EFFECTS OF BIOLOGICAL AND PHYSICOCHEMICAL VARIABLES ON THE VALVE MOVEMENT RESPONSES OF FRESHWATER BIVALVES TO MANGANESE, URANIUM, CADMIUM AND COPPER

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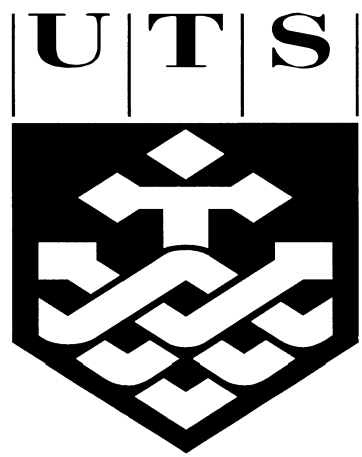
Thesis submitted for the degree of
Doctor of Philosophy



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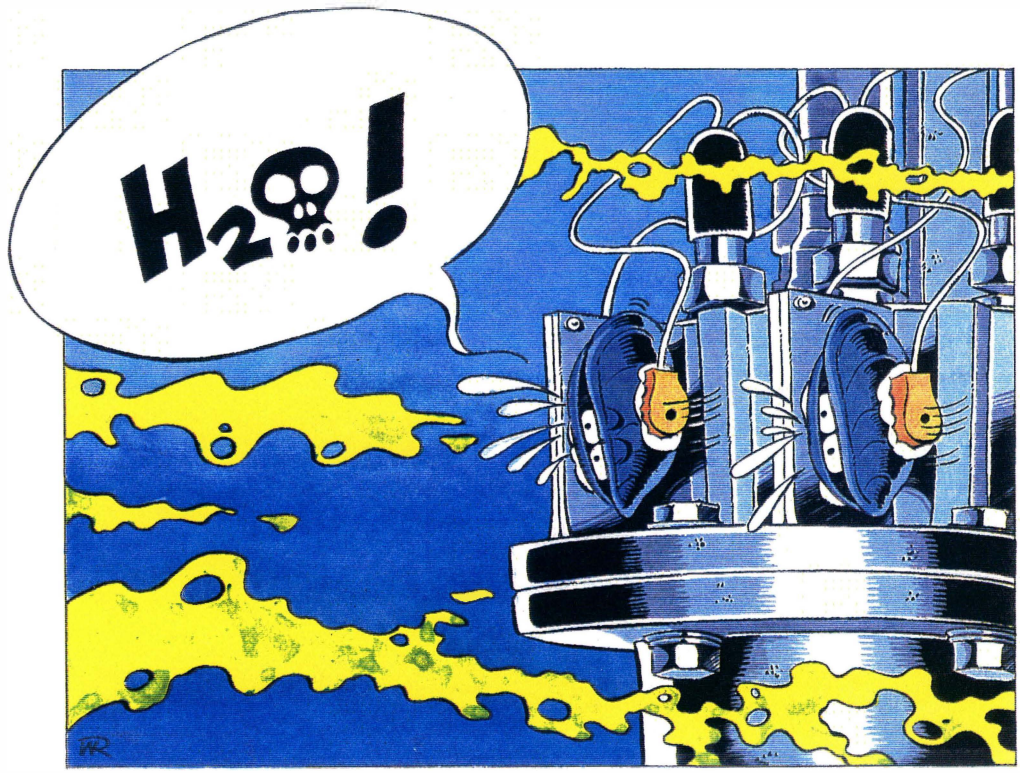

"Bivalves: Guardians of water quality."
(Courtesy of Delta Consult, Kapelle, The Netherlands)

To my family,
Judith, Ivan, Sean, Rebecca and Jeremy

"A man would accomplish nothing if he waited til he could do it so well that no one could find fault with it."

Cardinal John Henry Newman
(1801–1890)

CERTIFICATE

I certify that this thesis has not already been submitted for any degree and is not being submitted as part of candidature for any other degree.

I also certify that the thesis has been written by me and that any help that I have received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

Scott John Markich

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PUBLICATIONS ARISING FROM THIS THESIS

The following papers have been published, or submitted for publication, from material contained in this thesis:

Brown, P.L., Markich, S.J. and **Jeffree, R.A.** 1994. Migration of uranium: Integrating geochemistry with biomonitoring to evaluate and predict its environmental impact. *Radiochimica Acta* **66/67,** 351–357.

Markich, S.J. 1995. Behavioural responses of the tropical freshwater bivalve *Velesunio angasi* as real-time early-warning measures of uranium exposure. In: *Wetlands Research in the Wet-Dry Tropics of Australia* (C.M. Finlayson, Ed.). Supervising Scientist, Darwin. pp. 247–257.

Markich, S.J., Brown, P.L. and **Jeffree, R.A.** 1996. The use of geochemical speciation modelling to predict the impact of uranium to freshwater biota. *Radiochimica Acta* **74,** 321–326.

Markich, S.J., Brown, P.L., Batley, G.E., Apte, S.C. and **Stauber, J.L.** 1997. *Incorporating Metal Speciation and Bioavailability into Guidelines for Fresh and Marine Waters in Australia and New Zealand.* ANSTO/C503. Australian Nuclear Science and Technology Organisation, Sydney. 88 pp. (Integrated into the 1998 draft Australian water quality guidelines).

Markich, S.J. and **Brown, P.L.** 1998. Relative importance of natural and anthropogenic influences on the fresh surface water chemistry of the Hawkesbury-Nepean River, south-eastern Australia. *The Science of the Total Environment* **217,** 201–230.

Markich, S.J. and **Brown, P.L.** 1999. *Thermochemical Data for Environmentally-Relevant Elements. 1. Na, K, Ca, Mg, Mn, Fe, Al, U, Cu, Cd, Zn and Pb with Model Fulvic Acid (Aspartate, Citrate, Malonate, Salicylate and Tricarballyate).* ANSTO/E735. Australian Nuclear Science and Technology Organisation, Sydney. (In press).

Markich, S.J., Brown, P.L., Jeffree, R.A. and **Lim, R.P.** Evaluation of the free ion activity model of metal-organism interaction. 1. Valve movement responses of *Velesunio angasi* (Bivalvia: Hyriidae) to Mn and U. Submitted to *Aquatic Toxicology*.

Brown, P.L. and **Markich, S.J.** Evaluation of the free ion activity model of metal-organism interaction. 2. Extension of the conceptual model. Submitted to *Aquatic Toxicology*.

Reprints (where permitted) of published papers are given in Appendix L.

OTHER RELATED RESEARCH PUBLICATIONS

Jeffree, R.A., Markich, S.J. and **Brown, P.L.** 1993. Comparative accumulation of alkaline-earth metals by two freshwater mussel species from the Nepean River, Australia: Consistencies and a resolved paradox. *Australian Journal of Marine and Freshwater Research* **44,** 609–634.

Markich, S.J. and **Jeffree, R.A.** 1994a. Absorption of divalent trace metals as analogues of calcium by Australian freshwater bivalves: An explanation of how water hardness reduces metal toxicity. *Aquatic Toxicology* **29,** 257–290.

Markich, S.J. and **Jeffree, R.A.** 1994b. *Investigation on the Use of Freshwater Bivalves to Monitor the Bioavailable Levels of Elements in South Creek, Hawkesbury-Nepean River System.* ANSTO/C363. Australian Nuclear Science and Technology Organisation, Sydney. 36 pp.

Jeffree, R.A., Markich, S.J. and **Brown, P.L.** 1995. Australian freshwater bivalves: Their application in metal pollution studies. *Australasian Journal of Ecotoxicology* **1,** 33–41.

Jeffree, R.A., Markich, S.J., Lefebvre, F., Thellier, M. and **Ripoll, C.** 1995. Shell microlaminations of the freshwater bivalve *Hyridella depressa* as an archival monitor of manganese water concentration: Experimental investigation by depth profiling using secondary ion mass spectrometry (SIMS). *Experientia* **51,** 838–848.

Brown, P.L., Jeffree, R.A. and **Markich, S.J.** 1996. Kinetics of ^{45}Ca, ^{60}Co, ^{210}Pb, ^{54}Mn and ^{109}Cd in the tissue of the freshwater bivalve *Velesunio angasi*: Further development of a predictive and mechanistic model of metal bioaccumulation. *The Science of the Total Environment* **188,** 139–166.

Markich, S.J. 1996. *Element Concentrations in the Soft Tissues of a Freshwater Bivalve Species from the Mammy-Johnson River, NSW.* ANSTO/C485. Australian Nuclear Science and Technology Organisation, Sydney. 29 pp.

Twining, J.T., Ferris, J.M. and **Markich, S.J.** 1996. Bioaccumulation of ^{137}Cs and ^{85}Sr by an Australian sub-tropical freshwater teleost. *The Science of the Total Environment* **192,** 245–257.

Markich, S.J. and **Camilleri, C.** 1997. *Investigation of Metal Toxicity to Tropical Biota: Recommendations for Revision of the Australian Water Quality Guidelines.* Supervising Scientist Report 127. Supervising Scientist, Canberra. 87 pp.

Barata, C., Baird, D.J. and **Markich, S.J.** 1998. Influence of genetic and environmental factors on the tolerance of *Daphnia magna* to essential and non-essential metals. *Aquatic Toxicology* **42,** 115–137.

Franklin, N., Stauber, J.L., Markich, S.J. and **Lim, R.P.** 1999. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (*Chlorella* sp.). *Aquatic Toxicology* (In press).

ABSTRACT

The valve movement responses (VMR) of freshwater unionid bivalves to increasing concentrations of total Mn, U, Cd or Cu, under conditions of varying pH and/or dissolved organic carbon (model fulvic acid) concentrations, were experimentally measured using a computer-based data acquisition system. Specifically, *Velesunio angasi*, from Magela Creek in tropical northern Australia, was exposed to Mn and/or U in synthetic freshwater between pH 5.0 and 6.0, both with and without model FA (3.15 or 7.91 mg L^{-1}). In contrast, *Hyridella depressa*, from the Hawkesbury-Nepean River in temperate eastern Australia, was exposed to Cd and/or Cu in synthetic freshwater between pH 6.5 and 7.5, both with and without model FA (4.20 or 11.2 mg L^{-1}). Despite differences in bivalve species, geographical location (climate) and water chemistry, valve movement patterns and concentration-response curves were similar for all metals. The results showed that VMR is a quantifiable, sensitive and rapid, real-time endpoint for assessing the toxic effects of metal exposures. For Mn or Cd, VMR was independent ($P > 0.05$) of pH and/or model FA concentration. In contrast, VMR to U or Cu was highly dependent ($P \leq 0.05$) on pH and/or model FA concentration; individuals were more sensitive to U or Cu at low pH and model FA concentrations. Additionally, results from the concentration-response experiments were used to evaluate the free ion activity model (FIAM). Drug-receptor theory was used to develop an extended form of the FIAM to obtain a more rigorous conceptual model. The developed model explains the conditions under which the FIAM will be effective in explaining biological response (BR), but more importantly, precisely quantifies the interaction of metal species at cell receptor sites. Valve movement responses to Mn or Cd were directly proportional to the activity of the free metal ion (Mn^{2+} or Cd^{2+}), which is consistent with both the original and extended FIAM. In contrast, VMR to U or Cu were regarded as an 'exception' to the original FIAM, since they were a weighted function of the activities of the free metal ion and the 1:1 metal hydroxide species (UO_2^{2+} + UO_2OH^+ or Cu^{2+} + $CuOH^+$). However, this result is consistent with the extended FIAM, with BR primarily dependent on the activity of UO_2^{2+} or Cu^{2+}, and secondarily dependent on the activity of OH^-. Based on the extended FIAM, this study proposes, for the first time, a quantitative method of uncoupling the biological effects of a metal hydroxide species from that of amelioration of the free metal ion by H^+. This is a major outcome, since the activities of metal hydroxide and H^+ cannot be independently varied. Additionally, concentration-response data obtained from the literature, that are considered to be 'exceptions' to the original FIAM, were re-examined and found to be consistent with the extended FIAM. Overall, the extended FIAM provides a potentially more useful tool for evaluating metal-organism interaction than the original FIAM.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
AC	Alternating Current
AD	Analog to Digital
ANOVA	Analysis of Variance
ANSTO	Australian Nuclear Science and Technology Organisation
ANZECC	Australian and New Zealand Environment and Conservation Council
APHA	American Public Health Association
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
ARR	Alligator Rivers Region
Asp	Aspartic Acid
ASTM	American Society for Testing and Materials
ASV	Anodic Stripping Voltammetry
AVO	Amplitude of Valve Opening
BEC_{10}	10% Bounded Effect Concentration
BEWS	Biological Early Warning System(s)
Bq	Becquerel
BR	Biological Response
°C	Degrees Celsius/Centigrade
CCREM	Canadian Council of Resource and Environment Ministers
CI	Confidence Interval(s)
Cit	Citric Acid
CL	Confidence Limit(s)
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CV	Coefficient of Variation
CVC	Complete Valve Closure
DAVO	Duration and Amplitude of Valve Opening
DC	Direct Current
DEAE	Diethylaminoethyl-
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DRT	Drug Receptor Theory
DTW	Dry Tissue Weight
DUAP	Department of Urban Affairs and Planning
DVO	Duration of Valve Opening
EC_{50}	Median Effect Concentration
EDTA	Ethylenediaminetetraacetic Acid
E_H	Redox Potential (in mV)

EI	Exposure Index
EPRS	Electron Paramagnetic Resonance Spectroscopy
ERA	Energy Resources of Australia
ERISS	Environmental Research Institute of the Supervising Scientist
FA	Fulvic Acid
FIAM	Free Ion Activity Model
FVA	Frequency of Valve Adductions
FVOP	Frequency of Valve Opening Periods
Gly	Glycine
HA	Humic Acid
His	Histidine
HS	Humic Substances
I	Ionic Strength
IC	Ion Chromatography
ICPAES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICPMS	Inductively Coupled Plasma Mass Spectrometry
ISE	Ion Selective Electrode
IVA	Initial Valve Adduction
IVC	Immediate Valve Closure
K	Equilibrium Constant
KNP	Kakadu National Park
LC_{50}	Median Lethal Concentration
LFER	Linear Free Energy Relationship(s)
LOEC	Lowest Observable Effect Concentration
LVDT	Linear Variable Displacement Transducer
[M]	Free Metal Ion Activity
Mal	Malonic Acid
MCNC	Most Common Natural Concentration
MDEC	Minimum Detectable Effect Concentration
ML	Metal Complex
n	Total Number of Individuals or Variates
NBS	National Bureau of Standards
NEA	Nuclear Energy Agency
NMRS	Nuclear Magnetic Resonance Spectroscopy
NOEC	No Observable Effect Concentration
NSW	New South Wales
NTA	Nitriloacetic Acid
NT DME	Northern Territory Department of Mines and Energy
NTU	Nephelometric Turbidity Units
OECD	Organisation for Economic Co-operation and Development
P	Level of Significance (probability of incorrectly rejecting the null hypothesis)
PAR	Photosynthetically Active Radiation

pe	Measure of Redox Potential (at 25°C, pe = E_H/59)
pK_a	Acid Dissociation Constant
r^2	Coefficient of Determination
RP	Retention Pond
RRZ	Restricted Release Zone
RS	Reference Site
RUM	Ranger Uranium Mine
Sal	Salicylic Acid
SB	Shell Breadth
sd	Standard Deviation of the Mean
se	Standard Error of the Mean
SHNRW	Synthetic Hawkesbury-Nepean River Water
SL	Shell Length
SMCW	Synthetic Magela Creek Water
SPCC	State Pollution Control Commission
STP	Sewage Treatment Plant(s)
TDE	Truncated Davies Equation
TIVA	Time to Initial Valve Adduction
TOC	Total Organic Carbon
Tri	Tricarballylic Acid
TRLFS	Time-Resolved Laser-Induced Fluorescence Spectroscopy
TVC	Time to Valve Closure
UNESCO	United Nations Education, Scientific and Cultural Organisation
US EPA	United States Environmental Protection Authority
UTMIC	Unified Theory of Metal Ion Complexation
UTS	University of Technology, Sydney
VOP	Valve Opening Period(s)
YSI	Yellow Springs Instruments