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A site-specific tropical sediment toxicity test using *Chironomus crassiforceps* to investigate metal bioavailability in acid-sulphate sediments

Thesis submitted to
the University of Stirling
for the degree of
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July 2000

Mika Robert Peck

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DECLARATION

This thesis has been composed in its entirety by the candidate, and no part of this work has been submitted for any other degree

Candidate:

M.R. Peck

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Glossary of Acronyms

AAS – Atomic Absorption Spectroscopy

ANOVA – Analysis of Variance

ANZECC - Australian and New Zealand Environment and Conservation Council

AVS – Acid volatile sulphide

BEC - Bounded Effect Concentration

CEC – Cation Exchange Capacity ($c_+ molkg^{-1}$)

DOM – Dissolved Organic Matter

DTPA – Diethelyenetriaminepentacetic acid

EC_x - Test concentration of material to which test organisms are exposed that is estimated to effect x% of the test organisms (time and response dependent)

USEPA – United States Environmental Protection Agency

GS – Gauging Station

HC – Hazardous Concentration

HDPE – High Density Polyethylene

HPLC – High Performance Liquid Chromatography

ICP – MS – Inductively Coupled Plasma Mass Spectrometry

IWTU – Interstitial Water Toxicity Unit

IWCTU – Interstitial Water Criteria Toxicity Units

LC_x – Test concentration of material in water to which test organisms are exposed that is estimated to be lethal to x% of the test organisms (time-dependent)

LTRE – Life Table Response Experiment

LOEC – Lowest Observed Effect Concentration

NOEC – No Observed Effect Concentration

OECD – Organisation for Economic Co-operation and Development

PEC – Predicted Environmental Concentration

PNEC – Predicted No-Effect Concentration

SEM – Simultaneously extracted metals

TEA – Triethanolamine

ABSTRACT

In the wet-dry tropics of northern Australia physico-chemical factors and differing sensitivities of local tropical species may result in generic sediment metal toxicity guideline values, generally generated using temperate species under temperate conditions, being under-protective. To address this a site-specific sediment toxicity test was developed using a chironomid, *Chironomus crassiforceps*, identified from the Alligator Rivers Region. *Chironomus crassiforceps*, was found to have a similar acute sensitivity to copper as other chironomids used in toxicity testing. A four-day sublethal growth test was subsequently developed and found to be more discriminatory than emergence and demographic endpoints.

Risk to benthos of the Magela Plain is posed by potential deposition of metals from Ranger Uranium Mine within the Magela Creek catchment. On the floodplain acid-sulphate soils produce acidic pulses following re-inundation, and it was of interest to determine whether the resulting pH change altered metal bioavailability. Spiked sediment toxicity tests with copper and uranium demonstrated reduced toxicity at the lower sediment pH of 4 compared to sediment at pH 6. However, lowering pH increased porewater metal concentrations and overlying water concentrations in the laboratory bioassays.

Water quality criteria (WQC), adjusted for porewater hardness, have been applied to porewater metal concentrations to assess the toxicity of sediments. This thesis highlights the potential need to adjust the WQC for sediment pH. The lowering of pH can also release sediment bound metals to overlying water – posing a risk to water quality.

The need to understand and consider the effect of pH on toxicity in sediment risk assessment is highlighted by this thesis as is the need to take into consideration site-

specific environmental factors before the application of generic risk assessment methods.

1.1 General Introduction

Heavy metals have long been a subject of environmental concern and research interest in the context of the aquatic environment and their impact on aquatic ecosystems (Rand et al., 1995). While research has concentrated on the toxicity of metals in the aqueous phase the toxicity of sediments has received less attention. Historically, water quality guidelines have been used to interpret the discharge of metals to receiving waters overlooking the fact that sediments can concentrate metal contaminants to levels that can impair their function (Moriarty, 1983). At present, although generating a large amount of research interest, risk assessment of sediments is biased to environments and species from Europe and North America (Baird et al., 1995). Tropical species may differ markedly from these standard test organisms in their response to metals in sediments (Catallo, 1993). In addition, tropical sediments and tropical environments can be very different from temperate sediments and the standardised laboratory conditions currently used to generate sediment toxicity data.

In highly seasonal tropical wetland ecosystems environmental conditions can vary greatly, from a sedimentary system during the wet season to a soil system over the dry season. In regions of acid-sulphate sediments this fluctuation from anoxic to oxic conditions can result in the generation of acidity through the oxidation of sulphides (Dent, 1986). At present there are no data with which to predict bioavailability of in-place sedimentary metal pollutants to tropical benthic species under the influence of sediment-generated acidity.

This study has been developed in response to the lack of sediment-based tropical site-specific ecotoxicological data in the Alligator Rivers Region within the wet-dry tropics of northern Australia and the need to identify potential impacts from uranium mining

activity within its catchment. The main aims of the research were to develop a sediment toxicity assessment method using a local species and to determine whether acidity generated by acid-sulphate sediments underlying the Magela Plain had an effect on the bioavailability of potential metal contaminants such as uranium and copper.

This chapter gives an introduction to the wastes associated with mining activity and the sediment characteristics that play a major role in controlling the bioavailability of metals (Section 1.2). The effect of pH on toxicity of metals is reviewed (Section 1.3) followed by a summary of current risk assessment procedures (Section 1.4) with a focus on sediment toxicity testing (1.5). Site-specific issues of relevance to the Alligator Rivers Region of Northern Australia are outlined (1.6) and finally the aims of the thesis are defined (Section 1.7).

1.2 Metals and sediments

1.2.1 Mining activity and contaminants

Mining activity is a common source of metal pollution to aquatic systems. Water is the principal vector in transporting wastes from mine sites to local waterways and can be classified into three main groups (Noller, 1995):

- Mildly alkaline waters, often associated with pit dewatering, generally derived from groundwater sources that can contain contaminants from processes such as cyanide leaching or bauxite dissolution.
- Near neutral waters arising from surface runoff, which tend to contain higher levels of suspended solids.
- Acidic waters arising from acid mine drainage processes. Mine water acidity is often generated by the oxidation of mineral sulphides to give sulphuric acid. The acidic

solution dissolves other minerals and tends to become extremely metal rich. A common route of contamination is when metal sulphides in waste rock and mine tailings become exposed to air, generating acidity and dissolving metals in runoff (Moss, 1988)

The management of water in the mining context is essential to minimise ecological impacts. In wet-dry tropical regions where rain falls intensely over a short period of time the management of water takes on a critical importance (Noller, 1995). If a poor water management practice is in place downstream changes in water and sediment quality may be observed. Changes in sediment quality may or may not result in ecological impairment depending upon whether or not metals in sediment are in a bioavailable form. A recent review of mining impacts emphasised that there is at present a general lack of information regarding the bioavailability of metals in sediments (AQUAMIN, 1996).

1.2.2 Metals in the aquatic system

Once dissolved, metals tend to adsorb to particles or become entrained into organic and inorganic colloids. Since most biological surfaces and particles in natural waters carry a net negative charge, attributed to a layer of adsorbed humic material (Beckett, 1990; Beckett and Ngoc, 1990; Day et al., 1994), they attract positively charged metal ions. The sorption of metals to inorganic and organic suspended particulate matter and the settling of these particles (Sigg, 1985) plays an important role in the removal of metals from the water column (Hesslein et al., 1980). Physical transfer of metals in particulate or occluded form within the water column, or as bed-load during high flows, also transports potential contaminants to zones of deposition.

Subsequent chemical, mineralogical and physical changes after and during deposition (diagenesis) influence the fate of these deposited metals and can influence their uptake

by aquatic organisms. Examples of diagenetic processes involving chemical alteration include the formation of sulphides, iron and manganese oxyhydroxides and the decomposition of organic matter. In addition to diagenetic processes, benthic aquatic organisms can play an important role in influencing the fate of metals in sediments by working and reworking the sediment. This can change the depth of the oxic/anoxic border altering the form and affinity of sediment-metal binding phases (Boudou and Ribeyre, 1989).

1.2.3 Sediments – physico-chemical environment

Sediments influence the exchange of chemicals between the dissolved, particulate and biological phases. Sediment can be considered as a heterogeneous mixture of particles derived from rock, soil, biological sources and anthropogenic material. It consists of a wide range of particle sizes as defined by the size fractions of gravel, sand, silt and clay. The major inorganic components include clays, quartz, feldspars, various silicate minerals, oxyhydroxides and carbonates (Ferguson, 1990). The organic component of sediments results from microbial and chemical transformation of organic debris (Talibudeen, 1981). It is the surface chemistry of sediments that plays the major role in controlling the fate of pollutants.

Within sediments the major phases of metals are exchange sites, manganese oxides, iron oxides or hydroxy-oxides, organic matter, sulphides, carbonates and mineral lattices.

The exchange sites are governed by surface charge characteristics that depend on the mineralogical make-up of the sediment (Stumm, 1992). Ion exchange is the mechanism whereby one adsorbed ion is readily exchanged by another (Sposito, 1989). For metals, a measure of the exchangeable sites is the cation exchange capacity and is defined as the excess of counter ions (positively charged ions) that can be exchanged with the contact

solution for other cations in the diffuse layer of the double layer. The cation exchange capacity of a sediment reflects its mineralogy and solute composition (Stumm, 1992).

On oxidation at the oxic/anoxic boundary in sediment (or the water column in stratified lentic systems), iron and manganese oxides can precipitate forming small particles with very high surface areas. These particulates form complexes with, and have a strong affinity for, heavy metals. They play a significant role in trace metal uptake in aqueous systems even if present in low concentrations (Stumm, 1992). Under reducing conditions iron and manganese oxides go into solution, releasing metals previously incorporated in their structure.

Sedimentary organic matter, due to the nature of its origin, is extremely heterogeneous. It is generally divided into non-humic substances and humic substances that are further divided into fulvic acid, humic acid and humins. Organic matter also has a strong affinity for heavy metal cations (Jones and Jarvis, 1981).

Sulphide (S^{2-}) forms under reducing conditions and binds strongly to divalent metals, precipitating out of solution to form the solid (MS, where M is a divalent metal). The role of sulphides is discussed in more detail below.

It is the interaction of these trace metal sinks with interstitial water (porewater), ligands, physicochemical conditions and biota that determine the fate and biological effect of metals in sediment.

Highly weathered tropical soils and sediments of Northern Australia do not contain carbonate but do contain an abundance of sesquioxides – i.e. the aluminium and iron oxyhydroxides. Consequently they have an ability to sequester metals, particularly so if redox change promotes the formation of ferrihydrite and colloidal gibbsite to produce a high surface area with a high specificity for metal sorption. However, the lack of

carbonate means that there tends to be little buffering capacity to these sediments meaning that sediment properties can come under the influence of pH.

1.2.4 Sediment – biota interactions

The typical aquatic invertebrate can be considered to take up bioavailable heavy metals from solution and food. Metal uptake from solution can usually be explained by a passive facilitated diffusion process although some metals can be absorbed actively by transport pumps (Phillips, 1995). When the concentration gradient increases in favour of sorbed forms metals tend to remain within the invertebrate due to their high affinity for organic molecules via bonds to sulphur and nitrogen. If the metal remains in a metabolic form it can become toxic if it interferes with metabolic functioning. The point at which metals become toxic is that at which regulation breaks down (Rainbow, 1996).

Environmental factors and species-specific biological interactions with the contaminated environment determine the bioavailability. Luoma (1983) gives a detailed review of biological factors and concludes that '...the physiological characteristics of the environmental interface of organisms determine the metal forms of highest bioavailability.' Bioavailability is a prime consideration in sediment ecotoxicology since it has been found that dry weight metal concentration in sediment is a poor predictor of toxic effect (Ankley et al., 1996c; Borgmann et al., 1998). Within sediments, research has shown that toxicity can vary by up to two orders of magnitude between sediments for the same concentration of total metal. So, even when concentrations in sediments substantially exceed the background levels, metal bioavailability can be minimal and adverse impacts may not be observed (Ankley et al., 1996a).

Metals within contaminated sediment can interact with biota directly or indirectly. The direct route involves metal sorption onto food particles and subsequent ingestion and

has been emphasised as a potential exposure pathway for metal contaminants for fish and invertebrates (Dallinger et al., 1987; Hatakeyama, 1988; Munger and Hare, 1997; Taylor et al., 1998). The dietary uptake of contaminants is affected by a number of factors and includes physico-chemical conditions such as pH, hardness, the presence of complexing agents and metal speciation. Biological factors such as the metal assimilation efficiency of organisms, feeding behaviour and habitat preference also play vital roles (Hare, 1992).

Behavioural responses to the detection and avoidance of contaminated sediments have been reported for invertebrates by a number of authors (Wentsel et al., 1977; Kielty et al., 1988; West and Ankley, 1998). However, a number of sediment ingesting chironomid species tested by Hare and Schooner (1995) were found to be unable to detect moderate contamination by cadmium.

The indirect route involves partitioning to the aqueous phase (porewater) followed by uptake. The use of porewater metal concentrations as a measure of sediment toxicity has been the focus of much research.

1.2.5 Porewater and bioavailability

After a United States Environmental Protection Agency (USEPA) workshop in 1984 highlighted the fact that interstitial water concentrations determined the toxicity of the organochlorine pesticide kepone (Battelle, 1984) research was stimulated to find similar relationships for metals. Porewater metal concentration has been found to be a good predictor of toxic effect (Di Toro et al., 1990; Di Toro et al., 1991; Ankley et al., 1993a; Casas and Crecelius, 1994; Pesch et al., 1995; Besser et al., 1996). Total concentration of metals in interstitial water is controlled by sorption and precipitation-dissolution reactions with the solid phase. Major phases for metal uptake vary depending on whether the sediment is under oxic or anoxic conditions.

Under oxic conditions, sorption reactions onto sedimentary organic matter, iron-oxyhydroxides, and manganese-oxyhydroxides explain the take-up of metals and the subsequent equilibrium between the solid phase and the aqueous phase.

Under anoxic conditions precipitation-dissolution reactions involving amorphous sulphides have been implicated as the major phase controlling interstitial water concentrations and hence toxicity of metals to biota (Di Toro et al., 1990). Upon flooding, soils can quickly become anoxic as a consequence of the oxygen diffusion rate into soil becoming severely impeded and the consumption of oxygen by microorganisms. It has been shown that gas exchange in the saturated soil profile is slower than aerated soils by a factor of 300,000. After 1 to 3 days carbon dioxide composes most of the gas phase of the system with a transition zone of 1 to 3 cm depth at the surface where oxygen diffusion exceeds oxygen consumption (McKee and McKevlin, 1993).

Facultative and anaerobic microorganisms use a variety of substances as terminal electron acceptors for respiration. The reactions generally proceed in the following order, dependent upon pH and the lability of the mineral phase. After, or when, oxygen is nearly depleted, nitrate (NO_3^-) is reduced followed by Mn (IV). The ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}), sulphate (SO_4^{2-}) is then reduced to sulphide (S^{2-}). Sulphide reacts rapidly with cationic metals to precipitate out, most commonly as FeS (Hammer, 1989).

Divalent metals, such as Ni^{2+} which form sulphides that have a lower solubility product than FeS ($K_{sp}=10^{-19}$) can displace iron from its sulphide (Phillips and Kraus, 1965) and precipitate it from the interstitial water making the metal non-bioavailable.

Di Toro et al. (1990) theorised that since there is a 1:1 ratio in divalent metal to sulphide reactions ($M^{2+} + S^{2-} \Rightarrow MS$, where M is a divalent metal) metals will only become toxic

when no more sulphide is present to precipitate them from solution. Consequently, the molar ratio [SEM]/[AVS] has been defined where acid-volatile sulphide (AVS) is that fraction of sulphide extracted by weak cold hydrochloric acid (Wieder et al., 1985) and is represented primarily by amorphous iron monosulphides (FeS). SEM stands for 'simultaneously extracted metal' and is the concentration of metal in the acid used to determine the AVS concentration. It was suggested that only at [SEM]/[AVS]>1 would toxic effects due to metal presence in pore waters be exhibited (Di Toro et al., 1990; Di Toro et al., 1991).

[SEM]/[AVS] ratios have successfully predicted the absence of acute toxicity for Cd, Ni, Pb, Zn and Cu (Di Torro et al., 1990; Di Toro et al., 1991; Ankley et al., 1993a; Casas and Crecelius, 1994; Pesch et al., 1995; Besser et al., 1996; Berry et al., 1996; DeWitt et al., 1996). However it has been less successful in predicting toxic effects at ratios > 1 possibly due to the presence of other metal binding phases, such as organic matter or hydrous oxides of iron or manganese (Ankley et al., 1993a, Besser et al., 1996; Rule and Alden, 1996a, Rule and Alden, 1996b). Pesch et al. (1995) proposed a two-step complementary approach to predicting acute metal toxicity: AVS normalisation (as above) and evaluation of interstitial water metal concentration. By dividing the interstitial water metal concentration by known LC_{50} values (Equation 1.1) the interstitial water metal concentration can be expressed as interstitial water toxicity units (IWTU).

$$IWTU = [M_d]/LC_{50} \qquad [1.1]$$

where $[M_d]$ is the dissolved metal concentration in interstitial water.

Interstitial water measurements are needed for some metals (such as As, Cr, Ag and U) which are not bound by AVS. Berry et al. (1996) found that sediments with <0.5 IWTU

were seldom acutely toxic (3%) while sediments with >0.5 IWTU were toxic 94.4% of the time.

Since AVS can bind divalent metals on a molar equivalent basis, and other metals in proportion to their molar concentrations, Hansen et al. (1996) suggest expressing the concentrations of metals in sediments on a molar SEM - AVS (mol/g dry weight) basis as opposed to the [SEM]/[AVS] ratio. A value of SEM - AVS >0 (equivalent to [SEM]/[AVS] >1) shows that the sulphide binding phase is exhausted, yet Hansen et al. (1996) noted that tested field sediments could contain 1.0 to 1000 μmol of metal over that bound by sulphide but still remain non-toxic. This suggests that other binding phases, such as organic matter and oxy-hydroxides, play a significant role (Karsten et al., 1996). A paper by Mahony et al. (1996) correlated the binding of metals in excess of the AVS concentration to normalised sediment organic carbon and noted that the adsorption fitted a Langmuir curve suggesting a surface complexation system that is pH dependent.

1.2.6 Environmental variability and metals in sediments

Besser et al. (1996) concluded that freshwater sediments might act as sites of immobilisation of metals or sources of metals to pore water, overlying water and biota depending on seasonal cycles of AVS formation and oxidation. On a spatial scale the heterogeneous environment that makes up freshwater sediments can be a patchwork of oxic and anoxic areas (Di Toro et al., 1996). Temporal variability, such as fluctuations in oxic and anoxic conditions, results in dynamic phase partitioning in freshwater sediments. The effect of this has not been fully tested and would be expected to be significant in systems where fluctuations in oxic and anoxic conditions would affect the presence of the sulphide fraction. Work on tidal River Elbe sediments (Forstner, 1990) showed that seasonal and diurnal water level fluctuations on intertidal mudflats

concentrated cadmium in the anoxic zone (>20cm depth) with the regular influx of oxygenated waters causing release of bound cadmium and transfer to biota. This process of 'oxidative pumping' has been little studied and could play an important role in containing and releasing potential contaminants in fluctuating systems such as wetland ecosystems.

1.2.7 Acid sulphate sediments

An even more dramatic phenomenon associated with the inundation and drying of wetlands occurs in regions of acid sulphate soils/sediments. These sediments occur throughout the world from Holland to Sierra Leone, Vietnam, Indonesia and Australia (Dent, 1986). The total area of soils that are or may become acidic has been estimated at 12-14 million hectares (Dent and Pons, 1993).

Under anaerobic conditions, bacteria break down organic matter using energy from the reduction of sulphate ions and iron II oxides. The stable endpoint of this process is pyrite (FeS_2). Mangrove areas provide conditions for the formation of pyrite, with plentiful organic matter and sulphur from seawater. If these sediments become oxidised, by a lowering of the water table for instance, then acidity is generated as shown in equation 1.2.

$$FeS_2 \text{ (Pyrite)} + 15/4\ O_2 + 7/2\ H_2O \rightarrow Fe(OH)_3 + 2\ SO_4^{2-} + 4H^+ \qquad [1.2]$$

In soils with high levels of pyrite, drainage and subsequent inundation can reduce the pH to values less than 2 (McKee and McKevlin, 1993). This pH change affects the speciation and binding of metals in these poorly buffered sediments.

The implication is that bioavailability of metals may change dramatically with environmental conditions.

1.3 pH and toxicity

Biological surfaces are capable of binding metal ions, and it is thought that the concentration of metal at the biological surface is the first step in biological uptake from the aqueous phase (Morel, 1983). As the pH value decreases, hydrogen ions compete with metal ions for these surface-binding sites reducing the biological uptake of metals (Stary and Kratzer, 1982; Gerhardt, 1992; Witters, 1998). The lowered pH may also cause conformational changes in surface binding sites, reducing their affinity for metal ions, or cause a decrease in cell membrane potential. Whichever mechanism is involved it is clear that a decrease in pH causes a progressive decrease in adsorption (Campbell and Stoakes, 1985). To counter this effect metal toxicity may be seen to increase if the metal under investigation brought into solution or undergoes speciation changes to more toxic forms as a result of decrease in pH.

1.3.1 The effect of pH on metal toxicity in sediments

The pH effect on the toxicity of metals to organisms is strongly dependent on the particular organism and the environmental system. The effect of pH on the bioavailability of metal in sediment can come about in two main ways:

- Speciation changes (Buffle and De Vitre, 1994) and,
- Hydrogen ion competition (Crist et al., 1996).

The physico-chemical form of a metal in aquatic environments is termed its speciation. Species of metals include free hydrated ions, complexes with organic and inorganic ligands and species bound to colloids and particulates (Buffle and De Vitre, 1994). The speciation of a metal is dependent on concentration, pH, water hardness, other water quality characteristics and available ligands. The species of a metal plays a vital role in determining its affinity for the variety of ligands and surfaces available for sorption

within sediments and for surfaces determining biological uptake. Changes in pH can result in speciation and binding changes, which alter the bioavailability of metals within sediments thus influencing metal toxicity.

A decrease in pH implies an increase in hydrogen ions (protons) which can compete with metal cations for surface binding sites – thus an increase in hydrogen ions can result in increased porewater concentrations of metals as they out-compete them for sediment surface binding sites (Mahony et al., 1996).

To gain some quantitative insight into potential pH effects within oxic sediment, in which the toxic effect of a metal is assumed to occur through the liquid phase, the following is considered. For this analysis bioavailability will be defined along the lines of Morel (1983) as the mass of metal that can be sorbed by an organism in a given environment and it is assumed that the liquid phase is the route for all metal interaction.

To include pH in this simplified model, metal ion binding is considered as an equilibrium reaction with hydrogen ions by a site S_i on a sediment component (equations 1.3 and 1.4).

$$S_iH_x + M^{2+} \Leftrightarrow SiM^{2-x} + xH^+ \qquad [1.3]$$

$$K_i = \frac{(S_iM^{2-x})(H^+)^x}{(S_iH_x)(M^{2+})} \qquad [1.4]$$

K_i is the exchange constant where x represents the metal-proton exchange stoichiometry at the site and determines the pH dependence (Plette et al., 1999). (M^{2+}) represents free metal ion concentration in the solution. The character of the binding can be simplified and described by a two-component Langmuir-Freundlich equation (Temminghoff et al., 1994; Temminghoff et al., 1997). At low metal ion concentrations the equation can be simplified to the extended Freundlich equation:

$$Q_m = K(M^{2+})^n(H^+)^{-nx} \qquad [1.5]$$

Where Q_m is the amount of metal bound reversibly to the exchange sites, and K is a composite constant:

$$K = S_{total}(\tilde{K})^n \qquad [1.6]$$

Where S_{total} is the amount of sites available for metal binding; $\tilde{K}$ is the median exchange constant and n describes the width of the distribution of exchange constants. As K is a conditional constant the equation is valid only for the conditions under which it is defined.

Each component can be described by a separate extended Freundlich equation:

$$Q_{M\text{ - sediment}} = Ks(M^{2+})^{a_s}(H^+)^{b_s} \qquad [1.7]$$

$$Q_{M\text{ - biota}} = K_b(M^{2+})^{a_b}(H^+)^{b_b} \qquad [1.8]$$

$$Q_{M\text{-total}} = Q_{M\text{-sediment}} + Q_{M\text{-biota}} + Q_{M\text{-solution}} \qquad [1.9]$$

At typical concentrations, copper can be considered to be 99% bound to sediment (Plette et al., 1999) and the assumption is made that:

$$Q_{M\text{-total}} \approx Q_{M\text{-sediment}} \qquad [1.10]$$

Now considering free metal ion concentration in equilibrium with sediment solid phase and combining equations 1.7, 1.8 and 1.10:

$$Q_{M\text{ - biota}} = K_bK_s^{-a_b/a_s}(Q_{M\text{ - total}})^{a_b/a_s}(H^+)^{b_b - b_sa_b/a_s} \qquad [1.11]$$

K_b and the exponents a_b and b_b are biota specific, K_s and exponents a_s and b_s are soil specific, thus sediment specific and biota specific parameters will define the exponents. The values of which parameters will determine toxicity effects in relation to pH change. If the exponent above hydrogen ion activity (b_b-b_sa_b/a_s) is positive, increased metal ion binding to biota occurs with increasing pH as biological sites out-compete soil for metals. The exponent above the total metal concentration (a_b/a_s) determines the shape of the sorption isotherm. Values of a, b and K have been determined for simple systems and highlight potential toxic scenarios.

In the case of maize (considering soil as a saturated sediment), less copper binds to root cell walls as pH increases, due to soil ligands out-competing the roots for copper, and thus toxicity increases with decreasing pH. On the contrary, for yeast in the same soil/sediment system increased toxicity is observed with increasing pH. The effect of pH on toxicity is determined by the proton exponent (b_b-$b_s a_b/a_s$) and is both environment and biota specific (Plette et al., 1999).

In a complex system the effect and magnitude of bioavailability change will be a function of the metal, the sediment type and the particular mechanism of uptake by the organism. For the cations copper, cadmium, lead, nickel and zinc increasing pH leads to an increase in negatively charged adsorption sites with subsequent increased electrostatic attraction of positively charged ions (Hassan et al., 1996).

The strength of binding sites available on sediment will determine metal released to porewater for a unit drop in pH and this will vary with sediment composition. Metal specific characteristics will also affect adsorption; for example lead undergoes speciation changes at circumneutral pHs resulting in the formation of Metal-$(OH)^+$ species that compete less effectively for binding sites as they are larger and have a lower charge (Hassan et al., 1996). Finally the mechanism of uptake by the organism will ultimately determine the toxic effect.

1.4 Risk assessment

Having identified metal contamination as a potential risk it is important to evaluate the level of risk. Standard laboratory tests or literature data are currently used to estimate risk to ecosystems. A problem lies in the fact that the information has generally been gained in the Northern Hemisphere using temperate species, under temperate conditions. The applicability of standard test data, and data from the literature to

tropical freshwater ecosystems requires reviewing and, ideally, field verification (Catallo, 1993; Lemly, 1996; Widinarko and Van Straalen, 1997).

Risks to tropical ecosystems influenced by acid-sulphate sediments could potentially be miscalculated unless site-specific considerations and tropical species are used to generate the toxicological data used by risk assessment models (Everts, 1997). A site-specific approach involves characterising potential sources of risk and site-specific ecosystem characteristics likely to increase, or decrease, risk. It is important to understand the effects of pH change in a sedimentary system as present sediment toxicity tests, with their emphasis on circumneutral temperate sediments, form the basis of risk assessment models for both temperate and tropical systems.

The complex nature of sediment ecotoxicology means that risk assessment is still in its early stages.

1.4.1 Risk assessment: present status

1.4.2 Sediment quality criteria

A simple and common method for characterising risk is the use of quotients. These are the ratios of exposure and effects concentrations using the susceptibility to the most sensitive species with highest environmental concentrations. The predicted environmental concentration (PEC) is compared to the predicted no effect concentration (PNEC). A PEC/PNEC ratio greater than unity implies a potential risk. A variety of methods, for instance peepers (Bufflap and Allen, 1995), can be employed to measure or estimate predicted effect concentrations (PEC). Predicted no effect concentrations (PNEC) often take the form of sediment or water quality values. A number of concepts have been advanced for the development of sediment metal quality criteria: analytical

chemistry approaches, toxicity testing, and field survey data. A fourth integrative approach incorporates all three (Power et al., 1991; Jones et al. 1997).

The acid-volatile sulphide and the equilibrium-partitioning methods are examples of the analytical-chemistry approaches that aim to estimate porewater metal concentrations. The application of water quality criteria to estimated sediment porewater concentrations of single pollutants is then used to assess risk.

Toxicity testing methods follow methodology used for setting water quality criteria with organisms exposed to contaminated field sediments or spiked background sediments in the laboratory to establish dose-response relationships. (Chapman, 1989).

An example of a field survey method is the screening level concentration approach that uses field survey data to estimate the highest concentration of a particular contaminant in sediment that can be tolerated by approximately 95% of benthic infauna (Neff et al., 1988).

Combined methods include techniques that use field chemical data and either laboratory toxicity testing or field survey data. (EPA, 1996b).

Finally integrated approaches, such as the Sediment Quality Triad (Chapman, 1992) include results from sediment chemistry, bioaccumulation studies, toxicity testing and *in situ* observations to determine whether a particular sediment is hazardous and identifies the toxic components.

Recent work by Wildhaber and Schmitt (1998) suggests that toxicity units, obtained by dividing porewater concentrations by water quality standards, can predict the effect on community composition suggesting that two parts of the sediment triad could be predicted from chemical data. It is interesting to review the methods used to obtain the water quality standards, as this is the value used in the denominator (i.e. PNEC) in determining porewater toxic units.

1.4.3 Current approaches to setting water quality criteria

At present there are two principle approaches to determining water quality guidelines from data. The assessment factor method is the older method and involves dividing the lowest toxicity value obtained through testing by an assessment factor. This assessment factor is based on the quality and number of toxicity data. The objective is to protect all species from lifetime exposures to contaminants. The second approach, more recently proposed, involves statistical extrapolation methods. Using this method toxicity data from all species available are fitted to a particular distribution. The concentration level that protects a specified percentage of the population can then be estimated.

1.4.3.1 Safety factor methods

The lowest reported toxicity value is divided by a constant called an assessment, uncertainty, application or safety factor. This factor depends on the data available in such a way that the more sensitive the data the smaller the safety factor. Today the safety factor method is generally only used when insufficient data exists for statistical extrapolation methods. The strengths of the safety factor method include that it is simple to use, easily understood and that the safety factors can be easily modified. A weakness is that safety factors are purely empirical with no ecological or mechanistic base (Warne, 1998).

1.4.3.2 Statistical Extrapolation methods

Over the last decade alternative methods to safety factors have been developed. These methods fit single species toxicity NOEC (No Observed Effect Concentration) data to a statistical distribution to give a concentration that should protect a defined percentage of species in the environment. One such method being considered for the Australian and New Zealand Environment and Conservation Council (ANZECC) water quality guidelines is the approach set out by Aldenberg and Slob (1993). This method uses

NOEC data fitted to statistical distributions to determine the hazardous concentration (HC) to (normally) 5% of the population. The strengths of this method include the fact that it is a transparent method meaning that the level of protection and the uncertainty can be determined, and it uses toxicity data from single species tests that are the most commonly available data. Weaknesses of the method include the fact that NOEC data are used in the distribution and there is debate as to the validity of such data, and that it is more complex than the safety factor method to understand (Warne, 1998). Forbes and Forbes (1995) severely criticised the use of statistical distribution based methods by clearly showing that the underlying assumptions of the methodology have not been validated. One such assumption is that natural ecosystems have species sensitivity described by a theoretical distribution such as the log-logistic (Aldenburg and Slob, 1993). There has been no direct evidence to support this, and a fundamental point is that the term 'ecosystem' is not well defined. There is for example no explicit inclusion of the abiotic component in this definition.

A fundamental criticism of both methods is their over-reliance on single-species laboratory toxicity data, and the fact that both consider aquatic communities to be made up of non-interacting species. At the present state of knowledge it is impossible to model the competitive and symbiotic relationships between individuals and species that make up the community let alone the relationships between them and environmental factors and the toxicant effects on each of the species (Neuhold, 1986). Thus it could be argued that the development of more complicated risk assessment methods, such as the statistical distribution method, avoids addressing key fundamental ecological questions. Forbes and Forbes (1995) summed up the current state of risk assessment by stating that 'Extrapolation beyond the end of a fitted line is a bad idea when employing regression models and an equally bad idea for ecotoxicological models'.

The use of these water quality criteria, whichever method is used to determine them, to define sediment quality through comparison to porewater contaminant concentrations has merit. However there are potential weaknesses in the methodology. Benthic organisms can increase their exposure to contaminants though dermal adsorption and ingestion of sedimentary particles or decrease their exposure through interaction with overlying water, for instance by ventilating their burrows (Landrum and Robbins, 1990). Thus the correlation between toxic response and porewater concentration may be weakened.

1.4.4 Risk assessment alternatives

The following criteria have been put forward as reasonable requirements for research programs into assessing ecological effects from toxicity tests:

- The predictions should be focused on a particular habitat or community and be based on field studies of that community; i.e. a site-specific approach is required (Baird et al., 1995).
- Mortalities among key species should be predicted (Forbes and Forbes, 1995)
- Local environmental conditions such as seasonal phenomena should be incorporated into the risk assessment (Baird et al., 1995)

Calow et al. (1997) suggest a risk assessment method based on life history types. This method involves determining effects on population multiplication rate (lambda, λ) through toxicant effects on survival, time to reproduction and fecundity. The population growth rates are then associated with life history types and the risk of extinction to species within the community are estimated. The advantage over current risk assessment methods is that the method is grounded in ecological theory and testable hypotheses are generated in the concentration – lambda curve. In addition, it does not assume a single sensitivity distribution (i.e. log-logistic) for all communities and, in fact, the sensitivity

of a habitat is expected to vary with the distribution of life-history types. Finally, a basic understanding of the ecosystem under study is required, i.e. a species list. A weakness of the system is that it again assumes no species interactions. However, since it is based on a species list there exists the possibility for inclusion of interactions between species.

The data requirements for this type of risk assessment require a more standardised test system as data on the effects of contaminants on survival, time to reproduction and fecundity are required. Demographic parameters such as lambda (λ) mean that both lethal and sublethal effects are integrated in a single variable (Stark et al., 1997a; Walthall and Stark, 1997).

Forbes and Forbes (1995) propose a risk assessment framework for a marine sedimentary system that couples abiotic and biotic components within a modelling framework. Their interesting analysis focuses around the key role of bioturbation (the mixing of sediments by benthos) within sediments. It was suggested that the rate of bioturbation acts as an important measure of benthic community integrity providing a vital link between the biological and abiotic compartments.

More work is required to clearly formalise a risk assessment method that incorporates the ‘eco’ in ecotoxicology. However a combination of Calow et al.’s (1997) life-history method and Forbes and Forbes (1995) modelling method is potentially the way forward.

1.4.5 ANZECC sediment quality criteria guidelines.

The complexity of the sedimentary system has led to a tiered approach to sediment risk assessment being adopted in Australia. Total contaminant concentration is measured in sediment and if this value exceeds the lowest guideline value then the first step is to assess the normal background sediment concentrations. If these are exceeded then the bioavailable fraction is determined. At this point care must be taken to include any local site-specific factors that can have significant effects on bioavailability. If the guideline

values are still exceeded then acute and chronic toxicity testing are recommended (ANZECC, 1999).

1.5 Sediment quality assessment

1.5.1 Bioassays

The exposure of an organism or biotic system to a stimulus is known as a bioassay and includes both toxicity tests (which measure an effect) and bioaccumulation tests (that measure the body burden of the pollutant of interest) (Chapman, 1995b). To date, in ecotoxicology, survival, growth and reproduction endpoints have direct relevance for ecosystems and should be given more weight than other endpoints. Other endpoints such as behavioural endpoints and suborganismal effects (biomarkers such as mixed-function oxidases and metallothioneins) need further work to link them to ecologically relevant effects (Forbes and Forbes, 1995).

In sediment bioassays there are four major exposure routes, whole sediment, elutriate, interstitial or porewater and chemical extracts. Tests can be split into three generations of whole organism tests. First generation tests are water column tests adapted for sediments. Second generation tests are specifically designed for sediment testing, primarily measuring survival. Third generation tests are second generation tests that also measure chronic responses such as growth and/or fecundity. It is generally considered that third generation tests provide the most useful information (Chapman, 1995b) as the effect measured includes porewater exposure and dietary uptake of contaminants.

Within the laboratory, single species tests or multi-species tests can be used to measure response to contaminated systems. However, multi-species tests have come in for criticism with Moriarty (1983) remarking that ‘microcosms do appear to have the worst

of both worlds; they are too complicated to give results that are easily interpreted, but too artificial to be of immediate relevance to field situations'. A complaint often raised against microcosm experiments is that significant treatment effects cannot often be detected because there is a low 'signal-to-noise' ratio in system response (Forbes and Forbes, 1995). Some studies (e.g. Pratt et al., 1989) have suggested microcosms are less sensitive to pollutants than expected based on single-species tests.

However, single-species tests have also been criticised in risk assessment for measuring only 'the degree to which a pollutant prevents an organism from reaching its biological potential of life span and reproduction, if all other conditions approach the optimal' (Crow and Taub, 1979). Also, no single species is the most sensitive to all toxicants, and the probability of selecting the most sensitive species for testing a particular chemical is rather small (Forbes and Forbes, 1995). In addition, single species tests will always miss interactions between species (Lampert et al., 1989).

In spite of criticisms single species testing is currently the favoured methodology in use or development for sediment risk assessment. Freshwater organisms currently used in third generation laboratory toxicity tests with solid phase sediments include the oligocheate *Lumbriculus variegatus*, the amphipod *Hyalella azteca* and the midges *Chironomus riparius* and *C. tentans*. The tests typically measure survival and growth over a 10-day period (Ankley et al., 1993b). Chronic effects can have long term effects on population growth rates; for example Sibley et al. (1997) demonstrated that changes in growth, positive or negative, of *Chironomus tentans* can be used to make meaningful ecological predictions regarding population dynamics and reproduction.

1.5.2 Chironomids in sediment toxicity testing

Chironomids have been shown to be the major species involved in floodplain colonisation (McLachlan, 1974a; McLachlan, 1974b; Maher and Carpenter, 1984;

Batzer and Wissinger 1996; Gunn, 1997) and constitute a relevant and representative group of test organisms routinely used in sediment toxicity testing elsewhere.

1.5.2.1 Chironomids

Chironomids belong to the order Diptera (flies, midges and mosquitoes). This is the largest insect order with nearly 8000 species in Australia alone (Cranston, 1995). Adult dipterans are distinguished from other insects by having only one pair of functional wings, the other pair having diminished to a pair of knobs called halteres. As chironomids belong to the Endopterygota (or Holometabola) their lifecycle includes eggs, larvae, pupae and adult with the adult phase differing dramatically from the larvae. The larval stage usually consists of four instars. Instars are defined as the stages in development of an insect between two ecdyses (moults of the exoskeleton). Wings, legs and mouthparts of the adult develop in the larvae as internal imaginal discs and appear externally only in the pupal phase. The chironomid then emerges as the adult (imago). The egg, larval and pupal stages are normally aquatic, with mating and oviposition occurring in the adult phase (Figure 1.1). A distinguishing characteristic of the Chironomidae is the short duration of the pupal and adult phases compared with the egg and larval stage (Cranston, 1995). The larval stage is, for most chironomids, spent in tubes in the sediment from which they feed. It is their benthic existence that makes chironomids suitable for sediment toxicity testing. The major requirement for an organism ideal for sediment toxicity testing is one that feeds on and/or in the sediment in addition to living in sediment.

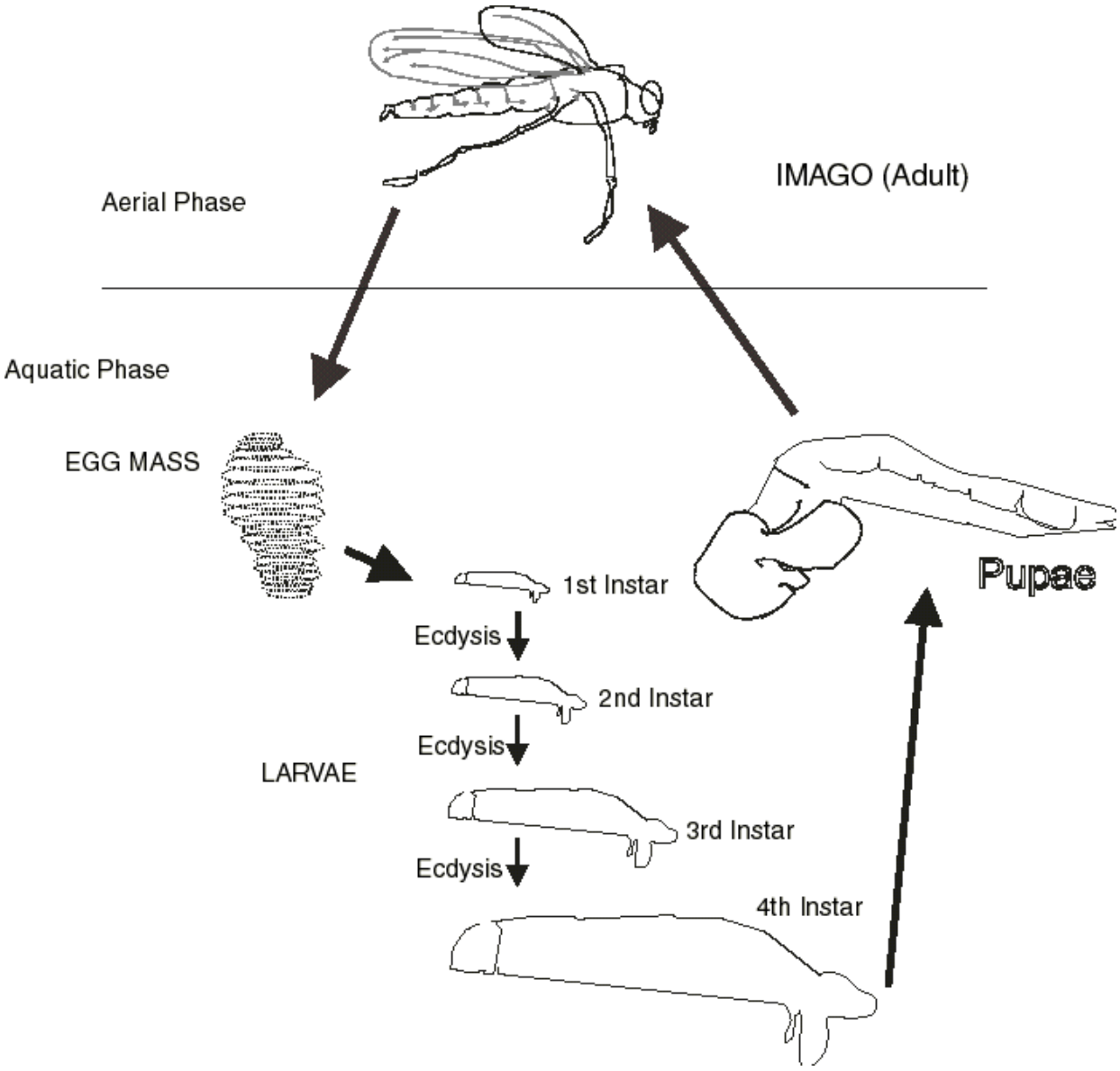


Figure 1.1. Life cycle of a typical chironomid

1.5.3 Chironomid toxicity tests

Advantages of using chironomids in sediment toxicity testing include the fact that chironomids generally:

- Remain in contact with the sediment;
- Tolerate a range of sediment physicochemical properties;
- Allow inter-laboratory comparisons of toxicity responses;
- Have relatively sensitive life stages;
- Are easy to handle and culture in the laboratory;
- Give a high probability of obtaining a valid test with relatively sensitive and accurate endpoints (Ankley et al., 1994; Giesy and Hoke, 1990; Ingersoll et al., 1995).

Also, the use of chironomids as test organisms is widespread with two laboratory standard species being *Chironomus tentans* (USA) and *Chironomus riparius* (Europe). Protocols and standards have been developed, the US Environmental Protection Agency having recently published standard guidelines for determining the acute toxicity, chronic toxicity and bioaccumulation of contaminated sediments to *Chironomus tentans* (EPA, 1996a). The OECD is also developing guidelines for the use of *Chironomus riparius* in sediment toxicity testing (OECD, 1998).

The data generated from a sediment toxicity test can be analysed in a number of ways, discussed below.

1.5.4 Quantitative measures from toxicity testing

Data on survival and sublethal effects can be treated in a number of ways. The predominant measures of survival are the median lethal concentration (LC_{50}) and the median effect concentration (EC_{50}). They are normally determined using the probit

method (Finney, 1971) or Spearman-Karber methods (Hamilton et al., 1977). These endpoints give relatively high values and are considered inappropriate as a measure to protect the environment.

A statistical method was introduced to measure low and no toxic effects using hypothesis testing to compare a control to treatment responses. With rejection of the null hypothesis multiple comparison testing (e.g. Dunnets, Newman-Keuls etc) is carried out to determine the no observed effect concentration (NOEC) and the lowest observed effect concentrations (LOEC). The use of LOECs and NOECs to measure a real 'no effect' concentration have been criticised (Bruce and Versteeg, 1992; Hoekstra and Van Ewijk, 1993a; Suter, 1996; Chapman et al, 1996) for a number of reasons including:

- The values of LOECs and NOECS are extremely dependent on experimental design, for example the value for the LOEC is inversely related to number of replicates. Hence, as experimental replicates increase, lower LOECs can be detected. As a result a poorly designed experiment with widely spaced concentration replicates will tend to give a higher LOEC value (Stephan and Rogers, 1985).
- The typical test has no set value for β. This means that the test may have a high probability of making a type II error, i.e. stating that there is no toxicity present when in fact there is.
- Risks derived from scenarios of varying severity cannot be estimated, as most of the information from the concentration response experiments is lost.

Most guidelines for toxicity testing have an experimental design favouring data collection for hypothesis testing (i.e. large number of replicates to few treatments), yet, as noted above, the odds favour the 'absence of toxic effect' hypothesis. Hoekstra and Van Ewijk (1993a, 1993b) proposed using a bounded effect concentration that involves

linear extrapolation from a concentration whose confidence intervals lie below a 25% effect, at most. The advantage is that the better the experimental design the lower the BEC (Bounded Effect Concentration) value obtained.

Moore and Caux (1997) proposed an alternative to hypothesis testing. They proposed the use of regression methods to interpolate or extrapolate to effect levels of interest, for instance EC_{10}, the concentration where a 10% effect is observed. The fitting of a model (e.g. probit, logistic) to concentration-response data addresses a number of the pitfalls associated with hypothesis methods by: using all the experimental information; attaining confidence intervals; and having the ability to reject poor data by using test statistics to determine model fits to data.

The regression-based approach also has its drawbacks. For example, most experimental design guidelines are based on obtaining data for hypothesis testing with high numbers of replicates per treatment. Changes in experimental design would be required to include more treatments and potentially fewer replicates to attain a better fit. Another criticism levelled at regression methods is that the choice of model fitted to the data is not necessarily based on a real mechanism of effect. The choice of regression model gives model-specific values for low effect levels, i.e. $<EC_{10}$, so care should be taken when extrapolating beyond the data (Caux and Moore, 1997).

Results from the methods outlined above are then used in risk assessment. Generally, a mix of sublethal and lethal data is used in risk assessment. However, a method exists which combines lethal and sublethal endpoints into one parameter. Data on survival, fecundity and time to reproduction can be quantified against pollutant concentration then integrated into effects on a population growth rate parameter (Caswell, 1989).

The choice of an optimal toxicity assay depends on several considerations, including study objectives and site characteristics (Burton, 1991).

1.6 Site-specific issues: wetlands in the wet-dry tropics

The wet-dry tropics cover a land mass of approximately a quarter of that defined as tropical and have been defined as areas with an annual rainfall of 600-1600 mm spread over 4 to 7 months (Finlayson, 1995). Northern Australia falls within the wet-dry tropics (Figure 1.2). The Magela Creek catchment is located in the wet/dry tropics within the borders of Kakadu National Park in Northern Australia (Figures 1.3 and 1.4). The Park was designated a site of World Heritage significance by the Australian Federal Government in 1979 owing to the unique natural diversity and cultural history occurring in this largely pristine area. The vegetation of the region is dominated by savannah and eucalypt woodland with large areas of natural wetland which are subjected to annual inundation.

The climate of tropical Australia can be classified into two seasons, the wet and dry season, although the local indigenous population recognises six distinct seasons. The wet season normally lasts for 3-4 months and commences in November or December. The mean annual total rainfall over Jabiru from 1971 to 1994 was 1460 mm. The temperature remains fairly constant over the year with a mean daily maximum of around 35°C. Annual potential evaporation exceeds rainfall in most years (Press et al., 1995). The constant rate of evaporation and the short, intense rainy season result in a landscape dominated by floods then almost desert-like dry conditions. During the dry season the groundwater level lowers, drying the floodplains. Critical to the survival of plants and animals are water-bodies such as billabongs (often periodically isolated water bodies in Australian river courses) and rock-pools that persist through to the next wet season.

1.6.1 History of the Magela Catchment

Around 6000 years ago, after the post-glacial rise in sea level, the Magela area consisted of a large mangrove forest (Woodroffe et al., 1989). With time the sea level dropped and decreasing tidal connections and increased deposition of sediment resulted in the plain rising above the tidal influence and marked the beginning of freshwater dominance around 2000 years ago. As the climate became wetter, around 1500 to 1000 years ago, the final steps towards freshwater conditions were made (Wasson, 1992). With this change from a marine, mangrove-influenced environment to one influenced by freshwater the composition of the sediment changed.

1.6.2 Sediments of Magela Creek and Floodplain

In its upper catchment Magela Creek is characterised by extensive, mobile sand sheets. Areas of deposition include backflow billabongs and swampy areas with dark brown, heavy clay soils. Downstream the floodplain area is the main deposition zone and consists of layers of sediment, the form of which are related to the historical development of the floodplain.

Wasson (1992) applied varimax-rotated principal factor analysis to selected chemical properties of Magela Plain to determine the origin of the soil/sediments. The Mudginberri corridor (Figure 1.5) is characterised by alluvium overlying unoxidised estuarine sandy clay sediments. In the Upstream Basin, freshwater sediments overly oxidised and slightly oxidised estuarine sediments containing jarosite indicative of oxidised pyrite from acid-sulphate sediments. The Upstream Basin is also the area of greatest organic matter accumulation on the plain.

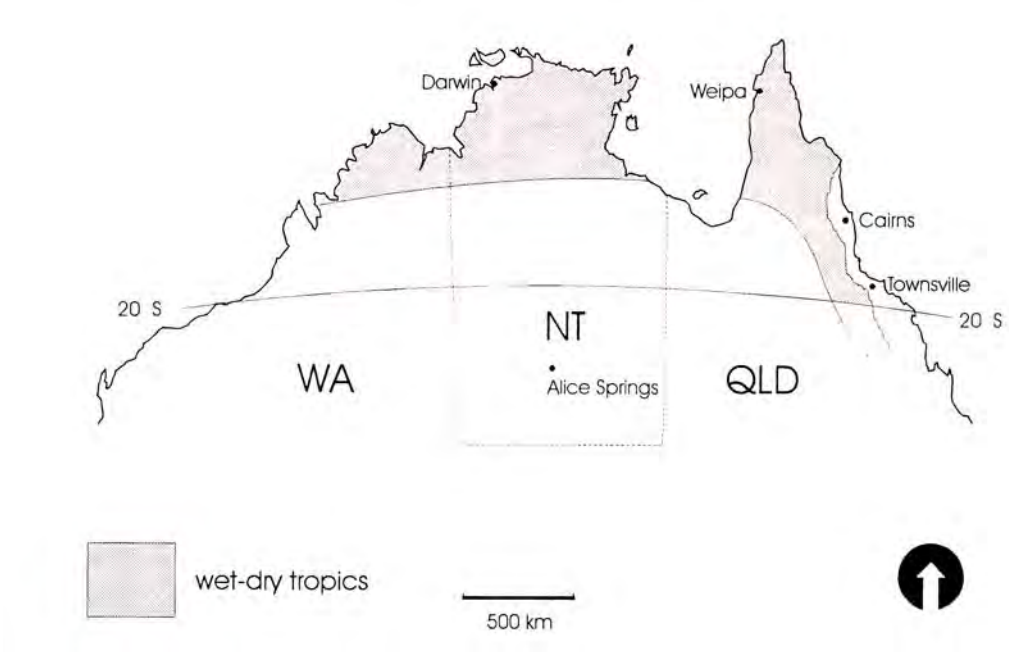


Figure 1.2. The wet-dry tropics of Northern Australia

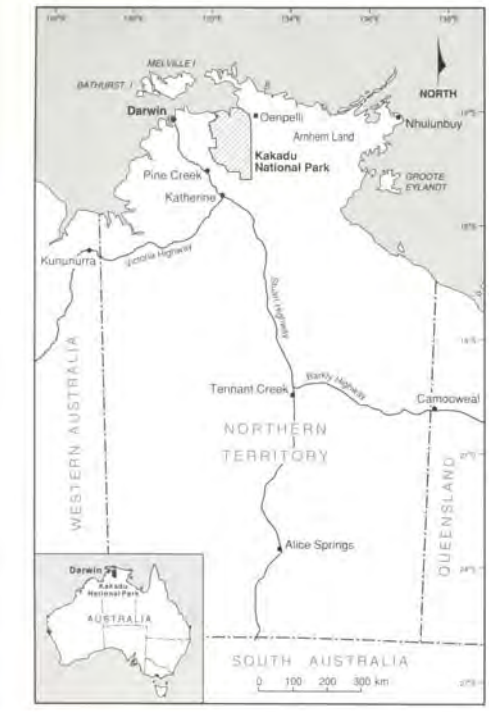
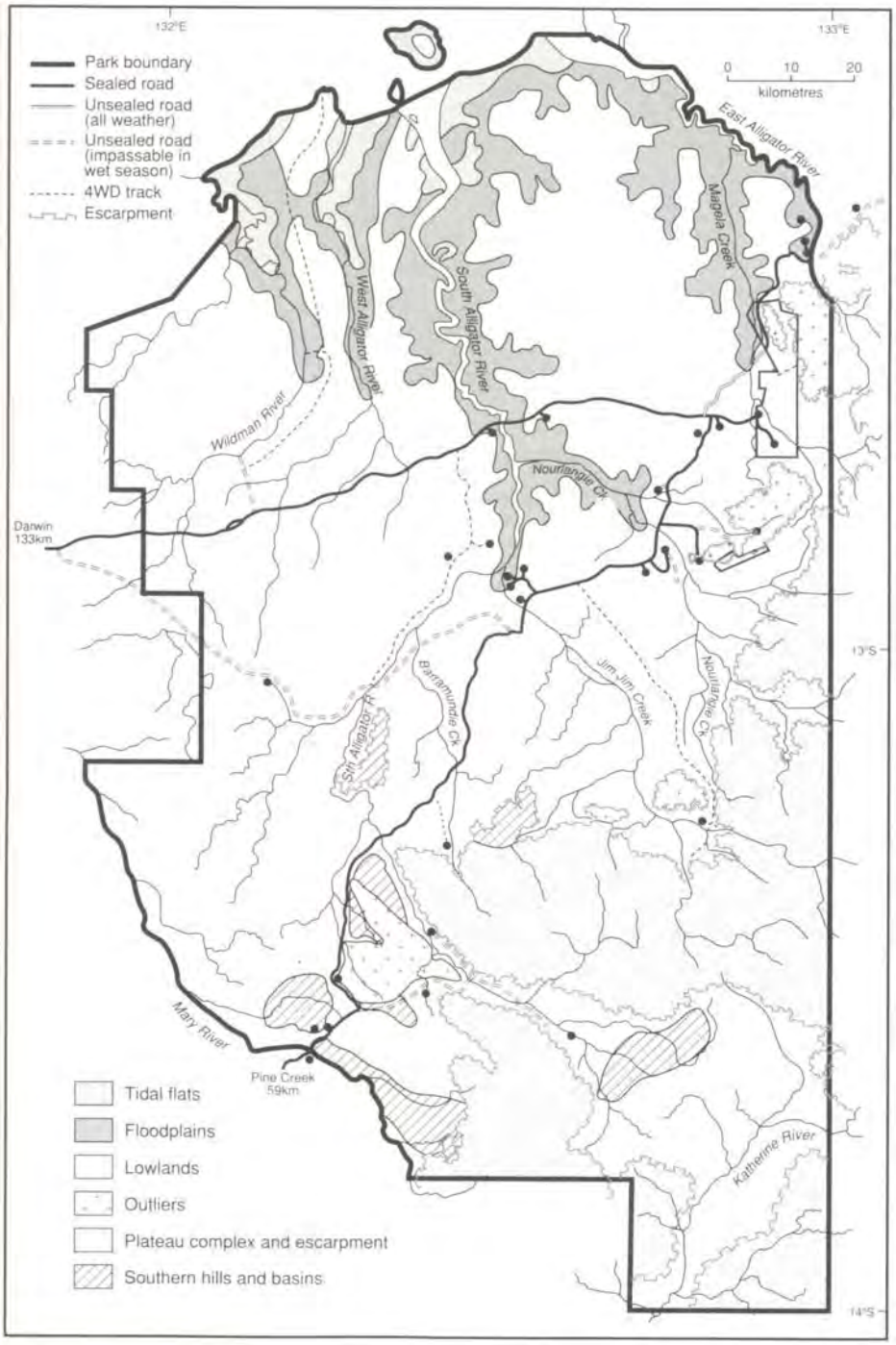
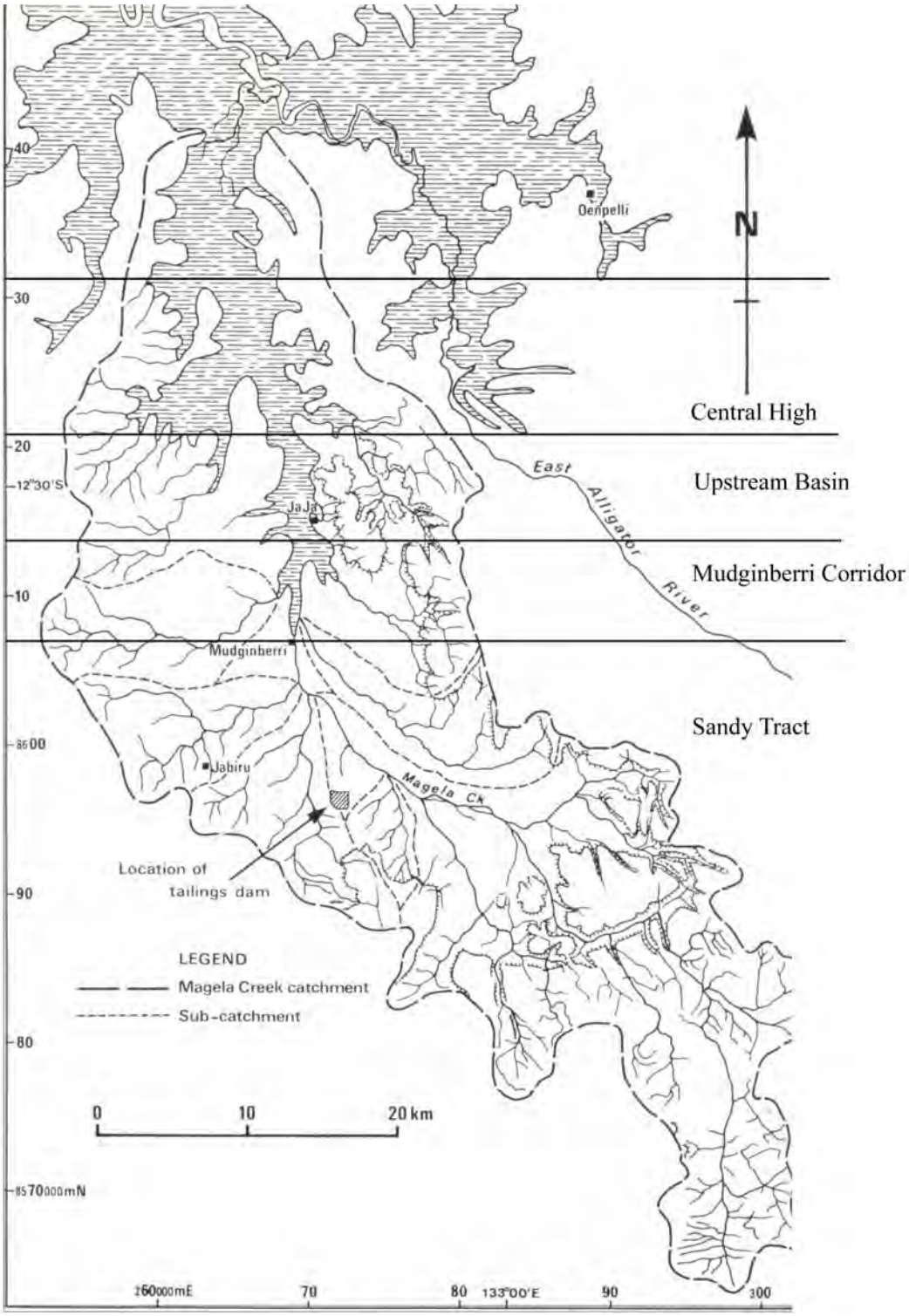


Figure 1.3. Location of Kakadu National Park



(From Press et al., 1995)

Figure 1.4. Landform of Kakadu National Park



(Adapted from Wasson, 1992)

Figure 1.5. Map of Magela catchment

1.6.3 The floodplain habitat

Since the signing in 1971 of the Convention on Wetlands of International Importance, commonly known as the Ramsar Convention, the realisation that wetlands serve vital ecological roles has been recognised by some policymakers (Dugan, 1990).

Riparian wetlands tend to form parallel to the channel, albeit a temporary channel in the case of the Magela, and can occupy most of the floodplain (Lemly, 1996). Floodplains are defined as 'areas that are periodically inundated by the lateral overflow of lakes or rivers, and/or by direct precipitation or groundwater; the resulting physicochemical environment causing the biota to respond by morphological, anatomical, physiological, phenological and/or ethological adaptations, and produce characteristic assemblage structures' (Junk et al., 1989).

The floodplain of Magela Creek becomes inundated after the onset of the wet season and covers an area of over 150 km^2 (Williams, 1979). The colonisation of these newly formed aquatic habitats is driven by the presence of nutrients deposited during the floods. These nutrients provide a valuable food source for colonising invertebrates, especially benthic invertebrates. These temporary waters tend to show a higher density of invertebrates but lower taxonomic diversity than permanent water bodies due to rapid colonisation by species with life-histories suited to the new conditions (Neckles et al., 1990). The temporary nature of the system means that there is no time for long-term succession and thus opportunistic fauna tend to dominate. The newly created aquatic habitat provides feeding areas for fish and bird species, with invertebrates on the floodplain providing a rich source of protein for egg production and subsequent juvenile growth for species of waterfowl, triggering breeding cycles (Maher and Carpenter, 1984; Armitage et al., 1995).

1.6.4 Invertebrates of the floodplain

The floodplain habitat includes temporary pools, semi-permanent backwaters and billabongs. Floodplain habitats often share insect taxa with their associated channels (Batzer and Wissinger, 1996). The movements of invertebrates between floodplains and channels have been documented in a few channel/floodplain systems. Some studies of invertebrate numbers have shown movement from channels to floodplain and others have recorded flushing from the floodplain to the channel although Smock (1994) found that there was no overall biomass transfer between the channel and floodplain system within a temperate system.

Gunn (1997) found that chironomid species are the most important invertebrate colonisers of the Magela floodplain habitat. The importance of chironomids in the early stages of colonisation has been well-documented (McLachlan, 1974a; McLachlan, 1974b; Maher, 1984; Batzer and Wissinger 1996).

The most important coloniser of the chironomids, and the preferred duck feed, has been shown to be species of the genus *Chironomus* (Batzer and Wissinger 1996). Reasons for the ability of this genus to succeed in newly inundated habitats include the following factors; rapid aerial colonisation of new habitats through the adult stage, high fecundity and the ability to tolerate low oxygen levels through the possession of haemoglobin (Batzer and Wissinger, 1996; Lahr, 1996).

The fact that adults are rapid aerial colonisers and can deposit eggs within a few days of inundation means that they are generally the most abundant and diverse insect group in these ephemeral habitats. The aerial colonisation from permanent water bodies is thought to be the major route of colonisation of seasonally inundated tropical floodplains. This phenomenon is termed ‘cyclic colonisation’ (Batzer and Wissinger, 1996).

Chironomids are also influenced by temporal and spatial dynamics through changes in physical, chemical and trophic conditions in the habitat (Botts, 1997) with several abiotic factors, including acidity, influencing wetland insects. Some insects have been shown to actively select certain low pH conditions and midge emergence was found to be unaffected by experimental reduction of pH in a fenland (Growns et al., 1992). The fact that low pH reduces the survival of insectivorous fish has led to the hypothesis that acidic environments can actually benefit insect communities by reducing predation (Batzer and Wissinger, 1996).

1.6.5 Risks to the floodplain: Mining operations

Within the Alligator Rivers Region reserves of uranium oxide of 360000 tonnes have been announced to date (Press et al., 1995). A major deposit within the catchment of the Magela Creek resulted in a mine being proposed and, in 1977, the Commonwealth Government announced its decision to allow mining and export of uranium to proceed providing strict environmental standards were implemented (Fox et al., 1977). The presence of Ranger uranium mine within the Magela catchment poses a potential source of contaminants to the Magela Floodplain.

1.6.6 Mine tailings

By 1991 the mining of ore body No. 1 at Ranger had produced approximately 10 million tonnes of tailings (location shown in Figure 1.5), which excludes gangue in the form of 28 million tonnes of waste rock and very low grade ore (Wasson, 1992). The tailings (crushed ore from which extractable uranium has been removed) have special containment requirements as they contain most of the radioactivity of the original ore body. They do not present an acute risk due to their low radioactivity but due to their large volume require containment.

The chemical composition of tailings from Ranger uranium mine is shown in Table 1.1. To identify metals of potential concern the ratio of metal concentration in tailings to metal concentration in background sediment was calculated (Willett, 1992). Table 1.2 shows the ratio of total elements in tailings to the average total from four soil types from the Magela catchment. Cu, Mn, Pb and U all show much higher concentration in tailings and are identified as being of potential concern.

Willett (1992) carried out experiments in which tailings were added to different sediments based on differing erosion and deposition scenarios. From the results it was concluded that Pb, Cu and Fe could be liberated under oxidising conditions from sulphides. In addition, a proportion of Mn, Cu and Pb was found in a reducible fraction in tailings suggesting that these metals might be liberated under anoxic conditions. The most marked result was that pyrite oxidation changed the distribution of metals between fractions to the extent that the presence of native sulphide in floodplain sediments was more important than any contribution made by tailings to metal availability (Willett, 1992).

Deposition of tailings on the Magela Floodplain is most likely to occur on the surface sediment made up of a Dark Brown/Black Clay layer (Clark et al. 1992). It is this layer that is the habitat of benthos. If this layer overlies an acid-sulphate horizon, as is common on the Upstream Basin, then it is likely to be affected by acidity generated from below upon re-wetting of the sediment and rising ground water. The conclusion of the study by Cull et al. (1992) was that sediment derived from the mine site should go no further than Jabiluka billabong; this corresponds to the Upstream Basin region. The implication of this is that any deposited material is likely to be influenced by acidity generated by the acid-sulphate horizon.

	Cu	Fe	Mn	Pb	U	Zn
Total (mg/kg)	312	36200	3070	1144	176	55

Table 1.1. Chemical properties of Ranger Uranium Mine Tailings (Willett, 1992)

	Cu	Fe	Mn	Pb	U	Zn
Ratio (tailings: soil) (from Wasson, 1992)	15:1	2:1	34:1	57:1	38:1	2:1

Table 1.2. Ratio of total element concentrations in tailings over background soil concentration (from Willett, 1992)

1.6.7 Acid-sulphate sediments of the Magela floodplain

Over the period of the dry season evaporation lowers the water table on the Magela floodplain resulting in oxidation of pyrites. In the Magela catchment, at the start of the wet season, during the first flushing of billabongs, fish and mussel kills are regularly recorded. A model has been proposed by Noller and Cusbert (1985) where high levels of acidity and labile aluminium and other elements are leached from the jarositic, acid-sulphate layer into pools and channels. The rain then flushes these acidic waters into billabongs leading to fish mortalities. With continuing rainfall the surface waters become diluted and mortalities cease. The point of relevance to this research is that the high levels of dissolved metals are associated with the fish kills (Ayers and Gillett, 1986). Another study (Roach, 1997) has shown complex and not easily predicted impacts of acid water inflow into estuarine systems.

The lowered pH itself has been shown to cause acute and sublethal effects, however associated metals such as aluminium, which comes into solution at low pH, have also been implicated in toxic effects (Morris et al., 1989).

In summary, it was of interest to determine whether pH changes in sediment effected bioavailability of potential metal contaminants to a local benthic organism.

1.7 Aims

The aims in the thesis were:

1. To develop a site-specific sediment toxicity test using a chironomid species from the wet/dry tropics.
2. To determine the impact of pH on the bioavailability of copper and uranium.

3. Evaluate a number of toxicity test endpoints in light of a review of current and proposed risk assessment methods.
4. Determine whether any special requirements are needed when trying to assess risk in regions of acid-sulphate sediments.

2 General Methods and Materials

This section contains general methods used in setting up the chironomid culture, defining life-cycle characteristics of *Chironomus crassiforceps,* sediment characterisation and general sediment spiking and toxicity test protocol.

2.1 Laboratory culture

A chironomid was chosen for use as a site-specific test organism. Chironomids were collected from the field, identified and screened for culture under laboratory conditions. Large numbers of individual species were initially collected to test their suitability for long-term laboratory culture. Once developed the collection methodology must be capable of re-establishing the culture from the wild if necessary.

2.1.1 Standard Field Collection Method

The standard method ultimately adopted was the light trapping of gravid female chironomids. Any egg masses oviposited were then tested for their suitability for long-term laboratory culture.

Many species of chironomid are drawn to light, with the intensity of light acting as the major attractant (Cranston, 1995). A light trap was constructed by mounting a 140 W halogen bulb in a car spotlight and illuminating a 2m x 1.5m polystyrene sheet using a car battery. Individual female chironomids landing on the sheet were collected using an aspirator or 'pooter' (Service, 1976) and placed individually in sealed plastic tubes. Approximately 10 ml of deionised water was added to the sealed tube and the location, time and number collected was recorded. The tubes were checked daily for signs of egg masses. If egg masses were present, the tubes were placed in an incubator at 27°C

(±1°C) until hatching occurred. This proved to be ideal for isolating single, fertile egg masses.

Using the above procedure a single egg mass was obtained and maintained at 27°C (±1°C) in 20 ml of deionised water. Egg masses were checked daily to see whether hatching had occurred. If no hatching occurred within 5 days then the egg mass was rejected. Upon hatching, the first instar larvae were added to a culture system comprising of a plastic container (16cm x 16cm x 9cm depth) with Magela Creek sand (sterilised at 400°C for 12 hours) to a depth of 0.5 cm, and deionised water to a depth of 4 cm.

A ration level of approximately 0.5 mg/individual per day was established (Sibley et al., 1997). The feed consisted of adding 3g of blended fish pellets (Aqua-lab aquarium products, Victoria) to 50 ml of deionised water. Two ml of this mix was added per culture container every second day. If excess feed or microbial growth was observed in the container feeding was temporarily suspended. The culture containers were checked daily for signs of pupation. Once pupae were observed, the culture container was set up for emergence by covering with mesh, and fourth instar larvae were collected and identified to genus.

Any egg masses oviposited by adult chironomids were collected with a soft paint brush and placed in a 100 ml plastic bottle with deionised water. If the eggs hatched within 5 days the species was deemed suitable for culturing in the laboratory.

2.1.1.1 Identification of Chironomids

Chironomids can be identified from the head capsule of the larvae, the pupae and from the adult male. To meet the objectives, a culture of the subfamily Chironomidae, and of genus *Chironomus* was required. Keys for the Alligator Rivers Region exist (Cranston,

1990) and it is relatively easy to identify the chironomid to genus. However, expert help was required to identify the species.

The procedure adopted in the screening of species for culture was to remove a fourth instar larva once a culture had exhibited emergence of adults. The head-capsule was mounted in Hoyers solution and identified to genus under a compound microscope. The distinguishing characteristic of the genus *Chironomus* was the presence of striations at the base of the mandible. Once an organism was deemed suitable for culture a fourth instar larva, pupa and an adult male were collected and sent to Dr Peter Cranston (CSIRO Division of Entomology) for expert identification to species.

The chironomid chosen for use as a standard organism was *Chironomus crassiforceps*. Re-establishing the culture involved repeating the steps outlined above. Table 2.1 shows the collection areas and the results of screening for a suitable species.

2.1.2 Laboratory culture of *Chironomus crassiforceps*

A stock culture was set up in 3 aquaria (75 cm x 25 cm x 35 cm) that were covered in mesh to contain adult chironomids. Sealable access points for hand access were cut into the mesh. Autoclaved Magela Creek sand and deionised water to a depth of 7 cm was added. The aquarium was aerated, and the water changed every two weeks. Two of the cultures were susceptible to outdoor fluctuations in temperature, but one was maintained at 27°C (± 2°C). The temperature controlled stock culture was used to provide egg masses for experimentation. The cultures were fed approximately 200 mg of feed per day.

Date collected	Time	Location	Number of females trapped	No. fertile egg masses	No. surviving culture and emerging	Number laying fertile eggs	Genus of potentially culturable chironomid and time to first emergence
2/4/97	19.00–21.00	Oenpelli Rd. Nr Mine Valley BB	1	0	XXXX	XXXX	XXXX
14/4/97	21.00–23.00	Jabiru Lake, Baralil Crk.	10	3	1	0	XXXX
16/4/97	19.00–21.00	Jabiru Lake	7	3	2	0	XXXX
22/4/97	19.00–21.00	009 Backflow	7	3	1	0	XXXX
20/5/97	19.00–21.00	Jabiru Lake	5	4	0	XXXX	XXXX
21/5/97	19.45–20.45	Jabiru Lake	1	1	1	1	Chironomus spp. (10 days to first emergence)
25/5/97	20.00–21.00	Jabiru Lake	0	XXXX	XXXX	XXXX	XXXX
27/5/97	19.30–21.00	Jabiru Lake	3	1	0	XXXX	XXXX
28/5/97	19.00–22.00	Ja Ja Floodplain	13	8	2	0	XXXX
30/5/97	19.00–21.00	Jabiru	1	1	0	XXXX	XXXX
31/5/97	19.00–21.00	0009 Backflow	20	13	7	XXXX	XXXX
1/6/97	20.30–21.30	0009 Backflow	1	1	0	XXXX	XXXX
5/6/97	19.00–21.00	0009 Backflow	16	10	5	XXXX	XXXX

Note: XXXX means no data recorded

Table 2.1. Locations and results of light trapping and test for culturability.

2.2 Observations on Life History and Development

Development of the bioassay required information on the life cycle of *Chironomus crassiforceps*. Information on growth, time to emergence and the time spent at each instar was needed. The fundamental variables controlling these factors are temperature and food quantity and quality (Hauer and Benke, 1991).

2.2.1 Methods

2.2.1.1 Temperature effect on life-cycle length

Holes were drilled in the top of 60 ml narrow-mouthed HDPE (Nalgene®) bottles to allow aeration and 10g sand (sterilised at 400°C for 12 hours) was added. Four replicates of five larvae were set up in 50 ml of artificial Magela Creek water (Appendix 1) in incubators with a light cycle of 12 hours light: 12 hours dark at the following temperatures: 27°C, 30°C and 33°C under a ration level of 1 mg/individual/day of ground and sieved (<500μm) fish pellets (Aqua-lab aquarium products, Victoria). Time to pupation was recorded and head capsule width of all remaining larvae after 5 days noted.

2.2.1.2 Growth of *C. crassiforceps* at 27°C

Thirty larvae were randomly collected from a hatched egg mass and placed in a tray with 1 cm of autoclaved sand. Synthetic Magela Creek water was added to a depth of 3 cm and aerated at 27°C. Larvae were provided with excess feed of ground and sieved (<500μm) fish pellets (Aqua-lab aquarium products, Victoria). Larvae were randomly sampled daily and placed under a binocular microscope fitted with a video camera. Video footage was taken and still images captured using a video capture card. The images were subsequently analysed using Image Tool, an image analysis system

available over the Internet (UTHSCSA *ImageTool* program - Developed at the University of Texas Health Centre at San Antonio, Texas and available on the net by anonymous FTP from ftp/maxrad6.uthsca.edu). This allowed determination of head capsule length, head capsule width, length, and area against time (Figures 2.1 and 2.2).

2.2.1.3 Growth of *Chironomus crassiforceps* under differing ration levels

Ten grams of Magela Creek sand (sterilised at 400°C for 12 hours) was added to the test bottles with 50 ml artificial Magela Creek water. Four replicates of five larvae were set up at the following ration levels: 0.1, 0.3, 0.5, 0.7, 0.9 mg/individual/day of ground and sieved (<500μm) fish pellets (Aqua-lab aquarium products, Victoria) at 27°C. After five days, survival was noted and live larvae were removed. Larvae were sexed by examining the developing 'Anlagen' on the inner ventral surface of the 8^{th} and 9^{th} abdominal segments at 25x magnification (Wulker and Gotz, 1968). The larvae were transferred individually to a Teflon drying tablet and dried for 12 hours at 60°C and desiccated for 12 hours. Their dry weight was subsequently measured on a microbalance (Mettler AT 261 Delta Range - sensitivity 0.01mg).

2.2.2 Results

At 33°C pupation and emergence was observed after 4 days; at 30°C pupation was observed after 5 days; and at 27°C all were still in the 4th instar of the larval stage after 5 days. Measurement of head capsule width showed all remaining larvae at all temperatures had reached the fourth instar.

At 27°C first instars have approximate head capsule widths of 0.10 mm, second instars of 0.17 mm, third instars of 0.30 mm and fourth instars of 0.50 mm (Figure 2.1).

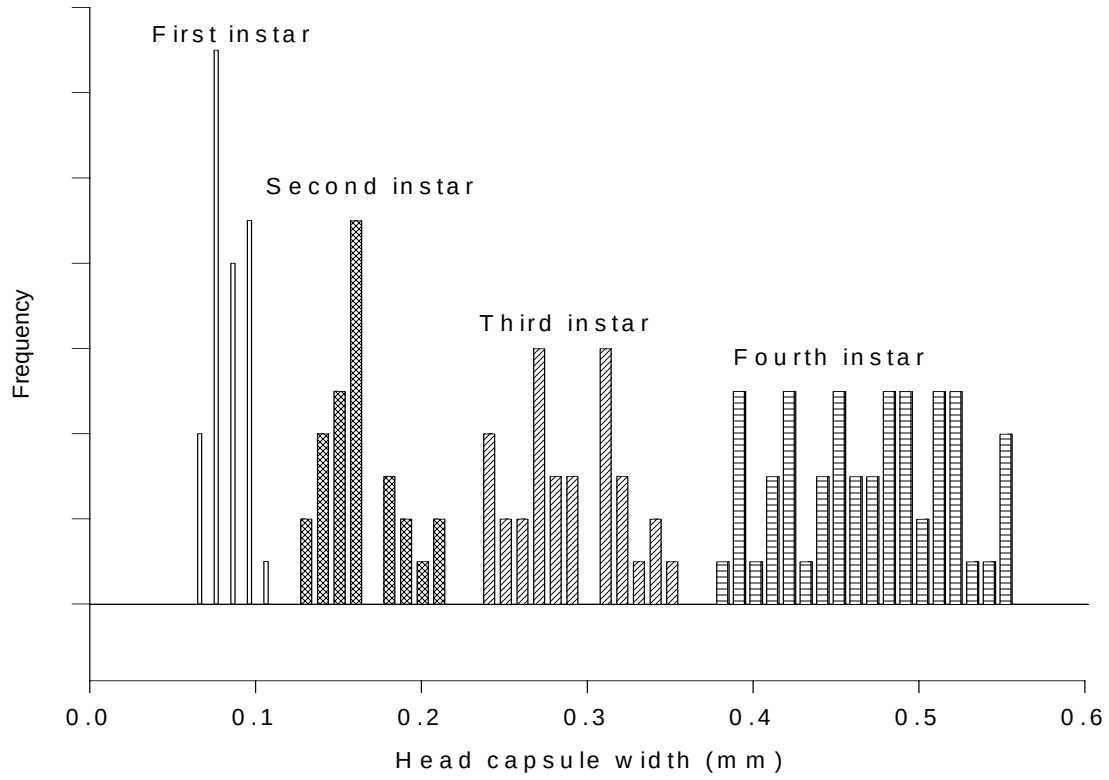


Figure 2.1. Head capsule width of instars for *C. crassiforceps* grown at 27°C

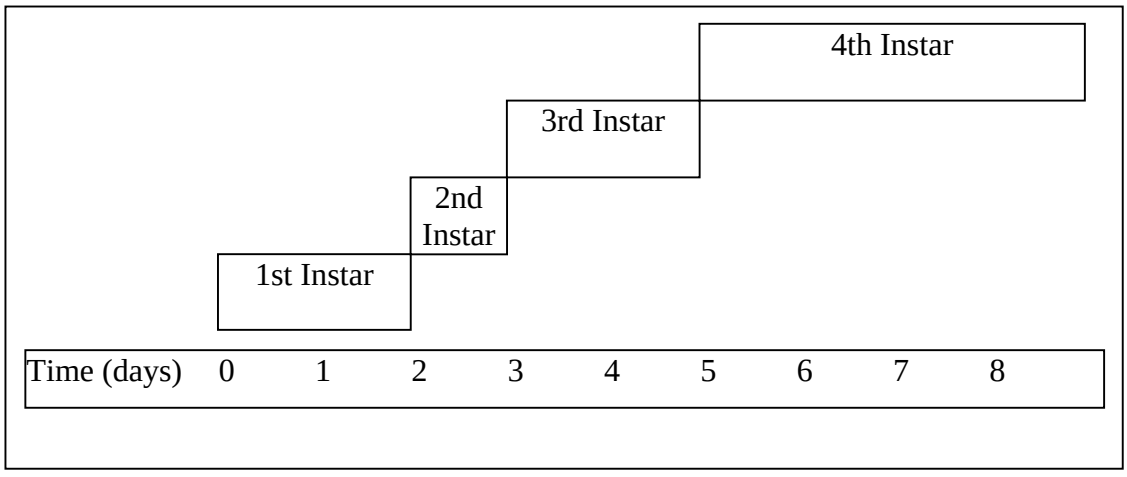


Figure 2.2. Growth of larvae of *C. crassiforceps* at 27°C

There is an approximate doubling of head capsule width at each ecdysis. A timeline representation of instar development over time is shown in Figure 2.2. After 8 days pupation occurred which was followed within 24 hours by emergence as a mature adult. After pupation, adults emerged in a 2:1 male to female ratio and lived for 2-3 days. Following mating an egg mass was laid from which first instars hatched in 2 to 3 days at 27 °C.

At a ration level of 0.9mg/individual/day larvae showed survival of 80% with no variability between replicates. As ration level was reduced, survival was also reduced and became more variable.

From the surviving larvae the distribution of dry weight after five days offered a valuable insight into the natural variability in growth and provided data with which to examine the potential sensitivity of the proposed toxicity test.

The sexual dimorphic weight difference common in chironomids (Day et al., 1994) with female chironomids heavier than males are clearly shown in Figure 2.3.

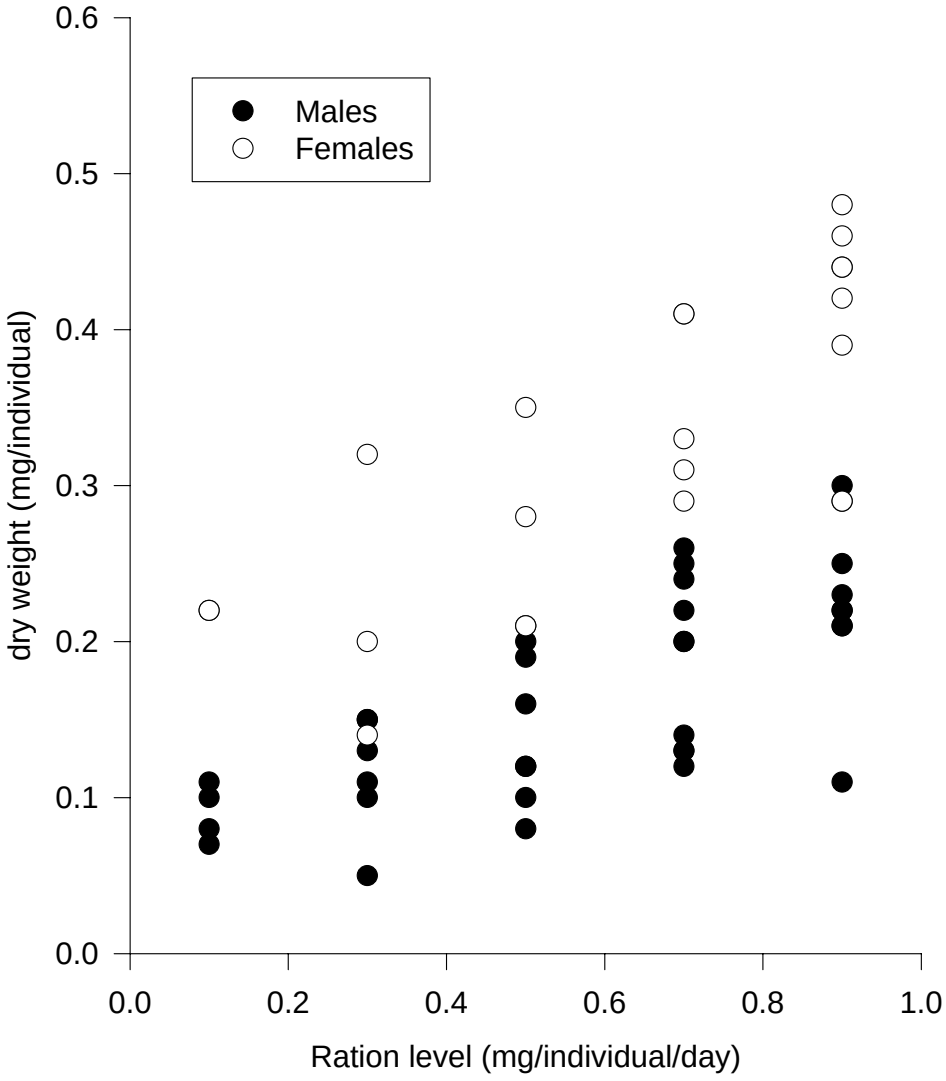


Figure 2.3. Graph of body mass of individual larvae after five days against ration level showing sexual dimorphic weight difference

2.2.3 Discussion

From the experiments a growth test, in which chironomids emerging from an egg mass were introduced into the test chamber, and in which the size or mass of the fourth instar was measured in response to sediment conditions, could be terminated after 8 days at 27°C. A full life-cycle test could be carried out in approximately 14 days.

This is an extremely short test period when compared to standard tests using *Chironomus riparius* and *C. tentans,* that require a period of 20 days from hatching to the end of the survival and growth tests (EPA, 1996a). The short test period reflects the increased metabolism under the higher temperatures of the tropical toxicity test.

Sexual dimorphic weight differences raise an important issue in using body mass as an endpoint in sediment toxicity testing. If male and female weights are grouped in one treatment and compared by weight to another treatment the variance is inherently large and the power of an ANOVA to detect differences in treatment effects is lowered. The problem exists that it is difficult to sex the larvae when starting the test as sexual characteristics are only developed during the fourth instar, and it is only in the adult that it is possible to tell male from female easily by sight. This problem has been encountered before with *Chironomus riparius* (Day et al., 1994a). To minimise this effect individuals must be allocated to replicates in a randomised manner to avoid bias. Without randomisation the danger exists in picking larger individuals first, as they are easier to spot, leading to systematic errors in interpreting data (Davis et al., 1998).

The effect of ration level on body mass is shown in Figure 2.4. From data on body mass against ration level, the experimental design of a toxicity test protocol was investigated. The EPA guidelines recommend a test system, using *Chironomus tentans*, with 6

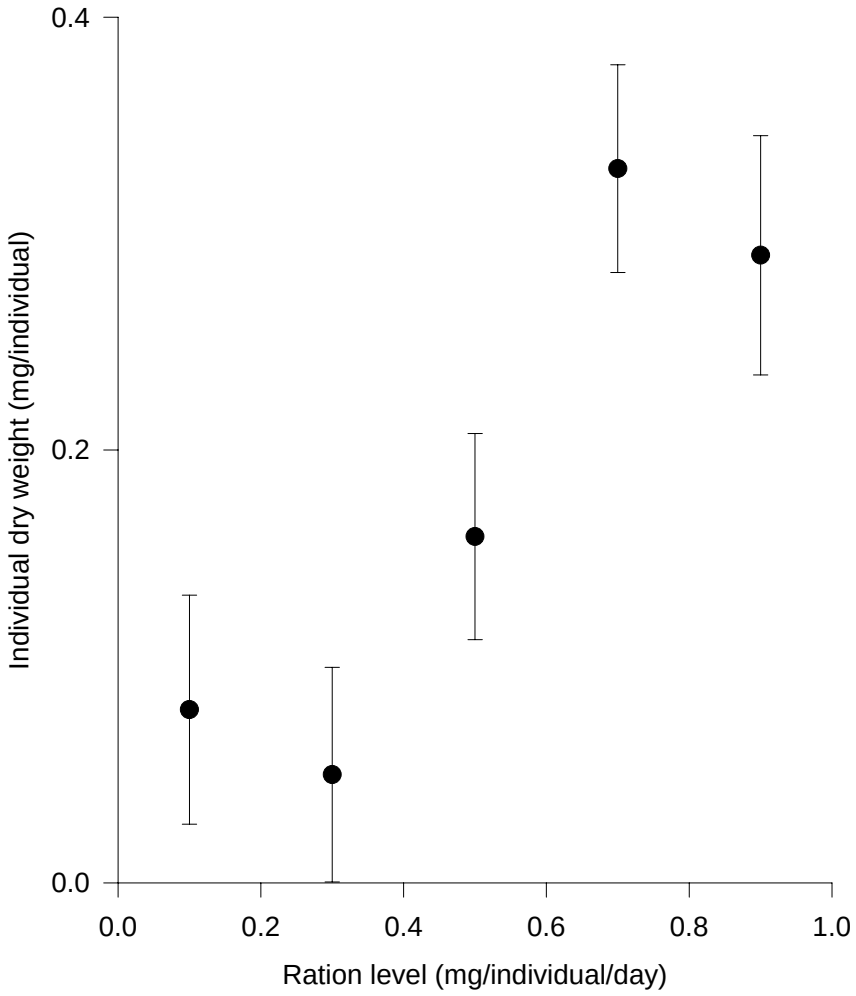


Figure 2.4. Graph of mean body mass of individual larvae after five days against ration level with 95% confidence intervals

treatments, 8 replicates per treatment and 10 individuals per replicate. This design can be evaluated using experimental data from body mass against ration level.

Considering an ANOVA involving k groups at an α level of significance with *n* data or replications per group then an estimate of the power can be obtained if we have an estimate of σ^2 - the variability within the k populations (Zar, 1996).

Information on individual dry weights were already available, thus, by randomly sampling the dry weight of all larvae at the ration level of 0.9 mg/individual/day the mean could be estimated for 8 replicates for the control, each containing 10 individuals. From a random sample the following data set was obtained (in mg);

0.307, 0.289, 0.275, 0.3, 0.301, 0.303, 0.307, 0.332, 0.331, 0.312

The mean is 0.31 mg, and the standard deviation is 0.017 mg.

The estimated minimum detectable difference for an α of 0.05, power of 80%, and a sample size of 8 (n) for 6 treatments (k) can be calculated from

$\nu_1 = k-1$

$\nu_2 = k\,(n-1)$

And referring to Zar (1996) for the solution of $\phi \approx 1.58$

where δ is the minimum detectable difference in means

$$\partial = \sqrt{2ks^2\phi^2 / n}$$

Hence, the minimum detectable difference = 0.03 mg

Using 6 treatments with 8 replicates with ten individuals per replicate a reduction of approximately 11% in weight between a control and a treatment could be detected. To ensure control replicates have the required level of survival (>80%) a minimum ration level of at least 1mg feed/individual/day was required.

2.3 Tropical Sediment Toxicity Test Protocol using *Chironomus crassiforceps*

Sediment toxicity testing was carried out using sediment collected from the Magela catchment spiked with the metals under investigation. Methodology for sediment collection and characterisation, sediment spiking and toxicity test protocol are described below.

2.3.1 Sediment collection and characterisation

An airboat was used to access a site overlying an acid sulphate horizon on the floodplain on 17/4/97. The site was chosen based on work carried out by East et al. (1992) on the floodplain near Jabiluka Billabong (12°27.809′ S 132° 52.233′ E).

An aluminium cage was lowered into the water of depth 70-cm. The cage provided protection from potential attack by crocodiles while sampling. An auger sample was collected to a depth of approximately 3 metres and the pH of the sediment profile taken (Rayment and Higginson, 1992) to ensure an acid-sulphate horizon lay below the sample. Surface sediment to a depth of approximately 10cm was cut with a spade and stored in a sealed plastic drum. A second sample was collected on 22/4/97 north of Island Billabong (12°32.644′ S 132° 53.041′ E) from a water depth of a few centimetres. The third sample was collected from a small backflow billabong approximately 300m south of gauging station (GS) 8210009, located several kilometres downstream of Ranger Uranium Mine. All the samples were stored in sealed plastic drums at 4°C.

A subsample of sediment was air-dried at 40°C then ground and sieved to <2 mm. A further subsample was ground and sieved to < 0.015 mm for total metals analysis. The air-dried sediment may still contain moisture if it is high in organic matter so air-dry

moisture content was determined to correct from air-dry to oven-dry mass (Rayment and Higginson, 1992). The electrical conductivity in a 1:5 soil/water extract was determined followed by the pH (Rayment and Higginson, 1992). Total organic matter by loss on ignition was determined by first drying at 100°C then heating for 8 hours at 375°C (Hesse, 1971). Cation exchange capacity and exchangeable bases were determined using silver thiourea followed by atomic absorption spectroscopy (Rayment and Higginson, 1992). Organic carbon was determined by dichromate digestion (Yeoman and Bremner, 1988). Total phosphorus was determined by digestion in acid persulphate followed by analysis using the molybdenum blue spectrophotometry method (Nelson, 1987). Microwave digestion, method 3051, (EPA, 1994) followed by ICP-MS allowed the determination of total metals. Total nitrogen was determined using a Kjeldahl digestion followed by alkaline distillation (Rayment and Higginson, 1992). Ammonium concentration was determined by 2M KCl extraction (Rayment and Higginson, 1992). Reference sediments 11, 15, 30 and 35 from the Australian Soil and Plant Analysis Council (ASPAC) were analysed in all batches. Lake sediment reference materials (Canada Centre for Mineral and Energy Technology, CANMET) LKSD–1 and LKSD–4 were used as references for microwave total metal acid extraction.

2.3.1.1 Observations

General sediment characteristics are shown in Figure 2.5. All results except pH and electrical conductivity are corrected for air-dry moisture content. Nitrogen levels of 0.61 mg/g for the floodplain, 0.52 mg/g for the Island billabong and 0.38 mg/g for the (GS) 8210009 were obtained. Copper levels of 14.7 mg/kg for the floodplain, 20.2 mg/kg for the Island billabong and 22 mg/kg for the (GS) 8210009 sediment were obtained. The texture of the sediment is shown in table 2.2.

Three factors contribute to the low pH of the sediments (pH 4.11 – pH 4.98). First the oligotrophic status of the overlying water, secondly the accumulation of base deficient organic matter and, in the case of floodplain samples, the influence of groundwater.

Electrical conductivity gives an indication of free salt and the ionic strength of porewater. There is an increase from 37.1 μScm^{-1} in the upstream backflow billabong of Magela Creek to 293 μScm^{-1} on the floodplain. It is hypothesised that the increase in electrical conductivity is due to influx of sulphate due to rising groundwater. The soil cation exchange capacity (CEC) is a measure of the soil's ability to retain cations. The high cation exchange capacities (12.72–19.64 $c_{+}molkg^{-1}$) are indicative of humified organic matter. The sum of exchangeable base cations (Ca, Mg, Na and K) gives a base saturation of the cation exchange capacity of approximately 50%. This fits well with the relationship between pH and base saturation which predicts decreasing base saturation below pH 7.

The organic carbon levels are relatively high (5–8%) and confirm the role of organic matter in determining a number of chemical properties. Total nitrogen and phosphorus levels are at the low levels associated with unfertilised Australian soils (Rayment and Higginson, 1992).

Sediment	Clay (%)	Silt (%)	Fine sand (%)	Coarse sand (%)
Floodplain	45	38	16	<1
Island Billabong	39	39	22	<1
Upstream Billabong	14	57	28	<1

Table 2.2. The texture of sediment collected from the Magela catchment (percentage mass)

Figure 2.5. Chemical characteristics of sediment from Magela Creek

	Floodplain	Island	Magela Creek Backflow
pH	4.42	4.11	4.98
Electrical Conductivity (μScm^{-1})	293	298	37.1
Total Volatile Solids (%)	15.31	19.64	19.01
Cation Exchange Capacity ($\mathbf{c_+molkg^{-1}}$)	18.34	17.82	13.34
Exchangeable Ca ($\mathbf{c_+molkg^{-1}}$)	2.01	1.27	1.00
Exchangeable Mg ($\mathbf{c_+molkg^{-1}}$)	7.00	3.86	2.40
Exchangable Na ($\mathbf{c_+molkg^{-1}}$)	1.03	0.21	0.14
Exchangeable ($\mathbf{c_+molkg^{-1}}$)	0.07	0.18	0.07
Total Exchangeable Base ($\mathbf{c_+molkg^{-1}}$)	10.11	5.52	3.61
Organic Carbon (%)	5.35	7.80	8.00

2.3.2 Sediment spiking

Sediment was spiked by dissolving the respective metal sulphate salt in 500 ml of water and then adding this to one litre of sediment in 5 litre HDPE containers. The mix was then shaken on a horizontal shaker for 12 hours at 25°C (ASTM, 1990). The sediment was left to settle for 6 hours and the overlying water then poured off after taking a sample for analysis of metal concentration. One hundred ml of sediment was added to 8 replicate vessels for each treatment and to 8 treatment replicates for analysis of porewater and then left to settle for 12 hours. The overlying water in each replicate was renewed 3 times by pouring deionised water gently onto a Teflon baffle then the replicates were placed into the test trays. The overlying water was changed in the static renewal system (described below) twice daily and once more just before addition of larvae. The pH and conductivity of overlying water was measured in each replicate before addition of test animals.

2.3.3 Sediment toxicity test methodology

The experimental design of the sediment toxicity test system consisted of 6 treatments of 8 replicates. The physical design of the system is shown in Figures 2.6 and 2.7. Aeration of individual replicates was necessary, as preliminary experiments showed that without aeration chironomids tended to spend extended periods near the surface in the overlying water in an effort to gain oxygen. The entire test system was designed to fit an incubator to ensure a constant temperature of 27°C and a 12-hour light: 12-hour dark tropical photo-cycle. Each replicate was set up in a 500 ml HDPE container designed to hold 100ml of sediment and 175ml of overlying water with an overflow covered by 250 μm mesh. The 175 ml of overlying water was renewed twice a day using a static renewal system.

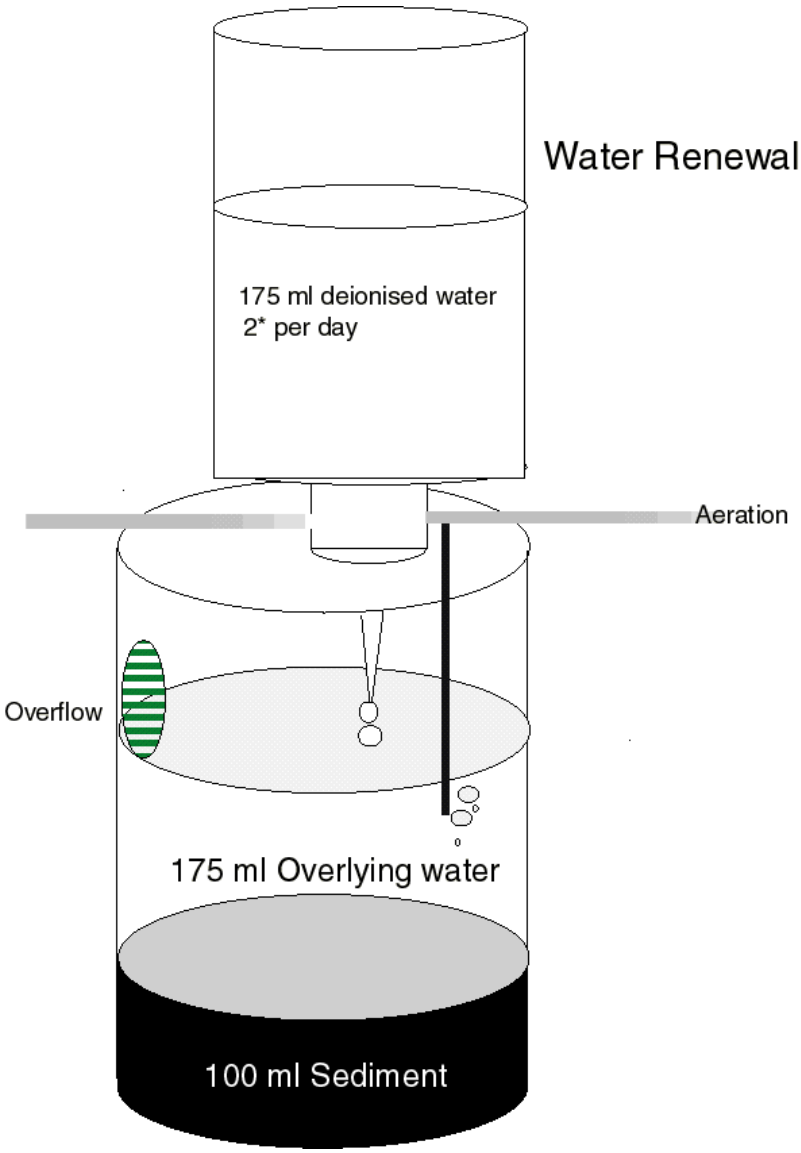


Figure 2.6. The whole-sediment toxicity test apparatus

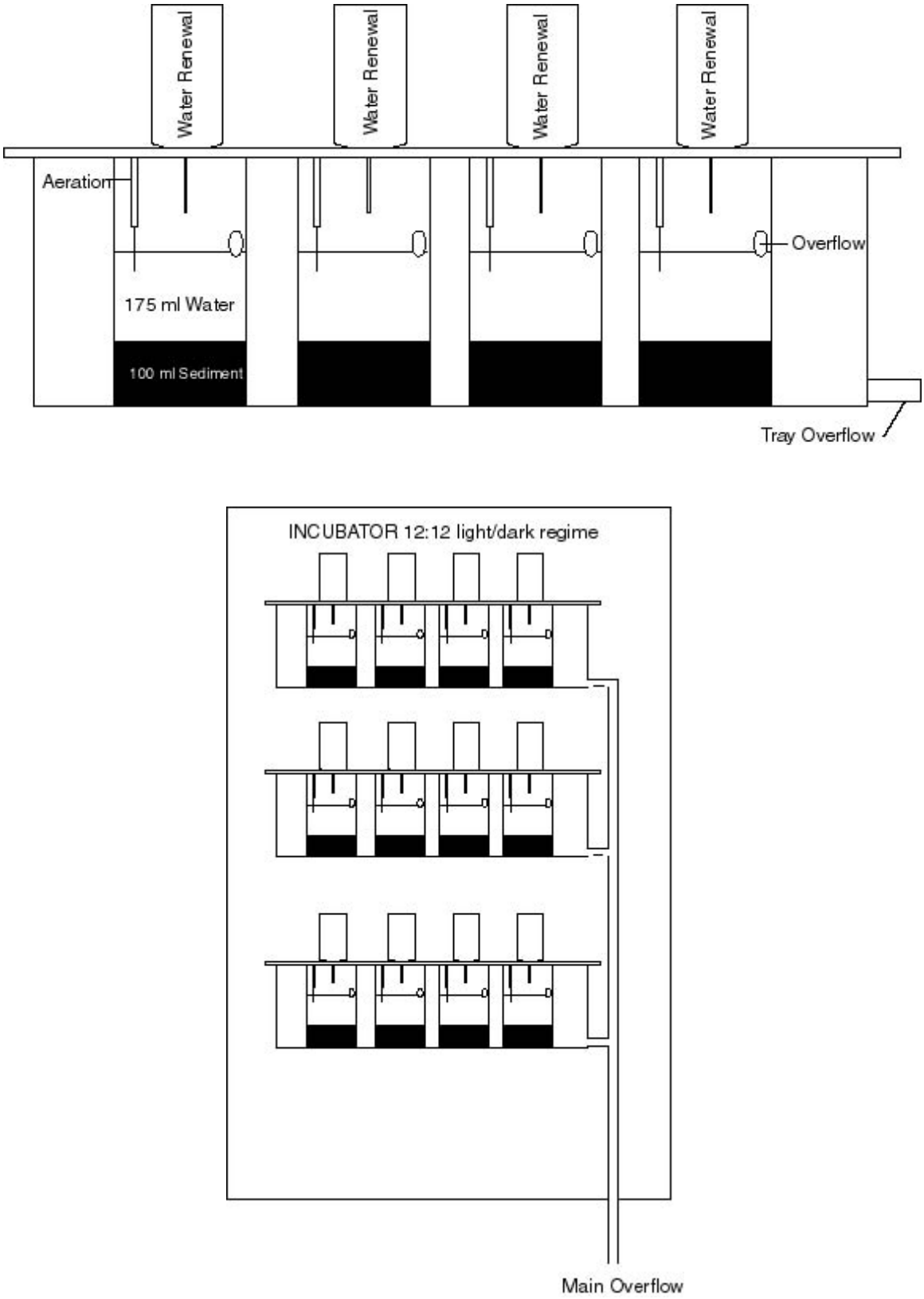


Figure 2.7. Design of the sediment toxicity test system

A test was initiated by the collection of at least 4 egg masses from the temperature regulated chironomid culture. Each egg mass was separately placed in deionised water in 50ml tubes. Approximately 48 hours later, upon hatching, larvae were transferred to separate plastic culture trays in deionised water with autoclaved sand to a depth of 1mm then fed 100mg of chironomid feed (ground and sieved (<250μm) fish pellets, Aqua-lab aquarium products, Victoria) then aerated. Every twenty-four hours 200 mg of feed was added per tray.

After 4 days the larvae were collected with a pipette and counted. At least 120 chironomids per egg mass from 4 egg masses are required for the test. 10 larvae were randomly allocated to each test chamber according to a randomised block design (Figure 2.8). The replicates were then randomly allocated to the incubator and the water renewal and aeration system attached. Overlying water was renewed twice daily (Ankley, 1993b).

A ration level of 2mg/individual/day was initiated to ensure control survival. 10g of blended and sieved fish pellets were made up to 500 ml with deionised water. 1 ml of this mixture was added per replicate. Timelines for carrying out the sediment toxicity test with metal spiked sediments are shown in Figure 2.9.

2.3.4 Termination of the sediment toxicity test

At the end of the experiment, metal concentrations in overlying water and porewater were measured in three replicates per treatment. In some experiments organically bound metal concentration was also analysed. The methodology is described in detail in the relevant chapter.

After water or sediment samples were taken for analysis, overlying water was decanted and ethanol to a depth of 2cm was added to all replicates to preserve chironomids. Chironomids were removed from sediment by sorting on a white tray, counted for

recovery then placed in 50ml containers representing each replicate. Chironomids were pooled, dried on a Teflon tablet at 75°C, cooled in a dessicator and weighed on a microbalance (Mettler AT 261 Delta Range - sensitivity 0.01mg).

2.3.4.1 Statistical analysis

The average mass of chironomids per replicate was calculated by dividing pooled dry mass by the number recovered. A two-way ANOVA was carried out to determine the contribution of treatment and parental origin (egg mass) to the dry mass of chironomids at the end of the experiment with egg mass treated as a blocking factor:

Dry Mass = parental origin (egg mass) + treatment

Homogeneity of variance was checked using plots of residuals versus fitted values.

Regression analysis was carried out by arcsine transformation of percentage data and linear regression carried out using GraphPad Prism (version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). The EC_{50} and its 95% confidence intervals were estimated (Zar, 1996).

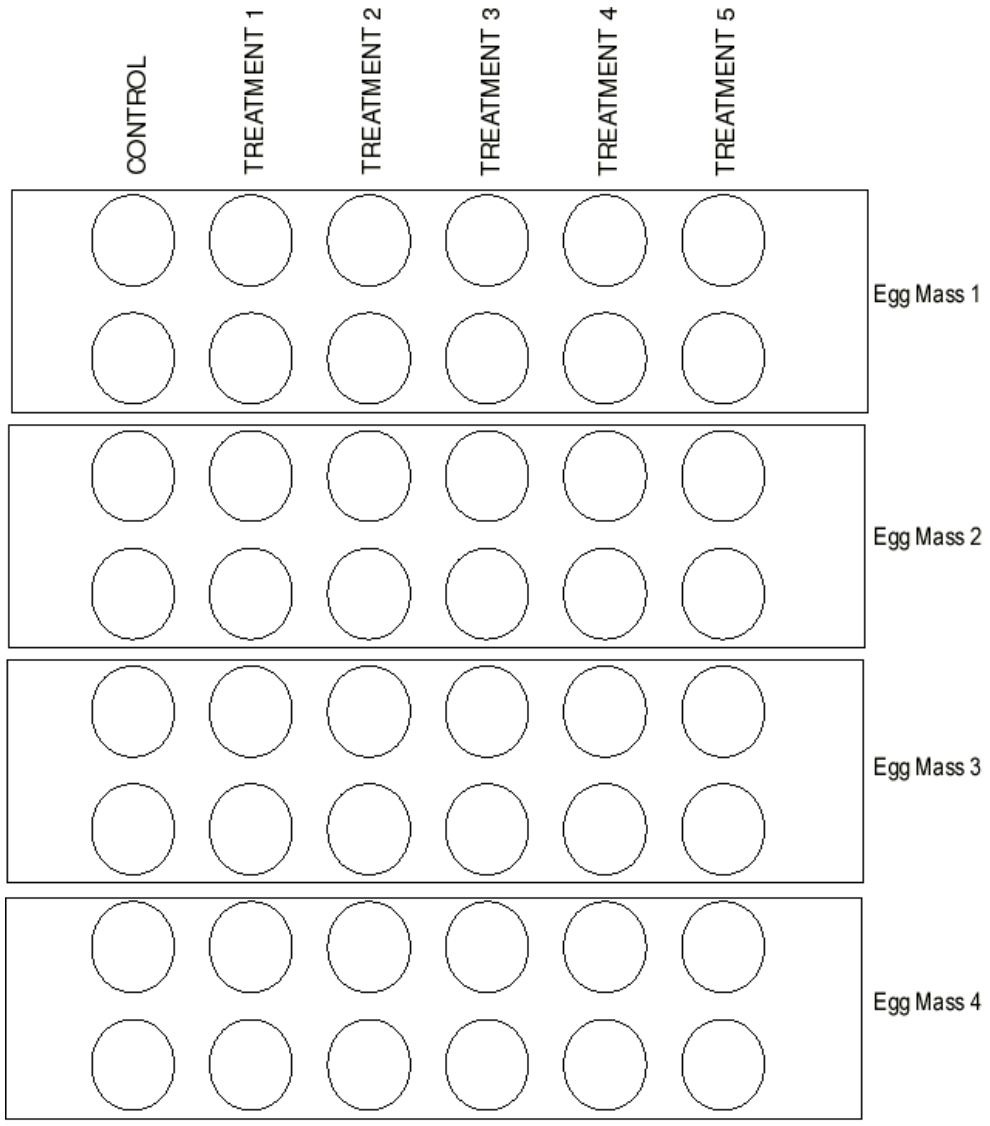


Figure 2.8. Experimental design of the sediment toxicity test

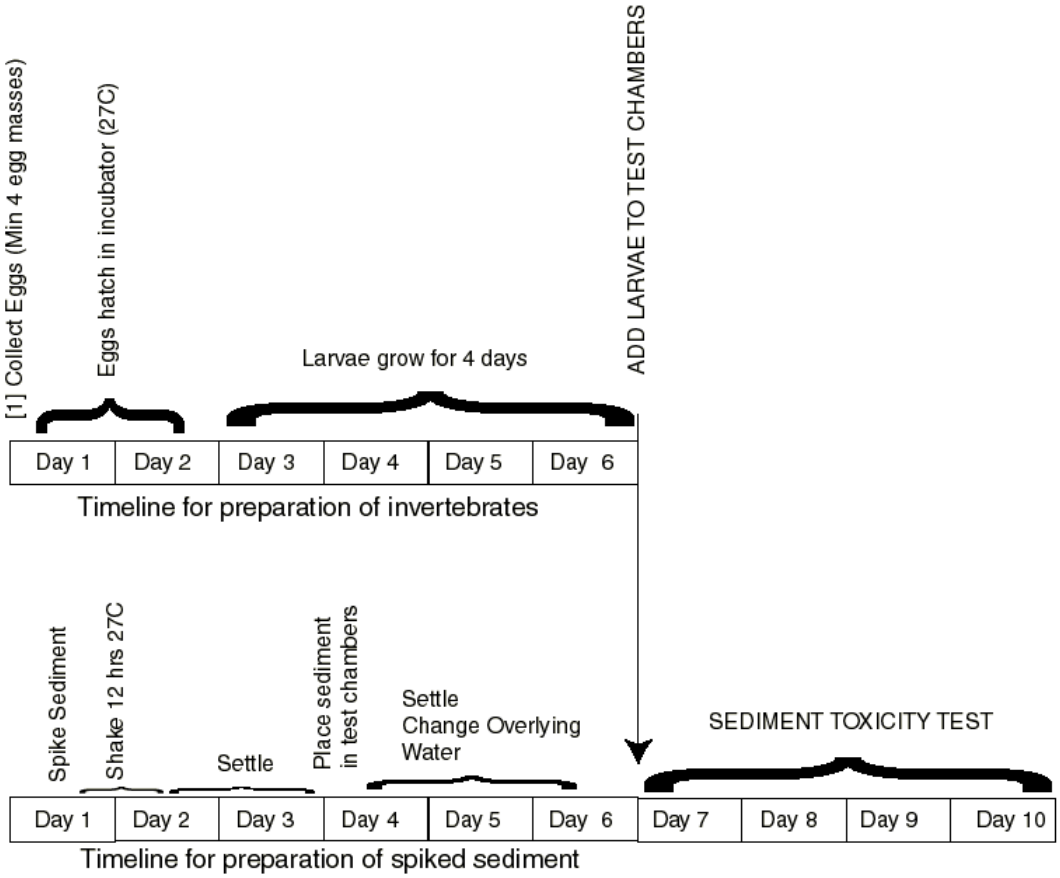


Figure 2.9. Timelines for carrying out the sediment toxicity tests with spiked sediment

2.4 Equipment preparation

All containers used in the experiments were soaked in 5% HNO_3 then rinsed in Millipore treated water (conductivity <0.05 μScm^{-1}). The items were then air dried before experimental work was undertaken.

3 Acute and sublethal responses of *Chironomus crassiforceps* to copper, uranium and pH

3.1 Introduction

The toxicity of copper and uranium to *Chironomus crassiforceps* were tested as they were considered metals of potential risk to the Magela Floodplain (Willett, 1992). Copper and uranium in solution differ in their reaction to pH change. Copper speciation in water is relatively simple at pH 6 with total copper predominating as the free cupric ion (Cu^{2+}) and a small contribution from ($CuOH^{+}$). At pH 4 the free cupric ion predominates. The predicted speciation of uranium contrasts with that of copper with a marked shift from polymeric uranyl species at pH 6 to the free uranyl ion UO_2^{2+} and UO_2OH^{+}, considered the toxic forms, at the lower pH of 4 (Markich and Camilleri, 1997). Toxicity of the metals to *C. crassiforceps* was assessed in water-only tests and in metal spiked sediments using the whole sediment toxicity test system developed and described in the previous chapter.

Before undertaking experiments to determine changes in sediment metal bioavailability in response to pH changes, an experiment to determine the effect of pH alone on the sublethal growth endpoint of *C. crassiforceps* was carried out.

To address the question as to whether sediment pH change can affect the toxicity of metal contaminants, sediment was spiked with metal then titrated to pH 4 and 6. A toxicity test using *C. crassiforceps* was then undertaken to determine whether toxicity increased or decreased with changing pH.

3.2 Methods

3.2.1 Acute tests

The LC_{50} of *C. crassiforceps* to copper in water was determined at pH 4 and pH 6. Sand was collected from Magela creek, sieved through a 500μm sieve; ashed at 400°C to remove any organic material then rinsed in deionised water. For each test the sand was titrated to the required pH using sulphuric acid or sodium hydroxide. Metal spiking involved first placing 3g of sand in a petri dish. Solutions containing nominal concentrations of 0.125, 0.250, 0.500, 1, 2 and 4 mg/l Cu were made up and the conductivity between copper spiking treatment solutions was equalised to 32 μScm^{-1} by titrating with 0.01 M sodium sulphate. Each treatment consisted of 4 replicates with 60 ml of treatment water that was changed twice daily. Ten four-day old chironomids were added to each treatment replicate and their survival recorded every 24 hours for 72 hours. The chironomids were fed at the same rate as in the sediment toxicity tests (2mg feed/individual/day). Water was collected from three replicates per treatment at the end of the experiment, acidified to 1% HNO_3 by volume, then analysed by flame atomic absorption spectroscopy. The results were analysed using the MINITAB statistics package (version 11, 1997) probit option. The LC_{50} of *C. crassiforceps* to uranium in water was determined at pH 4 and 6 by setting up replicates as above. Stock solutions with a nominal uranium concentration range of 0, 3, 5, 10, 25, 50 and 100 mg/l U were made up using uranyl sulphate. The conductivity between uranium spiking treatment solutions was again equalised to 32 μScm^{-1} by titrating with 0.01 M sodium sulphate.

3.2.2 Sediment toxicity range-finder tests

Sediment was collected from a backflow billabong approximately 300m south of gauging station (GS) 8210009, located several kilometres downstream of Ranger

Uranium Mine (12° 32.644′ S 132° 53.041′ E). Collected sediment was sieved through a 5mm sieve then stored at 4°C (ASTM, 1990). A range-finder test was undertaken without replication using copper spiked sediment (following the methodology described in section 2.3.3) with a nominal concentration range of 0, 150, 300, 1500, 3000, 6000 mg Cu/kg sediment dry weight. Ten chironomids per treatment were then tested for survival and mean individual mass. The test was repeated for uranium with a nominal concentration range of 0, 60, 600, 6000, and 8400 mg U/kg dry weight. The number of surviving chironomids were recorded and analysed using the probit option in the MINITAB statistics package (version 11, 1997).

3.2.3 Sediment copper toxicity test

Sublethal toxicity to copper was determine by spiking collected sediment with 0, 125, 250, 500, 1000 and 2000 mg Cu/kg dry weight sediment. Following this initial test a second test with a nominal range of 0, 200, 400, 800 and 1000 mg Cu/kg sediment dry weight was undertaken. At the end of the experimental run, pH and conductivity of the overlying water were recorded in three replicates from each treatment. Overlying water from 3 replicates in each treatment was sampled, acidified to 1% HNO_3 by volume and analysed for copper concentration. The sediment was treated using a two-stage extraction sequence as follows:

Porewater copper concentration

The remaining overlying water was decanted through a 250 μm sieve then approximately 30 ml of sediment from three replicates per treatment was transferred into a 50 ml centrifuge tube. Ten ml of 1,1,2-trichloro-trifluoroethane was added in a fume cupboard and the tube sealed. The sediment was centrifuged at 2500 rpm for 20 minutes then 5 ml of overlying water was collected and filtered through a 45μm filter. The use of a dense, inert hydrocarbon increases the recovery of pore water (Batley and

Giles, 1979). Preliminary experiments verified the inert nature of the hydrocarbon, which did not complex copper from solution. The filtrate was made up to 10 ml, acidified to 1% HNO_3 by volume, and analysed for copper on a Perkin-Elmer Atomic absorption spectrometer (AAS). Any chironomids remaining accidentally in the sediment were collected and placed in the relevant container in preparation for analysis.

Organically bound copper

A diethelyenetriaminepentacetic acid (DTPA) extraction method (Rayment and Higginson, 1992) was used to determine copper bound to organic matter. The 1,1,2-trichloro-trifluoroethane was poured into a storage container in a fume cupboard and 10cm^3 of sediment was washed once by shaking for 2 hours with 20 ml deionised water then centrifuged. The water was poured off and 20 ml of extractant solution (0.005M DTPA, 0.01M $CaCl_2$ and 0.1M TEA – triethanolamine adjusted to pH 7.3) was added. Extraction was undertaken on a horizontal shaker for 2 hours at 25°C. Samples were then centrifuged and 1 ml of supernatant collected before being made up to 10 ml, acidified to 1% HNO_3 and analysed for copper. The sediment was oven-dried overnight at 105°C, weighed and the results are given in mg copper/kg dry weight sediment.

Overlying water was decanted from the remaining replicates and ethanol added to a depth of 2 cm. Chironomids were removed from the sediment by sorting on a white sorting tray and periodically sieving the sediment through a 250 μm sieve. Chironomids were counted for survival and placed in 50ml containers representing each replicate. The chironomids from each replicate were then pooled and dried for 24 h on a Teflon drying tablet at 75°C. The chironomids were then cooled in a desiccator and weighed on a microbalance (Mettler AT 261 Delta Range - sensitivity 0.01mg). Data was analysed as described in section 2.3.4 using two-way ANOVA and by linear regression of arcsine

transformed percentage data against porewater, organically bound and nominal total metal concentrations in sediment.

3.2.4 Lethal and sublethal effect of pH on *C. crassiforceps*

A 0.05M solution of ANALAR H_2SO_4 was prepared and a series of stock solutions containing only deionised water were adjusted to pH 6.5, 6, 5, 4.5, 4 and 3.5. The conductivity of each solution was adjusted to 350 μScm^{-1} by titration with a solution of sodium sulphate. Washed and dried sand (50g) was added to each replicate in the test chambers and the overlying water added for each treatment from the stock solutions. The toxicity test was initiated and the water was renewed twice daily using the stock solutions. pH was measured twice daily to ensure pH stayed within 0.1 pH of the set level. After 4 days the test was terminated and the chironomids recovered, dried and weighed.

3.2.5 The effect of sediment pH changes on copper toxicity

The spiking methodology was changed from that described in the initial copper experiment. For each treatment 1 litre of sediment was added to 4.5 litres of deionised water and the copper sulphate for each spike dissolved in 500ml of deionised water. The copper solution was added to the sediment and stirred continuously for 5 minutes then left for 24 hours. A series of sediments were set up giving an approximate sediment concentration range of copper (in mg/kg) of 0, 400, 600, 1200, 1600, 1800. The pH of the sediment/water mixture was adjusted to pH 6 by titrating with NaOH while stirring continuously. The conductivity of the mixture was equalised between treatments at 1200 μScm^{-1} with sodium sulphate. The sediment was left to equilibrate for 48 hours and the pH readjusted if required. The overlying water was poured off (after samples for copper analysis were removed and filtered (<0.45µm)) and 100 ml was transferred to

the test chambers and the toxicity test carried out as above. The pH and conductivity of the spiked sediment was recorded in three replicates (Rayment and Higginson, 1992). The nominal copper concentration in sediment from spiking was calculated after accounting for loss in water poured off after spiking. Data was analysed as described in section 2.3.4 and NOEC, LOEC, regressions and EC_{50} values for the two pH regimes were compared.

3.3 Results

3.3.1 Acute tests

The acute toxicity of copper increased approximately three times from pH 4 to pH 6 (Figure 3.1). As with copper, the toxicity of uranium decreased at the lower pH of 4. The acute toxicity of uranium (72 hour LC_{50} 36 mgl^{-1} - pH 6) was low compared to that of copper (72 hour LC_{50} 0.64 mgl^{-1} - pH 6). Even in molar terms uranium is a factor of ten less toxic than copper (72 hour LC_{50} for uranium is 1.512×10^{-4} M as opposed to 1.007×10^{-5} M for copper). See Table 3.1 for a summary of the uranium toxicity results.

3.3.2 Sediment toxicity rangefinding tests

The estimated sediment nominal LC_{50} from the non-replicated tests was approximately 1291 mg Cu/kg sediment dry weight and 8800 mg U/kg sediment dry weight based on probit analysis using the MINITAB statistics package (version 11, 1997).

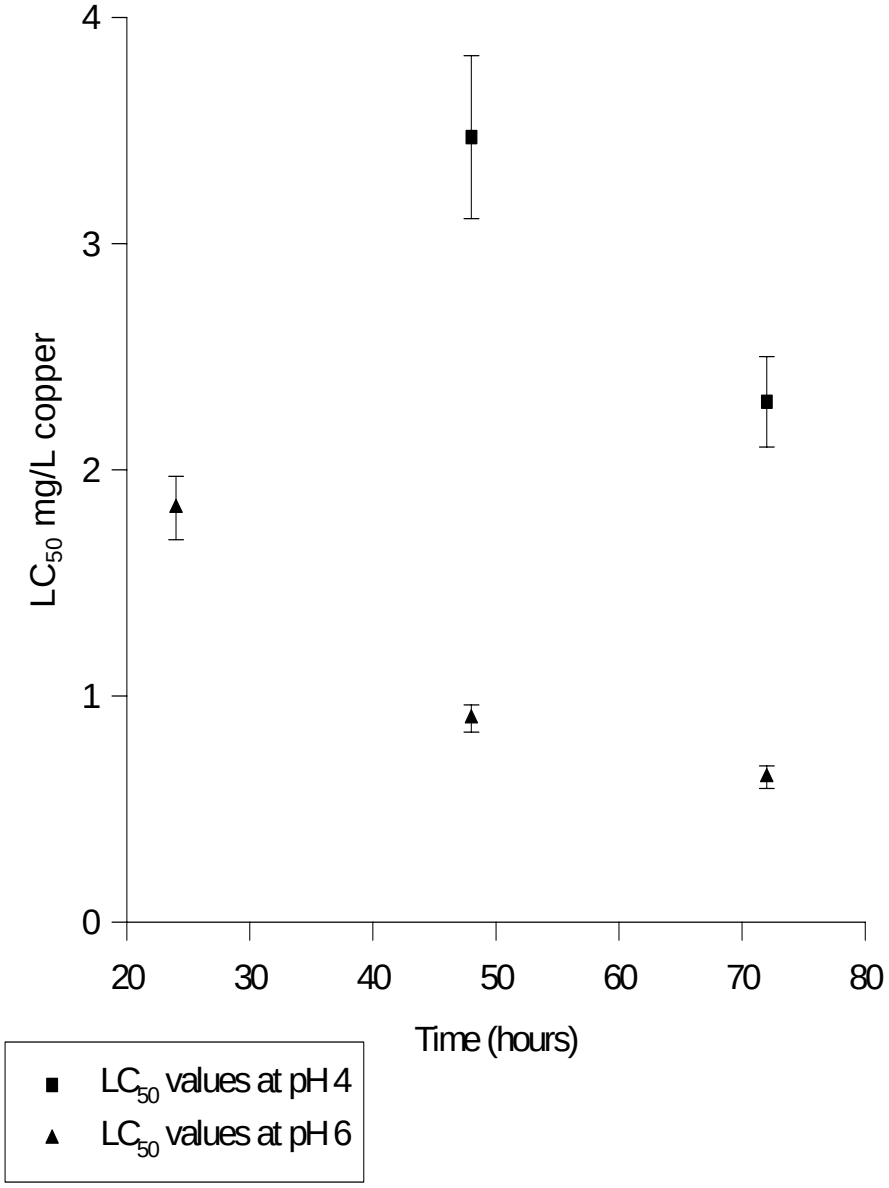


Figure 3.1. The aqueous LC_{50} of copper to C*hironomus crassiforceps* with 95% confidence intervals (n=4)

At pH 6 Time	72 hours
LC_{50} (mg/l)	**36** (95% CI 34 – 39)
At pH 4 Time	72 hours
LC_{50} (mg/l)	**58** (95% CI 52 – 64)

Table 3.1. Acute water-only toxicity of uranium with 95% confidence intervals

3.3.3 Sediment copper toxicity test

Two-way analysis of variance was carried out on the mean dry weight of chironomids in each replicate with parental source (egg mass) used as a blocking factor. Results are shown in Table 3.3. Parental source and treatment showed significant effects on body mass after 5 days ($p<0.001$). Dunnets test gave a LOEC of 400 mg Cu/kg sediment dry weight and a NOEC of 200 mg Cu/kg dry weight. In terms of porewater copper concentrations the LOEC was 0.30 mg/l copper with a NOEC of 0.08 mg/l copper.

Linear regression of arcsine transformed percentage response data (Figures 3.2, 3.3, 3.4) was carried out using GraphPad Prism (version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

A regression of arcsine transformations of percentage response, scaled to the highest control value (set at 100%), against porewater copper concentration gave a slope of -0.315 (±0.004) and intercept of 1.09 (± 0.04) with an $r^2=0.57$. The slope was significantly different to zero ($F=60.97$; $df_n = 1$, $df_d = 46$; $p<0.0001$).

For response against organically bound copper a slope of -0.00239 (±0.00026) and an intercept of 1.274 (±0.04876) was obtained with $r^2=0.6512$ and slope significantly different to zero ($F=85.87$; $df_n=1$. $df_d= 46$; $p<0.0001$).

For nominal copper concentration a slope of -0.0007819 (±0.000083) and an intercept of 1.301 (±0.0517) was obtained at $r^2=0.64$ and a slope significantly different to zero ($F=83.87$; $df_n=1$, $df_d=46$; $p<0.0001$). The EC_{50} values and their 95% confidence intervals were estimated (Zar, 1997) and are shown in Table 3.3.

Nominal sediment copper spike concentration against pH is shown in Figure 3.5. A trend of decreasing pH with increasing copper spike is observed. The linear regression (slope =-0.000927 (± 0.000116), y intercept 5.52 (± 0.07)) shows slope significantly

different to zero ($F=64.21$; $df_n=1$, $df_d=46$; $p<0.0001$) and an $r^2=0.58$. The deionised water used to renew the overlying water had a pH of 5.82.

Source of variation	d.f.	Sum of squares	F-value	p-value
Copper spiked sediment	5	0.690	29.53	<0.001
Egg mass	3	0.211	15.08	<0.001
Interaction	15	0.128	1.83	0.09
Error	24	0.112		
Total	47	1.141		

Table 3.2. Two-way ANOVA to test for the effect of copper spiked sediments and parental source (egg mass) on chironomid body mass on termination of the toxicity test.

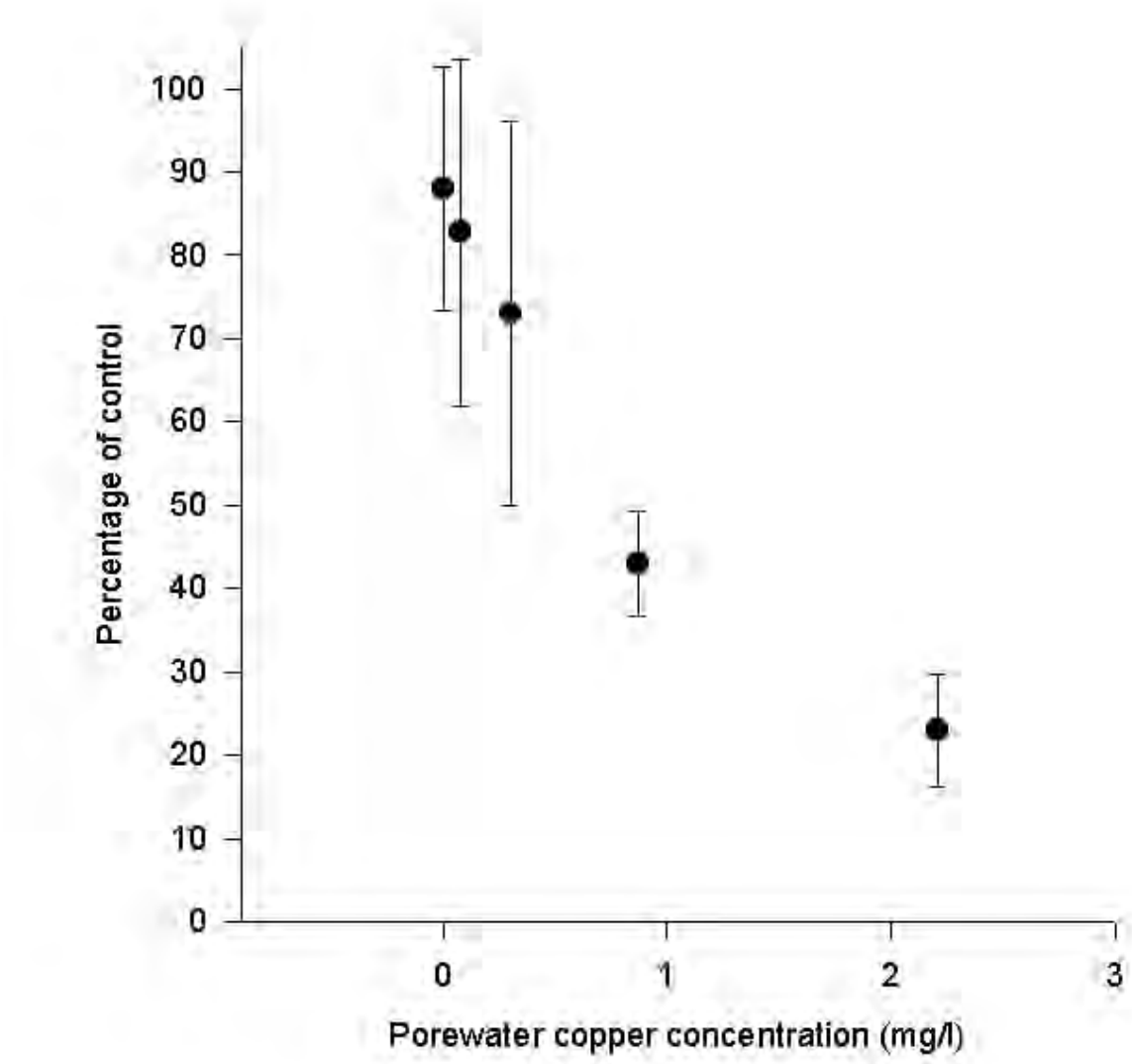


Figure 3.2. Graph of body mass after four days against porewater copper concentration with 95% confidence intervals (n=8)

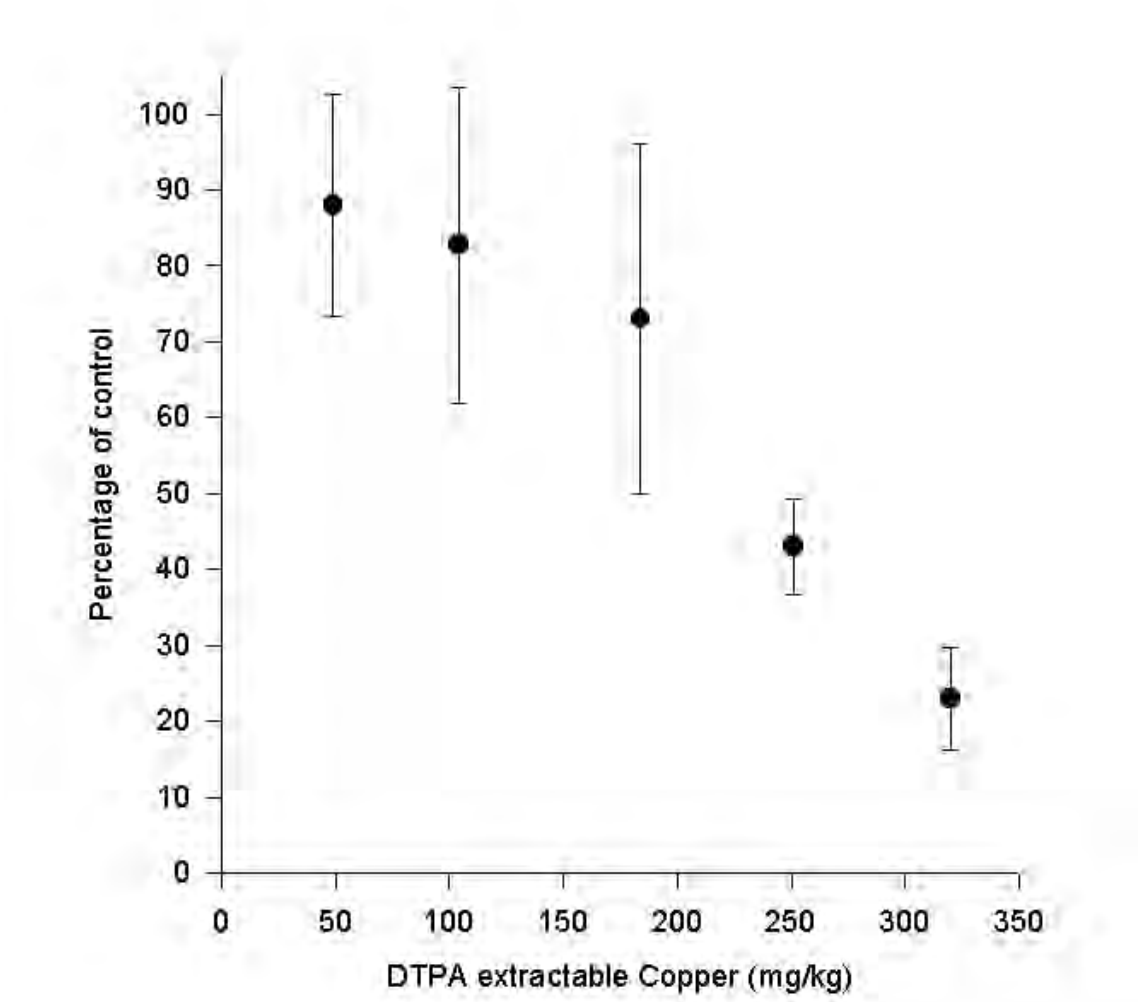


Figure 3.3. Graph of body mass after four days against organically bound (DTPA extractable) copper concentration with 95% confidence intervals (n-=8)

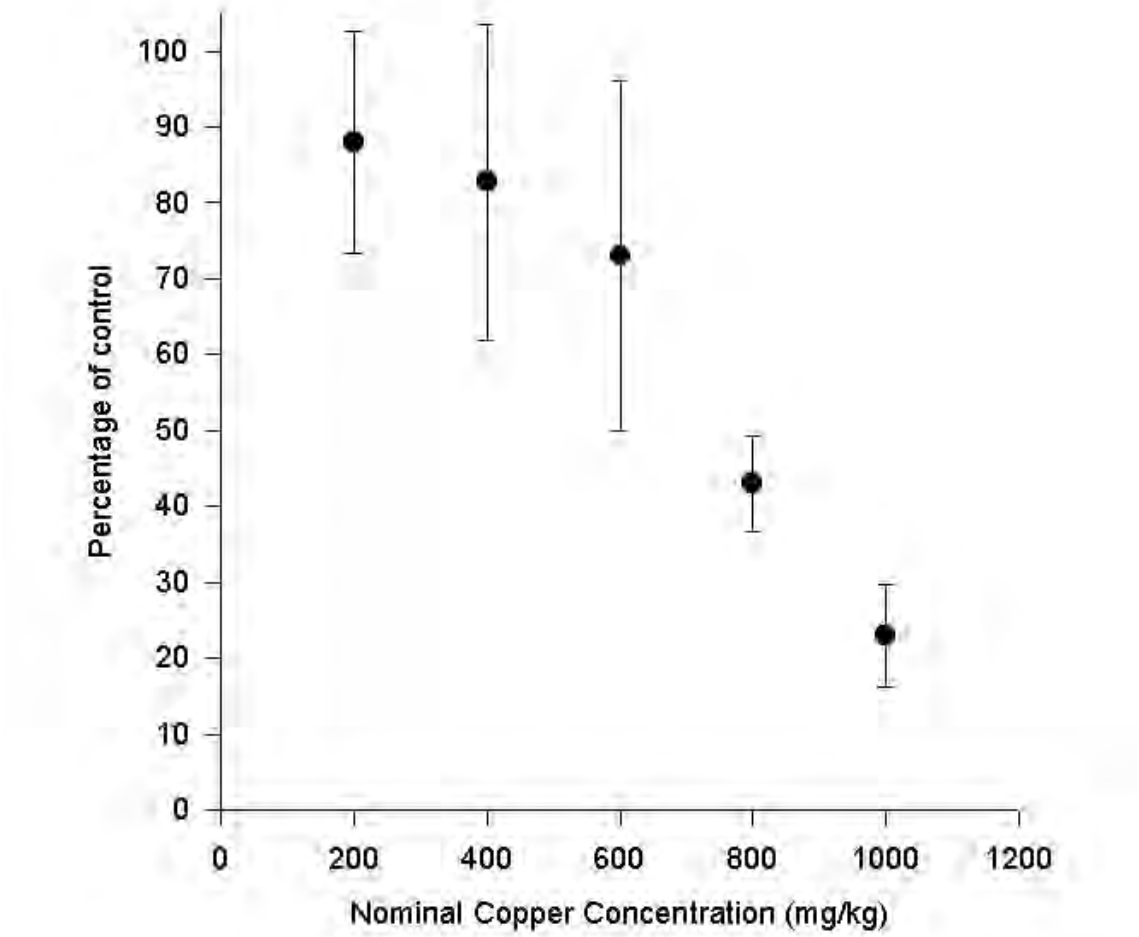


Figure 3.4. Graph of body mass after four days against nominal copper concentration with 95% confidence intervals

	Porewater copper concentration (mg/l) and 95% confidence intervals	**DTPA Extractable copper (mg/kg)**	**Nominal copper (mg/kg)**
EC_{50}	1.73 ($1.37<EC_{50}<2.25$)	265 ($227<EC_{50}<314$)	832 ($715<EC_{50}<981$)
LOEC	0.30	104	400
NOEC	0.08	48	200

Table 3.3. Copper EC_{50}, LOEC and NOEC results for *C. crassiforceps*

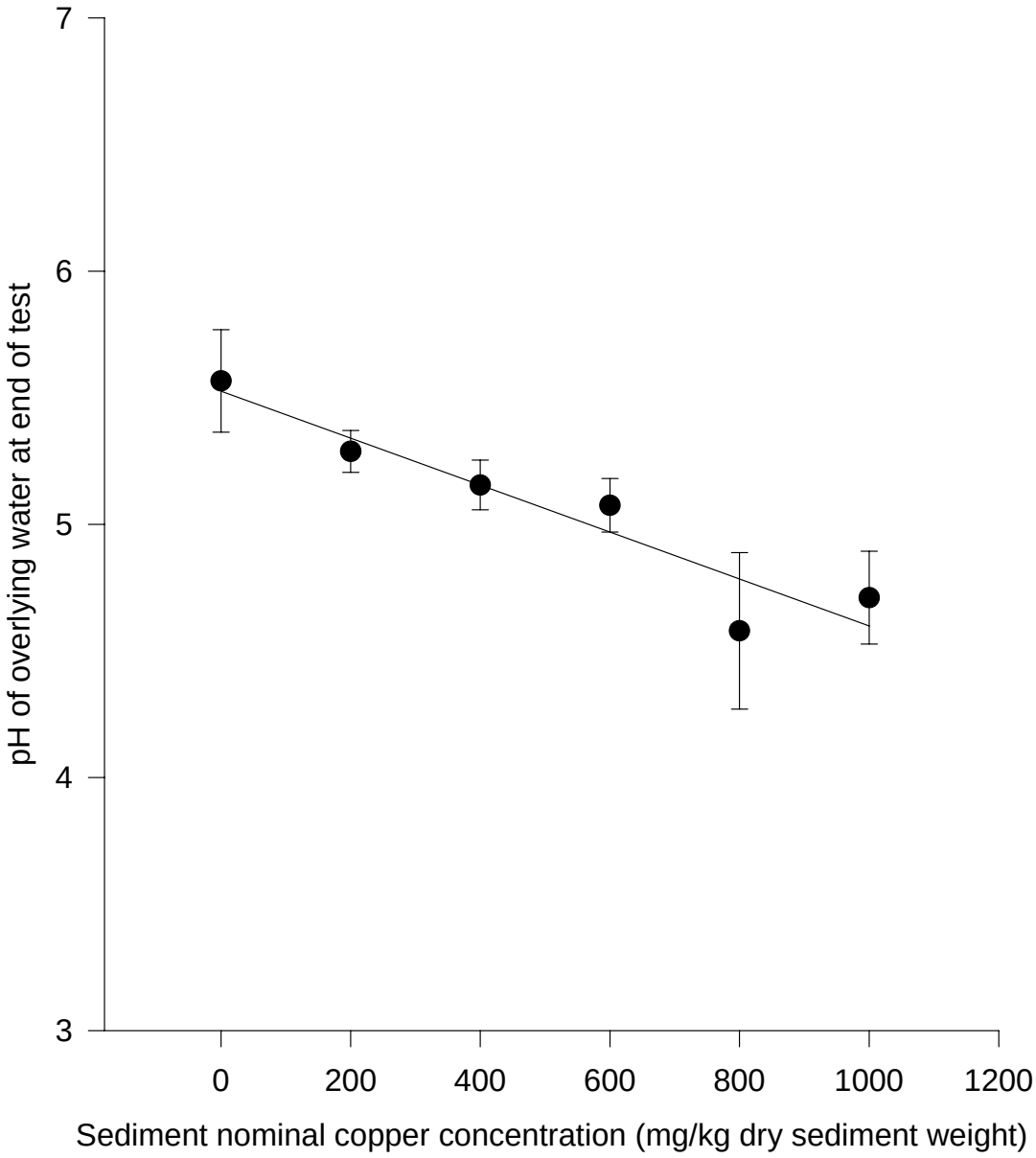


Figure 3.5. pH of overlying water at test termination against sediment nominal copper spike with 95% confidence intervals (n=8).

3.3.4 Lethal and sublethal effect of pH on *C. crassiforceps*

There was greater than 80% survival in all treatments so no mortality attributable to pH was observed even at pH 3.5. Figure 3.6 shows the mean weight per individual and the 95% confidence intervals. A trend of continuously increasing growth performance with pH can be observed. Linear regression of arcsine transformed data gives a slope of 0.172 (±0.0269) and an intercept of 0.246 (±0.1331) with a r^2= 0.4707. The slope is significantly different to zero ($F=40.91; df_n=1, df_d=46; p<0.0001$).

3.3.5 The effect of sediment pH changes on copper toxicity

3.3.5.1 Chemistry results

Porewater copper concentration against nominal concentration at pH 4 is shown in Figure 3.7. Fitting a linear regression through the points gave a r^2 value of 0.89 with slope significantly different to zero ($F=132.83$; $df_n=1$, $df_d=16$; $p<0.0001$).

However the data shows exponential character thus to ensure a more gaussian relationship and equalise variance across replicates the porewater copper concentration data was log transformed (log (porewater +1)), Figure 3.8. Linear regression of the log transformed data gave $r^2 = 0.97$ ($F=547.12$; $df_n=1$, $df_d=16$; $p<0.0001$). At pH 6 linear regression of porewater copper concentration against nominal concentration (Figure 3.9) gives $r^2=0.97$ ($F=586.94$; $df_n=1$, $df_d=16$; $p<0.0001$).

Porewater copper concentrations plotted against overlying water copper concentrations are shown in Figure 3.10. At pH 6 overlying water copper concentrations are extremely low in comparison to sediment porewater copper concentrations. At the lower pH of 4 overlying waters tend to reflect the concentration of porewater.

3.3.5.2 Biological response

Results of a two-way analysis of variance testing treatment and parental source as factors in chironomid dry mass at the end of the tests at pH 4 and 6 are shown in Tables 3.4 and 3.5. Results of Dunnets test to determine LOEC and NOEC values are shown in Table 3.6.

Percentage response (scaled to control dry weight) was arcsine transformed then regressed against the concentration variable (Figures 3.11 and 3.12). Linear regression was performed using GraphPad Prism (version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). For data at pH 6 linear regression gave an r^2=0.60 with slope significantly different to zero (F=67.99; df_n=1, df_d=46; p<0.0001). At pH 4 linear regression gave an r^2=0.358 with slope significantly different to zero (F=25.66; df_n=1, df_d=46; p<0.0001). Using analysis of covariance slopes were found to be significantly different to each other (F=4.61; df_n=1, df_d=92; p=0.03). Log transformation of porewater data had an insignificant improvement in fit (0.4%). EC_{50} values and 95% confidence intervals were estimated (Zar, 1996) for porewater copper and nominal copper sediment concentrations (Table 3.7). At pH 4 the EC_{50} value for porewater copper was 9.58 (with 95 % confidence interval 7.10 – 14.38). At pH 6 the EC_{50} value was 6.08 (with 95% confidence interval 5.00 – 7.40).

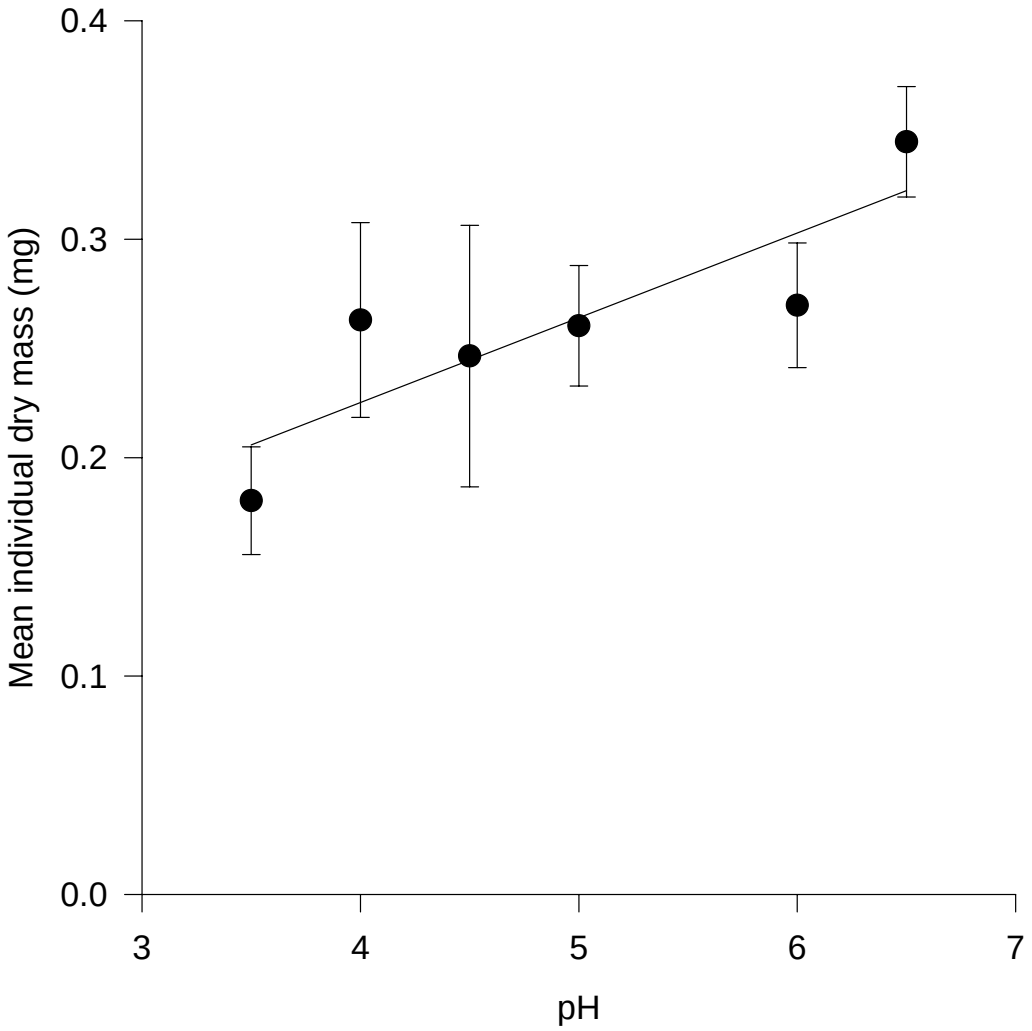


Figure 3.6. Graph of body mass against pH after 4 days with 95% confidence intervals (n=8)

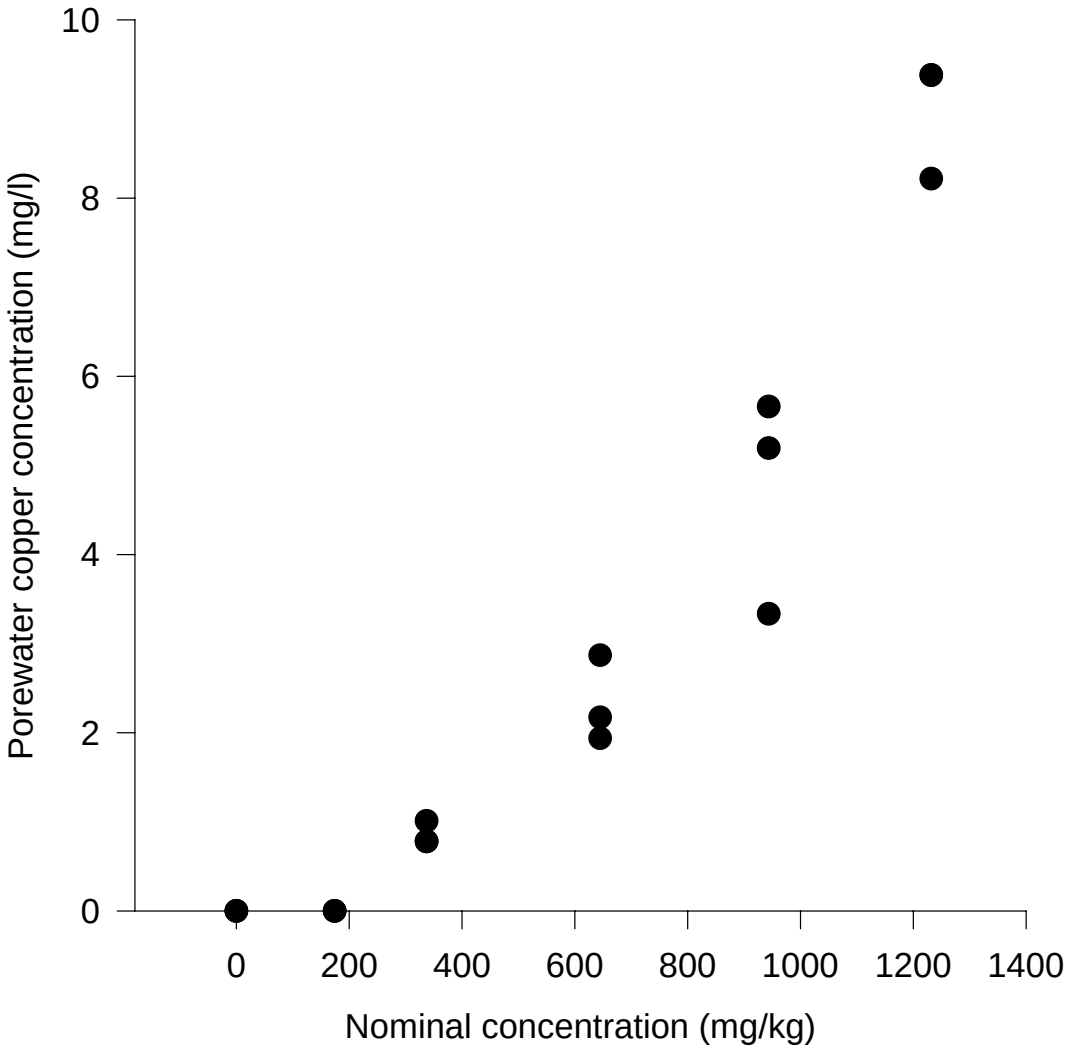


Figure 3.7. Porewater copper concentration against nominal spike at pH 4

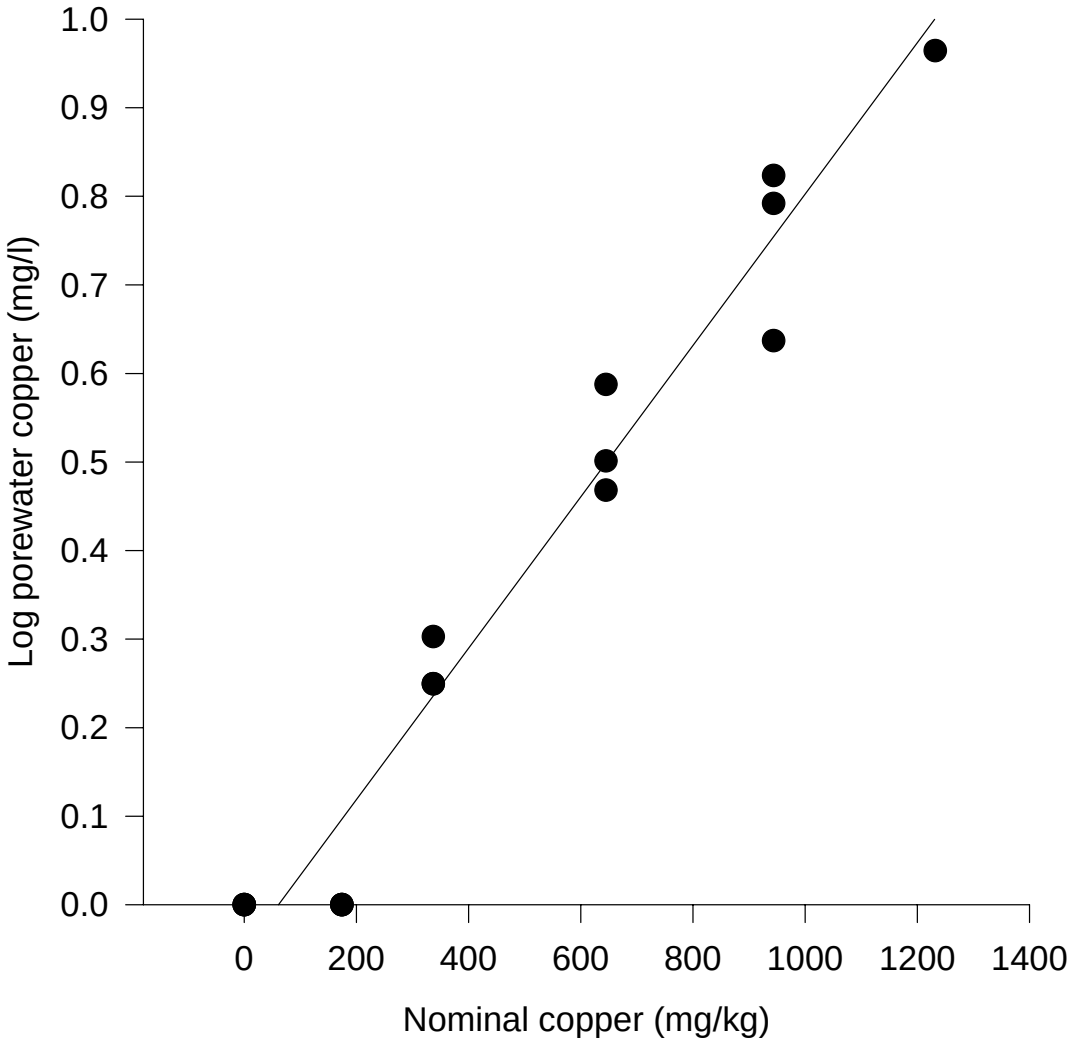


Figure 3.8. Plot of the log (porewater copper concentration +1) against nominal sediment copper concentration at pH 4

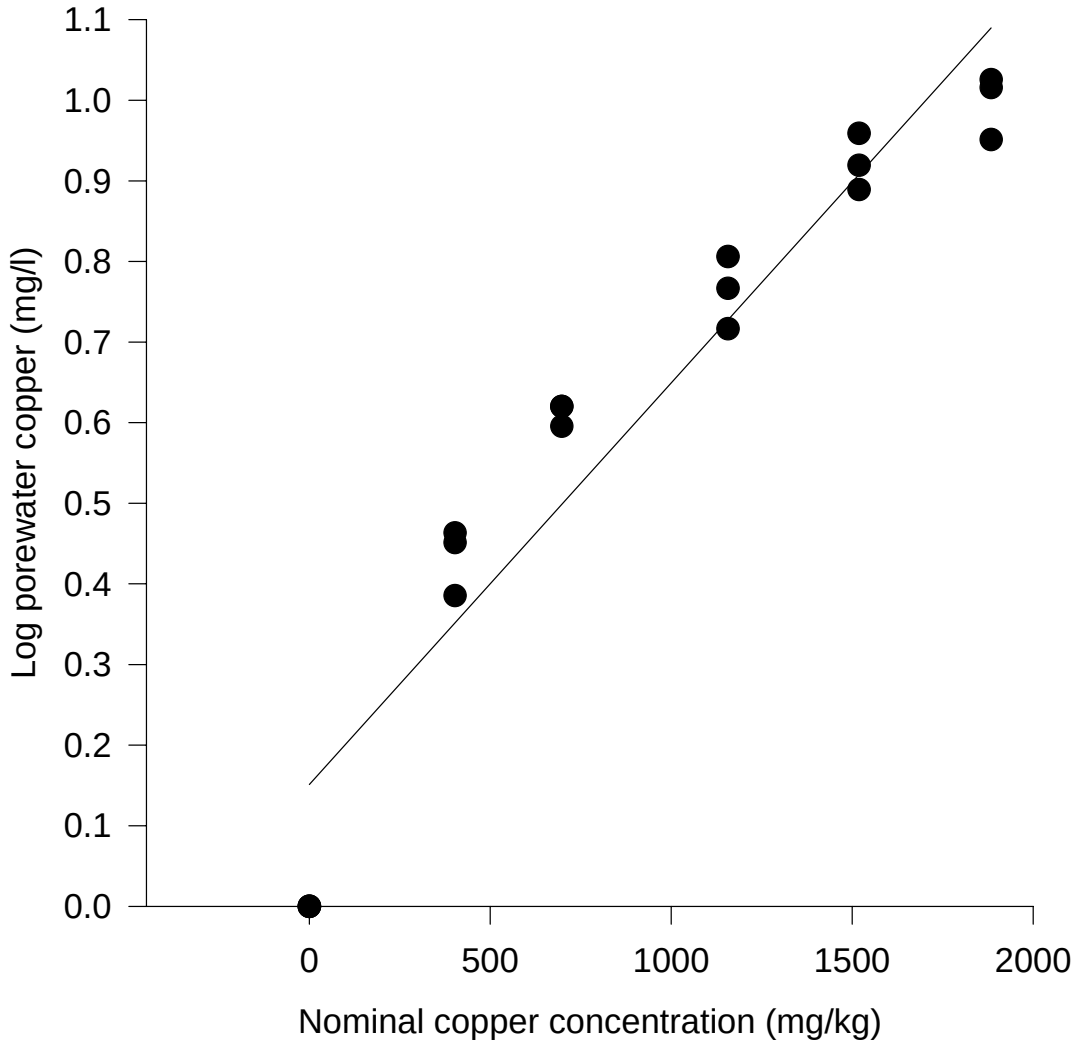


Figure 3.9. Plot of log porewater copper concentration against nominal sediment copper concentration at pH 6

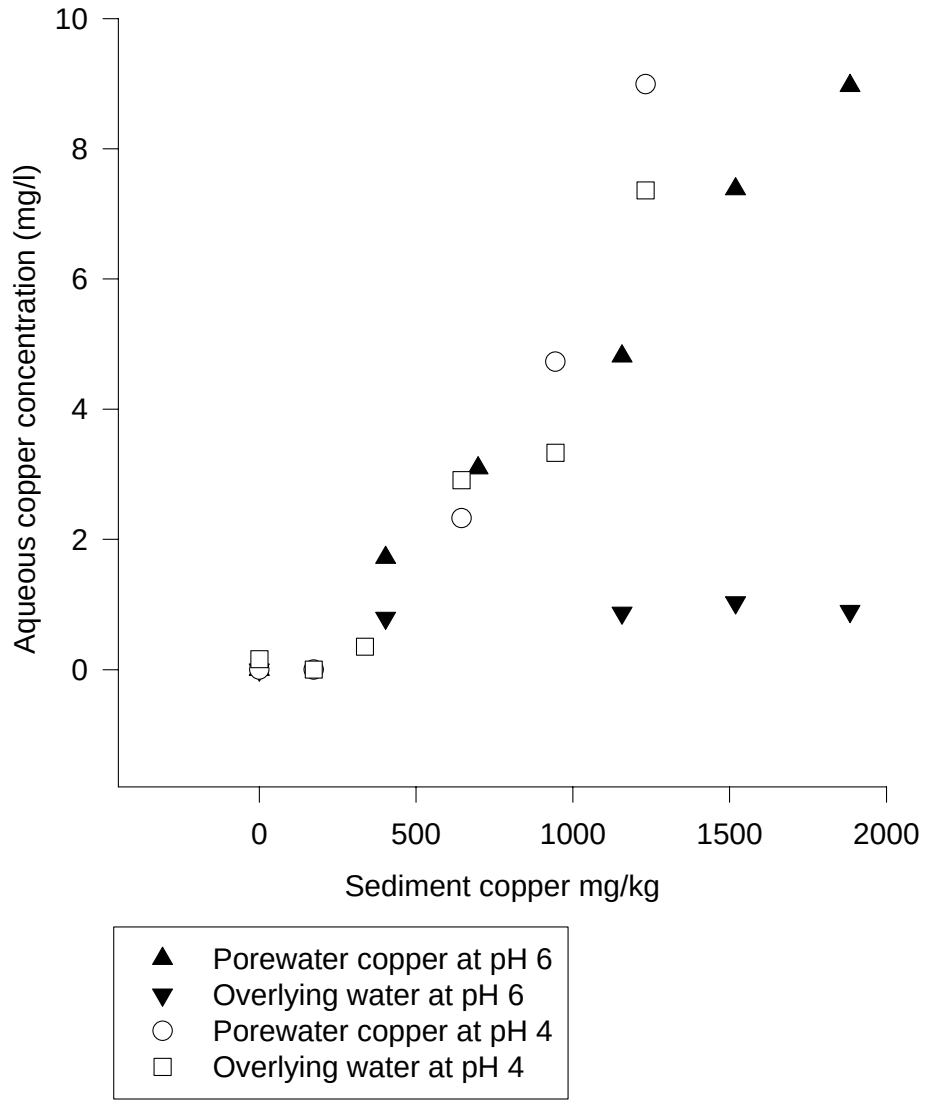


Figure 3.10. Graph of porewater and overlying copper concentration against total sediment copper concentration at pH 4 and pH 6

Table 3.4. Two-way ANOVA to test the effect of copper spiked sediments at pH 6 and parental source (egg mass) on chironomid body mass on termination of the toxicity test.

Source of variation	d.f.	Sum of squares	F-value	p-value
Copper spiked sediment	5	0.0495	21.46	<0.001
Egg mass	3	0.0011	0.78	0.514
Interaction	15	0.0074	1.07	0.427
Error	24	0.0111		
Total	47	0.0691		

Table 3.5. Two-way ANOVA to test the effect of copper spiked sediments at pH 4 and parental source (egg mass) on chironomid body mass on termination of the toxicity test.

Source of variation	d.f.	Sum of squares	F-value	p-value
Copper spiked sediment	5	0.113	13.12	<0.001
Egg mass	3	0.041	7.97	0.001
Interaction	15	0.0244	0.95	0.532
Error	24	0.0413		
Total	47	0.219		

	pH 4		pH 6	
	Porewater (mg/l)	Nominal (mg/kg)	Porewater (mg/l)	Nominal (mg/kg)
LOEC	4.73	944	1.72	403
NOEC	2.32	644	<0.01	22

Table 3.6. Porewater and Nominal LOEC and NOEC values for copper spiked sediments.

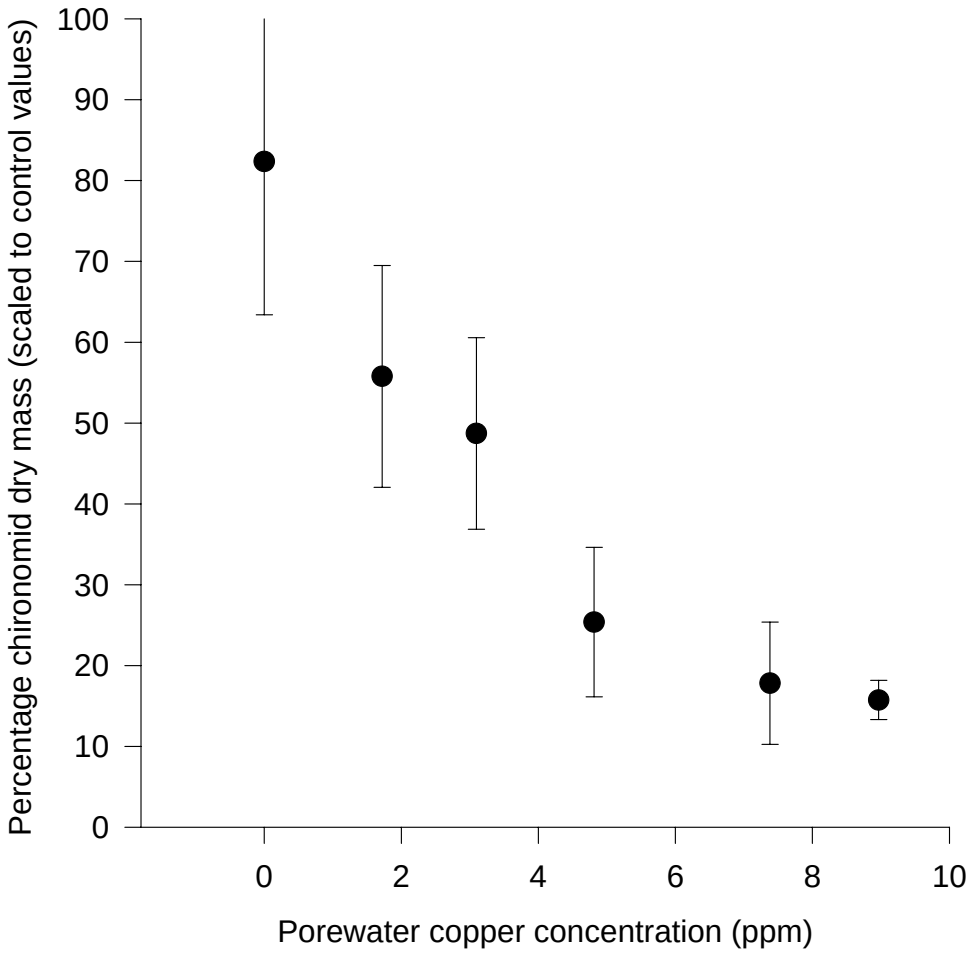


Figure 3.11. Graph of body mass against mean porewater copper concentration after four days at pH 6 with 95% confidence intervals (n=8)

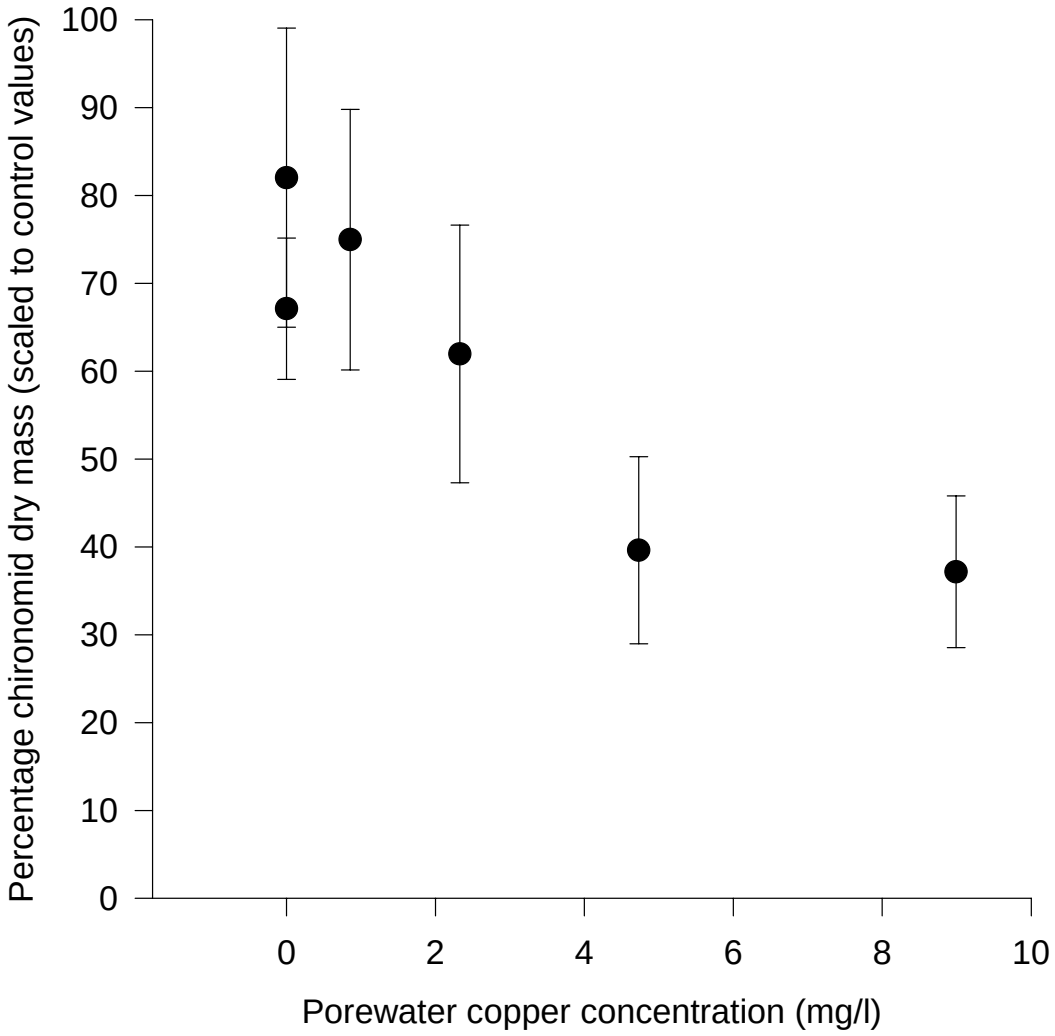


Figure 3.12. Percentage response against mean porewater copper concentration at pH 4 with 95% confidence intervals (n=8)

Sediment pH	Porewater EC_{50} (mg/l)	Nominal copper concentration (mg/kg)
pH 6	6.08 (95% Confidence intervals 5.00 – 7.40)	1270 (95% Confidence intervals 1056 – 1518)
pH 4	9.58 (95% Confidence intervals 7.10 – 14.38)	1292 (95% Confidence intervals 1024 – 1763)

Table 3.7. EC_{50} values for porewater and nominal sediment copper concentrations.

3.4 Discussion

A literature review of copper toxicity to chironomids was undertaken to compare the values obtained in the experiments to other studies and species of chironomid. The review is summarised in Table 3.8. To make the comparison clearer, only the 48 hour LC_{50} value for *C. crassiforceps* at pH 6 – to fit in with the fact that most tests are carried out around neutrality - was compared to literature values and is shown in Figure 3.13. As can be seen the sensitivity of *C. crassiforceps* falls between that of *C. riparius* and *C. tentans*, at a similar level to *Polypedilum nubifer* (also widely distributed throughout SE Asia and recorded from N. Australia - Hatakeyama, 1988). The chironomid is not very sensitive to uranium at either pH although the same trend as for copper can be observed with the toxicity of uranium increasing with an increase in pH.

Measurement of the pH of overlying water in sediment spiked with copper sulphate showed that an artefact of spiking sediment with copper sulphate is that the higher the copper sulphate concentration the lower the pH of the sediment (Figure 3.5). Experimental design should always consider this covariant and decide whether it is necessary to control pH or not, the choice being dependent on the objectives of the study.

	Temp (°C)	Acute Water-only (mg Cu/l)				Pore-water EC_{50} (mg Cu/l)		Sediment (mg Cu/kg)	Study
Chironomid		24 hr LC50	48 hr LC50	NOEC	Other	EC50	NOEC		
Chironomus crassiforceps	27°C	**1.83**	**0.90**		**0.64 (72 hrs)**	**1.37**	**0.08**	**400 (NOEC)**	**This study**
Chironomus riparius	21°C		1.2		0.7 (96 hrs)				(Roswalt and Knight, 1987)
	21°C	0.19			0.19 (10 day LC_{50})				(Taylor et al.,1991)
	21°C		1.17						(Dobbs et al., 1994)
Chironomus decorus	21°C				>2 (72 hrs)				(Knight and Kosalwat, 1987)
	21°C		0.7					5830 (LC50)	(Kosalwat and Knight, 1987)
Chironomus tentans.	21°C		0.53		0.630 (96 hr)	0.06		1026 (LC50)	(Suedel et al., 1996)
	21°C			<0.2		<0.02		<216	(Suedel, et al. 1996)
	21°C				0.054 (10 d)				(Phipps and Ankley, 1995)
	21°C					0.040		NOEC 600	(Suedel et al., 1996)
Polypedilum nubifer	21°C	7.91	0.63						(Hatakeyama, 1988)

Table 3.8. The sensitivity of *Chironomus crassiforceps* and other chironomids to copper

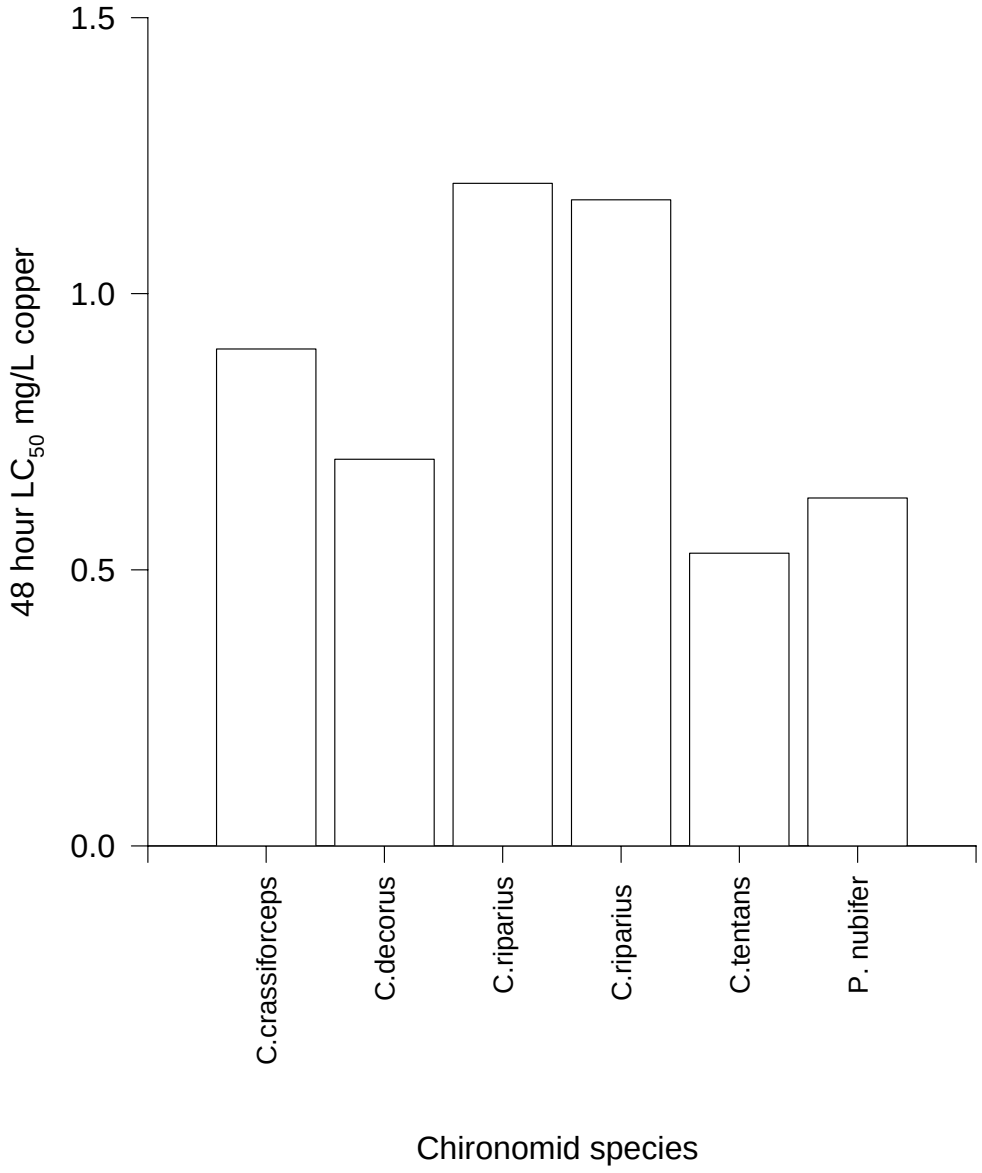


Figure 3.13. Acute toxicity of copper to *Chironomus crassiforceps* and other chironomid species

One of the assumptions of the regression analysis used here is that there is no variability in the independent variable, or that it is relatively small compared to the dependent variable. It is important to consider this assumption for two reasons:

- The regression analysis could be invalid if the independent variable has a high degree of variability
- Field measurements should be based on a concentration variable that is precise

Comparing the variability in measuring copper concentration in porewater, organically bound (i.e. DTPA extractable) or total copper offers an insight into choosing a precise measurement protocol.

The variability in porewater copper concentrations (calculated as 100*95% Confidence Interval/mean) was higher (30%) than DTPA extractable copper (10%) at concentrations causing sublethal effects. DTPA extractable copper would provide less variability in measurement however more work would be required to ensure that DTPA extractable copper is related to biological effects. It may be necessary to relate DTPA extractable copper to organic carbon content since, for the same total copper concentration, at low organic contents much of the copper would be present in porewater, whereas in sediments of high organic content that same copper may be bound and not bioavailable. In summary the use of DTPA to determine toxic sediment suffers from the same problems as using total copper in that it does not necessarily measure the bioavailable form. Porewater concentration is difficult to measure in the field, and has a high degree of variability even when measured in the laboratory, so models (empirical or mechanistic) to determine porewater from easier analyses such as DTPA concentration or total concentration would be useful.

Chironomus riparius larvae have been shown to suffer acute effects at pH 4 and below (Krantzberg and Stokes, 1988) although Gerhardt (1992) found that *C. riparius* has been shown to tolerate pHs down to 3. *Chironomus crassiforceps* can be seen to be relatively acid tolerant with no acute effects at the lowest test pH of 3.5.

Comparing the copper porewater sublethal EC_{50} value (1.73 mg/l – an effect over 4 days) to the 48 hour acute water-only LC_{50} (0.9 mg/l at pH 6, 3.47 at pH 4) it can be seen that copper is less bioavailable in porewater than water. There are potentially a number of reasons for this:

- The correlation between porewater copper concentration and a toxic effect on the chironomid is related to the degree of interaction of *C. crassiforceps* with porewater. The tubes that chironomids inhabit are irrigated with overlying water thus potentially reducing their direct contact with porewater copper.
- Copper forms extremely strong complexes with dissolved organic matter (DOM). The DOM-copper copper complexes are generally considered non-bioavailable (Palmer et al., 1998). Dissolved organic matter concentration in deionised water, in which the LC_{50} tests were carried out, was negligible however porewater, in equilibrium with the organic rich sediment, would be expected to contain dissolved organic matter. It has been observed that 98% of copper is normally complexed with organic matter in soil solution (Plette et al., 1999). This could potentially reduce toxicity in terms of total porewater copper concentration.

The LOEC value for porewater copper at pH 4 (4.73 mg/l) is more than twice that at pH 6 (1.72 mg/l), reflecting results obtained for water-only acute toxicity that also showed increasing toxicity at increased pH.

In terms of nominal sediment copper concentration a LOEC of 944 mg Cu/kg dry sediment at pH 4 compares to 403 mg Cu/kg dry sediment at pH 6. The NOEC value for pH 4 is 644 mg Cu/kg and 22 mg Cu/kg dry sediment at pH 6. Regression analysis expressed in terms of porewater copper concentration again shows the higher copper toxicity at the higher pH.

At pH 6 the relationship between nominal spike and porewater copper concentration is linear, however at the pH of 4 a non-linear relationship is observed. The linear relationship observed is a common experimental observation in sorption mechanisms at low metal concentrations (Hassan et al., 1996). The non-linear relationship (such as that observed at pH 4) is common at higher concentrations. To allow comparison of the two curves, data for porewater copper concentration against nominal concentration at pH 6 was log transformed and regressed showing significant deviation from zero ($F=175$; $df_n=1$, $df_d=16$; $p<0.0001$). The two slopes of the regressions were found to be significantly different ($t=4.9$; $df=32$; $p<0.01$).

It was hypothesised that the major reason for the difference in slopes is that the higher proton concentration at pH 4 in comparison to pH 6 competes for organic binding sites on the sediment with copper thus resulting in the greater metal concentration in the porewater. At pH 6 the porewater EC_{50} is approximately halved, showing increased toxicity of porewater copper as the pH increased.

4 The use of emergence and demographic endpoints

4.1 Introduction

Since the 1980s, research has been undertaken in which the impact of pollutants on population growth rates of indicator species has been measured (Daniels and Allan, 1981; Lenski and Service 1982; Allan and Daniels, 1982; Coniglio and Baudo, 1989; Sibley et al., 1997). Data on survival, fecundity and time to reproduction are quantified against pollutant concentration then integrated into effects on the population growth rate (λ), or the intrinsic rate of population increase (r). Studies of the effects of pollutants on these parameters are termed Life Table Response Experiments (LTRE). They are generally carried out under low, fixed population density and the survival, fecundity and time to reproduction quantified. The LTRE measures the population-level effect of the treatment under the conditions of the experiment and may be regarded as a population-level bioassay of the treatment effects (Newman and Jagoe, 1996).

The use of ecotoxicity testing to explore effects on population dynamics has been suggested by Calow et al. (1997) who found that species with differing life-histories, for instance semeloparous and iteroparous species, had different sensitivities to contaminants. Effects from laboratory testing on population parameters can then be used to estimate effects on different species, life-history types and thus the community. The use of population growth rate is useful in that it incorporates lethal and sublethal effects into a single parameter (Stark et al., 1997a; Stark et al., 1997b; Walthall and Stark, 1997). These effects are survivorship (l_x), time to reproduction (t_x) and reproductive rate (m_x).

From the Lotka equation

$$1 = \sum l_x \, m_x e^{-rmx} \qquad [4.1]$$

Where x is the age of the cohort, l_x is the proportion of individuals surviving to age x, m_x is the number of females produced per female of age x and r_m is the intrinsic rate of increase of the population.

Environmental stress on an individual can express itself in a number of ways that can ultimately result in a decrease in population growth rate. As pollution increases, the ability of an organism to maintain homeostasis becomes compromised and may result in an inability to compensate stress, eventually resulting in death (Sibley et al., 1997). The energy required to maintain homeostasis under environmental stress means that somatic growth tends to be impaired. The increase in mortality together with decreased somatic growth can lead to a reduction in production. Population parameters can integrate these effects.

The instantaneous rate of population increase (r) for a sexually dimorphic, semeloparous species such as *C. crassiforceps* can be estimated from

$$r = \log_e R_0/T \qquad [4.2]$$

where R_0 is the net reproductive rate or the sum of progeny produced by a female over all breeding events of a life cycle ($\sum l_x m_x$) and T is the mean generation time. For *C. crassiforceps* mating and egg laying occurs only once per generation so, for each individual female r can be calculated from survivorship (l), fecundity (number of daughters per female) and mean generation time (T) - time between birth of the parent generation and birth of the F1 generation (Sibley et al., 1997). Using an emergence test

and by measuring egg production it is possible to collect data so as to allow estimation of the demographic parameter.

In this example lambda (λ) is used, which is the finite rate of population increase and here it is expressed in terms of population increase per day. It is related to r as:

$$\lambda = e^{r} \qquad [4.3]$$

Lambda can be estimated from a series of experimental treatments forming the basis of a life table response experiment (Gotelli, 1995).

Demographic parameters have been used in toxicity testing (Daniels and Allan, 1981, Gentile et al., 1982; Stark et al., 1997a) but such estimates obtained are not normally stated with statistical confidence limits. The reason for this is that r or λ values cannot be calculated using closed form equations and, as such, algebraic expressions cannot be used to estimate the variance (Meyer et al., 1986). Since risk assessment demands a level of confidence for an estimate of risk it is important to develop methods to estimate the variability in r or λ. In this regard a number of computer intensive methods are available that make no assumptions about the distribution of λ. Two such procedures are the bootstrap and jack-knife methods (Taberner et al., 1993) which use re-sampling methods to estimate the distribution of the demographic parameters. Previous work (Meyer et al., 1986) has shown that, with high mortality values, the distribution of λ is skewed to low values, even becoming bimodal with a second mode at zero. If this is the case the use of normal-based statistical methods is inappropriate even for ANOVA which although generally robust to deviations from normality, is confounded by heterogeneity of variance. To address this, the data for λ generated in the emergence test was treated to a simple bootstrap procedure developed in EXCEL to graphically show the distribution types at different copper concentrations.

As the demographic parameter integrates lethal and sublethal effects of a contaminant, it is of interest to determine the mode of effect of the chemical under study. For instance is there a specific effect on survival, time to reproduction or on the number of young? Or is it a combined effect? A process known as the decomposition of the rate of population growth (Caswell, 1989) can be used to determine the relative effect of survival, fecundity and time to reproduction giving additional insight into important pollutant effects that may be missed by other endpoints. For example, over a range of concentrations, λ may remain stable. This may mean that the vital rates (l_x, m_x, T) also remain stable but, it could also suggest an increase in say, fecundity with a corresponding decrease in survival or increase in time to reproduction.

4.2 Methods

The set-up of the sediment toxicity test system was changed by sealing the top of each replicate thus enabling any emerged adults to be collected from the replicate with a pooter. Sediment was spiked with copper in the range 0, 200, 400, 600, 800 and 1000 mg Cu/kg sediment dry weight (from this point the term mg/kg will refer to mg Cu/ kg dry sediment). pH was not controlled in this test. The test was started with first instar larvae by adding ten individuals per replicate and following the protocol outlined in 2.3.3.

The number of males and females which emerged daily were recorded and the females transferred to oviposition chambers. The oviposition chambers were 100ml plastic tubes with deionised water to a depth of 1 cm. Males were collected from similar replicates and at least one male was added to the females in the oviposition chambers. The chambers were checked daily for egg masses. Any egg masses oviposited were placed under a binocular microscope and the number of eggs counted.

4.2.1 Data analysis

The data was analysed in terms of total emerged and development rate according to OECD guidelines (OECD, 1998). In addition mean time to emergence and the population multiplication rate (λ) were calculated.

Emergence at each treatment concentration is calculated as shown in equation 4.4.

$$E = \frac{n_e}{n_a} \qquad [4.4]$$

where E is emergence, n_e is the number emerged and n_a the total introduced. The data was arcsine transformed and a 2-way ANOVA performed.

Development rate is calculated as X_I, which is the development rate of midges emerged in interval I as follows;

$$X_i = 1/(day_i - l_i/2) \qquad [4.5]$$

where day_i : inspection day (days since application)

l_i : length of inspection interval I (days)

4.2.1.1 Calculation of the demographic parameter lambda

From the experimental values at each concentration lambda is calculated from

$$\lambda = e^{\log e(lm)/T} \qquad [4.6]$$

where l is survivorship, m is the number of daughters per female and T is generation time in days.

Average lambda is estimated together with the standard deviation. The distribution of lambda is effectively unknown so to explore the statistic further the technique known as bootstrapping is used to estimate bias and precision. A statistic is unbiased if it is centred at the population parameter and precision is a measure of the variation in the sampling distribution. Bootstrapping can be used to estimate bias, variance and confidence intervals (Rago and Dorazio, 1984; Alvarez-Buylla and Slatkin, 1991;

Scheiner and Gurevitch, 1993). It is a computer intensive method that involves two steps. First the unknown distribution of population values is estimated from the sample data then the estimated population is repeatedly sampled to estimate the sampling distribution of the statistic. An Excel macro was programmed in Visual Basic to generate 1000 bootstrap values (Appendix 2). This number of bootstrapped values is the minimum recommended to calculate confidence intervals (Caswell, 1989).

Since population multiplication rate is not usually normally distributed (Alvarez-Buylla and Slatkin, 1991) the mean and confidence limits are calculated by means of the bootstrap procedure by ordering the values in ascending order. The lower 95% confidence limit is estimated as the mean of the 25^{th} and 26^{th} value, and the upper 95% limit as the mean of the 975^{th} and 976^{th} value.

4.3 Results

The total number emerged is shown in Figure 4.1. It is clear that there are effects of copper concentration on mean number emerged and mean emergence time or numbers emerging. For total emerging at each concentration, a LOEC of 800 mg/kg and a NOEC of 600 mg/kg was obtained. Once split into male and female emergence the characteristic emergence of males before females is observed (Figure 4.2).

Fitting a linear regression to development rate and sediment copper concentration (Figure 4.3) a slope significantly different to zero is obtained ($F=19.66$; $df_n=1$, $df_d=4$; $p=0.01$) with a value for $r^2=0.83$. An EC_{50} of 941 mg/kg is obtained. Total pooled survivorship for males and females, measured as survival to emergence is shown in Figure 4.4 with maximum survival shown at around 50%.

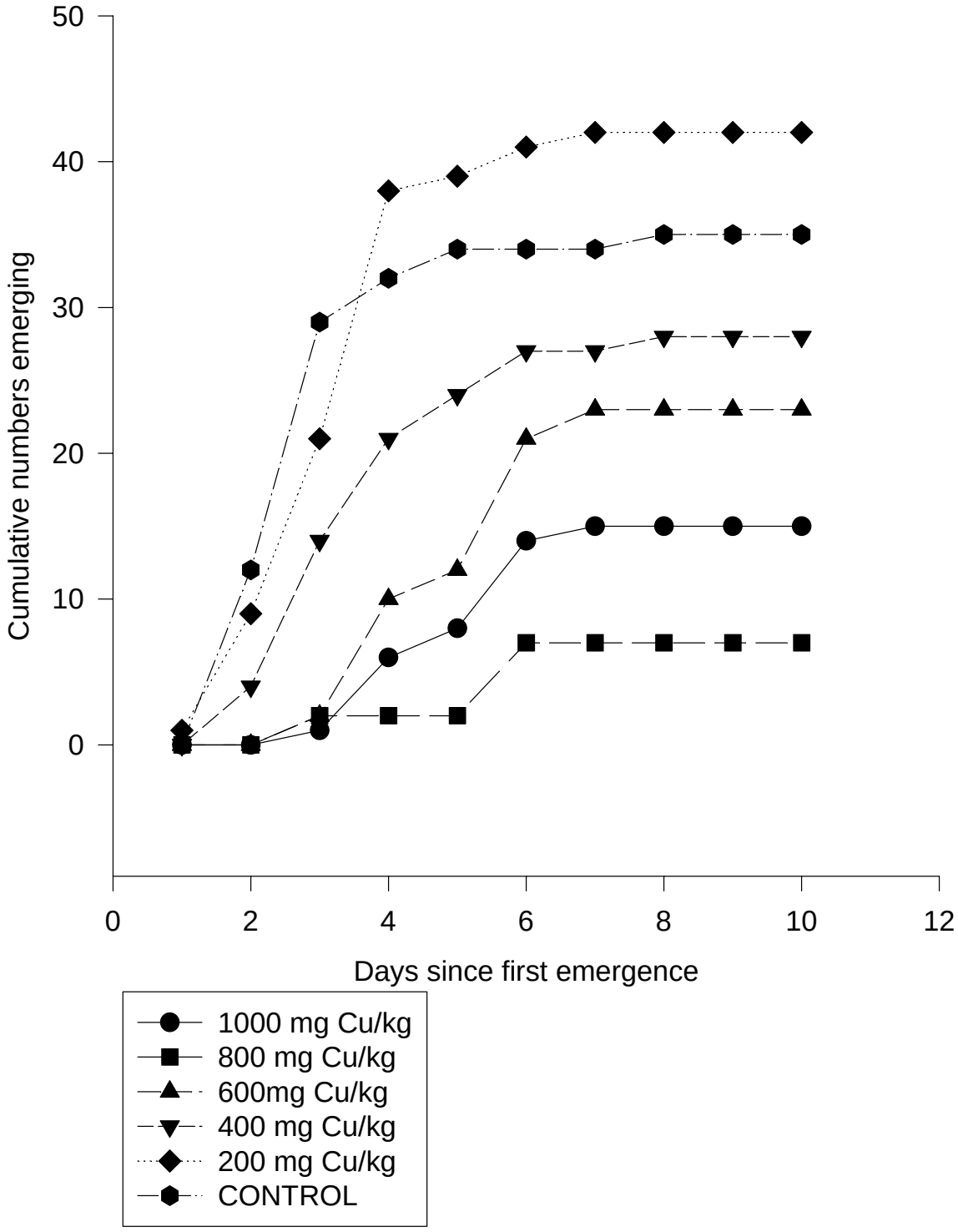


Figure 4.1. Total emergence against time for different treatments

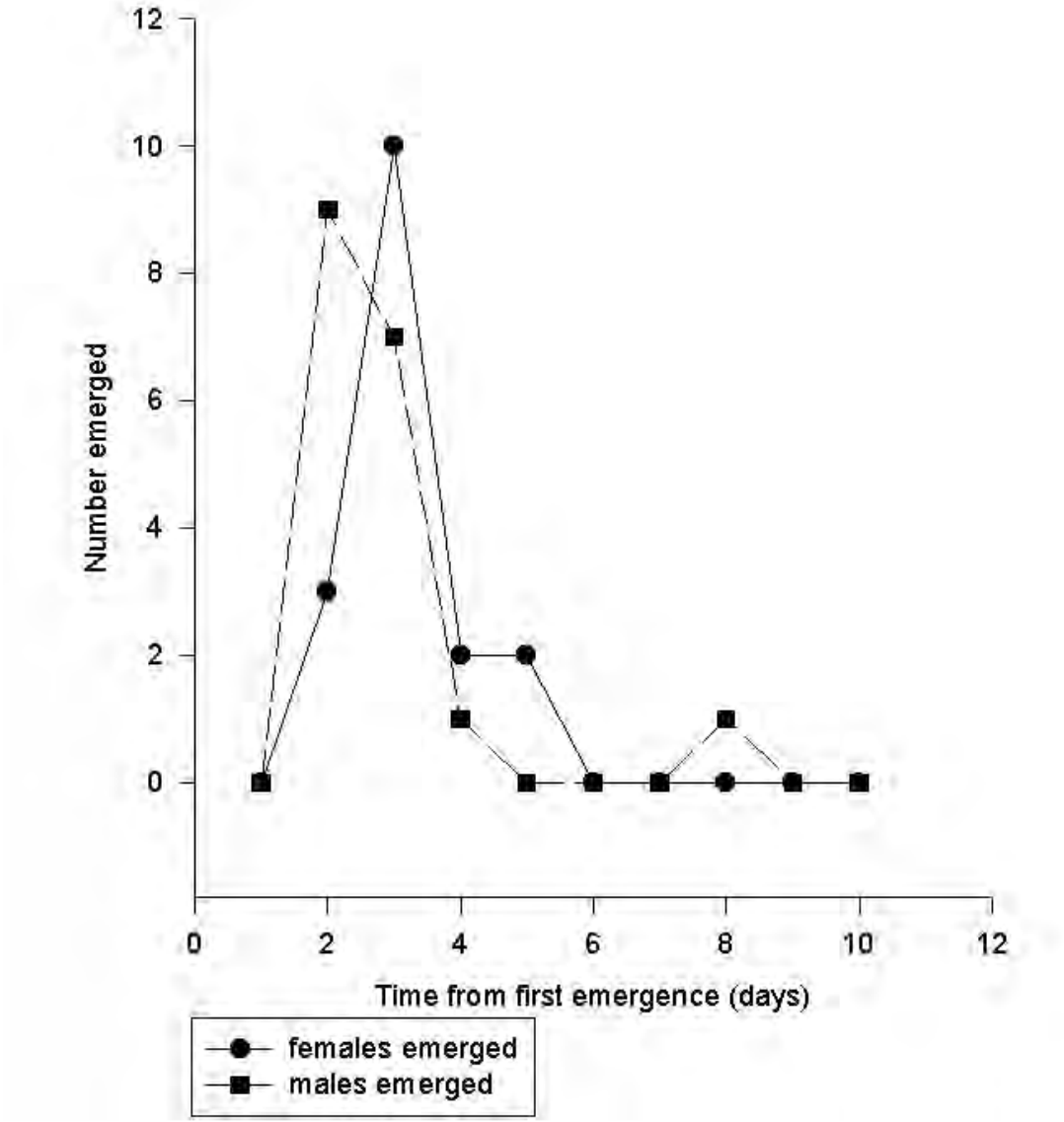


Figure 4.2. Male and female emergence patterns (days since hatching)

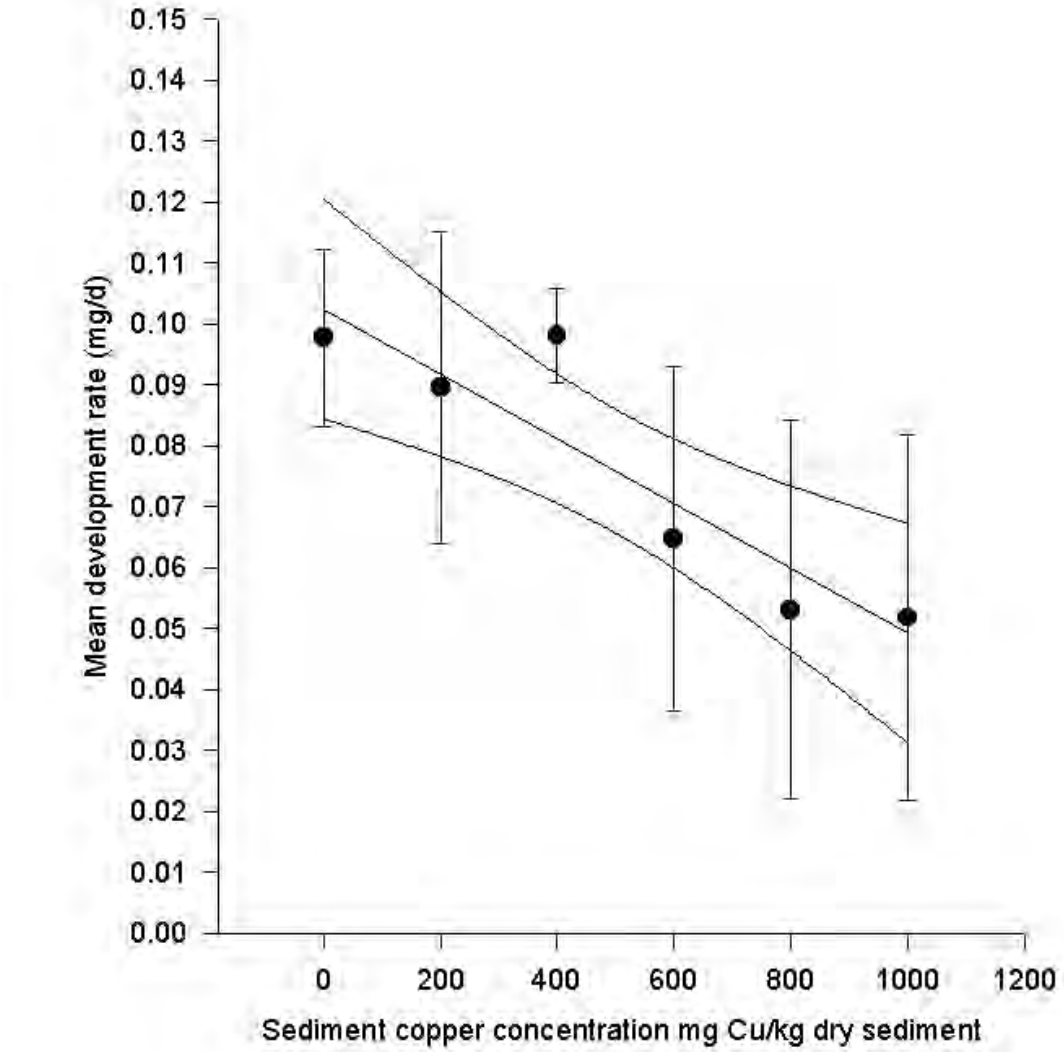


Figure 4.3. Development rate against sediment copper concentration with 95% confidence intervals (n=8)

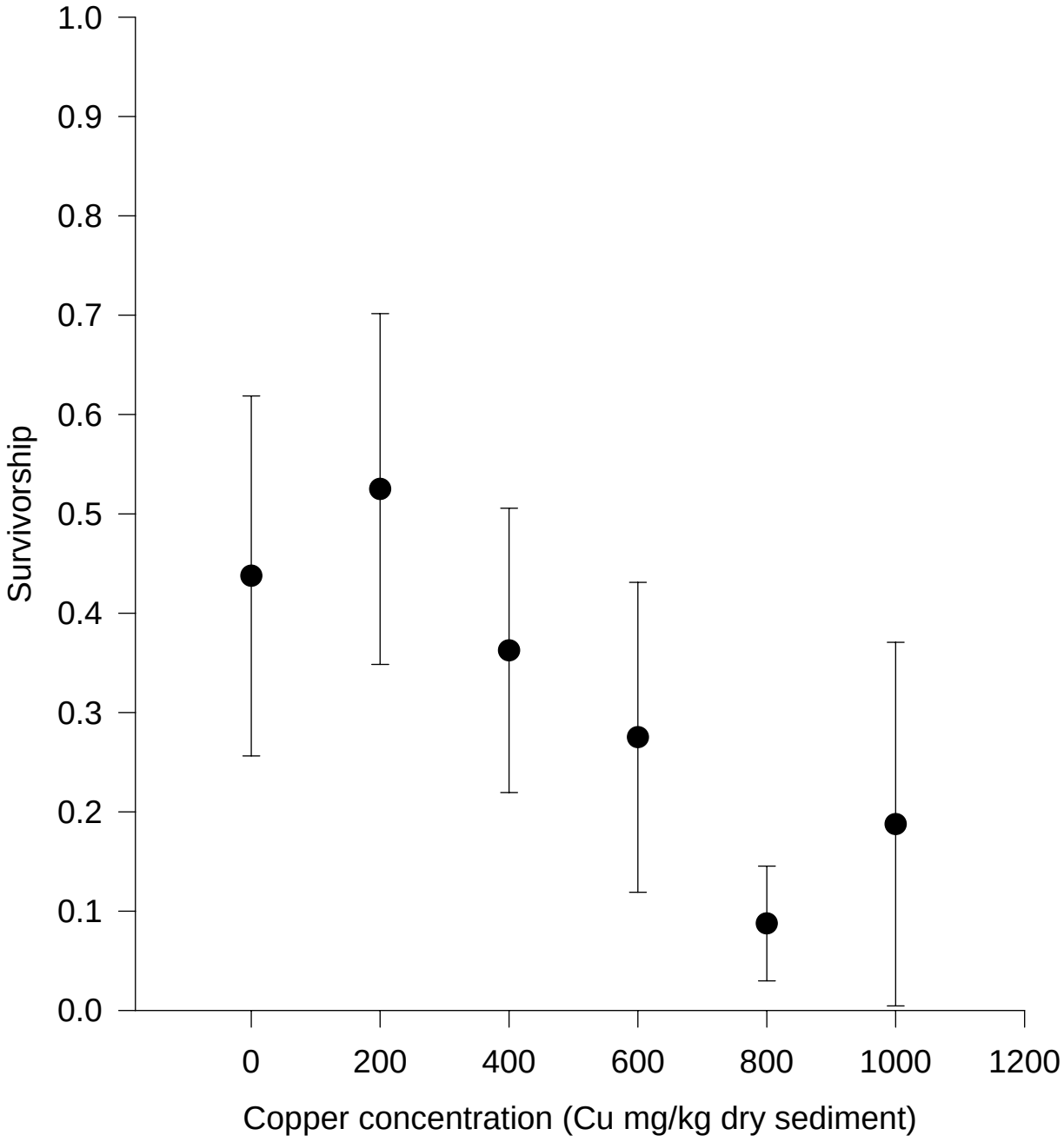


Figure 4.4. Graph of survivorship against sediment copper concentration with 95% confidence intervals (n=8)

Figure 4.5 shows mean emergence time for females only with all treatments being different ($p<0.05$) from the control except 800 mg/kg, due to the fact that only two emerged. Figure 4.6 shows the mean number of eggs per treatment with no significant difference between treatments ($p=0.28$).

The bootstrap process to estimate the distribution of lambda gives the distributions shown in Figure 4.7. The distributions are skewed to lower values when mortality increases with increased copper concentration. Figure 4.8 shows the means and 95% confidence intervals from the bootstrapped data (the larger confidence intervals) and 95% confidence intervals from an assumed normal distribution (smaller confidence intervals). By comparing the 95% confidence intervals from the bootstrapped data visually it can be seen that the only significantly different treatments are between 200 mg/kg and 800 mg/kg. If, however, a normal distribution is assumed and the standard deviation set at the highest value from all treatments (approximately 0.2) a minimum detectable difference of 0.37 is attained (Zar, 1996). With this treatments at 800 and 1000 mg/kg show significant differences to the mean (Figure 4.8).

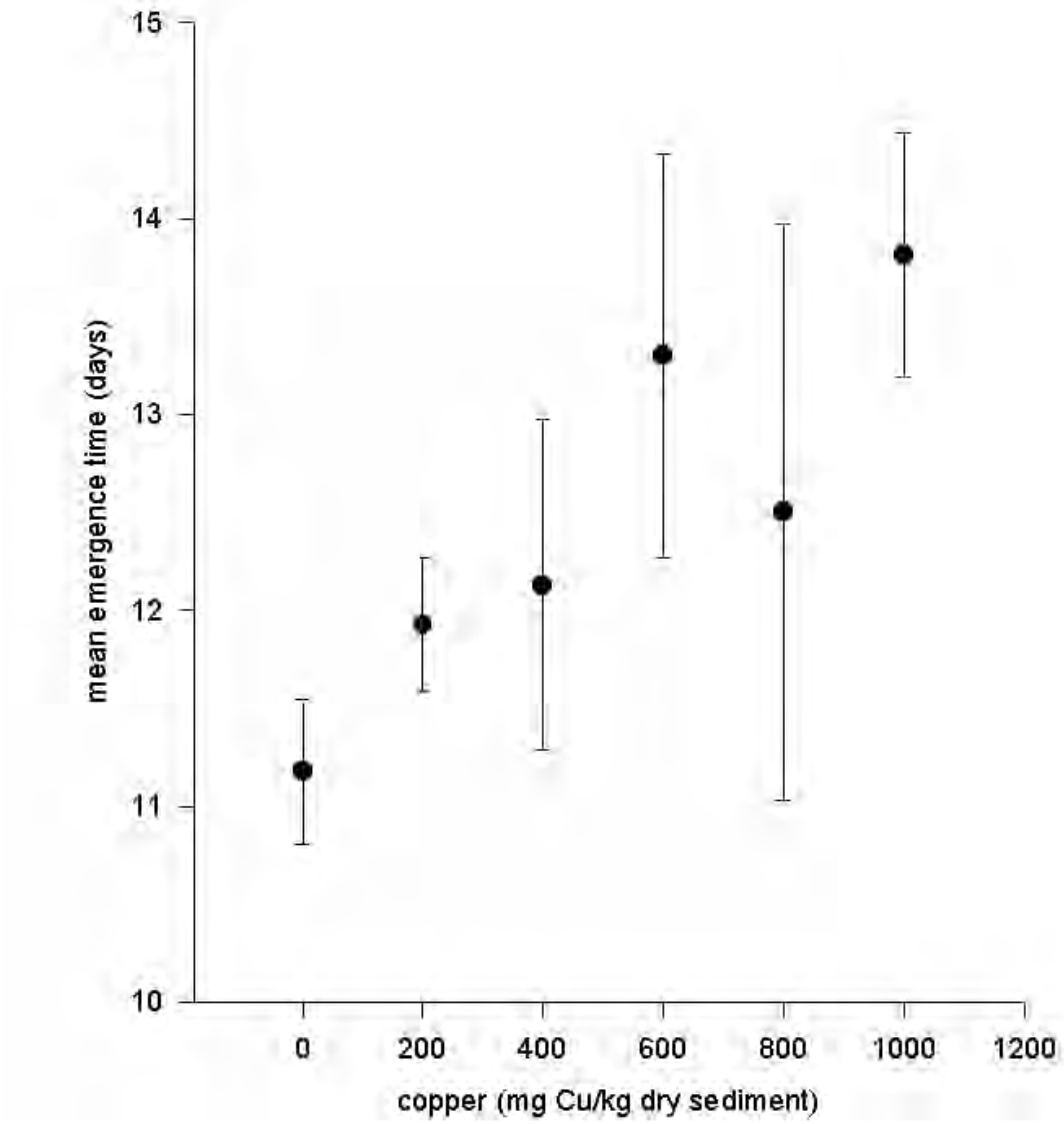


Figure 4.5. Graph of mean time to emergence against sediment copper concentration with 95% confidence intervals (n=8)

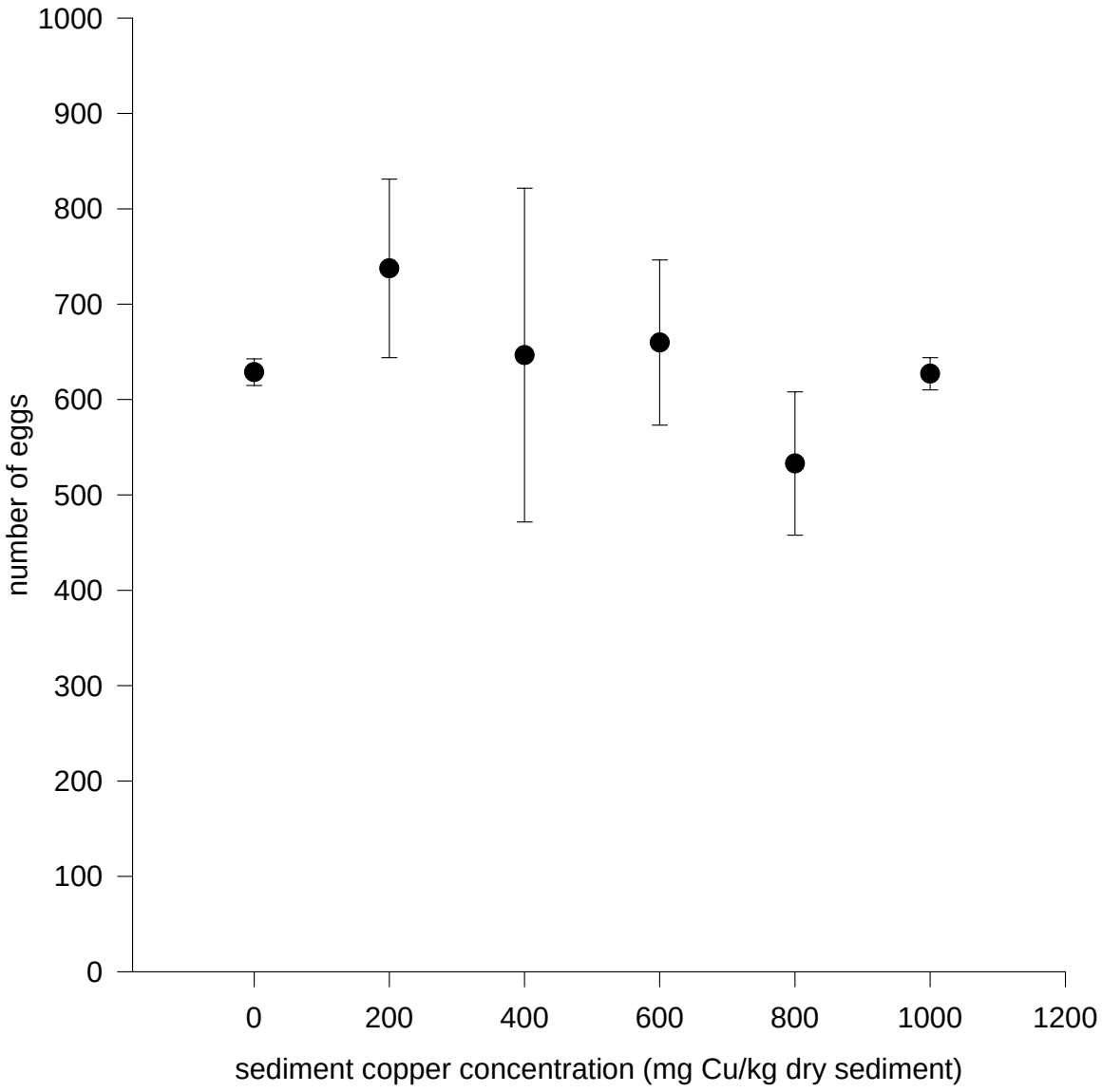


Figure 4.6: Graph of mean number of eggs per replicate against sediment copper concentration with 95% confidence intervals (n=8)

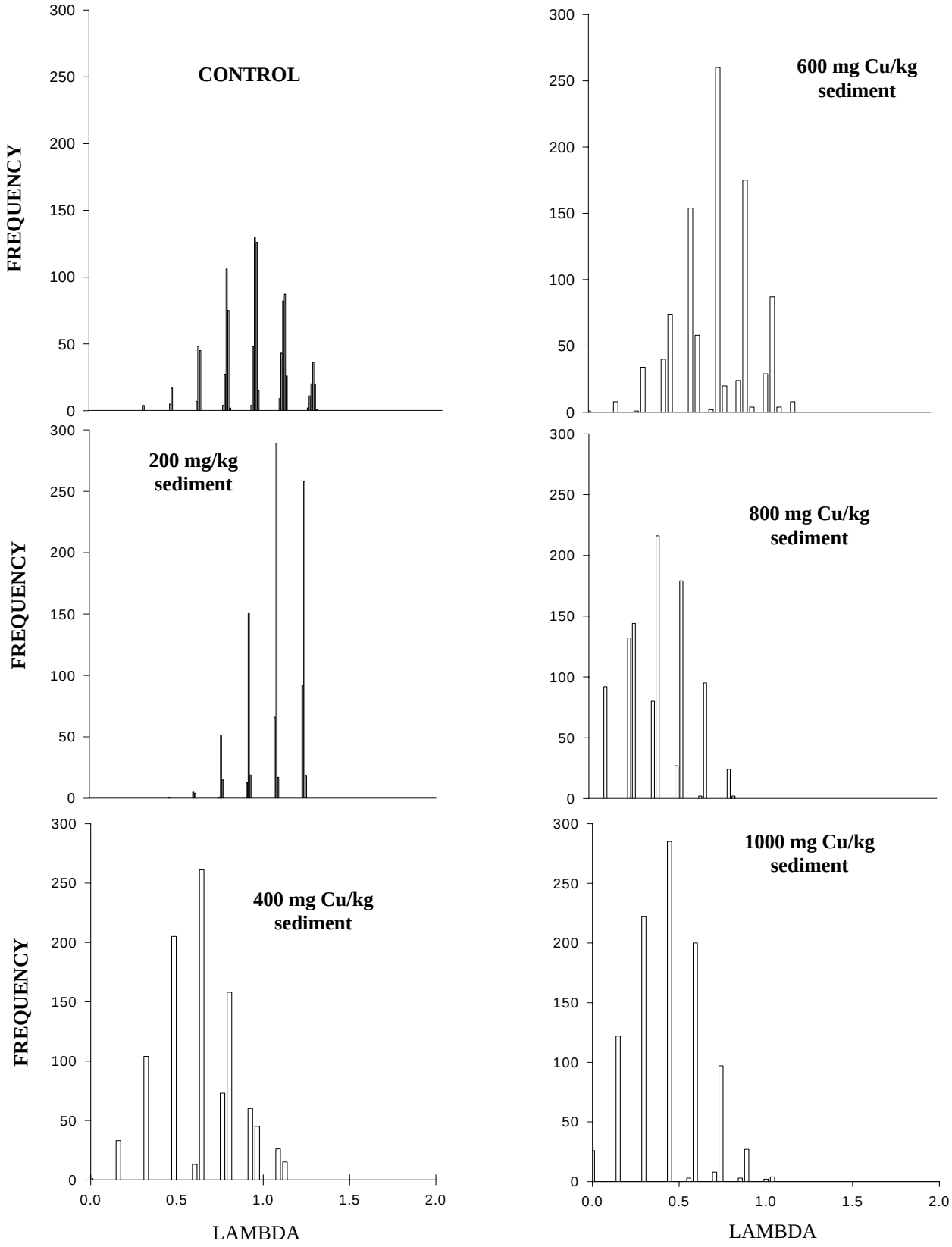


Figure 4.7: Bootstrap distributions of lambda

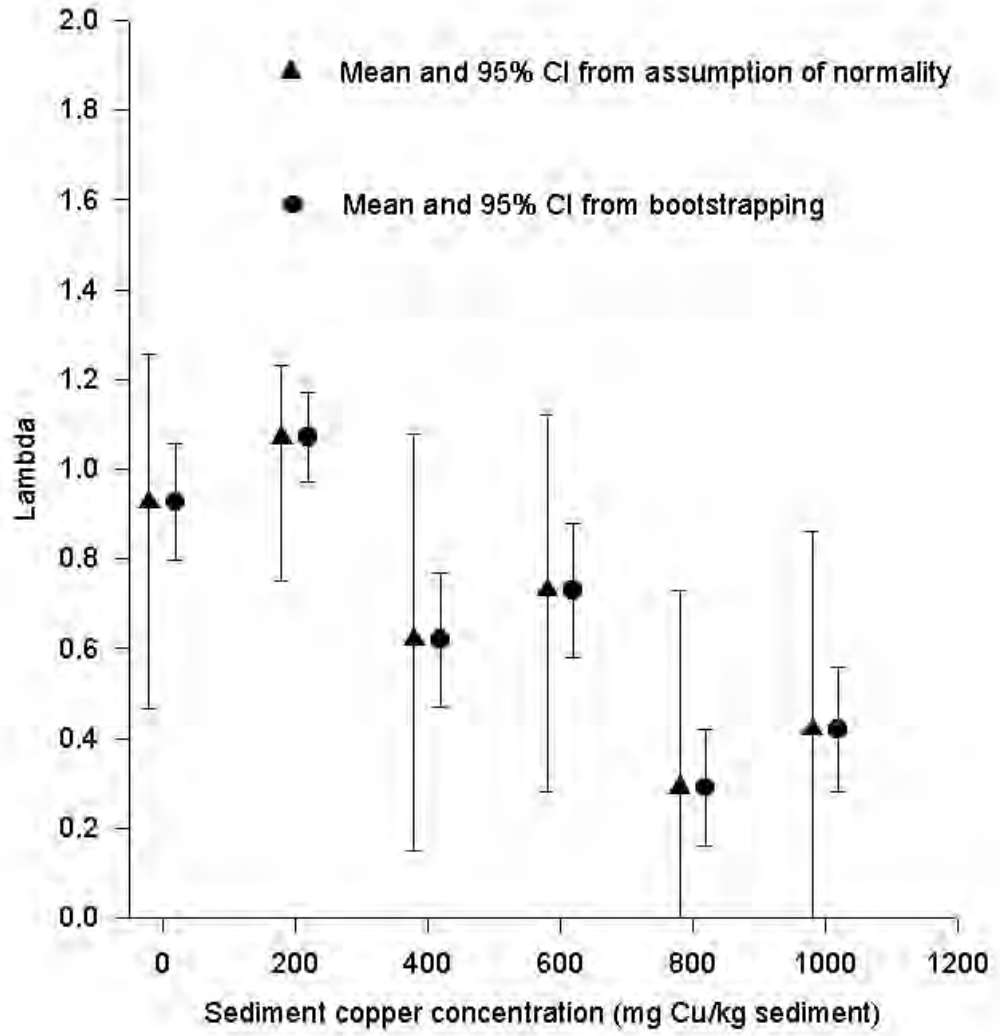


Figure 4.8: Graph of lambda against sediment copper concentration with 95% confidence intervals from bootstrapping and assumption of normality

4.4 Discussion

For a valid test the guideline figure of around 80% survival or success of the control is generally applied (OECD, 1998). Tests comparing the emergence of *C. riparius* to *C.tentans* showed greater than 90% and 15-43% emergence respectively (M. Watts pers comm.), and it was suggested that *C. tentans* is not a good test organism for emergence tests. Since only 50% of the chironomids emerged for *C. crassiforceps* it can be concluded that this test organism is not a good choice for an emergence test. In addition, when compared to the larval growth test that detected a LOEC of 600mg/kg and an EC_{50} of 702 mg/kg it can be seen that the emergence test is potentially less sensitive than the larval growth test. The larval growth test was also found to be more sensitive than emergence tests for *C. decorus* by Rosawalt and Knight (1987). For calculating the confidence intervals of lambda the bootstrap process allows assumption-free estimation of the intervals with the 95% intervals calculated using this experimental design almost twice as large as 95% confidence intervals obtained by assuming normality.

Holding two of the variables at their mean then varying a single factor at a time can show the contribution of each of the vital rates to lambda, this is termed the decomposition of lambda. The graphical analysis of this shows that lambda will tend to be greater than 1 for nearly all scenarios, which is due essentially to the extremely high reproduction rate. A species reproductive potential has been shown to be very important in terms of contaminant impact on populations (Stark et al., 1997a; Stark et al., 1997b). The low values of lambda from the experimental replicates are mainly due to the zero survivorship values from experimental replicates. The controlling factor on the effect of copper on demographic parameters is mortality. In replicates exhibiting zero emergence

lambda is skewed to zero to such an extent that decreases due to changes in emergence time are almost negligible. As a result confidence intervals attained by assuming normality underestimate the spread of data by two-fold for this particular experimental design.

In general, the following factors make the use of demographic parameters less than satisfactory with this experimental design.

- Low emergence rates (50%) in controls.
- The number of individuals per replicate is low and, with sexual dimorphism at a rate of 2:1 in favour of males, there is a high chance of getting zero females emerging from a single replicate thus biasing in favour of zero values. The inability to separate male from female at first instar means that there is a high possibility that no females will emerge from a replicate thus immediately biasing estimates of lambda.

The experimental design can significantly affect the analysis of demographic parameters. Increasing the number of individuals in each replicate would decrease the bias to lower values especially when using a species with inherently low survival to emergence.

In summary, for *Chironomus crassiforceps,* with low emergence rates, the value of demographic parameters as a good comparison statistic becomes questionable. This is due to a poor ability to detect differences between treatment and control. The advantage of using demographic parameters still lies in the fact that they are more ecologically relevant endpoints, and integrate sublethal and lethal effects (Stark et al., 1997a; Stark et al., 1997b). However, for a number of life history types, protocols for ecotoxicological tests would have to be re-evaluated. For example, in bioassays, neonates are typically introduced, whereas in nature a stable age distribution is normally seen. Stark and

Banken (1999) showed in experimental work that different results, in terms of population growth rate responses to pollutants, were obtained when starting the demographic bioassay with different starting age/stage structures.

In terms of sensitivity, growth measured as dry weight after 4 days is more sensitive than population parameters. This is not unexpected since if the individual parameters are measured without error it is impossible for lambda to be more sensitive, however a combined, statistically undetectable difference in all the individual traits could be detected by lambda (Forbes and Calow, 1999).

Comparing the emergence test to the results from the larval growth test in 3.3.3 it can be seen that the emergence test is less sensitive. Although the emergence test, using population parameters as an endpoint, may be more ecologically relevant the growth test could be considered as an early warning of potential population effects. However, when testing new contaminants it is recommended that the emergence test be used to ensure that egg production (and hatching) is not substantially affected.

5 General Discussion

The discussion will centre on each of the following main findings of this thesis:

- The sensitivity of the tropical chironomid *Chironomus crassiforceps* to copper was found to be similar to results obtained for a number of chironomids native to the Northern Hemisphere.
- *Chironomus crassiforceps* showed a high degree of tolerance to low pH.
- Acute toxicity of *Chironomus crassiforceps* to copper and uranium in water-only exposures was seen to increase with increasing pH.
- In whole sediment toxicity tests decreasing toxicity to porewater copper together with increasing porewater copper concentration was observed with decreasing pH.
- Uranium in sediment was not found to inhibit growth even at a nominal concentration of 5000 mg U/kg sediment (dry weight).
- As an endpoint, chironomid larval growth was found to be more sensitive than emergence or demographic parameters.

5.1 *Chironomus crassiforceps* as an indicator species

The biogeographical range of *C. crassiforceps* (Cranston, pers comm) means that this chironomid has potential as a standard test organism with local relevance to SE Asia, Indonesia and Micronesia. The chironomid is adapted to the wet-dry tropical conditions of the Northern Territory of Australia, with a short life cycle ideal for aerial colonisation of newly formed water bodies created during the wet seasons (Mahler, 1984; Batzer and Wissinger 1996). This short life cycle at 27°C makes the sediment toxicity test much faster (4 days) than current ten-day sediment toxicity tests using other species of chironomids at 21°C (EPA, 1996a).

Chironomus crassiforceps showed a high degree of tolerance to low pH with no acute effects down to pH 3.5. Chironomid tolerance to pH has been recorded in temperate studies showing that chironomids were the dominant species under acidic conditions (pH of 3.2). The chironomid *Chironomus riparius* has been shown to tolerate pHs down to 3, the lowest tolerance of invertebrates and fish in a review (Gerhardt, 1992), however, another study observed a reduction in survival of *Chironomus riparius* larvae at pH 4 (Rousch et al., 1997). From studies under temperate conditions it is generally thought that acidified streams show diminished species richness, increased abundance of tolerant species and lower densities due to lower productivity through decreased pH (Lindegaard, 1995). Studies undertaken in southern tropical waters suggest that the response to acidity may be different. An experiment by Cranston et al. (1997) within the Alligator Rivers Region showed that the responses to acid, heavy metal rich waters were a loss of some species typical of pristine conditions but overall an increase in species richness. The higher species richness at low pH is explained by the large tropical (Australian and SE Asian) pool of species that are tolerant of naturally occurring acidic aquatic habitats.

5.2 pH and the toxicity of copper and uranium

The acute toxicity data, showing increased toxicity of copper with increased pH broadly agrees with reviews on fish, algae, fungi, crayfish and benthos (Gerhardt, 1992; Albers and Camardese, 1993a; Albers and Camardese, 1993b). The reduced toxicity with decreasing pH is attributed to increased competition from protons on uptake sites on the biological membrane (Stary and Kratzer, 1982; Campbell and Stokes, 1985; Morel, 1983; Gerhardt, 1992; Parent and Campbell, 1994; Witters, 1998).

Using a chemical speciation approach the toxicity of copper might be expected to increase slightly with decreasing pH. As pH is lowered increasing copper dissociation from organic and inorganic ligands is expected resulting in increased concentrations of Cu^{2+} and $Cu(OH)^{+}$, thought to be the toxic species (Krantzberg and Stokes, 1988). In the simplest of solutions (ignoring the role of dissolved organic matter and other co-ions) the dominant forms of inorganic copper around neutrality would be Cu^{2+} and $CuOH^{+}$ i.e. from the formation constant for $CuOH^{+}$:

$$Cu^{2+} + H_2O = CuOH^{+} + H^{+} \qquad \log K° = -7.34$$

At pH 4, more than 95% of inorganic copper in solution is in the Cu^{2+} form. If speciation were the dominant factor in determining toxicity to the chironomids an increase in toxicity with decreasing pH should have been observed.

For uranium, marked speciation changes are expected to occur between pH 6 and 4. Markich et al. (1996) showed an exponential increase in uranium toxicity when pH was reduced from 6 to 5 for the bivalve V*elesunio angasi*. This toxicity was associated with a speciation change favouring the uranyl ion (UO_2^{2+}). This pattern was not observed for *C. crassiforceps* and highlights the fact that mechanisms of toxicity between species are variable (Luoma, 1983).

Hydrogen ion competition was proposed to explain the findings. Several mechanisms can be put forward to explain the way H^{+} ions reduce toxicity. One is that as pH decreases, the concentration of H^{+} ions increases logarithmically, competing with metal ions for biological uptake sites on the membrane surface of the chironomid. This fits with a biological surface complexation model where uptake sites are occupied by either metal ions or protons before being taken up by the organism (Campbell and Stokes, 1985; Krantzberg and Stokes, 1988; Crist et al., 1994). Changes in the surface potential of the cells of the surface of the chironomid may also contribute to increasing toxicity

with increasing pH. With increasing pH the electrostatic potential between the cell surface and the positively charged metal cations may increase with subsequently increasing metal sorption and uptake (Crist et al., 1994 Another possibility is that hydrogen ions interact with the plasma membrane altering transport (Macfie et al., 1994). The reasons for decreased toxicity with increasing hydrogen ion competition may be a result of one or a combination of the mechanisms.

5.3 pH effects on toxicity in sediment

Within the sediment system studied, decreasing pH resulted in increased porewater copper concentrations. This was attributed to hydrogen ion competition at the sediment-porewater interface.

The increased porewater EC_{50} and LOEC values for *C. crassiforceps* at the lower pH of 4 compared to pH 6 were hypothesised to be due primarily to proton competition at the biological uptake sites of the chironomid (Stary and Kratzer, 1982; Campbell and Stokes, 1985; Morel, 1983; Gerhardt, 1992; Parent and Campbell, 1995; Witters, 1998). Krantzberg and Stokes (1988) found that chironomids accumulated metals to a greater degree with increasing pH. However the effect may have arisen from surface adsorption as opposed to an intake mechanism. This thesis used ecologically relevant endpoints and clearly demonstrated that sublethal chronic toxicity of copper to *Chironomus crassiforceps* increased with increasing porewater pH.

In terms of nominal sediment copper concentrations, hypothesis testing gave lower LOEC and NOEC values for copper at pH 6 than at pH 4 reflecting the trend for toxicity observed in porewater data. Using regression analysis the nominal sediment copper concentrations associated with porewater EC_{50} values did not differ significantly over the two pHs. The effect of decreased porewater copper toxicity at lower sediment pH

was offset by the increased porewater metal concentrations. The net effect on *C. crassiforceps* was that toxicity, in terms of nominal sediment copper loading (EC_{50} nominal), remained the same at both pHs. This highlights the importance of evaluating the bioavailable phase when assessing sediment toxicity. If, using regression analysis, the nominal concentrations alone had been used to assess whether there was a change in toxicity to copper from sediments the conclusion would have been that pH played no role in affecting the bioavailability of copper to the chironomid.

Uranium was found to have no sublethal effect on the chironomid at all levels expected in the environment (up to 5000mg U/kg dry sediment). This was attributed to a low affinity for protein binding sites as the UO_2^{2+} ion exhibits ionic (hard acid) characteristics in soft/hard Lewis acid theory (Shriver et al., 1992). The implication is that it would not form strong bonds with the soft bases (such as sulphur) that tend to make up protein binding sites. Another implication is that uranium distribution does not appear to be clearly associated with any major authigenic precipitates of sulphur. However, deposition may be increased in regions of acid-sulphate sediments as the oxidation of sulphides and the associated acidity have been implicated in the uncoupling of the stable uranyl carbonate complex and subsequent scavenging by humic acids and iron oxides (Church et al., 1996). Copper on the other hand is classified as a soft Lewis acid and forms complexes (often very stable chelate complexes) with sulphur and nitrogen donor atoms of proteins, changing their conformation, and either degrading gas exchange and/or altering fluid retention/exclusion (Rainbow, 1996).

5.4 Toxicity test endpoints

The adaptation of the larval growth sediment toxicity test for use as an emergence test or the use of demographic parameters as endpoints was problematic because

Chironomus crassiforceps exhibited low emergence rates in controls. Using demographic parameters as a measure of toxic effect required information on the emergence rates and egg production of female chironomids. A combination of low emergence rates and the fact that females constituted a third of the population increased the possibility of replicates exhibiting zero emergence of females. This skewed the demographic parameter and resulting in non-normality of the test statistic. This in turn resulted in a poor ability to detect differences between treatment and control.

In terms of sensitivity, growth measured as dry weight after 4 days is more sensitive than population parameters and emergence. Although population parameters may be more relevant to the field (Calow et al., 1997), the results of the thesis suggest that decreased growth provides an early warning of population effects. However, when testing new potential contaminants it is recommended that a life-cycle test be undertaken to ensure that the contaminant does not target egg production and hatching success.

Protocols for ecotoxicological tests with *Chironomus crassiforceps*, using demographic parameters, would have to be re-evaluated should demographic endpoints be deemed necessary or useful. Experimental design would have to minimise the number of replicates exhibiting zero emergence.

5.5 Implications for risk assessment

Two major findings of the research have implications for risk assessment in regions of acid-sulphate sediment.

- The effect of pH on the toxicity of porewater metal
- The release of metal from the sedimentary compartment to overlying water

Normally, contamination of the environment from mining activity results in a mixture of metals entering the ecosystem (Harrahy and Clements, 1997). Individual metals within mixtures can interact to change toxic response in three possible ways (Gerhardt, 1992):

- Additivity: where the effect of two metals is the sum of their individual effects
- Synergy: where the effect of two metals is greater than the sum of the effect of the individual metals
- Antagonism: where the effect of combined metals is less than that expected from the sum of the metals

Mixtures generally demonstrate additivity (Ross, 1996; Warne, 1998) and based on this assumption a method of assessing the risk from metals in sediment has been proposed (Ankley et al., 1996). Porewater metal concentrations, or predicted porewater concentrations (PEC – predicted environmental concentration), for all metals of concern are converted to interstitial water criteria toxicity units (IWCTUs) for each metal. The IWCTU are calculated by dividing the porewater metal concentration or PEC by the chronic water quality guideline value (PNEC – predicted no effect concentration). Summing the Interstitial Water Criteria Toxicity Units (IWCTUs) for each metal gives an estimation of risk. A risk is posed if a value greater than unity is obtained.

Wildhaber and Schmitt (1998) evaluated this model as a predictor of benthic community structure and laboratory measured toxicity. They found that an additive model using IWTCU, based on estimated porewater metal concentration divided by chronic toxicity values from ambient water quality criteria, was able to predict both laboratory-measured toxicity and benthic community structure.

Estimating the porewater metal concentration can be achieved through models such as the Two Species Freundlich or the Non-ideal Competitive Adsorption Models

(Temminghoff et al., 1997). If these models are applied in areas affected by acid-sulphate sediments the pH effects must be considered. One important factor is that the low pH generated by the oxidation of sulphides has the potential to dissolve even occluded metals, generally not considered bioavailable (Willett et al., 1992). This should be considered if developing any model of porewater metal concentrations for such regions.

Hassan et al. (1996) studied the effect of pH on porewater metal concentrations using chromatographic sand as the substrate to determine maximum expected porewater concentrations. Calculated porewater metal concentrations were used to screen sediment. The estimated porewater concentrations were used to predict whether sediment would pass sediment quality criteria in terms of predicted interstitial water criteria toxic units. The interstitial water criteria toxic units (IWCTU) were calculated by dividing the estimated porewater concentrations by a chronic water quality guideline value that was first adjusted for the measured pore-water hardness to account for hardness effects on toxicity (Leonard et al., 1996). This thesis highlights the fact that adjustment for porewater pH may also be necessary. However, one of the problems in considering the effect of pH change on toxicity is that contradictory results have been obtained for different organisms (Markich and Camilleri, 1997). For other organisms the pattern seen here for the chironomid *C. crassiforceps* - of decreasing toxicity of copper with decreasing pH - has not been observed. For example, the root systems of plants often react to pH in the opposite way to other biota (Lexmond, 1980; Suave et al., 1998). As a tropical wetland ecosystem can exist as a soil system for a number of months of the year it is also important to assess risk to the ecosystem in its phase as sediment and as a soil (Pascoe, 1993; Lemly, 1996).

Another aspect of acid-sulphate sediments of concern is the potential impact on overlying water quality when generated acidity releases sediment-bound contaminants. Work by Willett et al. (1992) examining the oxidation of sulphidic material from acid-sulphate soil from the Magela floodplain showed that pH levels could reach a low of 1.5. Soluble manganese, iron and aluminium concentrations increased suggesting that silicate clay minerals were dissolved under such extremely acidic conditions. The implication for risk assessment of acid-sulphate sediments is that even occluded metals may become bioavailable following the generation of acidity and the dissolution of mineral matrices. From the experimental work in this study sediment-associated metals were found to be released to the overlying water column with decreasing sediment pH. Water quality can be affected as a result of this phenomenon and it is suggested that water quality assessment in regions of acid-sulphate sediments consider the sediment as a potential source of metal contamination.

As highlighted by this thesis, understanding the effect of contaminants on a particular ecosystem requires a thorough understanding of both the effect of pollutants on species and physico-chemical and environmental factors that may modify the bioavailability of contaminants. Highly seasonal wetland ecosystems in the wet-dry tropics, such as those in Kakadu, present a particular challenge since environmental conditions vary greatly both spatially and temporally.

Although recognised as a major influence on speciation and bioavailability of metals in freshwater systems pH is currently not used to modify guidelines values for metals, primarily due to contradictory findings and poor quality data (Markich et al., 1997). Rather than ignore the role of pH in influencing bioavailability this thesis highlights the

need to understand and include the effect of pH on metal toxicity, especially for sediment quality assessment in acid-sulphate soil regions.

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Appendix 1

Composition of Synthetic Magela Creek Water

To make up a 10 litre volume **Add 0.44 ml/10L of the following stock solutions**	
$MgSO_4.7H_2O$	138.10 g/L
$CaCl_2.2H_2O$	37.46 g/L
KCl	16.01 g/L
$Al_2(SO_4)_3.18H_2O$	39.23 g/L
$NaHCO_3$	82.20 g/L
0.147 ml/20L of $FeCl_3$	68.09 g/L
0.22 ml/10L of Trace element Stock Solution **<u>Trace Stock Solution</u>** Make up Trace stock solution from:	
$CuSO_4.5H_2O$	0.1249 g/L
$Pb(NO_3)_2$	0.0087 g/L
$MnSO_4.H_2O$	1.3504 g/L
$UO_2SO_4.3H_2O$	0.0082 g/L
$ZnSO_4.7H_2O$	0.1397 g/L

Make up volume with deionised water. Bubble overnight to allow gas exchange

Adjust to pH 6 using 0.05M H_2SO_4 or 0.05M NaOH.

Appendix 2

Visual Basic program used to bootstrap values of Lambda

```
'
Sub BootstrapCONTROL()
   'set the bootstrapped lambda values to start at the first column
      Sheets("Bootstrap CONTROL").Select
      Range("A2").Select
   'set up the bootstrapping procedure to repeat 1000 times
      Let b = 0
      Do Until b = 1000
      ' reset all variables
      Let survival = 0
      Let eggs = 0
      Let T = 0
      Let survivorship = 0
      Let Lambda = 0
      Let AverageLambda = 0
      ' Use the temporary work sheet to store 6 randomly chosen values - with replacement
      Sheets("Temporary").Select
      Range("A2").Select
      For Repeat = 1 To 8
      Sheets("Simulated data").Select
      Range("B5").Select
      Let vertical = Int(8 * Rnd)
      ActiveCell.Offset(vertical, 0).Activate
      Let survival = Application.ActiveCell
      ActiveCell.Offset(0, 1).Activate
      Let eggs = Application.ActiveCell
      ActiveCell.Offset(0, 1).Activate
      Let T = Application.ActiveCell
      Sheets("Temporary").Select
      ActiveCell.FormulaR1C1 = "" and survival and ""
      ActiveCell.Offset(0, 1).Activate
      ActiveCell.FormulaR1C1 = "" and eggs and ""
      ActiveCell.Offset(0, 1).Activate
      ActiveCell.FormulaR1C1 = "" and T and ""
      ActiveCell.Offset(1, -2).Activate
      Next
      ' collect survivorship value from temporary data sheet
      SumLambda = 0
      Sheets("Temporary").Select
      Range("A13").Select
      Let survivorship = Application.ActiveCell
      ' if the survivorship value equals zero then no data to add up
      If survivorship = 0 Then
      AverageLambda = 0
      Else

      For c = 1 To 8
      Range("A1").Select
      ActiveCell.Offset(c, 0).Activate
```

```
        survival = Application.ActiveCell
        ActiveCell.Offset(0, 1).Activate
        eggs = Application.ActiveCell
        ActiveCell.Offset(0, 1).Activate
        T = Application.ActiveCell
        ActiveCell.Offset(1, -2).Activate
        If survival = 0 Then
        Lambda = 0
        ElseIf eggs = 0 Then
        Lambda = 0
        ElseIf T = 0 Then
        Lambda = 0
        Else
        ActiveCell.Offset(-1, 3).Activate
        Lambda = Application.ActiveCell
        ActiveCell.Offset(1, -3).Activate
        End If
        SumLambda = SumLambda + Lambda
        Next
        Range("A11").Select
        Number = Application.ActiveCell
        AverageLambda = SumLambda / 8
        End If
        ' Place the bootstrapped average for lambda in the Bootstrap control sheet for statistical analysis
        Sheets("Bootstrap CONTROL").Select
        ActiveCell.FormulaR1C1 = "" and AverageLambda and ""
        ActiveCell.Offset(1, 0).Activate
        Let b = b + 1
        Loop
End Sub
```