

3.5 Conclusions

The effect of increasing water hardness was variable, depending on the metal and test organism investigated. It was found that a 50-fold increase in hardness had no effect on Cu toxicity to *H. viridissima*, but decreased U toxicity by approximately 2-fold. The opposite was observed for *M. mogurnda*, where increased hardness resulted in a 2-fold decrease in the toxicity of Cu, while it had no effect (ie in the second investigation) on U toxicity. The observed toxicity effects of hardness occurred without any change in the speciation of Cu or U. The reduction in U toxicity to *H. viridissima* and Cu toxicity to *M. mogurnda* with increasing hardness may be explained by Ca-Mg competition mechanism, where the Ca and Mg ions compete with Cu/U ions for binding sites at the cell surface (Markich & Jeffree 1994, Erickson et al 1996). However, the competition mechanism is not amenable where Cu toxicity to *H. viridissima* or U toxicity to *M. mogurnda* was not reduced, suggesting there is another mechanism in place.

4 Effect of alkalinity on the toxicity of Cu and U

4.1 Rationale

Many authors have described alkalinity to be an influential factor on metal toxicity. In attempting to define the effects of alkalinity (ie carbonate concentration), several of these authors confounded their results with the effects produced by hardness (ie Ca and Mg concentration) and pH (eg Cu: Howarth & Sprague 1978, U: Parkhurst et al 1984, Barata et al 1998). Those studies that manipulated the carbonate concentration independently of the Ca and/or Mg concentration, and pH, found increasing alkalinity to reduce the bioavailability and toxicity of Cu (Andrew et al 1977, Miller & Mackay 1980, Laurén & McDonald 1986, Daly et al 1990a). Similarly, alkalinity has been found to attenuate the adverse effects of U toxicity to a freshwater bivalve (*Vesunio angasi*), under constant water hardness and pH conditions (Markich et al 1996). Hardness and alkalinity effects need to be separated as the two variables affect metal toxicity differently, as described previously (see section 3).

Thus, one objective of this study was to isolate and assess the effects of alkalinity, at constant hardness and pH, on the toxicity of Cu and U to *H. viridissima* (green hydra, population growth) and *M. mogurnda* (purple-spotted gudgeon, sac-fry survival).

4.2 Methodology

Toxicity testing materials and procedure are detailed in chapter 2. Only specific modifications made to these standard procedures are mentioned here.

4.2.1 Selection of alkalinity levels

Regional water quality information was gathered from the Water Resources Branch of the Department of Lands, Planning and Environment and the Environmental Research Institute of the Supervising Scientist to determine a relevant range of alkalinity for tropical Australian freshwater systems (fig 2, table 4). Three levels of alkalinity were selected – 4.0, 102 and 205 mg CaCO₃ L⁻¹. The baseline alkalinity of 4 mg CaCO₃ L⁻¹ represents the mean alkalinity of Magela Creek water (see fig 2). The rationale for using Magela Creek water as a baseline reference in this study is outlined in Riethmuller (2000) Appendix B.1.4. The other alkalinity levels were calculated to compliment the hardness levels studied (ie 165 and 330 mg CaCO₃ L⁻¹) and represent tropical Australian waters (see fig 2, table 4).

4.2.2 Isolating alkalinity effects

Alkalinity was manipulated using NaHCO_3 , while $\text{Ca}(\text{NO}_3)_2$ and $\text{Mg}(\text{NO}_3)_2$ continued to be added to the synthetic diluent water as detailed in chapter 3. The rationale was that the difference in toxicity with the addition of carbonate to test waters at known hardness could be attributed as alkalinity effects. All other physico-chemical parameters were held constant (ie pH 6.0 ± 0.3 and conductivity within 10% error, over 24 h).

Solutions containing the corresponding alkalinity and hardness levels were prepared, and examined for the formation of precipitates. A white precipitate formed at an alkalinity of $205 \text{ mg CaCO}_3 \text{ L}^{-1}$ after 72 h, while the other solutions appeared free of precipitates.

A preliminary test was conducted to ensure the pH, conductivity, dissolved oxygen (DO) and alkalinity of the test solutions remained within an acceptable range over 24 h. Physico-chemical parameters were measured as per section 2.4. It was observed that the pH deviated beyond the acceptable range of 6.0 ± 0.3 . This can be explained by the direct logarithmic relationship between pH and alkalinity, where the pH increases with the addition of carbonate and when the pH was lowered the carbonate is converted to CO_2 (Stumm & Mogan 1981). A few techniques were examined to stabilise pH, and these are detailed in Riethmuller (2000) Appendix E. As a result of these investigations it was obvious a biological buffer was needed to maintain pH so that the effects of alkalinity would not be confounded. While biological buffers have proved successful in controlling pH in experimental systems (Stauber et al 1994, Franklin et al 1998), caution must be exercised as they have been shown to complex metals (Good et al 1966), and thus alter metal toxicity (Lage et al 1996). Consequently, the use of buffers in metal toxicity tests is generally avoided. MES (2-morpholinoethanesulfonic acid) biological buffer appeared suitable for this study as its pKa at 20°C is 6.15 (Good et al 1966), which is ideal for stabilising pH in the range of 6.0 ± 0.3 . The suitability of MES was assessed as detailed below.

4.2.3 Incorporation of MES biological buffer into toxicity protocols

A series of tests were conducted to determine the concentration at which MES buffer maintained pH without having adverse effects on or altering the toxicity of Cu and U to the test organisms. Three buffer concentrations were selected – 2.5, 5.0 and 10 mM – based on Franklin et al (1998). Each buffer concentration was added to each of the selected alkalinity solutions. In the presence of 2.5 mM buffer, the pH of each treatment increased by 2.0 units over 24 h. The 10 mM buffer maintained the pH of all treatments within 6.0 ± 0.3 , but reduced the population growth of *H. viridissima* by 12–33% compared with growth measured in non-buffered treatments. The 5 mM buffer maintained the pH of 4 and 102 $\text{mg CaCO}_3 \text{ L}^{-1}$ alkalinity solutions within 6.0 ± 0.3 , but the pH of 205 $\text{mg CaCO}_3 \text{ L}^{-1}$ solution increased by two pH units over 24 h. *Mogurnda mogurnda* sac-fry survival showed no observed effect when exposed to 5 mM buffer solution. However, *H. viridissima* population growth decreased by 20% compared with growth in non-buffered treatments. Subsequently, a MES concentration of 4 mM was found to maintain the pH of 4 and 102 $\text{mg CaCO}_3 \text{ L}^{-1}$ alkalinity solutions within 6.0 ± 0.3 . In addition, *H. viridissima* population growth and *M. mogurnda* survival in buffered treatments was similar to growth in non-buffered treatments. The alkalinity level of 205 $\text{mg CaCO}_3 \text{ L}^{-1}$ was excluded from this study as the 4 mM MES was unable to maintain solution pH within an acceptable range, and MES concentrations >4 mM reduced *H. viridissima* population growth. During the 4 mM buffer trial, the effects of NaHCO_3 and conductivity were also examined and found to have no effect on either *H. viridissima* or *M. mogurnda* control responses.

Tests were conducted to investigate the effect of 4mM MES buffer on the toxicity of Cu and U to *H. viridissima* and *M. mogurnda*. A range of metal concentrations was selected based on the results reported in section 3. Two tests were run in parallel – one containing the buffer solution and one without. The 4 mM buffer solution did not significantly ($P \geq 0.05$) affect the toxicity of either Cu or U to *H. viridissima* (ie overlapping 95% confidence intervals of the EC₅₀ values, table 8). These results suggest 4 mM MES buffer had no effect on Cu and U toxicity to *H. viridissima*. In contrast, *M. mogurnda* sac-fry showed a decrease in sensitivity to Cu with the incorporation of MES buffer at a concentration of 4 mM. At 110 µg L⁻¹ Cu, 3.3% survival was recorded in non-buffered water while 80% survival was recorded in buffered water, indicating that Cu toxicity to *M. mogurnda* was reduced by 4 mM MES buffer. For this reason, the effect of alkalinity on the toxicity of Cu and U to *M. mogurnda* was not investigated. Subsequently, this study focused on the effect of alkalinity on Cu and U toxicity to *H. viridissima*.

Table 8 Population growth of *H. viridissima* exposed Cu and U in the presence and absence of 4 mM MES biological buffer

Metal	EC ₅₀ (95% CI)	
	MES absent	MES present
Cu	8.1 (7.8–8.4)	6.7 (4.5–8.9)
U	230 (198–267)	210 (194–225)

4.3 Results

This study was designed to assess the effects of alkalinity (carbonate concentration) on the toxicity of Cu and U to *H. viridissima*, at constant water hardness (165 mg CaCO₃ L⁻¹) and pH (6.0 ± 0.3). Raw data for each test-series of a given metal-organism exposure are provided in Riethmuller (2000) Appendix F, tables 5 and 6.

4.3.1 Influence of alkalinity on Cu toxicity to *H. viridissima*

The concentration-response relationship for *H. viridissima* exposed to Cu at two alkalinity levels is shown in figure 10. Summary data for the concentration-response curve are given in Riethmuller (2000) Appendix G, table 5. The calculated BEC₁₀, MDEC, NOEC, LOEC, and EC₅₀ values for *H. viridissima* exposed to Cu at two alkalinity levels (4 and 102 mg CaCO₃ L⁻¹) are given in table 9.

A 25-fold increase in alkalinity (ie from 4.0 to 102 mg CaCO₃ L⁻¹) at a hardness of 165 mg CaCO₃ L⁻¹ did not significantly ($P > 0.05$) affect the toxicity of Cu to *H. viridissima* (ie overlapping 95% confidence intervals of the EC₅₀ values, table 9). The trend observed for the EC₅₀ values is consistent with the BEC₁₀, MDEC, NOEC and LOEC values given in table 9, such that all endpoints suggest there is no difference in Cu toxicity with a 25-fold increase in alkalinity.

Table 9 Toxicity endpoints (BEC₁₀, MDEC, NOEC, LOEC, EC₅₀) calculated for *H. viridissima* exposed to Cu (µg L⁻¹) at two alkalinity levels, under constant hardness (165 mg CaCO₃ L⁻¹) and pH (6.0 ± 0.3) conditions, for 96 h

Alkalinity (mg CaCO ₃ L ⁻¹)	BEC ₁₀	MDEC	NOEC	LOEC	EC ₅₀ (95% CI)
4	1.1	1.4	0.9	1.7	5.0 (4.5–5.5)
102	1.2	1.4	0.7	1.8	6.0 (5.5–6.5)

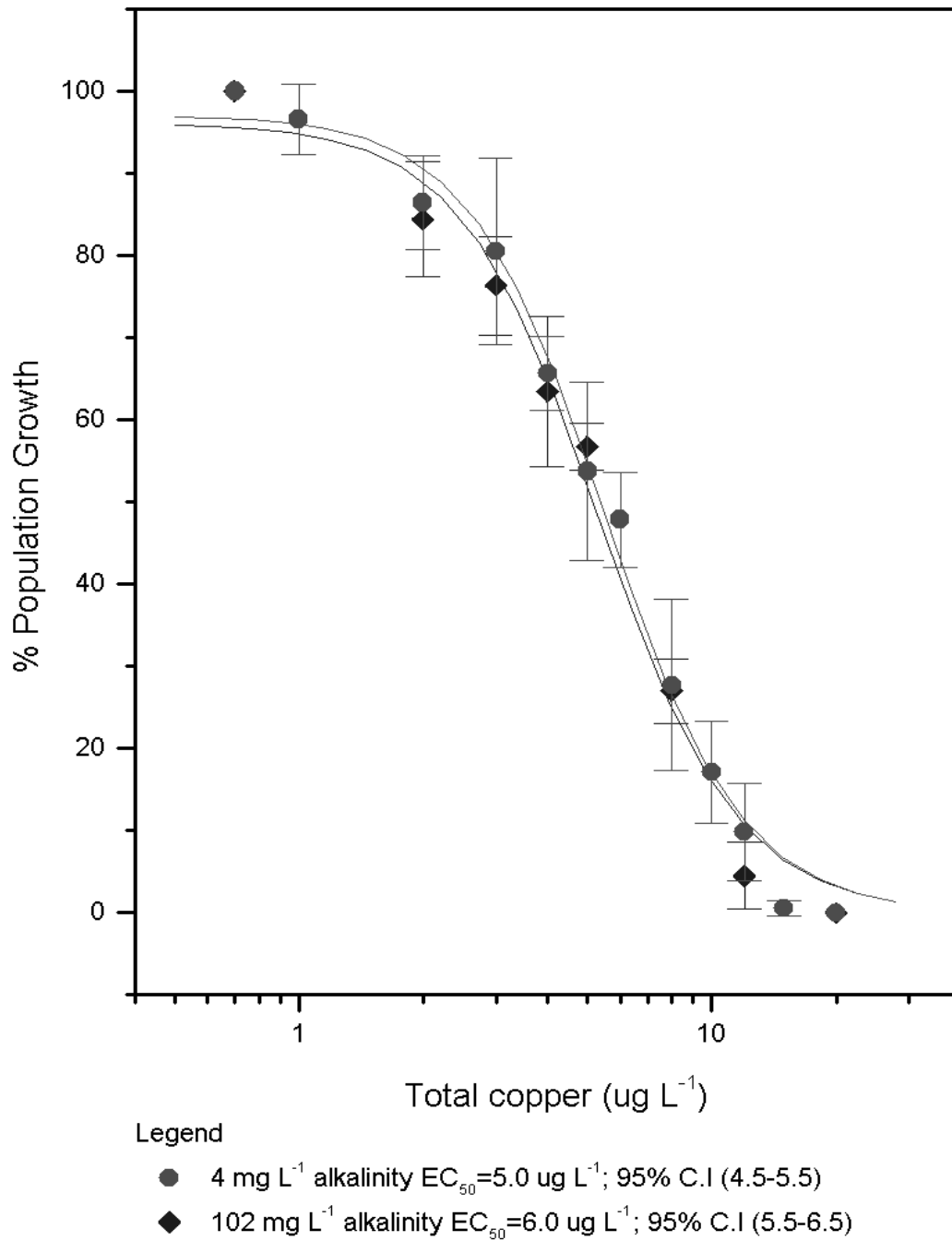


Figure 10 Population growth of *H. viridissima* exposed to Cu over 96 h at two alkalinity levels (4 and 102 mg CaCO₃ L⁻¹). Data points represent the mean of six or nine replicates ± 95% CI.

Cu speciation

The predicted speciation (% distribution) of Cu in the test waters at pH 6.0 at the two alkalinity levels (ie 4.0 and 102 mg CaCO₃ L⁻¹) is given in figure 11. Copper(II) was found to be the dominant species in both alkalinity solutions. In absolute terms, the free cupric ion (Cu²⁺) was 5% more available in the 4.0 mg CaCO₃ L⁻¹ alkalinity solution than the 102 mg CaCO₃ L⁻¹ alkalinity solution (see Riethmuller (2000), Appendix H, table 3).

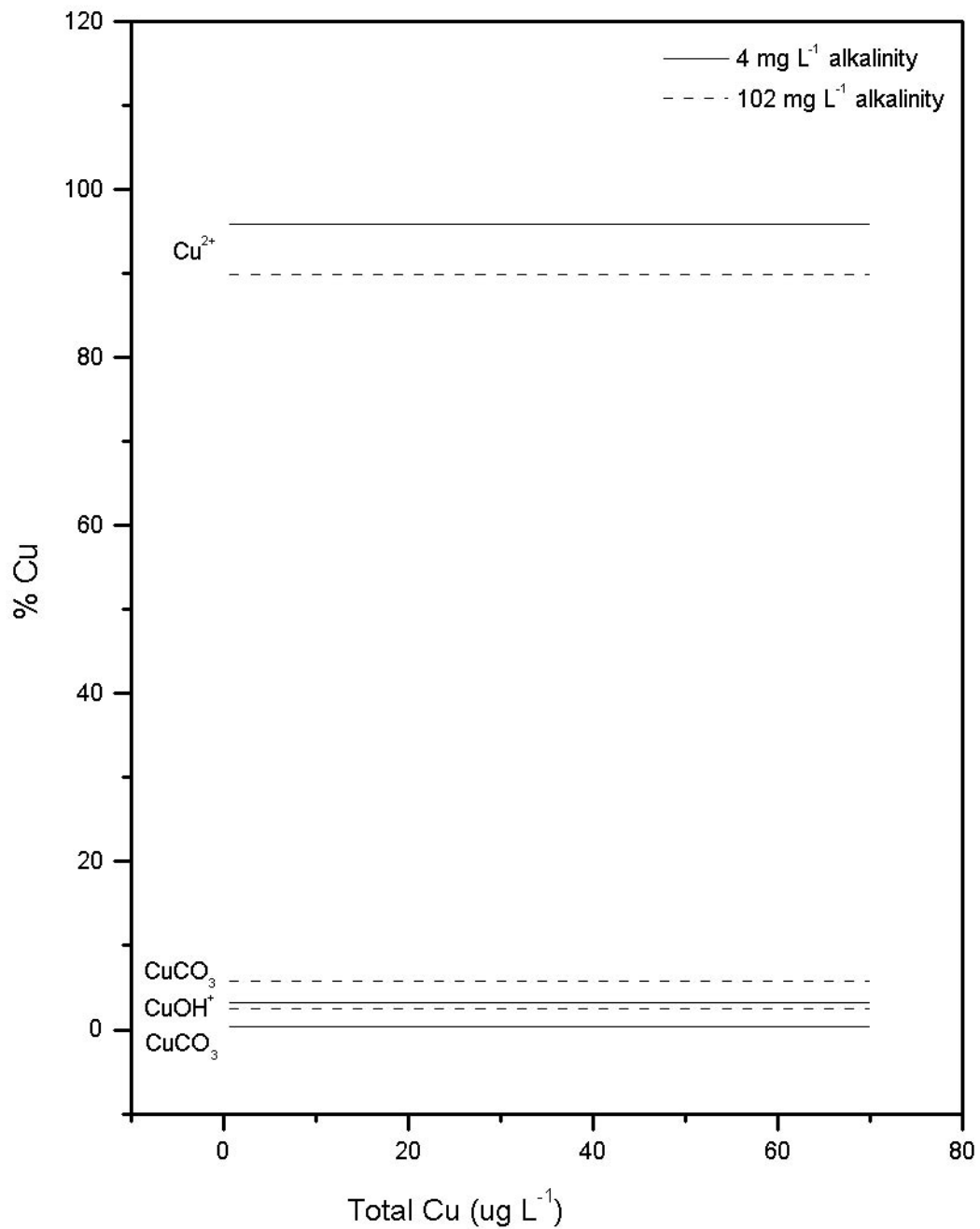


Figure 11 Predicted speciation (% distribution) of Cu in test water (pH 6.0) at two alkalinity levels (4 and 102 mg CaCO₃ L⁻¹)

4.3.2 Influence of alkalinity on U toxicity to *H. viridissima*

The concentration-response relationship for *H. viridissima* exposed to U is shown in figure 12. Summary data for the concentration-response curve are given in Riethmuller (2000) Appendix G, table 6. The calculated BEC₁₀, MDEC, NOEC, LOEC, EC₅₀ values for *H. viridissima* exposed to U at two alkalinity levels (ie 4.0 and 102 mg CaCO₃ L⁻¹) are given in table 10.

Table 10 Toxicity endpoints (BEC₁₀, MDEC, NOEC, LOEC, EC₅₀) calculated for *H. viridissima* exposed to U (µg L⁻¹) at two alkalinity levels, under constant hardness (165 mg CaCO₃ L⁻¹) and pH (6.0 ± 0.3) conditions, for 96 h

Alkalinity (mg CaCO ₃ L ⁻¹)	BEC ₁₀	MDEC	NOEC	LOEC	EC ₅₀ (95% CI)
4	81	90	150	162	177 (166–188)
102	25	42	130	171	171 (150–192)

Based on EC₅₀ values, a 25-fold increase in alkalinity (ie from 4.0 to 102 mg CaCO₃ L⁻¹), at a hardness of 165 mg CaCO₃ L⁻¹ did not significantly ($P > 0.05$) affect the toxicity of U to *H. viridissima* (ie overlapping 95% confidence intervals of the EC₅₀ values, table 10).

The slopes of the two alkalinity concentration-response curves differ, thus precluding a reasonable comparison of the BEC₁₀ and MDEC values (fig 12). The BEC₁₀ and MDEC at 102 mg CaCO₃ L⁻¹ alkalinity (25 and 42 µg L⁻¹, respectively) are lower than at 4.0 mg CaCO₃ L⁻¹ alkalinity (81 and 90 µg L⁻¹, respectively) (table 10), due to the concentration-response curve of the latter having a steeper slope. Note also, that the LOEC at an alkalinity of 102 mg CaCO₃ L⁻¹ is equivalent to the EC₅₀ (table 10).

U speciation

The predicted speciation (% distribution) of U in the test waters at pH 6.0 at two alkalinity levels (4.0 and 102 mg CaCO₃ L⁻¹) is given in figure 13. A 25-fold increase in alkalinity (ie carbonate concentration) altered the calculated U speciation through inorganic complexation. At ~175 µg L⁻¹ U (ie EC₅₀ value, see Riethmuller (2000), Appendix H, table 4), the percentages of UO₂CO₃ increased by a factor of four at 102 mg CaCO₃ L⁻¹ (compared with the baseline alkalinity of 4.0 mg L⁻¹ as CaCO₃), whilst the percentages of UO₂²⁺ and UO₂OH⁺ decreased by a factor of six. The polymeric U species, (UO₂)₂(OH)₃CO₃⁻, was also calculated to decrease by a factor of two. The increased alkalinity also substantially increased the percentage of UO₂(CO₃)₂²⁻ from <1 to 20%.

4.4 Discussion

4.4.1 Influence of alkalinity on Cu toxicity to *H. viridissima*

The toxicity of Cu to *H. viridissima* did not differ with an increase in alkalinity from 4.0 to 102 mg CaCO₃ L⁻¹, contrary to previous reports in the literature. Daly et al (1990b) reported Cu toxicity to the Australian freshwater shrimp, *Paratya australiensis*, decreased in solutions of increasing alkalinity. Likewise, Andrew et al (1977) found that the sensitivity of *D. magna* to Cu decreased when the alkalinity of the test solution was increased. These authors attributed the formation of copper-carbonate complexes to the reduction of Cu²⁺ activity, subsequently decreasing the uptake and toxicity of Cu.

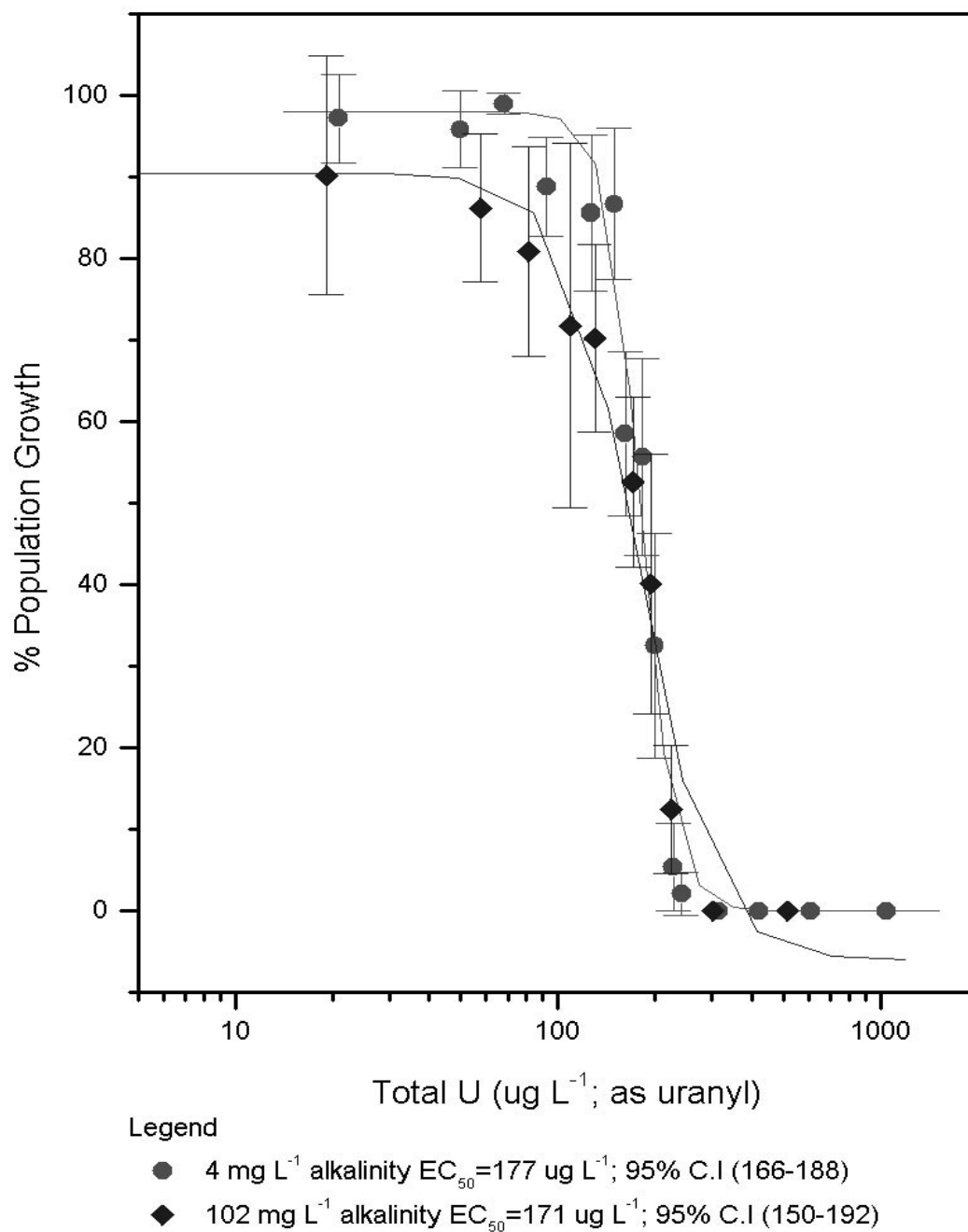


Figure 12 Population growth of *H. viridissima* exposed to U over 96 h at two alkalinity levels (4 and 102 mg CaCO₃ L⁻¹). Data points represent the mean of six or nine replicates ± 95% CI.

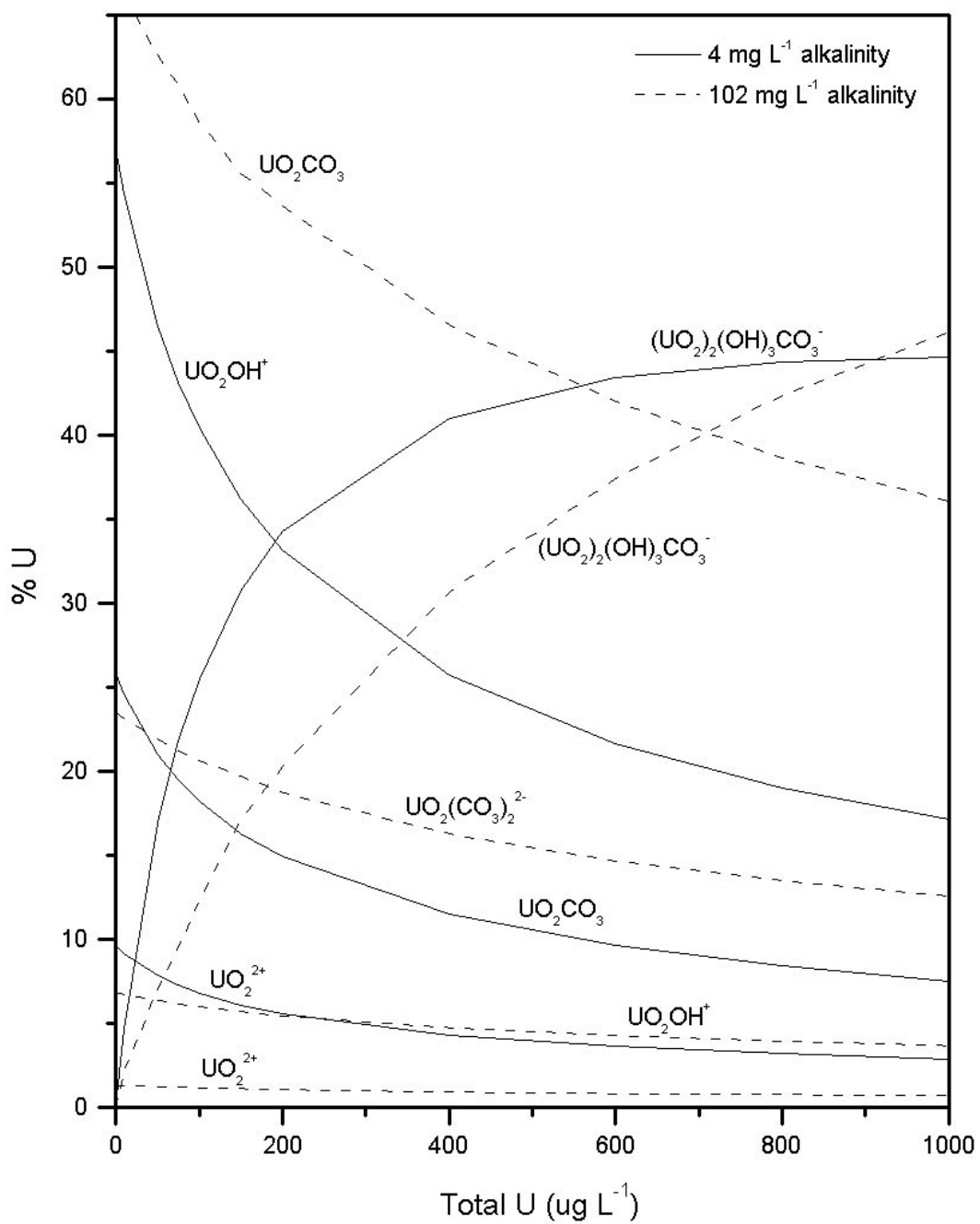


Figure 13 Predicted speciation (% distribution) of U in test water (pH 6.0) at two alkalinity levels (4 and 102 mg CaCO₃ L⁻¹). Uranyl species comprising <2% total U are excluded for clarity.

The percent distribution of Cu^{2+} in the test waters differed by 5% between 4.0 mg L⁻¹ and 102 mg L⁻¹ alkalinity, supporting the fact that alkalinity did not affect Cu toxicity to *H. viridissima* at the pH and increased alkalinity used in this experiment. More specifically, the addition of carbonate ions did not alter the proportional relationship between Cu species, therefore not influencing the toxicity of Cu.

4.4.2 Influence of alkalinity on U toxicity to *H. viridissima*

The present study found that a 25-fold increase in alkalinity did not affect the toxicity of U to *H. viridissima*, at pH 6.0. This contrasts with the results of Markich et al (1996) who found a 5-fold increase in alkalinity reduced U toxicity by 20% in the freshwater bivalve, *Velesunio angasi*, at pH 5. The different observations made in these studies may be attributed to the use of different pH levels and/or use of different test organisms. Evidence of such reasoning is provided by Markich et al (1996), who found U toxicity decreased with increasing pH from 5.0 to 6.0, while the relative proportions of UO_2^{2+} and UO_2OH^+ declined, and several uranyl carbonates and hydroxides increased.

In contrast to the effects of increased Ca and Mg concentration, the increased alkalinity (ie bicarbonate concentration) altered the calculated U speciation through inorganic complexation. Despite the changes in the calculated U speciation, there was no change in U toxicity. The absolute percent change in UO_2^{2+} from 6 to 1% is minimal, given the errors associated with the selected stability constants used in the calculations. Therefore, according to the FIAM, which interprets that the toxic effect of U to *H. viridissima* is governed by UO_2^{2+} , then a minimal change in U toxicity would be expected. The FIAM could be further tested by creating a larger absolute percentage difference between the calculated activity of UO_2^{2+} , by slightly reducing the pH of the test waters.

4.5 Conclusions

MES biological buffer (4 mM at pH 6.0; alkalinity 4 and 102 mg CaCO_3 L⁻¹) was found to be a suitable and practical option of controlling the pH in the bioassay protocols used in this study. Although the buffer enhanced *M. mogurnda* survival in elevated levels of Cu, the buffer did not affect *H. viridissima* population growth or toxicity of Cu and U. A 25-fold increase in alkalinity at constant hardness and pH, was found to have no effect on the toxicity of either Cu or U to *H. viridissima*. The toxicity effects of Cu occurred without any change in speciation. In contrast, U speciation was altered with increasing alkalinity through inorganic complexation, but with no change in U toxicity. These results indicate that carbonate alkalinity does not affect Cu and U toxicity under the experimental conditions of this study.

5 General discussion

Metal speciation and bioavailability in fresh surface waters may be influenced by a variety of physico-chemical variables, particularly water hardness, alkalinity, pH, natural organic matter and redox potential (Hamelink et al 1994, Markich et al 2000). Quantitative relationships (algorithms) have only been established to describe the reduction in the bioavailability of Cd, Cr(III), Cu, Ni, Pb and Zn as a function of increasing hardness. Such algorithms have been incorporated into the water quality guidelines of several countries for the protection of aquatic organisms (CCREM 1991, US EPA 1995, ANZECC & ARMCANZ 2001).

Although several studies have found water hardness to reduce Cu (Gauss et al 1985, Belanger et al 1989) and U (Parkhurst et al 1984, Barata et al 1998) toxicity to freshwater biota, insufficient and/or inconsistent data have precluded an algorithm being established. These and