval development of *Callianassa kraussi*. Comparing the toxicity of the three concentrations of metals to *Callianassa kraussi* brood and larval development, it was found that individual Pb concentrations exhibited the least mortality (LC<sub>50</sub> Pb-eggs-35ppm = 1.580 mg·ℓ<sup>-1</sup> whilst individual Zn concentrations displayed considerably higher mortality (LC<sub>50</sub> Zn-eggs-35ppm = 0.066 mg/ℓ) but that the combination of Zn and Pb concentrations produced the highest mortality (LC<sub>50</sub> Pb&Zn-eggs-35ppm = 0.036 mg·ℓ<sup>-1</sup>). Varying salinities in permutation with varying metal concentrations exhibited a significant detrimental influence on the brood and larval development of *Callianassa kraussi*, notably at the lowest salinities in combination with the highest metal concentrations.

**Keywords:** heavy metals, Pb, Zn, mortality, toxicity, brood and egg development, estuarine environment, *Callianassa kraussi*, sediment, sand prawn

## Introduction

Although human activities have always impacted on coastal areas, it is only within the last two centuries that the effects of industrialization, intensive agriculture and coastal engineering (including tourism) have seriously begun to threaten marine life (His et al., 1999). Most of these impacts have led to environmental pollution, i.e. the introduction of substances or energy by man into the environment, which may put living resources and human health at risk. Forstner and Wittmann (1981) stated that compared with land systems, the relatively small biomass in aquatic environments generally occurs at a greater variety of trophic levels. This correlates to the particular sensitivity of aquatic systems with regard to pollution influences. Unfortunately, this distinctive trophic structure enhances accumulation of xenobiotic and poisonous substances.

Clark (1989) maintained that the problem is further extrapolated in the sense that rivers carry their pollutants (either in dissolved, colloidal or particulate form) to estuaries and finally to coastal oceans, where harmful substances enter the food chain and become concentrated in fish and other edible organisms (known as bioaccumulation), particularly in near-shore areas (His et al., 1999).

Many substances pollute the marine environment, but non-biodegradable compounds are the most dangerous due to their innate ability to constantly remain with the ecosystem (Hernandez-Hernandez et al., 1990; Tyler, 1972). Heavy metals are notable for

their high toxicity, as are organochlorine compounds such as pesticides and PCBs, amongst others. According to Van Vuuren et al. (1999), metal pollutants are currently considered to be some of the most toxic contaminants present world-wide. Furthermore, Carvalho et al. (1999) states that heavy metals are some of the most toxic, persistent, and widespread contaminants in estuarine systems in the sense that dissolved or suspended metals become available to plankton, nekton, and benthic filter and deposit feeders. The release of metal ions into a river system poses a serious threat to aquatic life and causes secondary effects upon the water quality. According to Robinson and Avenant-Oldewage (1997) the two factors which contribute principally to the damaging effect of metals as environmental pollutants are, firstly, their inadequate biological degradation to inert metals (as in the case of most organic pollutants), and secondly, the trend of metals to accumulate and largely remain in the aquatic environment. Some heavy metals in trace concentrations are normal constituents of marine organisms, but are potentially toxic at elevated concentrations (Ober et al., 1987). Metal pollution has become a major international issue since the 1960s when thousands of people were poisoned in Minamata, Japan, after consuming mercury –polluted fish (Fergusson, 1990).

Ober et al. (1987) and the WRC (1999) affirmed that pollution of the marine ecosystem by heavy metals is a world-wide problem, and the main sources of metal pollution are domestic/industrial sewage, industrial effluents, oil and chemical spills, combustion emissions, mining operations, metallurgical activities and non-hazardous landfill sites. According to Hernandez-Hernandez et al. (1990) the presence of metals in the marine environment is partly due to natural processes such as volcanic activity and erosion, but mostly results from industrial processes, with metals mainly

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entering the sea suspended in both liquid and solid industrial wastes, and in solid particles carried by winds, and eventually deposited in the sea.

Hernandez-Hernandez et al. (1990) stated that with regard to metal bioaccumulation in marine organisms, several authors have proved their high accumulation ability in crustaceans, molluscs and fish, which generally depends on their exposure time and the concentrations of metals in the water. According to Robinson and Avenant-Oldewage (1997), Hernandez-Hernandez et al. (1990), Avenant-Oldewage and Marx (2000), Carvalho et al. (1999) and Koli et al. (1977) several factors affect the toxicity of pollutants to aquatic organisms and can be divided into biotic factors such as physiological condition, tolerance, growth and reproduction, species variation, inter- and intra-specific variation in life history stages, adaptive capabilities and behavioural responses; and abiotic factors such as metal species in the water, the presence of other metals or pollutants, nature of dissolved organic matter, pH, temperature, alkalinity and hardness, metal interactions and dissolved oxygen and interactions between them all. Robinson and Avenant-Oldewage (1997) highlighted the fact that the effect of two or more toxicants may be additive, antagonistic or even synergistic. Heavy metals, such as Cu and Zn, are being mobilised by man at ten times the natural rates expected from geological weathering. Studies conducted in the urban environment in Sydney, Australia, for example, showed that Cu, Pb and Zn are among the main pollutants from urban runoff (Stark, 1998).

Binning and Baird (2001) reported an increase in heavy metal concentrations in the sediments of the Swartkops River and its estuary since 1979. Of particular interest is the substantial increase of both Pb and Zn along the estuary over the assessment period of 1979 to 1999 by 270% and 96% respectively (mean value from six sites). Both Pb and Zn are known to be particularly toxic to most animals (Oehme 1978), and therefore further research into their effects on the ecology of the Swartkops Estuary due to their drastic increases will prove to be invaluable in the future when prevalent metal concentrations may escalate further. According to Binning & Baird (2001), it is estimated that approximately 1 million people presently live and work in the Swartkops River catchment which not only contains the greatest part of the metropolitan population, but is also an area where the greatest diversity of urban users is found and where urban growth is most rapid. Binning & Baird (2001) maintained that the river is subject to the pressures and impacts of these rapidly expanding urban areas, and due to the social and economic importance of the Swartkops River the effects of heavy metal pollution on the biota is of particular interest and concern. Pb has been identified as a pollutant of particular importance due to its relatively high toxicity to humans, especially causing brain retardation in children (Sadiq, 1992). Harte et al. (1991) reported a dramatic three to five orders of magnitude increase in the degree of Pb contamination since prehistoric times which they attributed to extensive mining ( $>3 \times 10^6 \text{ t} \cdot \text{a}^{-1}$ ) and its extensive use by industry. Sadiq (1992) reported that the combustion of oil and gasoline alone accounts for 50% of all anthropogenic emissions of Pb and thus constitutes a major component of the global cycle of Pb.

Pb enters the aquatic environment through erosion and leaching from the soil, Pb-dust fallout from the atmosphere, combustion of petrol, domestic and industrial waste discharges, runoff of fallout deposits from roads and other surfaces, as well as in precipitation (DWAF 1996; Harte et al. 1991). Pb appears ubiquitous in aquatic ecosystems and bioaccumulates in aquatic organisms (Moriarty, 1990). Furthermore, studies both *in vitro* and *in vivo* showed that Pb concentrations as low as  $0.2 \text{ mg} \cdot \text{L}^{-1}$  adversely affect aquatic biota (Sadiq, 1992). In natural water the total Pb concentrations generally

range between  $0.05$  and  $10.0 \text{ mg} \cdot \text{L}^{-1}$  (DWAF 1996).

Pb occurs in the environment in a wide range of physical and chemical forms that greatly influence its behaviour and its effects on the ecosystem. Most of the Pb in the environment is in the inorganic form and exists in several oxidation states (0, I, II and IV). According to Nussey et al. (2000), Pb (II) is the most stable ionic species present in the environment, and is thought to be the form in which most Pb is bioaccumulated by aquatic organisms. In addition, Pb is also present in the organic form, such as alkyl Pb from auto emissions. Seymore et al. (1995) reported that Pb appears to be metabolised via the Ca metabolic pathways and therefore accumulates in the skeletal tissues. However, Pb is also known to accumulate in the tissues of fish, including skeletal bones, gills, kidneys, liver and scales (Nussey et al., 2000). Seymore et al. (1995) stated that the uptake of aqueous  $\text{Pb}^{2+}$  across the gills into the blood stream is the primary mode of uptake in freshwater fish. According to Nussey et al. (2000), the toxicity of Pb is dependent upon the life stage of the fish, pH and hardness of the water, in addition to the presence of organic materials. Seymore et al. (1995) stated that as the pH of the water decreases, the ionic state of the metal becomes more prevalent and toxicity increases acutely.

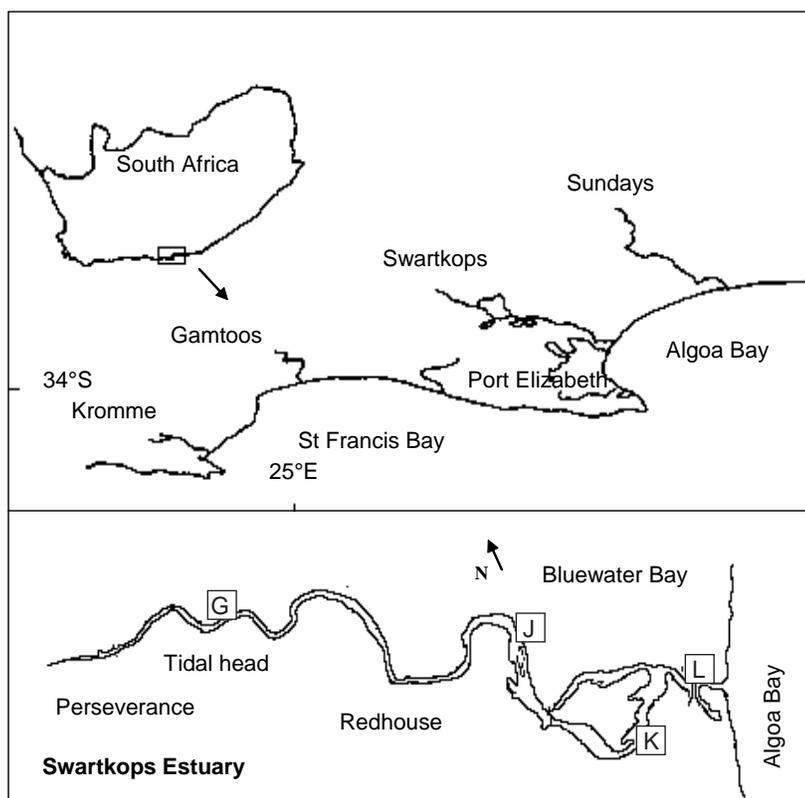
Due to the fact that Zn is a naturally occurring trace element actually required for human and animal health, more research has been focused on Zn deficiency rather than Zn overload (Harte et al., 1991). According to Wilber (1969) lethal concentrations of Zn compounds to aquatic organisms, as reported in the literature, show great variation as a result of different experimental designs, analytical methodology, and times of exposure. Harte et al. (1991) cited reports of tens of thousands of fish that have been killed from Zn pollution. Although not many studies on metal toxicity have included Zn, it still remains to a large extent more toxic than Pb (His et al., 1999; MacDonald et al., 1988). The mechanism of toxic action of Zn is essentially unknown, but it is speculated that it causes direct damage to the gills of fish and thus inhibits respiratory function (Wilber 1969). Wilber (1969) also stated that fish can detect Zn solutions as low as  $10 \text{ mg} \cdot \text{L}^{-1}$  and that after exposure to even lower concentrations of Zn, fish bioaccumulate the metal in their gills, gut, and liver. The effect of heavy metals and other toxicants on various animal taxa and life stages of them have been extensively reported in the literature (MacDonald et al., 1988; McKenney and Neff, 1979; Ober et al., 1987; Odendaal and Reinecke, 1998; Stark 1998).

The objectives of this study were to determine whether the presence of varying concentrations of Pb and Zn since these are respectively escalating within the Swartkops River sediment (Binning and Baird, 2001) in combination with various salinities could have a significant impact on brood and larval development in the sand prawn, *Callinassa kraussi* (Crustacea). We tested the following hypotheses:

- Increasing concentrations of the metals Pb and Zn (singularly and combined) cause significant increases in the mortality in both brood and larvae of *C. kraussi*;
- Increasing Pb and Zn concentrations (singularly and combined) in permutation with varying salinities exhibit a significant negative influence on brood and larval development of *C. kraussi*;
- The synergistic effect of Pb and Zn result in significantly higher mortalities on brood and larvae of *C. kraussi* than these metals on their own.

This euryhaline organism was chosen because of the widespread global occurrence and abundance of the genus in tropical and temperate waters. *C. kraussi* occurs in South African estuaries, has commercial value as a popular bait organism for recreational fishers,

**Figure 1**  
Map of Swartkops River estuary, Port Elizabeth



has two larval phases which remain in the burrow (i.e. no planktonic phase), and its important role in the food web of the estuarine ecosystem (Baird and Ulanowicz 1993). *C. kraussi* is regarded as an important bioturbator owing to its deep burrows, abundance, and vast impact on sediments by increasing oxygenation and mineralisation (Vorsatz, 2000).

## Methods and materials

### Collection and handling of ovigerous *Callianassa kraussi*

Ovigerous *C. kraussi* females were collected from the Swartkops Estuary (32°52' S 25°38'E) near Port Elizabeth, Algoa Bay, South Africa, for laboratory experiments.

Ovigerous females carrying embryos in an advanced state of development (detected by distinguishing eye-spots visible) were collected with the aid of a prawn pump from the sand banks, adjacent to the estuary mouth (Site "L" in Fig. 1). This was identified as an area less impacted by prevailing metal concentrations according to Binning (1999). The ovigerous females were transported to the laboratory in large sealed plastic buckets containing 15 l of water from the collection locality. They were transported to the laboratory within 30 min, where the ovigerous females were transferred to glass jars containing a culture medium made up of 1 l of 0.5 µ filtered UV irradiated natural seawater preset at a temperature (20°C) and salinity (35 mg/l) similar to those measured in the field. These holding jars were placed within water baths (0.5°C accuracy) and housed within environmentally controlled rooms to maintain a temperature of 20°C. The ovigerous females were left for 12 h to acclimate to these conditions before experiments proceeded. Experiments were then carried out at a photoperiod of 12 h light: 12 h darkness.

Larvae hatched in the holding jars approximately 3 to 4 d after

collection. After hatching, the larvae were transferred within 12 h to 50 ml plastic vials (single larva per vial), containing the respective culture medium at which hatching occurred (as described below in the experimental procedure). Furthermore, these vials were exposed to the same environmental conditions used in brood development described above (i.e. 20°C, 12 h light: 12 h darkness).

## Experimental procedures

An experiment was firstly performed to assay the effects of 3 metal solutions, namely the influence of Pb only, to Zn only, and to a combination of Pb and Zn on embryo survivorship and hatching. Brood development was monitored over a period of 96 h (4 d) to record all [potential] hatchings. A second experiment was then executed to determine larval survival by using embryos not previously exposed to metals. These larvae were exposed to Pb only, to Zn only, and to a combination of Pb and Zn (refer to Table 1 for respective concentrations) at a constant temperature of 20°C. The larval development phase was monitored over a period of 7 d to ensure larvae had developed through all larval stages.

The respective experiments for brood and larval development were performed for each of three metal analyses namely Pb only, Zn only, and a combination of Pb and Zn, at varying salinities (20, 24, 30 and 35 mg·l<sup>-1</sup>) and at a constant temperature of 20°C. The experimental design used in this study was a 4 x 7 factorial with salinities of 20, 24, 30 and 35 mg·l<sup>-1</sup>, and seven respective metal concentrations for each of the three analyses, each including a zero metal concentration serving as a control. These concentrations are given in Table 1. The respective metal solutions were prepared every third day from primary stock solutions of 1 g Pb and Zn per litre respectively.

The respective experimental metal concentrations (given in Table 1) were decided upon, firstly, a comprehensive literature survey to establish the influences and respective concentrations of

<b>TABLE 1</b> <b>Respective metal concentrations used in the analyses of brood and larval development at constant temperature (20°C) and varying salinities (20, 24, 30 &amp; 35 mg·L<sup>-1</sup>) (* each concentration of both Pb and Zn used equally in combination)</b>							
<b>Metal</b>	<b>Metal concentrations (in mg·L<sup>-1</sup>)</b>						
<b>Brood development</b>							
Pb	0	0.125	0.250	0.500	1.00	2.00	4.00
Zn	0	0.010	0.025	0.050	0.100	0.250	0.500
Pb and Zn *	0	0.010	0.025	0.050	0.100	0.200	0.400
<b>Larval development</b>							
Pb	0	0.250	0.500	1.00	1.50	3.00	4.50
Zn	0	0.025	0.050	0.100	0.250	0.500	0.750
Pb and Zn *	0	0.010	0.025	0.050	0.100	0.200	0.400

Pb and/or Zn on other related species (crustaceans) (MacDonald et al., 1988; McKenney and Neff, 1979; Ober et al. *date?*, Odendaal and Reinecke 1998), and secondly, test experimentation using the methodologies and concentrations, gleaned from the literature, for each respective metal solution on eggs and larvae of *C. kraussi*. Results obtained from these trial runs were only used to establish the analytical procedures, and were not included in the final results or ensuing statistical analysis.

The respective salinities were chosen within the range normally prevailing at or near the collection site in the Swartkops River estuary (Binning & Baird, 2001). Ovigerous females used in the testing were acclimated to the appropriate salinity combination by adjusting the salinity of the culture medium at a reduction rate of 1 every 4 h. Dilutions were obtained by the addition of distilled water to the culture medium. Experiments were conducted for all salinities at one metal concentration at a time. All experiments were performed at a constant temperature of 20°C comparable to field temperatures at the time of collection.

### Impact of Pb and Zn on brood development

After the collection and acclimation of the ovigerous females, the number of females required for each respective metal analysis equated to 112 (7 concentrations x 4 salinities x 4 replicates). These animals were removed from the holding jars and placed in 300 mL glass bowls filled with a culture medium that was adjusted to the correct salinities and metal concentrations, placed in water baths. Finely crushed commercial fish flakes were used as food for the brooding females. Careful consideration was taken to monitor the water balance (pH & salinity) to ensure the food did not interfere with these parameters or water quality. If variations in the water balance occurred, they were corrected to those of the original culture medium. The culture medium within the bowls was renewed every second day and care was taken not to stress the ovigerous females and disturb the broods. Overlapping dark covers were placed over the bowls to prevent evaporation and to reduce light conditions in the bowl to mimic that of the prawn burrows. The brooding females were observed daily for hatching and the number of dead/discarded eggs (noted by their opaque appearance) were noted. After a period of 4 d the total number of egg mortalities was calculated as a percentage of the total number of eggs, determined at the end of each run.

### Impact of Pb and Zn on larval development

After the collection of the newly hatched larvae, the total number of individuals required for each respective metal analysis equated to 1120 (10 individuals x 7 concentrations x 4 salinities x 4 replicates). The culture medium in the vials containing the larvae were adjusted to the correct salinities and metal concentrations, and placed in the water baths. No food was made available to the larvae. The larvae were placed in new vials every day containing fresh culture medium, and care was taken not to injure the vulnerable larvae. Dark covers were placed covering the vials to reduce light conditions.

Larvae were examined daily under a dissecting microscope to determine the presence of a heartbeat. Microscopic analysis continued until the larvae had died or the experiment had been concluded. Larvae were considered to be dead when no heartbeat was observed and/or they appeared opaque. After a period of 7 d the total number of larval mortalities was calculated as a percentage of the total number of larvae (10) used in each run.

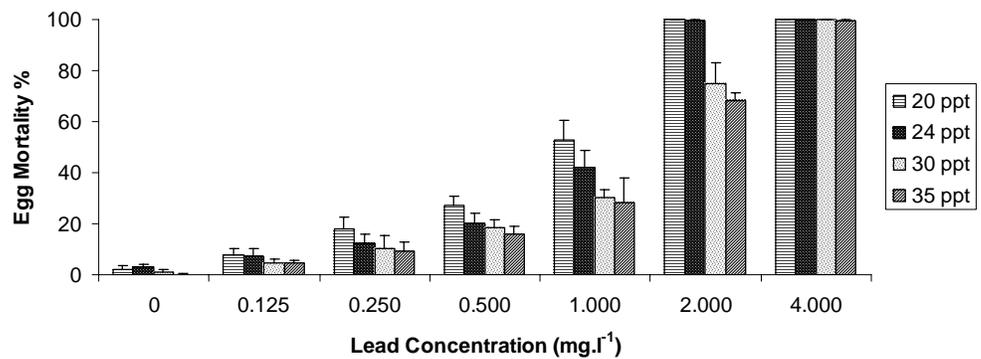
### Statistical analysis

Egg and larval mortality was calculated as percentages of total eggs/larvae used in the respective experiments. Egg mortality was defined as eggs that were discarded from females and/or appeared opaque. Larval mortality was defined as those individuals appearing opaque and exhibiting no heartbeat. Mortality data were analysed for both eggs and larvae over three variables for significant differences in survival as a result of salinity and concentration treatments. These included: differences amongst all metal concentrations, differences amongst salinities within a single metal concentration, and differences amongst salinities at all metal concentrations

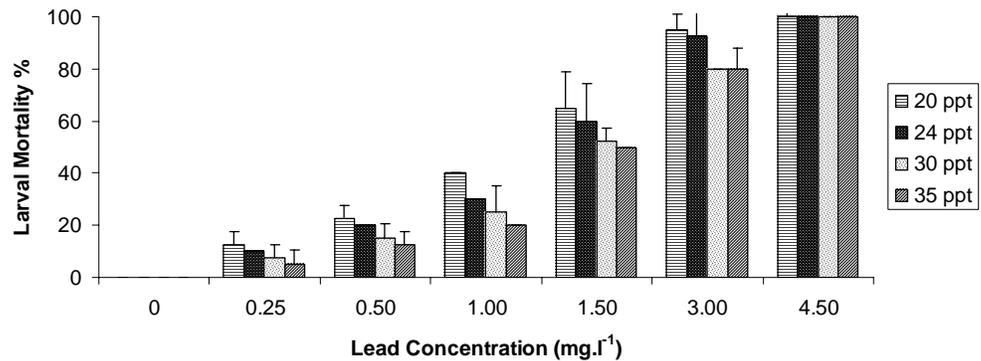
The statistical analysis for the egg and larval mortality firstly employed the use of  $F_{\max}$  (homogeneity of variance) test to check that all four sample variances were similar to each other. If this test produced a positive result, an ANOVA (Analysis of Variance) test was used in calculating significant differences ( $p < 0.05$ ) between these aforementioned groups. This specific test was used due to its robust nature, yet capable of yielding a certain amount of flexibility in comparing more than one set of means.

If the homogeneity of variance test ( $F_{\max}$ ) indicated that there were significant differences between the experimental data obtained, a Tukey HSD (honest significant difference) test was employed,

**Figure 2**  
Graphical representation of *Callianassa kraussi* egg mortality, (expressed as percentages) exhibiting deaths at respective concentrations of lead ( $\text{mg}\cdot\text{L}^{-1}$ ) and salinities (ppt) at a constant temperature of  $20^\circ\text{C}$ . Bars above columns indicate standard deviation (STD)



**Figure 3**  
Graphical representation of *Callianassa kraussi* larval mortality, (expressed as percentages) exhibiting deaths at respective concentrations of lead ( $\text{mg}\cdot\text{L}^{-1}$ ) and salinities ( $\text{mg}\cdot\text{L}^{-1}$ ) at a constant temperature of  $20^\circ\text{C}$ . Bars above columns indicate standard deviation (STD)



which is more sensitive for distinguishing significant differences (Fowler and Cohen 1990).

## Results

The experimental procedure was split into six components, namely the impact of:

- Pb on brood development of *C. kraussi* at various salinities
- Pb on larval development of *C. kraussi* at various salinities
- Zn on brood development of *C. kraussi* at various salinities
- Zn on larval development of *C. kraussi* at various salinities
- Pb and Zn (in combination at equal respective concentrations) on brood development of *C. kraussi* at various salinities
- Pb and Zn (in combination at equal respective concentrations) on larval development of *C. kraussi* at various salinities.

The tabulated data recorded from these analyses as well as the degree of variation between sample data are given by Jackson (2002). The data were used to construct mortality graphs (see Figs. 3.1 to 3.6) comprising the percentage mortality (number of deaths as a percentage of total eggs/larvae used) vs. the respective concentration for each metal solution used in the experimental component at respective salinities and at a constant temperature of  $20^\circ\text{C}$ .

## Statistical analysis

Most of the analyses (eggs and larvae at 3 different metal solutions, at 7 concentrations and 4 different salinities) exhibited distinct homogeneity of variance (i.e.  $F_{\max}$  passed), utilising all four samples between the groups. The only analyses that produced indistinct homogeneity of variance results utilising the  $F_{\max}$  test were in assessment of significant differences over all the salinities in a group. Further investigation into the specific troublesome data values illustrated that all were related to mortality at the lowest salinity (20) and specific results were discarded/ignored (for reasons explained below), namely the exposed controls for Zn and Pb and Zn and the

Pb concentration of 0.125. The remainder was then utilised to perform a Tukey HSD over the respective groups that subsequently produced distinct significant differences (all  $p < 0.05$ ).

The majority of the subsequent statistical determinations then proceeded utilising the ANOVA test on data of both egg and larval mortality (detailed results are given by Jackson, 2002). These results illustrated that significant differences existed (i.e.  $p < 0.05$ ):

- Over the whole range of Zn and Pb concentrations (see Table 1)
- Amongst salinities within a single concentration
- Amongst salinities of all concentrations.

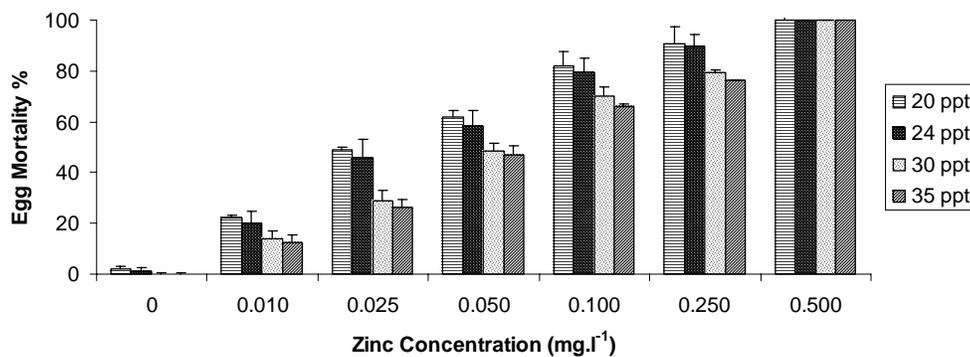
The results indicated that each experimental series yielded significantly unique sets of data that are graphically given in Figs. 2 to 7.

Other complications transpired due to the inability of the statistical software (SigmaStat 6.0) to process certain groups of data once again within the comparison of the salinities over all concentrations. Upon investigation it was realized that the occurrence of this problem was due to clusters of data in which all values were equal. This generally occurred in some instances at the lowest or highest concentrations in which all mortality percentages were equal (0 or 100%) and clearly no significant difference would then exist (i.e.  $p < 0.05$ ).

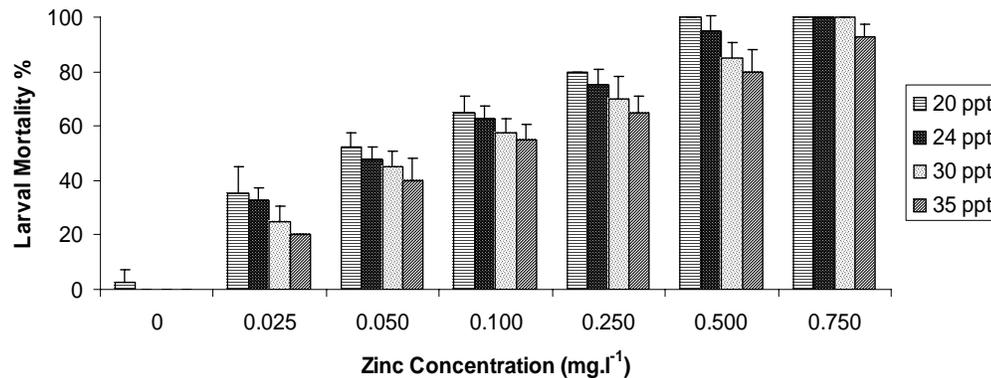
## Impact of Pb on brood development of *C. kraussi*

Figure 2 represents the percentage mortality of *C. kraussi* eggs at respective Pb concentrations and salinities at constant temperature.

Certain trends can be observed from Fig. 2. Firstly, in comparisons at the same respective salinities, egg mortality generally escalated as the Pb concentration increased to a concentration, which was clearly sufficient to induce 100% mortality over the whole range of salinities. Secondly, at most of the respective Pb concentrations, egg mortality was always higher at the two lower salinities of 20 and



**Figure 4**  
Graphical representation of *Callianassa kraussi* egg mortality, (expressed as percentages) exhibiting deaths at respective concentrations of zinc ( $\text{mg}\cdot\ell^{-1}$ ) and salinities (ppt) at a constant temperature of  $20^\circ\text{C}$ . Bars above columns indicate standard deviation (STD).



**Figure 5**  
Graphical representation of *Callianassa kraussi* larval mortality, (expressed as percentages) exhibiting deaths at respective concentrations of zinc ( $\text{mg}\cdot\ell^{-1}$ ) and salinities (ppt) at a constant temperature of  $20^\circ\text{C}$ . Bars above columns indicate standard deviation (STD).

24 than at the higher salinities of 30 and 35. This was evident at all concentrations, except where the  $[\text{Pb}] = 4.00 \text{ mg}\cdot\ell^{-1}$  at which the Pb solution was apparently too concentrated and all eggs expired (100% mortality).

#### Impact of Pb on larval development of *C. kraussi*

Figure 3 illustrates the percentage mortality of *C. kraussi* larvae at respective Pb concentrations and salinities at constant temperature, and shows that larval mortality generally escalated at increasing Pb concentrations. Secondly, at most of the respective Pb concentrations, larval mortality was always higher at the two lower salinities of 20 and 24 compared to the two higher salinities of 30 and 35. This was evident at all concentrations, except at  $[\text{Pb}] = 4.50 \text{ mg}\cdot\ell^{-1}$  at which the Pb solution was apparently too concentrated and all eggs expired (100% mortality) a concentration which was clearly sufficient to induce 100% mortality over the whole range of salinities.

#### Impact of Zn on brood development of *C. kraussi*

Figure 4 illustrates the percentage mortality of *C. kraussi* eggs at different Zn concentrations and salinities at constant temperature.

Figure 4 shows similar trends as observed for Pb in Fig.2 Firstly, in comparisons between the same respective salinities, egg mortality generally increased at higher Zn concentrations. Secondly, at most of the respective Zn concentrations, egg mortality was always higher at the two lower salinities of 20 and 24 compared to two higher salinities of 30 and 35. This was evident at all concentrations, except at a  $[\text{Zn}]$  of  $0.500 \text{ mg}\cdot\ell^{-1}$  at which the Zn solution was apparently too concentrated and 100% mortality was observed a concentration which was clearly sufficient to induce 100% mortality over the whole range of salinities.

#### Impact of Zn on larval development of *C. kraussi*

Figure 5 represents the percentage mortality of *C. kraussi* larvae at respective Zn concentrations and salinities at constant temperature, which illustrates that larval mortality increased with increasing Zn concentrations at all salinities, and that larval mortality was always higher at the lower salinities of 20 and 24 than at the higher salinities of 30 and 35 (units). This was evident at all Zn concentrations, except where the  $[\text{Zn}] = 0.750 \text{ mg}\cdot\ell^{-1}$  at which 100% egg mortality occurred, a concentration which was clearly sufficient to induce 100% mortality over the whole range of salinities.

#### Impact of Pb and Zn on brood development of *C. kraussi*

Figure 6 illustrates the percentage mortality of *C. kraussi* eggs at respective combinations of Pb and Zn concentrations and salinities at constant temperature.

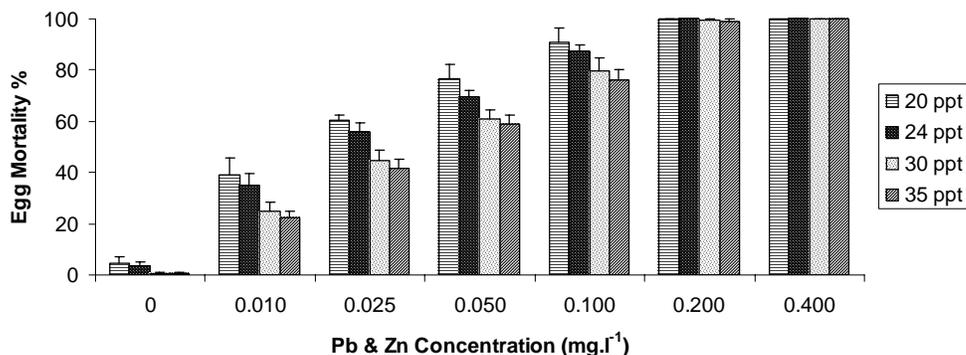
Figure 6 shows an increase in egg mortality at all salinities with increasing Pb and Zn concentrations, and that egg mortality was always higher at the two lower salinities of 20 and  $24 \mu\text{g}\cdot\ell^{-1}$  compared to salinities of 30 and 35 units. This was evident for all concentrations, except for  $[\text{Pb}]$  and  $[\text{Zn}] = 0.200 \text{ mg}\cdot\ell^{-1}$  and  $0.400 \text{ mg}\cdot\ell^{-1}$ , concentrations which were clearly sufficient to induce a 98% to 100% mortality.

#### Impact of Pb and Zn on larval development of *C. kraussi*

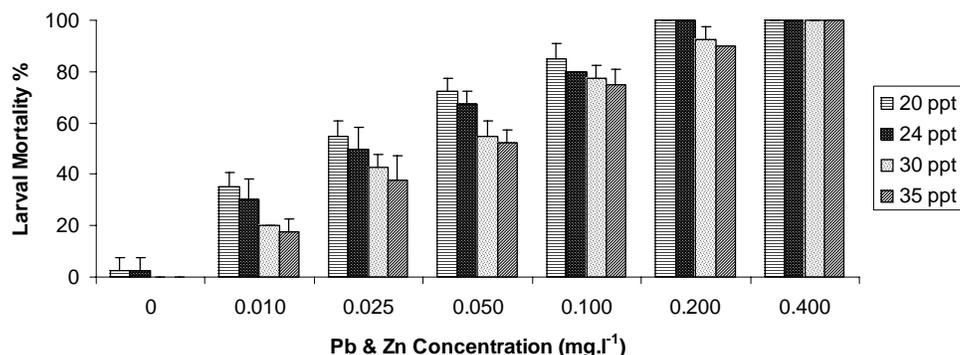
Figure 7 illustrates the percentage mortality of *C. kraussi* larvae at respective equal combinations of Pb and Zn concentrations and salinities at constant temperature.

It can be seen from Fig. 7 that larval mortality generally escalated with increasing Pb and Zn concentrations for all salinities, and that

**Figure 6**  
Graphical representation of *Callianassa kraussi* egg mortality, (expressed as percentages) exhibiting deaths at equal respective combined concentrations of lead & zinc ( $\text{mg}\cdot\text{L}^{-1}$ ) and salinities (ppt) at a constant temperature of  $20^\circ\text{C}$ . Bars above columns indicate standard deviation (STD).



**Figure 7**  
Graphical representation of *Callianassa kraussi* larval mortality, (expressed as percentages) exhibiting deaths at equal respective combined concentrations of lead & zinc ( $\text{mg}\cdot\text{L}^{-1}$ ) and salinities (ppt) at a constant temperature of  $20^\circ\text{C}$ . Bars above columns indicate standard deviation (STD).



Metal	Development stage	Salinity			
		20	24	30	35
Pb	Eggs	0.850	1.050	1.140	1.580
	Larvae	0.680	0.740	1.000	1.130
Zn	Eggs	0.031	0.035	0.054	0.066
	Larvae	0.047	0.049	0.075	0.087
Pb and Zn	Eggs	0.012	0.021	0.031	0.036
	Larvae	0.019	0.022	0.037	0.042

larval mortality was always higher at the two lower salinities of 20 and 24 compared to two higher salinities of 30 and 35 at most of the respective Pb and Zn concentrations. This was evident for all concentrations, except where the  $[\text{Pb}] = 0.400 \text{ mg}\cdot\text{L}^{-1}$  at which all eggs expired and except for  $[\text{Pb}]$  and  $[\text{Zn}] = 0.200 \text{ mg}\cdot\text{L}^{-1}$  and  $0.400 \text{ mg}\cdot\text{L}^{-1}$ , concentrations which were clearly sufficient to induce a 94% to 100% mortality.

#### LC 50 determination

From the data for egg and larval mortalities of *C. kraussi* mortality  $\text{LC}_{50}$  values, i.e. concentrations of respective metal concentrations at which 50% of the sample population died over a defined time period, were determined by Probit analyses and given in Table 2. The concentrations of the metal solutions at which 50% of the sample population died are given in the columns under the relevant salinity. The results show that 50% mortality occurs for all salinities at much lower concentrations of Zn, and of Zn and Pb combined with the lowest  $\text{LC}_{50}$  values calculated for Zn, followed by the Zn and Pb in combination, followed by that for Pb.

#### Discussion

Although the hypothesis that environmental variables exert a greater effect on certain life history stages was first put forward over 75 years ago (Vorsatz, 2000), it was only in the 1960s that studies incorporating this concept into bioassays mushroomed, and presently a large body of information exists on the influence of various environmental factors on ecosystems and their inhabitants (Oehme, 1978; Ravera, 1991). In particular, the dominant influence of varying salinities and temperatures on estuarine invertebrates has been clearly established by many authors (McKenney and Neff, 1979), and it is well documented that environmental parameters may not only individually modify the function, distribution and/or community structure of estuarine organisms, but that the interactions and synergism between certain environmental factors may jointly influence these biological properties.

Due to the nature of the experimental design, various results over different groupings of brood development (eggs and larvae) against salinities and metal concentrations may now validate the hypotheses stated for this investigation (given in the

**Introduction**), namely:

- Increasing concentrations of the metals Pb and Zn (singularly and combined) cause significant increases in the mortality in both brood and larvae of *C. kraussi*;
- Increasing Pb and Zn concentrations (singularly and combined) in permutation with varying salinities exhibit a significant negative influence on brood and larval development of *C. kraussi*.
- The synergistic effect of Pb and Zn result in significantly higher mortalities on brood and larvae of *C. kraussi* than these metals on their own.

The statistical analyses performed on the results mostly produced conclusive and significant results in terms of the mortality of eggs and larvae at various metal concentrations and salinities.

Robinson and Avenant-Oldewage (1997) stated that in metal toxicity bioassays the sample size should be large, but when employing so many dynamic variables (4 x 7 factorial) these assays can become increasingly complex and difficult to assess. Robust statistical analysis proved that significant distinction existed over all three levels of treatments, namely:

- Differences amongst over all ranges of concentrations
- Differences amongst salinities within a single concentration
- Differences amongst salinities at all concentrations.

These results indicated that each experimental series yielded significant results.

His et al. (1999) noted that the marine species used most often in assessments of pollutant toxicity and aquatic environmental quality include sea urchins (used since the late nineteenth century), rotifers, crustaceans (e.g. copepods, brine shrimps, mysids, barnacles) and bivalves. According to His et al. (1999) the life history of the species used in pollutant toxicity studies should exhibit the following characteristics:

- Year-round availability
- Ease of handling
- Sensitivity to contaminants
- Reliability and accuracy of evaluation of the response to the pollutant
- Relatively short exposure times
- Exhibit a wide range of salinity tolerance
- At relatively low cost.

The sand prawn, *C. kraussi*, firstly not only conforms to all of the conditions listed above, but also is a benthic species with a non-pelagic larval stage (Forbes, 1974). The latter characteristic is important for two reasons. Firstly, benthic organisms are more exposed to heavy metal contamination since it mainly accrues within the sediment (heavy metals have a tendency to adsorb to sediment particles) and their concentrations in marine sediments may be several orders of magnitude higher than in the overlying water column (Livett, 1988). Secondly, the association between pollutants and sediments can be of a very long duration and it may have critical deleterious effects on organisms living on and within the bottom sediments (His et al., 1999). Consequently, the embryos of *C. kraussi* are exposed to the prevailing benthic conditions, whereas the planktonic larvae escape to the water column, a more variable and pollutant-diluted environment. Therefore embryos may be exposed over a long period of time to high concentrations of metals found in sediments (MacDonald et al., 1988), and this obviously

poses a much higher risk of metal toxicity compared to species exhibiting a life cycle that includes pelagic planktonic phases.

The results of this study confirm the hypotheses that at zero metal concentration the control exposure displayed very little to no mortality, whereas increasing concentrations of metals accelerate the mortality of *C. kraussi* in both the brood and larval stages. This occurred in all six experiments (Pb, Zn and Pb and Zn for both brood and larval development). Results of experiments in which Pb and Zn were equally mixed to various concentrations, demonstrated that in combination the metals are increasingly toxic to both the eggs and larvae of *C. kraussi*. This verifies the statement of Robinson and Avenant-Oldewage (1997) that metal ions are important factors of water pollution either individually or in combination, with the resultant effect of two or more toxicants being additive, antagonistic or even synergistic. Since the increase in toxicity of the two metals in combination compared to individual effects are significantly different (see Table 2), it is proposed that these two metals produce synergistic toxic distress to estuarine biota. These observations support the statements of Binning (1999) and Binning and Baird (2001) that since the estuarine sediments and adjoining marshes serve as reservoirs for heavy metals, their increase in the Swartkops River is definitely a cause for concern as these metals may adversely affect the survival of eggs and larvae of various estuarine biota.

Many studies expressed the opinion that early developmental stages are thought to represent a sensitive period in the complex life cycle of marine invertebrates (His et al., 1999; MacDonald et al., 1988; McKenney and Neff 1979; Ober et al., 1987). The effect of heavy metal contamination may well represent acutely lethal consequences for the developmental stages of estuarine biota. The comparison of the two developmental stages of *C. kraussi* (eggs and larvae) exposed to increasing metal concentrations indicated that the mortality values are significantly higher for earlier developmental stages (eggs) than of those of the hatched larvae, under the conditions of this experiment (see Figs. 2 to 7). This trend was evident at three of the metal solutions used, which means that *C. kraussi* larvae may survive in environments with elevated metal concentrations *in situ*, than the *C. kraussi* eggs which tend to expire at lower contamination levels. (It must be noted though that this experiment was *in situ* on laboratory-based experiments using natural sea-water with only three parameters involved namely Pb, Zn and salinity. In nature both the eggs and larvae are exposed to many other potentially hazardous contaminants)

Fifty per cent mortalities of eggs and larvae were obtained at different metal concentrations and salinities. The mortality of eggs and larvae occurred at higher concentrations of Pb than for Zn and Pb-Zn combined, while the toxicity of all three concentrations of metals increased at lower salinities. His et al. (1999) and Gintenreiter et al. (1993) stated that in the comparison of the toxicities of heavy metals Pb and Zn, the latter is significantly more toxic than the former (i.e. Zn >> Pb), with LC<sub>50</sub> values of Zn frequently 4 times less than those of Pb. The comparison of the LC<sub>50</sub> values for Pb and Zn in this study (see Table 2) showed that Zn is indeed considerably more toxic, with 50% mortality occurring at Zn concentrations more than 10 times less (for eggs and larvae) relative to the those of Pb. However, the synergistic mortality effect of Pb and Zn is clearly evident from the results presented in Table 2, which shows that 50% mortality is inflicted on eggs and larvae at lower concentrations than for Zn and Pb separately. It would also appear that the sensitivity of the life stages (such as eggs and larvae) of typical estuarine organisms that reproduce in an estuary becomes greater as the salinity decreases. This implies that both the quality and quantity of inflowing freshwater could have a significant **effect?** on the survival rates of sensitive life stages of estuarine invertebrates.

Long, MacDonald, Smith and Calder (1995) reported on the percentage incidence of biological effects in concentration ranges (defined between the effects range-low, ERL, and effects range-medium, ERM) were determined for various heavy metals, including Pb and Zn. According to Long et al. (1995), metal concentrations equal to and above the ERL value represent a possible-effects range within which effects would occasionally occur, whereas concentrations equal to and above the ERM value represent a possible-effects range within which effects would frequently occur. Long et al. (1995) reported these respective values for Pb to be between 46.7 (ERL) to 218 (ERM) mg·ℓ<sup>-1</sup> and Zn between 150 (ERL) to 410 (ERM) mg·ℓ<sup>-1</sup>. According to Binning and Baird (2001), the mean respective levels of Pb and Zn present in the sediments of the Swartkops River estuary are: Pb 32.9 (SE<sub>x</sub> 10.5) mg·ℓ<sup>-1</sup> and Zn 35.9 (SE<sub>x</sub> 10.1) mg·ℓ<sup>-1</sup> with highest concentrations of both metals occurring at sites G, J, and K [see Fig. 1]. The present levels of Pb and Zn in the Swartkops River estuary sediment are both less than the ERL guideline concentrations where possible adverse effects on the sediment-associated biota can be expected. However, immobilised Pb and Zn concentrations in the sediments of the Swartkops River estuary have increased substantially by 270% and 95% respectively over the past 20 years (Binning and Baird, 2001), and further increases could trigger biological effects within the Swartkops Estuary.

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