NOTE

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.
Trace metal behaviour in an industrialised estuarine system and the toxicity of pulsed copper exposures

Brad M. Angel

B. Sc. Hons.

A thesis submitted in fulfilment of the requirements for the award of the degree

Doctor of Philosophy

from

University of Wollongong

Department of Chemistry

May 2009
Certification

I, Brad M. Angel, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institute.

Brad M. Angel

May 2009
Acknowledgements

- From my CSIRO Land and Water group at Lucus Heights, I would like to thank everybody, but special thanks goes to Leigh Hales and Simon Apte (for expert advice and training in ultra-trace metal analyses), Anthony Chariton (for assistance with the statistical analysis of results), Merrin Adams and Monique Binet (for assistance and training in the handling of algae and toxicity test methodology), David Spadaro and Ian Hamilton (for assistance and training in the handling of amphipods and toxicity test methodology), Graeme Batley (for Thesis editing and advice), and Jenny Stauber (for advice on bioassays, presentations, and my thesis;
- I would like to thank the following for technical and financial assistance received during the TEM work with *P. tricornutum*:
  - an Australian Institute of Nuclear Science and Engineering Grant (AINSE GRANT #05082); and,
  - Tania Ballind, Michael Collela and Kath Smith from the Australian Nuclear Science and Technology Organisation.
- I would like to thank the following for technical and financial assistance received to perform the field trips at Port Curtis:
  - The CRC for coastal zone, waterway and estuary management for partial funding of my PhD and field trips required to complete this thesis;
  - Damon Shearer for organisation of field trip logistics and help with presentation of data; and,
  - Leonie Anderson for advice on industrial activities in the region and local contacts.
- I would like to thank my parents John and Diane Angel for their love and support;
- I would like to thank my wife Taryn Angel for her love, support and help; and,
- Last and most importantly I would like to thank my supervisors Stuart Simpson and Dianne Jolley for all the time and effort they have contributed towards my thesis. They have always shown support and encouragement, which has greatly assisted my personal development and helped me to become a better scientist. I am eternally grateful for all their hardwork, which often went beyond the normal requirements of a supervisor.
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APDC</td>
<td>Ammonium pyrrolidine dithiocarbamate</td>
</tr>
<tr>
<td>ASS</td>
<td>Acid Sulfate Soils</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BLM</td>
<td>Biotic Ligand Model</td>
</tr>
<tr>
<td>CBT</td>
<td>Critical Body Threshold</td>
</tr>
<tr>
<td>DDC</td>
<td>Diethylthiocarbamic acid</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved Organic Carbon</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration that causes 50% toxicological response</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ERA</td>
<td>Ecological Risk Assessment</td>
</tr>
<tr>
<td>FIAM</td>
<td>Free-Ion-Activity Model</td>
</tr>
<tr>
<td>GBRMP</td>
<td>Great Barrier Reef Marine Park</td>
</tr>
<tr>
<td>GF-AAS</td>
<td>Graphite Furnace-Atomic Absorption Spectrometry</td>
</tr>
<tr>
<td>GPS</td>
<td>Global Positioning System</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration that causes 50% inhibition</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Inductively Coupled Plasma-Atomic Emission Spectrometry</td>
</tr>
<tr>
<td>ISQG</td>
<td>Interim Sediment Quality Guidelines</td>
</tr>
<tr>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration that causes 50% lethality</td>
</tr>
<tr>
<td>LOEC</td>
<td>Lowest contaminant concentration tested that causes a significant toxicological effect relative to controls</td>
</tr>
<tr>
<td>Milli-Q water</td>
<td>Deionised water (18 MΩ cm, Millipore, Australia)</td>
</tr>
<tr>
<td>NOEC</td>
<td>Highest contaminant concentration tested that does not cause a significant toxicological effect relative to controls</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
</tr>
<tr>
<td>SNK</td>
<td>Student-Newman-Keuls test</td>
</tr>
<tr>
<td>SPM</td>
<td>Suspended Particulate Matter</td>
</tr>
<tr>
<td>STP</td>
<td>Sewage Treatment Plant</td>
</tr>
<tr>
<td>TAC</td>
<td>Time-Averaged Concentration</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscope</td>
</tr>
<tr>
<td>TPM</td>
<td>Total Particulate Metals</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States - Environmental Protection Authority</td>
</tr>
<tr>
<td>WQG</td>
<td>Water Quality Guidelines</td>
</tr>
</tbody>
</table>
Publications/presentations

Research Publications


Oral Presentations


Poster Presentations


Abstract

The concentrations of metal contaminants may fluctuate in estuarine waters due to the erratic nature of sources and various physico-chemical parameters that influence concentrations. Standard toxicity tests use continuous contaminant exposure to assess organism toxicity even though organisms may respond differently when exposed to fluctuating concentrations. An investigation was made of the spatial distribution and short-term temporal fluctuation trace metals in an industrialised estuarine system and the influence of short-term fluctuations in metal concentrations on the toxic effects elicited to aquatic organisms. Copper was determined to be the metal of greatest concern due to the elevation of this metal above concentrations representative of regional waters and because copper is known to be relatively toxic to aquatic organisms. The toxic effects and mechanisms of toxicity in a marine algae and amphipod elicited by pulsed copper exposures were thoroughly investigated in laboratory bioassays.

Spatial and temporal sampling and analysis of trace metals in waters and sediments was undertaken in the highly industrialised central Queensland harbour of Port Curtis, Australia, and surrounding waters. The trace metals measured in Port Curtis were significantly higher (p<0.05) than those measured offshore in and adjacent to the Great Barrier Reef Marine Park, indicating the harbour had elevated metals compared to regional concentrations. Dissolved concentration maxima of copper and zinc were measured in the inner harbour of Port Curtis near anthropogenic input sources. Dissolved manganese and nickel exhibited more pronounced concentration maxima than copper and zinc, and were located in the Narrows, a large distance from anthropogenic sources and probably due to natural, but localised, input sources. Temporal monitoring of trace metals near a suspected industrial input to the middle harbour did not detect fluctuating metal concentrations. However, like many industrial harbours, temporal variations in metal concentrations to the waters of Port Curtis are likely, and copper was selected for the pulse-exposure effect studies.

Various aspects of pulsed copper exposure (concentration and duration, the duration between pulses, and the frequency and timing of pulses, delayed toxicity, and recovery following pulses) were investigated using new bioassay procedures that were developed in the laboratory specifically for the marine amphipod, Melita plumulosa and the marine alga, Phaeodactylum tricornutum. Mortality and biomass inhibition endpoints were
used for *M. plumulosa* and *P. tricornutum*, respectively. The pulses were generated by spiking with dissolved copper sulfate into seawater and terminated by replacing the copper-spiked water with clean seawater.

The bioassays with *M. plumulosa* employed dissolved concentrations in the range 30-1200 µg Cu/L and exposure durations of 2-240 h. This species exhibited delayed mortality, of which, the majority of effects occurred 48-96-h post-exposure. The results from the current study indicated that short (e.g. 4-d) bioassays may underestimate toxicity, as individuals may be counted as unaffected at the end of tests, but would eventually succumb to effects in the post-exposure period if tests lasted 10-d. The copper pulse concentration and duration were both important parameters that affected the mortality of *M. plumulosa*. The lack of significant difference in the mean morality of *M. plumulosa* exposed to multiple pulses separated by different durations in clean water, or to pulses of different frequency but similar time-averaged concentration (TAC), indicated the organism was not able to recover in the period between pulses.

The bioassays with *P. tricornutum* employed dissolved concentrations in the range 4-600 µg Cu/L and exposure durations of 0.3-72 h. Short-term pulses were observed to elicit significant biomass inhibition of *P. tricornutum*. However, the rate of cellular division of *P. tricornutum* was similar to that of controls after 24-48-h post-exposure, indicating the alga recovered from exposure after this duration in clean water. The timing of single pulsed exposure in the 72-h bioassay did not generally effect the biomass inhibition of *P. tricornutum*.

Different exposure scenarios with equivalent TACs of dissolved copper generally elicited similar toxic responses in *M. plumulosa* and *P. tricornutum*, indicating that toxicity was related to the TAC of dissolved copper exposure over the duration of the bioassays. This research supports the use of standard toxicity tests that utilise continuous contaminant exposure to predict toxicity in the field, as negative effects elicited by continuous exposure were similar to those elicited by pulsed exposures with similar TACs. The findings suggest single time-point measurements in the field may not adequately assess eco-system contamination, because this only reveals the exposure concentration at the instant the sample was collected, when it is the time-averaged concentration that is related to organism response.

A mechanistic study of the effects of copper exposure on *P. tricornutum* was performed using exposure concentrations in the range 10-50 µg Cu/L for durations in the range
0.3-72 h. The *P. tricornutum* cells were observed to increase in size and clump together following copper exposure. Cell surface-bound (extra-cellular) copper was measured by extraction with a solution of EDTA, and internalised (intra-cellular) copper was measured by digesting the EDTA-washed algal cells with nitric acid. Copper rapidly bound to the surface of *P. tricornutum* cells and then surface-copper remained relatively constant. A linear rate of copper internalisation within cells was measured over the exposure duration, indicating copper internalisation was the rate limiting step of metal uptake into *P. tricornutum* cells. The concentrations of intra- and extra-cellular copper were only observed to increase significantly (p<0.05) above control cells when significant (p<0.05) biomass inhibition was observed. The copper bound to the surface of *P. tricornutum* cells decreased rapidly when cells were placed in clean seawater. However, the internalised copper per cell did not decrease significantly (p<0.05) until 18-h post-exposure, after which, it exhibited a linear decrease with exposure time.

Although the intra-cellular copper concentration per *P. tricornutum* cell decreased, the total copper measured within all cells in the test population remained relatively constant during the post-exposure period. This indicated that the decrease in intra-cellular copper per cell was due to dilution of copper when divided between daughter cells produced by cellular division, and was not the result of copper efflux from cells.

For short-term pulsed copper exposures to *P. tricornutum*, surface-bound copper increased rapidly, but the short durations did not allow significant internalisation. Between pulses, copper rapidly desorbs from cell surfaces. The short pulsed exposures of 0.3-4 h employed in Chapter 5 were not expected to result in significant copper being internalised within cells, but these exposures still resulted in significant (p<0.05) biomass inhibition. It is hypothesised that the toxicity of short-term copper pulses to *P. tricornutum* cells is an acute response exerted by copper bound to the surface of cells, rather than a chronic response to internalised copper.
Table of contents

Certification ................................................................................................................................. ii
Acknowledgements .................................................................................................................... iii
List of abbreviations .................................................................................................................. iv
Publications/presentations ........................................................................................................ vi
Abstract .................................................................................................................................... vii
Table of contents ...................................................................................................................... x

1 GENERAL INTRODUCTION ................................................................................................. 1
  1.1 Contaminants in the environment .................................................................................. 1
  1.2 Metal contaminants in waters ....................................................................................... 2
  1.3 The response of organisms exposed to metals ............................................................. 3
  1.4 Metal transport to the surface of micro-organisms ....................................................... 5
  1.5 The influence of metal speciation on bioavailability .................................................... 6
  1.6 Metal speciation in waters ............................................................................................. 8
  1.7 Metal speciation in sediments ....................................................................................... 9
  1.8 Factors which affect metal speciation and bioavailability .......................................... 10
  1.9 Predicting metal toxicity .............................................................................................. 12
  1.10 Regulation of contaminants in Australia ..................................................................... 13
      1.10.1 Regulation of industrial discharges ..................................................................... 15
  1.11 Assessing contaminants using toxicological testing ................................................. 17
      1.11.1 Uses of toxicity tests ......................................................................................... 18
      1.11.2 Criteria for successful toxicity tests ................................................................. 18
  1.12 Effects of fluctuating metal concentrations on aquatic organisms ............................. 19
      1.12.1 Time-averaged doses and choice of test exposure scenario ............................. 20
      1.12.2 Delayed toxic responses and the exposure duration required to elicit irreversible effects ................................................................................................................. 21
1.12.3 Effects of pulse concentration and duration ............................................. 23
1.12.4 The effect of time intervals (recovery time) between multiple pulses .... 26
1.12.5 Effects of pulse frequency ...................................................................... 27
1.12.6 Other factors that influence effects induced by pulse exposures ......... 28
1.12.7 Implications for environmental risk assessment ..................................... 28
1.13 Thesis aims and outline ................................................................. 29

2 GENERAL METHODS .............................................................................. 33
  2.1 General analytical methods ................................................................. 33
  2.1.1 Acid-washing of experimental equipment ........................................... 33
  2.1.2 Measurement of physico-chemical parameters in the laboratory .......... 33
  2.1.3 Sampling of dissolved metals from waters in laboratory experiments ... 33
  2.1.4 Microwave assisted acid digestions of sediment and biological samples 34
  2.2 Analytical measurements .................................................................... 34
  2.2.1 Dissolved metal analyses by inductively coupled plasma-atomic emission spectrometry (ICP-AES) ................................................................. 34
  2.2.2 Dissolved metal analyses by graphite furnace-atomic absorption spectrometry (GF-AAS) ............................................................................ 35
  2.3 Quality control / quality assurance ...................................................... 35
  2.4 Procedures used to culture and conduct bioassays with Phaeodactylum tricornutum ................................................................................. 36
  2.4.1 Seawater used as test media ................................................................. 36
  2.4.2 Test species and stock maintenance .................................................... 36
  2.4.3 Culture conditions and media preparation ........................................... 36
  2.4.4 Counting algal cell densities using flow cytometry ............................ 38

3 METAL BEHAVIOUR AND FACTORS AFFECTING EXPOSURE PATHWAYS IN PORT CURTIS, QUEENSLAND .................................................... 39
3.1 Introduction .................................................................................................................. 39
  3.1.1 Objectives and Scope .......................................................................................... 40
  3.1.2 Site Description and Background Information ................................................. 41
3.2 Methods .................................................................................................................... 42
  3.2.1 General Field Work .......................................................................................... 42
  3.2.2 Sample collection ............................................................................................ 47
  3.2.3 Sample Processing and Analyses .................................................................... 49
  3.2.4 Data Analysis .................................................................................................. 53
3.3 Results ..................................................................................................................... 55
  3.3.1 Salinity, pH, and suspended particulate matter in Port Curtis seawater .......... 55
  3.3.2 Spatial trends in dissolved cadmium, copper, lead, manganese, nickel, and zinc 60
  3.3.3 Analysis of variance of dissolved metals between different areas .............. 65
  3.3.4 Analysis of variance of dissolved metals between different surveys ......... 67
  3.3.5 Short-term temporal variability in dissolved metal concentrations .......... 67
  3.3.6 SPM-bound metals ......................................................................................... 68
  3.3.7 Sediment acid-extractable metals and the effect of decreasing pH on sediment-bound metal remobilisation ................................................................. 70
3.4 Discussion ................................................................................................................. 72
  3.4.1 Physico-chemical parameters in Port Curtis and surrounding waters ....... 72
  3.4.2 Factors affecting metal concentrations in Port Curtis and surrounding waters and comparison with previous studies .......................................................... 75
3.5 Conclusion ................................................................................................................. 82
  3.5.1 Future recommendations ................................................................................ 84
4 EFFECTS OF FLUCTUATING COPPER EXPOSURES ON THE MARINE AMPHIPOD, MELITA PLUMULOSA .................................................................................. 85
  4.1 Introduction ....................................................................................................... 85
5.2.4 Data analysis ................................................................. 134
5.3 Results ........................................................................... 135
5.3.1 Sensitivity of test species ............................................. 135
5.3.2 The effect of copper pulse concentration and duration on the biomass inhibition of *P. tricornutum* ......................................................... 138
5.3.3 Tests investigating the effects of different pulse and continuous exposures of equivalent TAC of dissolved copper. ................................. 142
The recovery of *P. tricornutum* following single pulses and the effect of varying the exposure period ........................................................................ 151
5.4 Discussion ...................................................................... 156
5.5 Conclusions ..................................................................... 159
5.5.1 Future recommendations ............................................. 160
6 MECHANISMS OF COPPER TOXICITY IN *PHAEODATYLUM TRICORNUTUM* ................................................................. 161
6.1 Introduction ...................................................................... 161
6.2 Methods .......................................................................... 163
6.2.1 Sizing of control and copper-exposed *P. tricornutum* cells .............................................................. 163
6.2.2 Transmission electron microscopy of control and copper-exposed *P. tricornutum* cells .............................................................. 164
6.2.3 Measurement of intra- and extra-cellular copper concentrations in the alga, *P. tricornutum* .................................................................. 165
6.2.4 Data Analysis ................................................................. 168
6.3 Results ............................................................................. 169
6.3.1 Changes in algal size following copper exposure ......... 169
6.3.2 Copper uptake in *P. tricornutum* cells following exposure .............................................................. 171
6.3.3 72-h copper uptake following daily pulsed and continuous exposures (tested in Chapter 5.3.3.2) ................................................................. 172
6.3.4 Kinetics of copper uptake for continuous copper exposure .............................................................. 173
6.3.5 Kinetics of copper elimination following 72-h exposure to 15 µg Cu/L

6.4 Discussion .................................................................................................................. 180
6.5 Conclusion .................................................................................................................. 186
6.5.1 Recommendations ............................................................................................... 187

7 GENERAL DISCUSSION ............................................................................................. 188
7.1 Investigation of metal behaviour in the industrialised harbour, Port Curtis... 189
7.2 Comparison of the toxicity of pulsed copper exposures ........................................... 191
7.3 Influence of cellular concentrations on toxic response ......................................... 193
7.4 Recommendations for future work ........................................................................... 195

REFERENCES .............................................................................................................. 197

Appendix A1. intermittent exposure studies................................................................. 218
Appendix A2. Trace metal detection limits on ICP-AES ................................................. 224
Appendix A3. Sample Site conditions and description ................................................. 225
List of Figures

Figure 1.1. Concentration-response relationships for (A) synthetic organic chemicals, (B) essential metals and metalloids, and (C) non-essential metals and metalloids (Chapman and Wang, 2000). .......................... 4

Figure 1.2. Conceptualisation of metal transport from the bulk solution to the cell surface and transport across the plasma membrane, with the diameters of typical particles found in each layer listed at the top. M\(^{2+}\) = metal cations, ML = metal ligand complex in solution, X-M = metal complex with organism surface-bound ligand, K = equilibrium constant of dissociation of metal from ML complex, K\(_d\) and K\(_{fr}\) = equilibrium constants for formation of metal complex with organism surface-bound ligand (X), K\(_d\) and K\(_{fr}\) = dissociation constant for metal from organism surface-bound ligand (X), and K\(_{int}\) = internalization constant for metal (Campbell, 1995). .................................................. 6

Figure 1.3. Hypothetical profiles of major compounds in sediment porewater (Buridge, 1993). ...................... 10

Figure 1.4. Decision tree for the assessment water quality (ANZECC/ARMCANZ, 2000). ............................... 15

Figure 1.5. The marine amphipod, Melita plumulosa .......................................................... 31

Figure 1.6. The marine alga, Phaeodactylum tricornutum ................................................................. 31

Figure 3.1. Map of Port Curtis estuary and surrounding waters showing positions of sampling sites during the axial transect sampling (survey 1, ○), targeted sampling (survey 2, ◊), and during both surveys (▲). Where PC refers to the Port Curtis sampling zone, TN refers to The Narrows sampling zone, and KB, FI, and RB refer to the Keppel Bay, Facing Island, and Rodd’s Bay offshore sampling zones, respectively. .................................................. 46

Figure 3.2. Salinity of Port Curtis and surrounding waters ................................................................. 56

Figure 3.3. The salinity (●) measured along the transect directed from the outer harbour of Port Curtis into the Narrows during survey 1 and/or 2. The entrance to the harbour was defined as site A1.17 (survey 1) and site B1.15 (survey 2), and the initials FL, SEN, RC, and NEN refer to the Yarwun trade waste outlet at Fisherman’s Landing, the southern entrance to the Narrows, Ramsay’s Crossing, and the northern entrance to the Narrows, respectively .................................................. 56

Figure 3.4. The pH measured along the transect directed from the outer harbour of Port Curtis into the Narrows during survey 1 and/or 2. The entrance to the harbour was defined as site A1.17 (survey 1) and site B1.15 (survey 2). The initials FL, SEN, RC, and NEN refer to the Yarwun trade waste outlet at Fisherman’s Landing, the southern entrance to the Narrows, Ramsay’s Crossing, and the northern entrance to the Narrows, respectively .................................................. 59

Figure 3.5. The pH of Port Curtis and surrounding waters ................................................................. 59

Figure 3.6. Dissolved copper, nickel, and zinc concentrations (ng/L) in Port Curtis estuary and surrounding waters (dissolved cadmium and lead are shown in Appendix Table 3.5). ......................... 64

Figure 3.7. The concentrations of dissolved copper (▲, △), manganese (●, ○), nickel (◆, ◊), and zinc (■, □) measured along the transect directed from the outer harbour of Port Curtis into the Narrows during survey 1 (closed symbols) and survey 2 (open symbols). The entrance to the harbour was defined as site A1.17 (survey 1) and site B1.15 (survey 2), and the initials FL, SEN, RC, and NEN refer to the Yarwun trade waste outlet at Fisherman’s Landing, the southern entrance to the Narrows, Ramsay’s Crossing, and the northern entrance to the Narrows, respectively .................................................. 65

Figure 3.8. Temporal fluctuations in dissolved Cu (▲), Ni (◆), and Zn (■) near Ramsay’s Crossing and Fisherman’s Landing, where Time = 0 is the mid-point of the ebb tide and low tide occurred at a time of approximately 3h ................................................................. 68

Figure 3.9. The concentrations of suspended particulate matter (SPM) and SPM-Fe and -Zn (SPM-bound aluminium, copper, and manganese are shown in Appendix Table 3.6). .................................................. 69

Figure 3.10. Dissolved metals measured following 5 min re-suspension and 24-h storage of central Narrows (sampling site B1.5) sediment for (A) 24 h water pH’s 4-9 and (B) 24 h water pH’s 6.5-9.5 .................................................. 72

Figure 3.11. The pH (A) and salinity (B) of waters in Port Curtis and the Narrows (Apte et al., 2006) .... 74
Figure 3.12. The relationship between the dissolved manganese and nickel measured in Port Curtis and the Narrows during sampling in surveys 1 and 2. .................................................. 80
Figure 4.1. Bioassay vessels in environmental chambers. ......................................................... 89
Figure 4.2. Example of single pulse exposures with the same concentration (different exposure duration and TAC). .................................................................................. 92
Figure 4.3. Example of pulse exposures conducted for comparison of single and multiple pulses with the same concentration (different total exposure durations and TAC). .................................................. 93
Figure 4.4. Example of pulse exposures conducted to investigate the effects of varying pulse concentration (same duration and frequency, different concentration and TAC). .................................................. 93
Figure 4.5. Pulse exposure scenarios tested to investigate the effects of varying pulse duration for multiple pulses (same concentration and frequency, different duration and TAC) .................................................. 94
Figure 4.6. Examples of pulse exposure scenarios tested to investigate the effects of varying the interval between pulses (same concentration, exposure duration and TAC). .................................................. 94
Figure 4.7. Pulse exposure scenarios tested to investigate the effects of varying pulse frequency (same concentration, exposure duration, and TAC) ......................................................................... 95
Figure 4.8. Examples of pulse exposure scenarios tested to investigate the effects of different pulse exposure scenarios with equivalent exposure (different concentration and exposure duration, same TAC). .................................................................. 95
Figure 4.9. The relationship between survival of 16-d old *M. plumulosa* and the dissolved copper TAC in 10-d continuous exposure bioassays. .................................................. 97
Figure 4.10. Example of single pulses used to compare mortality of *M. plumulosa* exposed to equivalent net exposures (A; 100 µg/L, 96 h, and B; 900 µg/L, 10 h). .................................................. 97
Figure 4.11. The survival (mean ± standard deviation, n=3) of *M. plumulosa* at the end of 10-d bioassays in which organisms were exposed to single copper pulses with dissolved concentrations of 100, 200, 300, 400, 600, and 900 µg Cu/L for varying durations in Figures A, B, C, D, E, and F, respectively. Where * refers to mean mortality which was significantly different (p<0.05) to controls. ........................................................................................................... 99
Figure 4.12. The relationship between the duration of a single pulse required to cause significant *M. plumulosa* mortality (mean ± standard deviation, n=3) compared to the pulse concentration (20 individuals, 3 replicates per treatment). .................................................. 100
Figure 4.13. The mean survival of *M. plumulosa* compared to the TAC of pulse treatments 100-900 µg Cu/L pulses for durations of 4-160 h. .................................................................................. 102
Figure 4.14. Examples of delayed mortality of *M. plumulosa* (20 individuals per replicate, 3 replicates per treatment) following single pulse exposures of dissolved copper (filled bars) with concentrations of (A and B) 600 µg Cu/L, (C, D, E and F) 400 µg Cu/L, and (G and H) 300 µg Cu/L (mean ± standard deviation, n=3). .................................................................................. 103
Figure 4.15. The (i) measured concentrations of dissolved copper and the (ii) survival (mean ± standard deviation, n=3) following the 10-d bioassays of (A) 300 µg/L 48 h, (B) 400 µg/L 24 h, and (C) 400 µg/L 32 h (bars with symbols indicate treatments were significantly different from controls, treatments with the same symbol were not significantly different (p>0.05) different, treatments with different symbols were significantly different). .................................................................................. 105
Figure 4.16. The (i) measured concentrations of dissolved copper and the (ii) survival (mean ± standard deviation, n=3) following the 10-d bioassays of (A) 150-1200 µg/L, 5×2-h and (B) 150-1200 µg/L, 3×6-h, (symbols above bars interpreted the same as Figure 4.15). .................................................................................. 108
Figure 4.17. The (i) measured concentrations of dissolved copper over the durations of different pulses exposure scenarios (4 and 8-h pulses in one figure, 24 and 48-h pulses and continuous pulses in separate figure) and the (ii) survival (mean ± standard deviation, n=3) following the 10-d bioassays of 300 µg/L 3 × (4-48)-h and continuous exposure (symbols above bars interpreted the same as Figure 4.15). .................................................................................. 110
Figure 4.18. The (i) measured concentrations of dissolved copper and the (ii) survival (mean ± standard deviation, n=3) following the 10-d bioassays of single and double pulses of (A) 300 µg/L, (B) 400
µg/L, (C) 400 µg/L, and (D) 600 µg/L (symbols above bars interpreted the same as Figure 4.15).

Figure 4.19. The (i) measured concentrations of dissolved copper and the (ii) survival (mean ± standard deviation, n=3) following the 10-d bioassays of 400 µg/L for different pulse frequencies of total duration (A) 20 and (B) 32 h (symbols above bars interpreted the same as Figure 4.15). ..........115

Figure 4.20. The (i) measured concentrations of dissolved copper and the (ii) survival (mean ± standard deviation, n=3) following the 10-d bioassays of (A) 56-d old organisms exposed to 150-375 µg/L for 3 × (24-48)-h and continuous exposure, and (B) 16-d old organisms exposed to 150-1160 µg/L for 3 × (6-48)-h and continuous exposure (symbols above bars interpreted the same as Figure 4.15). ..........117

Figure 4.21. The relationship between the mean 10-d survival of M. phumulosa and the dissolved copper time-averaged concentration (TAC) for (A) all exposures and for (B) single, double, triple, and continuous exposures (B) tested in this study, where the TAC is the time-averaged concentration. ..........119

Figure 5.1. Example of pulse exposures conducted to investigate the effects of varying pulse duration (same concentration and frequency, different exposure duration and time averaged concentration (TAC)). The dissolved copper concentration during the period between pulses was designed to be approximately 1% of the concentration during pulses. ..........131

Figure 5.2. Example of exposure scenarios used to investigate the effects of pulsed exposures with different exposure concentrations and durations, but equal TAC. The study included an investigation of the effect of replacing 80, 90, and 99% of the bioassay solution, and the pulse concentrations in the period between pulses were 20, 10, and 1% of the pulse concentration. ..........132

Figure 5.3. Example of tests investigating the effect of the timing of single copper pulses (equivalent TAC) on biomass inhibition, and the algal recovery once the pulse was removed. The dissolved copper concentrations during the period between pulses were 1% of the concentration during pulses. ..........132

Figure 5.4. The relationship between biomass inhibition of P. tricornutum and continuous exposure dissolved copper concentration for (A) linear and (B) logarithmic scales. Note: the data in these figures was combined from 12 sensitivity tests. ..........136

Figure 5.5. Examples of the concentration-effects data used to calculate 72-h biomass inhibition of P. tricornutum exposed continuously to dissolved copper in seawater. ..........137

Figure 5.6. The (i) measured pulse exposure concentrations and the (ii) biomass inhibition (mean ± standard deviation, n=4) at the end of the 72-h bioassays for the (A) 30, (B) 100, (C) 200, and (D) 600 µg Cu/L pulse exposures (bars with symbols indicate treatments were significantly different from controls, treatments with the same symbol were not significantly (p<0.05) different, treatments with different symbols were significantly different). ..........141

Figure 5.7. The nominal pulse exposure scenarios for the (A) 99, (B) 90, and (C) 80% water replacement tests used to investigate the effects of different exposure scenarios with equivalent time-averaged concentrations (TACs) of dissolved copper. ..........142

Figure 5.8. The (i) measured pulse exposure scenario and the (ii) biomass inhibition (mean ± standard deviation, n=4) at the end of the 72-h bioassays of the three tests that investigated the effects of different exposure scenarios of equivalent TACs by employing 95% water replacement (symbols above bars interpreted the same as Figure 5.6) in (A) tests 1, (B) test 2, and (C) test 3. ..........146

Figure 5.9. The (i) measured pulse exposure scenario and the (ii) biomass inhibition (mean ± standard deviation, n=4) at the end of the 72-h bioassays of the three tests that investigated the effects of different exposure scenarios of equivalent TACs by employing 90% water replacement (symbols above bars interpreted the same as Figure 5.6) in (A) tests 1, (B) test 2, and (C) test 3. ..........149

Figure 5.10. The (i) measured pulse exposure scenario and the (ii) biomass inhibition (mean ± standard deviation, n=4) at the end of the 72-h bioassays employing 80% water replacement (symbols above bars interpreted the same as Figure 5.6). ..........151

Figure 5.11. The (i) measured pulse exposure scenario and the (ii) biomass inhibition (mean ± standard deviation, n=4) at the end of the 96-h bioassays of two tests that investigated the effects of timing
and algal recover using different exposure scenarios of equivalent TACs and employing 99% water replacement (symbols above bars interpreted the same as Figure 5.6) in (A) test 2 and (B) test 3. 155

Figure 5.12. The relationship between 72-h biomass inhibition of *P. tricornutum* cells and the dissolved copper TAC in tests investigating (A) the effect of pulse concentration and duration, and (B) all continuous and pulse copper exposure treatments tested in this study. .................................................. 157

Figure 6.1. The visualised shape of *P. tricornutum* cells as two cones joined at the base, which allowed measurements of length and width to be used in calculations of cell surface area and volume. ........................................... 163

Figure 6.2. The extra-cellular copper of copper-exposed *P. tricornutum* cells measured following different durations of extraction by 0.01 M EDTA. .................................................................. 167

Figure 6.3. The (A) length, (B) width, (C) surface area, and (D) volume of *P. tricornutum* cells following exposure to dissolved copper determined by light microscopy and Equations 6.1 and 6.2 (mean ± standard deviation, n≥15). .................................................................................. 170

Figure 6.4. Images of (A) control and (B) copper-exposed (15 µg Cu/L, 72 h) *P. tricornutum* cells generated by TEM. ........................................................................................................ 171

Figure 6.5. Images of (A) control and (B) copper-exposed (15 µg Cu/L, 72 h) *P. tricornutum* cells generated by TEM with higher magnification. ........................................................................................................ 171

Figure 6.6. The (A) extra-cellular and (B) intra-cellular copper following 72-h exposure to 0-50 µg Cu/L (mean ± standard deviation, n=3). ........................................................................................................ 172

Figure 6.7. The relationship between the biomass inhibition of *P. tricornutum* cells and the (A) extra- and (B) intra-cellular copper following 72-h exposure to 0-50 µg Cu/L (mean ± standard deviation, n=3). ........................................................................................................ 172

Figure 6.8. The relationship between the biomass inhibition of *P. tricornutum* cells and the (A) extra- and (B) intra-cellular copper following various 72-h pulse and continuous exposures (mean ± standard deviation, n=3). ........................................................................................................ 173

Figure 6.9. Relationships between extra-cellular copper and the duration of exposure to (A) 10 µg Cu/L (3 tests), (B) 30 µg Cu/L (2 tests), and (C) 50 µg Cu/L (1 test) in 72-h bioassays (mean ± standard deviation, n=3). ........................................................................................................ 174

Figure 6.10. Relationships between extra-cellular copper measurements pooled for equal concentrations (10 (▲), 30 (●), and 50 (●) µg Cu/L) and the duration of exposure in 72-h bioassay (mean ± standard deviation, n=3). ........................................................................................................ 175

Figure 6.11. Relationships between intra-cellular copper and the duration of exposure to (A) 10 µg Cu/L (3 tests), (B) 30 µg Cu/L (2 tests), and (C) 50 µg Cu/L (1 test) in 72-h bioassay tests (mean ± standard deviation, n=3). ........................................................................................................ 176

Figure 6.12. Relationships between intra-cellular copper measurements pooled for equal concentrations (10 (▲), 30 (●), and 50 (●) µg Cu/L) and the duration of exposure in 72-h bioassay (mean ± standard deviation, n=3). ........................................................................................................ 176

Figure 6.13. The relationship between the (A) extra-cellular (2 tests) and (B) intra-cellular (2 tests) copper and the duration of post-exposure for *P. tricornutum* cells exposed to 15 µg Cu/L (mean ± standard deviation, n=3). ........................................................................................................ 178

Figure 6.14. The relationship between (A) total intra-cellular copper in *P. tricornutum* pellets, and (B) cell density and the post-exposure duration (mean ± standard deviation, n=3). ........................................................................................................ 179

Figure 6.15. The inverse relationship between the intra-cellular copper per cell and the cell density in the two replicate tests. ........................................................................................................ 185

xix
List of Tables

Table 2-1. Summary of parameters employed in cultures and bioassays using the marine alga, *P. tricornutum*.

Table 3-1. Metal concentrations that were dissolved and bound to suspended particulate matter (SPM), SPM, and salinity in Port Curtis estuary and surrounding waters: Survey 1.

Table 3-2. Metal concentrations that were dissolved and bound to suspended particulate matter (SPM), SPM, salinity, and pH in Port Curtis estuary and surrounding waters: Survey 2.

Table 3-3. Quality control data for the dissolved metals analysed by solvent extraction (Section 3.2.3.2) (mean ± SD).

Table 3-4. Total particulate metal (TPM) concentrations in suspended particulate material retained in algal nets from Port Curtis estuary and surrounding waters (survey 2).

Table 3-5. Aqua-regia extractable metal concentrations measured in sediments from the mid-harbour and the central and southern Narrows collected in May 2005.

Table 3-6. Concentration of trace metals in waters around the world.

Table 4-1. The physico-chemical properties of sediment collected from Bonnet Bay (Sydney, NSW, Australia) for use in culturing *M. plumulosa*.

Table 4-2. Bioassay conditions employed in pulse and continuous dissolved copper exposure toxicity tests using *M. plumulosa*.

Table 4-3. Single pulse concentrations and durations tested, the time-averaged concentrations (TACs) achieved and the resulting organism survival.

Table 4-4. The time-averaged concentration (TAC) of dissolved copper required to cause significant mortality of *M. plumulosa* for each single pulse concentration.

Table 4-5. Data generated in 10-d bioassays investigating the effects of 48-h single and multiple pulse exposures on the survival of *M. plumulosa* (20 individuals per replicate, 3 replicates per treatment).

Table 4-6. Data generated in 10-d bioassays that employed multiple 6-h pulses to investigate the effect of concentration on the survival of *M. plumulosa* (20 individuals per replicate, 3 replicates per treatment).

Table 4-7. Data generated in 10-d bioassays that employed multiple pulses of 300 µg Cu/L to investigate the effect of pulse duration on the survival of *M. plumulosa* (20 individuals per replicate, 3 replicates per treatment).

Table 4-8. Data generated in 10-d bioassays that employed two pulses of 300 µg Cu/L to investigate the effect of the duration of the interval between pulses on the survival of *M. plumulosa* (20 individuals per replicate, 3 replicates per treatment).

Table 4-9. Data generated in 10-d bioassays that employed multiple pulses of 400 µg Cu/L to investigate the effect of pulse frequency on the survival of *M. plumulosa* (20 individuals per replicate, 3 replicates per treatment).

Table 4-10. Data generated in 10-d bioassays that employed multiple pulses of varying concentration and duration to investigate the effect of different pulse exposure scenarios of equivalent TAC of dissolved copper on the survival of *M. plumulosa* (20 individuals per replicate, 3 replicates per treatment).

Table 5-1. Summary of 72-h algal biomass recovery from daily centrifugation and/or water replacement at various speeds and durations.

Table 5-2. The dissolved copper TACs (µg Cu/L) calculated for bioassays where different exposure scenarios (exposure concentration and duration) were designed to deliver equivalent TACs.

Table 5-3. The 72-h biomass IC_{50} results for continuous exposure bioassays with *P. tricornutum*.
Table 5-4. Data generated from 72-h tests employing daily pulses to investigate the effect of pulse concentration and duration on the biomass inhibition of \textit{P. tricornutum}. ........................................ 140

Table 5-5. Data generated in 72-h tests that employed 99% water replacement to investigate the effect of different pulse exposure scenarios with equivalent dissolved copper TACs on the biomass inhibition of \textit{P. tricornutum}. ................................................................. 145

Table 5-6. Data generated in 72-h tests that employed 90% water replacement to investigate the effect of different pulse exposure scenarios with equivalent dissolved copper TACs on the biomass inhibition of \textit{P. tricornutum}. ................................................................. 148

Table 5-7. Data generated in 72-h tests that employed 80% water replacement to investigate the effect of different pulse exposure scenarios with equivalent dissolved copper TACs on the biomass inhibition of \textit{P. tricornutum}. ................................................................. 151

Table 5-8. Data generated in tests investigating the rates of cell biomass increase over successive 24-h intervals of 72-h bioassays that employed single 18- and 24-h pulses and continuous exposure... 153

Table 6-1. Extra- and intra-cellular copper following the different 72-h copper pulse exposure scenarios. .................................................................................................................. 173

Table 6-2. The 72-h uptake data for \textit{P. tricornutum} exposure to 10, 30, and 50 µg Cu/L. ................. 177