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Valve movement responses of *Velesunio angasi* (Bivalvia: Hyriidae) to manganese and uranium: An exception to the free ion activity model

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Abstract

The veracity of the free ion activity model (FIAM) was tested by examining the acute (48 h) valve movement responses (VMR) (measured in terms of the duration of valve opening) of the Australian tropical freshwater unionid bivalve, *Velesunio angasi* to increasing concentrations of total Mn or U, in a standard synthetic water under conditions of varying pH (5.0–6.0) and/or dissolved organic carbon (model fulvic acid, FA) concentrations (0–8.9 mg l^{-1}). Valve movement behaviour, measured using an automated data acquisition system, was shown to be a quantifiable and rapid, real-time endpoint for assessing the toxic effects of Mn and U exposures. For Mn, the VMR of *V. angasi* were independent ($P > 0.05$) of pH and/or model FA concentration. In contrast, VMR to U exposures were highly dependent ($P \leq 0.05$) on pH and/or model FA concentration; individuals were more sensitive to U at low pH and model FA concentrations. Valve movement responses to Mn were directly proportional to the activity of the free metal ion (Mn^{2+}), which is consistent with the FIAM. In contrast, VMR to U were regarded as an 'exception' to the FIAM, since they were a weighted function of the activities of the free metal ion and the 1:1 metal hydroxide species (i.e. $1.86 \times {UO_2}^{2+} + UO_2OH^+$). Additionally, the effect of U on *V. angasi* demonstrates the importance of examining VMR at more than one pH. At a fixed pH, the results for U were consistent with the FIAM (i.e. response was directly proportional to ${UO_2}^{2+}$); only when pH was altered, were the results inconsistent with the FIAM. The inconsistency in the VMR of *V. angasi* to U exposures in this study, together with similar examples from other studies using different metals (e.g. Al or Zn), raises questions regarding the veracity of the FIAM. A detailed examination of the conceptual development of the FIAM is required to probe its apparent failure to describe several metal–organism interactions.

Keywords: Free ion activity model; Valve movement; Bivalve; Metal speciation; Uranium; Fulvic acid

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1. Introduction

Metals in aquatic systems exist in a variety of physicochemical forms, including the free hydrated metal ion (M^{z+}) and metal complexes with organic and inorganic ligands in dissolved, colloidal and/or particulate forms (Pickering, 1995). A convincing body of experimental evidence supports the free ion activity model (FIAM) (Morel, 1983; Campbell, 1995), which postulates that the biological effects of metals are best predicted by the activity of the free hydrated metal ion (M^{z+}), rather than the concentration of total metal. Therefore, knowledge of the distribution of a metal amongst its various physicochemical forms (i.e. speciation) is paramount to understanding the interaction of the metal with the cell surfaces of aquatic organisms (i.e. bioavailability).

A metal must first interact with and/or traverse the cell membrane surface to elicit a toxic response from a target organism and/or accumulate within the organism. Within the construct of the FIAM, the interaction of the metal with the cell membrane surface, involving either the free metal ion, M (charges here, and subsequently are omitted for brevity), or a metal complex, ML, as the reactive species, is represented in terms of the formation of cell surface complexes, M–X-cell, where X-cell is a cellular ligand present at the cell membrane surface and L is a metal binding ligand in solution (Campbell, 1995). For example, the interaction of the free metal ion, M, with the cell membrane surface, X-cell, can be expressed by the following reaction:

$$\mathrm{M} + \text{X-cell} \overset{K_1}{\leftrightarrow} \mathrm{M} - \text{X-cell} \quad (1)$$

from which the surface activity of M can be determined, namely

$$\{\mathrm{M} - \text{X-cell}\} = K_1\{\text{X-cell}\}[\mathrm{M}] \propto \mathrm{BR} \quad (2)$$

In Eq. (2), K_1 is a conditional equilibrium constant, and {} and [] refer to the activities of surface and dissolved species, respectively. The biological response (BR), whether it be (lethal or sublethal) toxicity or uptake/accumulation, is assumed to be proportional to the activity of the cell surface complex, {M–X-cell} (Campbell, 1995). It is evident from Eq. (2) that if {X-cell} remains approximately constant, then {M–X-cell} will be a linear function of [M], hence, BR is directly proportional to [M].

Almost without exception, experiments designed to test the FIAM have been performed at a fixed pH (i.e. [H]). The few data available for freshwater organisms (represented by a few algal, crustacean and fish species) exposed to metals under conditions of varying pH (Campbell, 1995), clearly indicate that [M] is insufficient to predict BR. The competition between [H] and [M] for metal binding sites on the cell surface has been suggested as a mechanism to explain this apparent departure from the FIAM (Campbell and Stokes, 1985). Furthermore, few studies have tested the FIAM in the presence of natural dissolved organic matter (DOM).

Natural DOM, in the form of fulvic and humic acids, is an important complexing agent for some metals, such as Cu and U, in aquatic systems (Buffle, 1988; Moulin et al., 1992). Although numerous studies have reported the effects of natural DOM on metal bioavailability to aquatic organisms, the vast majority have not determined metal speciation, and thus, are qualitative in nature (Campbell, 1995). Consequently, they are not suitable for testing the FIAM. The few quantitative studies that do exist are evenly divided between examples that are consistent with the FIAM, and others that appear to be contradictory (Campbell, 1995). Thus, the veracity of the FIAM in waters containing natural DOM remains to be confidently demonstrated.

Manganese and U have been identified as the dominant ecotoxicological contaminants in waste waters (Noller, 1991) entering Magela Creek from the ranger uranium mine (RUM), Alligator Rivers Region, Northern Australia (12°40′S, 132°57′E) [see Fig. 1 in Brown et al. (1994)]. The potential impact of these waste waters on aquatic ecosystems downstream of the RUM are of particular concern, since these wetland areas is an important part of the Kakadu National Park, which has been included in the World Heritage List. Therefore, any waste water releases from the RUM into Magela Creek need to be carefully controlled to minimise ecological detriment (NT DME, 1982).

Manganese and U have a high propensity for solubilisation and migration in natural waters (Morse and Choppin, 1991; de Vitre and Davison, 1993), and are present in RUM waste waters at concentrations up to 15 and 450 times higher, respectively, than their background concentrations in the surface waters of Magela Creek during the main wet season (Noller, 1991). Thus, both metals pose a potential hazard to aquatic organisms. However, few data are available on the effects of Mn and/or U on aquatic organisms, particularly macroinvertebrates [see review by Markich and Camilleri (1997)].

Velesunio angasi, a freshwater unionid bivalve, is ubiquitous and abundant throughout the Magela Creek system (Humphrey and Simpson, 1985), and its filter-feeding habit ensures that large volumes of water, containing metals, flow through the body on a daily basis. However, when exposed to toxic concentrations of metals, bivalves have the ability to reduce the exposure of their soft tissues to the aquatic environment, for extended periods, by valve closure (Manley and Davenport, 1979; Kramer et al., 1989; Salánki and Balogh, 1989; Huebner and Pynnönen, 1992). As such, standardised testing procedures, including fixed period median lethal (96 h LC_{50}) exposures, for assessing the effects of toxicants to bivalves are not suitable (Naimo, 1995).

Nevertheless, the toxicity of metals to bivalves can be assessed by measurement of valve movement behaviour (e.g. valve closure). A number of studies (e.g. Manley and Davenport, 1979; Kramer et al., 1989; Huebner and Pynnönen, 1992; Markich et al., 1996) have confirmed that this behaviour is a quantifiable, sensitive and rapid real-time biological endpoint for assessing the toxicological effects of metal exposures. Valve movement behaviour is an integrative measure that can be used to indicate physiological rate functions (e.g. feeding rate). For these reasons, bivalves are potentially useful as biological early warning systems of water quality (Matthias and Römpp, 1994; de Zwart et al., 1995).

Only one study (Schenck et al., 1988) has tested, and subsequently, provided evidence to support the FIAM with respect to Mn (i.e. *Chlamydomonas variablis*, a freshwater green microalga). None have been reported for U. The FIAM has not been tested using freshwater molluscs. Overall, there is a clear requirement to further test the veracity of the FIAM with metals of ecotoxicological relevance, under conditions of varying water chemistry, and using a larger range of freshwater organisms. The specific aim of this study was to test the FIAM using the freshwater bivalve, *V. angasi*, exposed to Mn or U, under conditions of varying pH and/or fulvic acid (FA) concentration. Additionally, the speciation of Mn and U in the experimental waters was determined using analytical and/or geochemical modelling procedures and related to valve movement response.

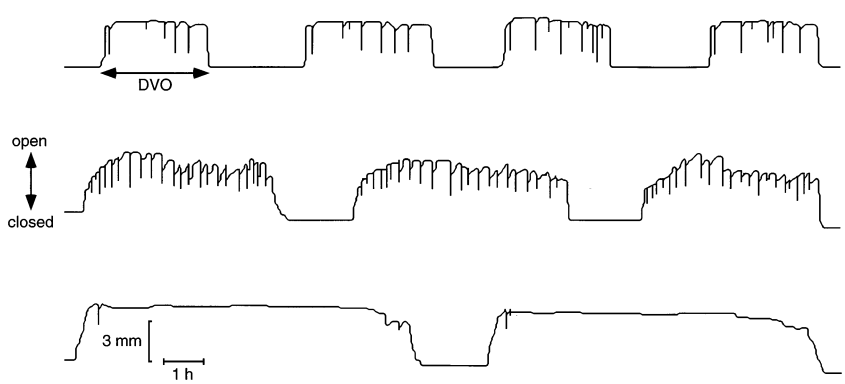

Fig. 1. Real-time valve movement patterns of three specimens of *V. angasi* exposed to synthetic Magela Creek water (SMCW). DVO, duration of valve opening.

Table 1
Measured inorganic composition of background synthetic Magela Creek water (SMCW)[a]

Parameter	SMCW[b]
Na (mg l^{-1})	1.03 ± 0.03
K (mg l^{-1})	0.38 ± 0.03
Ca (mg l^{-1})	0.47 ± 0.02
Mg (mg l^{-1})	0.62 ± 0.02
Cl (mg l^{-1})	1.37 ± 0.04
SO_4 (mg l^{-1})	2.87 ± 0.07
HCO_3 (mg l^{-1})	2.66 ± 0.11
NO_3 (μg l^{-1})	18.2 ± 1.3
PO_4 (μg l^{-1})	8.8 ± 1.5
Fe (μg l^{-1})	85 ± 5.7
Al (μg l^{-1})	74 ± 5.4
Mn (μg l^{-1})	9.9 ± 0.3
Cu (μg l^{-1})	0.76 ± 0.04
Zn (μg l^{-1})	0.77 ± 0.04
U (as UO_2) (μg l^{-1})	0.127 ± 0.018
Pb (μg l^{-1})	0.092 ± 0.018
Cd (μg l^{-1})	0.037 ± 0.006

[a] Without elevated levels of Mn or U. pH and temperature were tightly regulated (see Section 2.2).
[b] Mean $\pm$ 95% confidence limits; $n = 45$.

2. Methods and materials

2.1. Collection of animals

Specimens of *V. angasi*, covering a wide size range [shell length (SL), 13.05–71.70 mm], were collected manually and/or by an air–vacuum pump from Mudginberri Billabong (0.15 km^2), a minimally-polluted, permanent, waterbody in Magela Creek, located 12 km downstream of the RUM. The chemical composition of surface water from Mudginberri Billabong during the wet season (typically, November–March) is typically retained throughout the dry season (Humphrey and Simpson, 1985), allowing the constant sampling of *V. angasi* throughout the year. Animals were air-transported to the laboratory in an insulated container (15–20°C) within 36-h of collection.

2.2. Acclimation and feeding of animals

On arrival at the laboratory, animals were acclimated to a synthetic Magela Creek water (SMCW) (Table 1) in a 70-l Perspex holding tank (30-l of water without substrate) under flow-through conditions (95% replacement every 12 h; i.e. 7.5 l h^{-1}) (Sprague, 1973) for at least 7 days. The composition of SMCW represents the mean inorganic composition of the fresh surface waters of Magela Creek during the wet season [i.e. based on 20 years (1974–1994) of continuous water chemistry data; see review by Markich (1998)]. The wet season is the time when Magela Creek flows and when discharges from the RUM may occur. Many of the metals, including Mn and U, were added as sulfates, resulting in sulfate concentrations of 10–120-fold higher than those measured in natural Magela Creek water (NMCW). Nevertheless, sulfate is predicted to be elevated in Magela Creek water due to waste water releases from the RUM during the wet season (Noller, 1991).

Dissolved oxygen (DO) concentrations in the SMCW were maintained at near-saturation (88–95%) using filtered compressed air. Water temperature was regulated at 28.0 ± 0.1°C (mean water temperature for Magela creek during the wet season) using an immersion heater (Julabo PC4), that kept the water well circulated. The pH was maintained at pre-determined levels (i.e. 5.0, 5.3, 5.5, 5.8 or 6.0 ± 0.1) by metered additions of 0.05 mol l^{-1} NaOH and/or H_2SO_4 using a pH controller (Chemtrix model 45 AR). The selected pH range (5.0–6.0) typically reflects that of surface water in Magela Creek during the wet season, when waste water releases from the RUM, if permitted, are likely to occur. Photoperiod was kept constant at 12 h dark: 12 h subdued light (3 μmol photons PAR m^{-2} s^{-1}).

Since animals were maintained in the holding tanks for 2–5 months (experimental time course for each batch), it was essential that optimum feeding conditions be established to ensure a constant valve movement behaviour of individuals over this period. Optimum feeding conditions were found to be a combination of unicellular green algae (i.e. *Ankistrodesmus falcatus* and *Chlorella* sp.) (total density of ca. 10^7 cells per litre) and cattle manure leachate. This food combination was shown to maintain a constant valve movement behaviour [measured in terms of the duration of valve opening (DVO)] in animals held

under laboratory conditions for at least 210 days, and up to 10 days after cessation of feeding (Markich, 1998). Mortality of animals was negligible ($<2\%$) during maintenance in the laboratory.

2.3. Measurement of valve movement behaviour

The valve movement behaviour of immobilised specimens of *V. angasi* was continuously measured using a computer-based data acquisition system, as described by Markich (1995). Briefly, the left valve of each individual was cemented using cyanacrylate to a Perspex plate attached to the bottom of an experimental tank. A nylon thread attached to a clasp on the right valve was connected to the spring-loaded lever arm of a linear variable displacement transducer (LVDT) (Watson Victor). The induced AC signal from the LVDT was filtered so that underlying changes in the DC level, due to valve movement, could be accurately measured. The analog DC signal was then acquired and digitised by a MacLab (ADInstruments) (Macknight and Macknight, 1987; Stephens and Doherty, 1992). An acquisition rate of two readings per second was the minimum required to accurately measure the most subtle valve movement patterns (Markich, 1995). This acquisition rate was adopted for all subsequent experiments. Animals were allowed to resume normal activity for at least 2 h before measurement of valve movement. All measurements were initiated at the same time of day (i.e. 2–3 h before darkness).

Barnes (1962) questioned the environmental relevance of the valve movement behaviour of immobilised specimens maintained in the laboratory. However, Salánki and Véró (1969) later showed that the valve movement behaviour of immobilised, laboratory specimens (i.e. where one valve was fixed to a substrate) of the freshwater unionid bivalve *Anodonta cygnea*, did not significantly ($P>0.05$) differ from unrestrained activity in the field. This finding supports the methodology used in this study.

2.4. Experimental design

Eleven separate experiments were performed. Bivalves were exposed to constant concentrations of Mn or U at five pH levels (5.0, 5.3, 5.5, 5.8 and 6.0) without model FA, and at three pH levels (5.0, 5.5 and 6.0) with model FA (3.15 and 7.91 mg l^{-1}; equivalent to 3.7 and 8.9 mg l^{-1} as dissolved organic carbon, DOC). A FA model was employed to simulate the metal binding capacity of natural DOM in Magela Creek water from pH 3.0 to 7.0, by addition of finite mixtures of simple organic acids (Table 2), as described by Markich and Brown (1999). Two FA concentrations (3.15 and 7.91 mg l^{-1}) were simulated in the present study, typical of the mean DOC concentrations in Mudginberri Billabong during low and high flow conditions (Markich, 1998).

The valve movement responses of *V. angasi* to Mn or U were measured over two consecutive 48-h exposure periods (i.e. a control phase followed by an exposure phase). A previous study (Markich, 1995) showed that 24–36 h was the minimum period necessary to adequately characterise the valve movement behaviour of an individual, in the context of displaying several specific, rhythmic and reproducible valve movement patterns (Fig. 1). Markich (1995) also found a high inherent variability between individuals, with respect to the duration and amplitude for each valve opening period (VOP), and the frequency of valve adductions (FVA) per unit time for each VOP, when exposed to background con-

Table 2
Composition of the FA models used to simulate natural FA in Magela Creek water during low (3.7 m^3 s^{-1}) and high (30 m^3 s^{-1}) flow conditions

Organic acid	FA model (μ mol l^{-1})	
	Low flow[a]	High flow[b]
Aspartic acid	1.78	4.29
Citric acid	0.31	0.75
Malonic acid	1.82	4.38
Salicyclic acid	1.82	4.38
Tricarballylic acid	1.41	3.38

[a] Equivalent to 3.15 mg l^{-1} FA (3.7 mg l^{-1} DOC) or 14.2 μmol l^{-1} COOH.

[b] Equivalent to 7.91 mg l^{-1} FA (8.9 mg l^{-1} DOC) or 34.1 μmol l^{-1} COOH.

centrations of Mn and U (Fig. 1). To minimise this variability, all experiments were conducted using individuals as self-controls.

For experiments with Mn or U, the control phase consisted of measuring the valve movement characteristics of six individuals exposed to SMCW, containing background concentrations of Mn (9.7 μg l^{-1}) or U (0.11 μg l^{-1} as UO_2) (see Table 1), in 70 l Perspex tanks (30 l of water) under flow-through conditions (95% replacement every 12-h; i.e. 7.5 l h^{-1}). The exposure phase consisted of measuring the valve movement behaviour of the same individuals exposed to constant concentrations of Mn (0.0097–120 mg l^{-1}) or U (0.11–4000 μg l^{-1}) under identical experimental conditions. The range of Mn or U concentrations used was based on preliminary range-finding experiments, where the nature of the concentration–response relationship for each metal could be sufficiently characterised. Based on studies by Choppin and Clark (1991), Garnier et al. (1997), it was assumed that equilibrium was rapidly established for U and Mn species after their addition to SMCW.

Initial spike additions of Mn or U were used only when the valves of *V. angasi* were closed. Several experimental runs consisting of consecutive control phases were performed to adjust for any changes in temporal valve movement behaviour not associated with Mn or U concentrations elevated above background. All experimental runs were conducted with animals that had been exposed to background Mn or U concentrations only. Animals were neither physically handled, disturbed, nor fed throughout the experiments.

Due to the negatively skewed size-frequency distribution of bivalves (i.e. bias to larger individuals), it was necessary to allocate specimens to arbitrary size classes, based on increasing SL, to obtain an even size distribution for each experiment. Size classes for *V. angasi* were defined as: A, 13.05–48.45 mm (age, 0.1–1.9 years); B, 48.50–56.45 mm (age, 2.0–7.9 years) and C, 56.50–71.70 mm (age, 8.0–30 years). An identification code was scribed onto the shell of each individual. Specimens from the holding tank were then allocated to their corresponding size class (i.e. A, B or C). For all experiments, two individuals were randomly selected from each size class (i.e. resulting in a group of six individuals). Each group was randomly assigned to a given Mn or U concentration.

2.5. Evaluation of valve movement behaviour

The valve movement patterns of *V. angasi* (Fig. 1) exposed to constant concentrations of Mn or U, were evaluated using several characteristics (Markich, 1998). For brevity, only the results for the DVO for each VOP (Fig. 1) will be discussed here. The results for the DVO, however, are typical of the other valve movement characteristics (Markich, 1998). The DVO is commonly used in valve movement studies and provides a basic measure of metal exposure.

An exposure index (EI) was used to evaluate the DVO. This was calculated for an individual exposed to a given Mn or U concentration, by dividing the mean value of the DVO for the exposure phase (E), by the mean value of the DVO for the control phase (C). Thus, $EI = E/C$. An EI of 1.0 indicates an identical mean value of the DVO for both the control and exposure phases. The smaller the overall mean of the DVO during the exposure phase, the smaller the EI, and therefore, the greater the reduction in valve movement response by an individual. For example, an EI of 0.10 would indicate a ten-fold reduction in the DVO, relative to the control response. For each individual, the mean value of the DVO was based on a minimum of four VOPs, for each phase.

2.6. Physicochemical analyses

In situ measurements of pH, conductivity, redox potential (E_H) and DO in SMCW were performed daily, as described by Markich and Jeffree (1994). Concentrations of Na and K were measured using a Varian Model AA-975 flame atomic absorption spectrophotometer. Concentrations of Al, Ca, Mg, Mn, Na and Fe were measured using a Labtam Model 8410 Plasmascan inductively coupled plasma atomic emission spectrometer (ICPAES), while Cd, Cu, Pb, U and Zn were

measured using a VG PQ2 inductively coupled plasma mass spectrometer (ICPMS).

For all analytical measurements employing ICPAES and ICPMS, a multi-metal calibration standard (matched to the sample matrix) and a reagent blank were analysed with every eight samples to monitor signal drift. For all metals, the signal typically varied by 3–5%, although variations of up to 8% were noted, throughout an analytical run. Additionally, where ICPMS was utilised, gallium, indium and rhenium were employed as internal standards to correct for any non-spectral interferences (Vanhaecke et al., 1992). Quality assurance procedures were followed for all analyses, with emphasis on Mn and U. A duplicate sample and a standard reference material (SRM) (National Research Council of Canada, SLRS-2 Riverine water for trace metals) were analysed with each batch of ten samples to measure method precision and accuracy, respectively. The CV (%) for duplicate sample analyses averaged 7% for all metals. The mean concentrations of Mn and U in the SRM were consistently within their certified concentration ranges.

Chloride and sulfate were determined by ion chromatography using an anion exchange column (Dionex AS4A) and suppressed conductometric detection (Hansbury and Dyke, 1992). Nitrate was measured using the cadmium reduction standard method 4500–NO_3-E (APHA et al., 1989). Phosphate was measured using the automated ascorbic acid reduction standard method 4500–P-F (APHA et al., 1989). Alkalinity was measured using an automated potentiometric titration facility (Brown et al., 1992) following standard method 2320B (APHA et al., 1989) for low alkalinity waters. Bicarbonate concentrations were determined nomographically from alkalinity measurements using standard method 4500–CO_2 (APHA et al., 1989). Organic carbon was measured using the photocatalytic oxidation method described by Matthews et al. (1990). For treatments where model FA was added, total carboxylic acid concentration (COOH) was determined by direct potentiometric titration following the method described by Cabaniss (1991).

2.7. Geochemical speciation modelling

The thermodynamic geochemical speciation code HARPHRQ (Brown et al., 1991), an extended version of PHREEQE (Parkhurst et al., 1980), was used to predict the speciation of Mn (e.g. Mn^{2+}) and U (e.g. UO_2^{2+}) in SMCW. The input parameters for HARPHRQ were based on measured physicochemical data (i.e. pH, redox potential and ion concentrations; e.g. Table 1). Geochemical speciation calculations were constrained to a fixed (input) pH.

Equilibrium constants for the inorganic U species used in the geochemical simulations were derived primarily from the Nuclear Energy Agency (NEA) critical review series (Grenthe et al., 1992, 1995), but also from original research publications (e.g. Choppin and Mathur, 1991; Palmer and Nguyen-Trung, 1995; Brendler et al., 1996), where data were deemed to be consistent. Uranium (VI), or the uranyl ion (UO_2^{2+}), was used to represent U in this study. Equilibrium constants for the inorganic Mn species, as well as other inorganic metal species, in the SMCW were derived primarily from critical literature compilations and/or reviews (e.g. Baes and Mesmer, 1976; Nordstrom et al., 1990; Smith et al., 1995). In contrast to the inorganic metal species, few critically evaluated data (e.g. Smith et al., 1995) were available for Mn or U complexes with the organic acids (Table 2) that comprise the model FA. As such, the literature was critically reviewed by Markich and Brown (1999).

2.8. Statistical analyses

The 10% bounded effect concentration (BEC_{10}), an alternative statistical measure to the no observed effect concentration (NOEC), was estimated using the approach described by Hoekstra and van Ewijk (1993). The minimum detectable effect concentration (MDEC) (similar to the EC_{10}), an alternative measure to the lowest observed effect concentration (LOEC), was estimated using the approach described by Ahsanullah and Williams (1991).

A four-parameter logistic model (Guardabasso et al., 1987; Seefeldt et al., 1995) was used to fit

sigmoidal (i.e. concentration–response) relationships between measured total concentrations of Mn or U and the EI of the DVO. The a priori, mechanistically-based, selection of this model is discussed by Markich (1998). The adequacy of the logistic model was evaluated using a χ^2 goodness of fit test (Sokal and Rohlf, 1995) and confirmed in all cases. From the fitted sigmoidal concentration–response curves, a series of toxic endpoints for the DVO, ranging from the EC_{10} through to the EC_{90}, were calculated for *V. angasi* exposed to Mn or U. Differences between concentration–response curves were tested using the generalised *F* test (Ratkowsky, 1990).

Simple linear and stepwise multiple linear regression analyses were performed to determine if Mn or U species (e.g. Mn^{2+}, $UO_2{}^{2+}$), were significant ($P \leq 0.05$) predictors of the valve movement response (DVO) of *V. angasi* exposed to given Mn or U concentrations. The assumptions of simple and multiple linear regression analyses were tested (Helsel and Hirsch, 1992) and model adequacy was obtained in all cases. Significance levels were tested at the $P = 0.05$ level, unless otherwise indicated.

3. Results and discussion

3.1. Water chemistry

A summary of measured physicochemical data for background SMCW, without model FA, is given in Table 1. For all experiments, the mean measured concentrations of major ions and trace metals (including Mn and U) in SMCW were all within 10% of their nominal concentrations, but usually within 5%. In experiments, where model FA was added, the mean measured COOH concentrations (a collective measure of the five organic acids; Table 2) were within 5% of their nominal concentrations.

3.2. Metal speciation

3.2.1. Manganese

The predicted speciation of Mn in SMCW, without model FA, was independent of pH from 5.0 to 6.0, with Mn^{2+} being the dominant species (87–99%) across the range of total Mn concentrations (0.0097–120 mg l^{-1}). The proportion of Mn^{2+} gradually decreased (i.e. from 99 to 87%) with increasing Mn concentration. This decrease was offset by a gradual increase in the proportion of $MnSO_4$(aq) (1–12%), due largely to the addition of total Mn as the sulfate salt. The addition of model FA (3.15 or 7.91 mg l^{-1}) produced a negligible change in the predicted speciation of Mn from pH 5.0 to 6.0. Organic Mn species (primarily MnMal, where Mal is malonate) comprised a maximum of only 0.7% of total Mn at pH 6.0 and 7.91 mg l^{-1} model FA. In summary, the predicted speciation of Mn in SMCW was essentially independent of both pH and the concentration of model FA used in this study.

To partially verify the predictions from the model, the percentage of Mn^{2+} in SMCW [pH 5.0 and 6.0, with (7.91 mg l^{-1}) and without model FA] was measured across a range of total Mn concentrations (1.0, 10, 30 and 60 mg l^{-1}) using electron paramagnetic resonance spectroscopy, as described by Chiswell and Mokhtar (1987). The agreement between the two techniques was very good ($\leq 1\%$ difference), indicating that geochemical speciation modelling provided a realistic estimate of Mn^{2+} in SMCW for the experimental conditions used. Electron paramagnetic resonance spectroscopy (EPRS) has been used by several investigators (Carpenter, 1983; Chiswell and Mokhtar, 1987; Noller, 1992) to demonstrate that the free hydrated Mn^{2+} ion is the major species (90–98%) of dissolved Mn for a range of freshwater systems (pH 5–8), including Magela Creek. Noller (1992) showed that Mn^{2+} comprised 93–98% of Mn in filtered (< 0.015 μm) surface water samples from the Magela Creek floodplain. Manganese typically forms weak complexes with natural DOM and, as such, Mn–DOM complexes usually comprise a minor component of dissolved Mn in most freshwater systems (Laxen et al., 1984; LaZerte and Burling, 1990; Lu et al., 1997).

3.2.2. Uranium

In contrast to Mn, the predicted speciation of U in SMCW (pH 5.0–6.0 at 0, 3.15 and 7.91 mg l^{-1} model FA) is complex, and highly dependent

on pH and/or model FA concentration. The speciation of U under these experimental conditions has been discussed in detail by Markich et al. (1996). In summary, at pH 5.0 without model FA, UO_2^{2+} (43–58%) and UO_2OH^+ (26–36%) were the dominant uranyl species predicted to form (Fig. 2a). The relative proportions of these two species decreased with increasing total U concentration; such decreases were offset by increases (0–12%) in the relative proportions of polymeric uranyl species [i.e. $(UO_2)_2(OH)_2^{2+}$ and $(UO_2)_3(OH)_5^+$]. However, at pH 6.0 without model FA, UO_2^{2+} was predicted to be a minor species (1–10%), while UO_2OH^+ was only important at low U concentrations (Fig. 2b). Polymeric uranyl species, such as $(UO_2)_2(OH)_3CO_3^-$, $(UO_2)_3(OH)_5^+$ and $(UO_2)_3(OH)_7^-$ were more dominant than at pH 5.0, and similarly, increased in importance with increasing total U concentration.

At pH 5.0 with model FA (7.91 mg l^{-1}), UO_2^{2+} (26–35%) and UO_2OH^+ (15–21%) remained the dominant uranyl species predicted to form, although at reduced proportions (Fig. 2c). This was due to the formation of four organic uranyl species [i.e. UO_2Cit^-, $UO_2(OH)Cit^{2-}$, UO_2Mal and $UO_2(OH)Mal^-$, where Cit is citrate]. The relative proportions of the organic uranyl species progressively declined with increasing total U concentration; such reductions were offset by increases in the relative proportions of inorganic uranyl species once the binding capacity of the organic ligands comprising the model FA was exceeded (Fig. 2c). The formation of organic uranyl complexes delayed the onset of the formation of polymeric inorganic complexes. Similar trends were observed for the speciation of U at pH 6.0 and 7.91 mg l^{-1} model FA (Fig. 2d), except that only three organic uranyl species [i.e. $UO_2(OH)Cit^{2-}$, UO_2Mal and $UO_2(OH)Mal^-$] were predicted to form. In contrast to pH 5.0 and 7.91 mg l^{-1} model FA, the proportions of the hydrolysed organic uranyl species [i.e. $UO_2(OH)Cit^{2-}$ and $UO_2(OH)Mal^-$] were relatively greater than the unhydrolysed uranyl-organic species (e.g. UO_2Mal).

To partially verify the predictions from the model, the percentage of UO_2^{2+} and UO_2OH^+ in SMCW (pH 5.0 and 5.5 without model FA and pH 5.0 with 3.15 mg l^{-1} model FA) was measured across a range of total U concentrations using time-resolved laser-induced fluorescence spectroscopy, as described by Moulin et al. (1995). The general agreement between the two techniques was good (2–5% difference), indicating that geochemical speciation modelling provides a reasonable estimate of UO_2^{2+} and UO_2OH^+ in SMCW for the experimental conditions used.

The speciation of U measured in freshwaters by Li et al. (1980), Giesy et al. (1986) was also consistent with predictions from our model (Table 3). Li et al. (1980) measured the speciation of U (50 μg l^{-1}) in a freshwater (hardness, 4 mg l^{-1} as $CaCO_3$; 20 mg l^{-1} FA) over a wide range of pH (2–9) using a combination of dialysis and ultrafiltration techniques. Similarly, Giesy et al. (1986) measured the speciation of U (10 μg l^{-1}) in a freshwater (pH 5.5; hardness, 12 mg l^{-1} as $CaCO_3$; 3.5 mg l^{-1} HA) using a combination of chromatography (chelex) and fluorescence techniques. Geochemical modelling of the water chemistry of both studies using the model FA as an analogue of natural FA (Li et al., 1980) or HA (Giesy et al., 1986), showed similar relative proportions of inorganic and organic uranyl complexes as obtained by the authors of both studies (Table 3). The close agreement between the measured and predicted data for both studies indicates that the model of U speciation used in this study provides reliable estimates of inorganic- and organic (natural DOM)-bound U in two different freshwater systems. In contrast to Mn, U typically forms strong complexes with natural DOM and, as such, uranyl–DOM complexes usually comprise a major component of dissolved U in most freshwater systems (Table 3).

3.3. Concentration–response relationships

Sigmoidal (S-shaped) concentration–response relationships were established for both Mn and

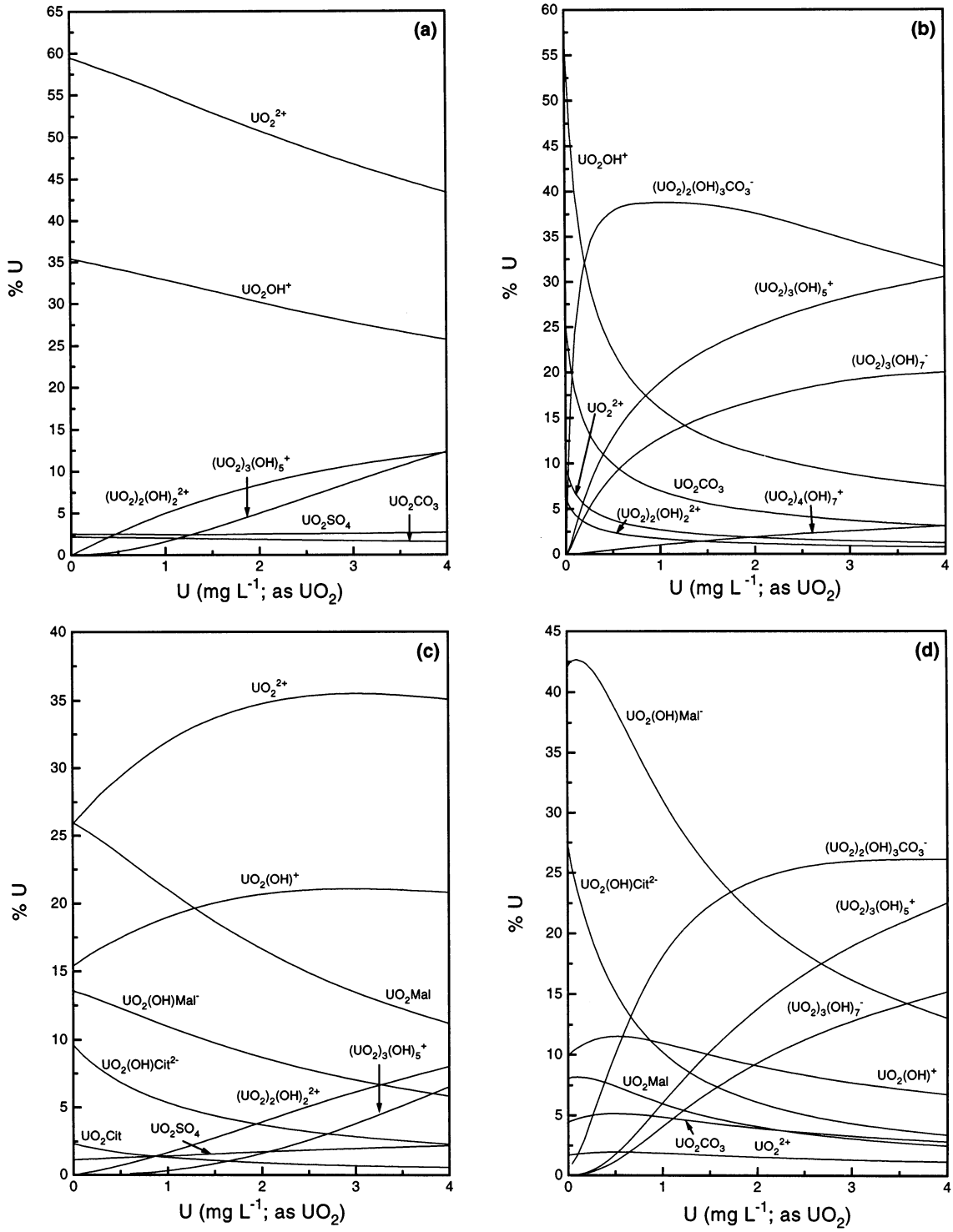

Fig. 2. Predicted speciation (% distribution) of U in SMCW at (a) pH 5.0 without model FA; (b)) pH 6.0 without model FA; (c) pH 5.0 + 7.91 mg l^{-1} model FA; and (d) pH 6.0 + 7.91 mg l^{-1} model FA. Uranyl species comprising $< 2\%$ total U are excluded for clarity.

U under all conditions of varying pH and/or model FA concentration (Markich, 1998). Values of the BEC_{10}, MDEC and EC_{50} for each relationship are given in Table 4. A summary of the concentration–response relationships, highlighting the range of DVO under conditions of minimum and maximum pH and/or model FA concentration (i.e. pH 5.0, pH 5.0 + 7.91 mg l^{-1} FA, pH 6.0 and pH 6.0 + 7.91 mg l^{-1} FA), is provided in Figs. 3 and 4 for Mn and U, respectively.

The valve movement response (DVO) of *V. angasi* to Mn exposures was independent ($P > 0.05$) of pH and/or the concentration of model FA, at least over the range used in this study (Fig. 3). Concentration–response curves were not significantly ($P > 0.05$) different (generalised *F* test) between treatments. This was also evident in the overlap of the mean and 95% CL for all EC_{50} values given in Table 4.

These results are consistent with those of several other studies (pH: Babich and Stotsky, 1981; Reader et al., 1988; Witters et al., 1990; Wepener et al., 1992; Lee et al., 1994; natural DOM: Rouleau et al., 1994). Babich and Stotsky (1981) established that the toxicity (growth rate) of Mn

Table 3
Comparison of the measured and predicted speciation of U in two freshwaters

U species	Li et al. (1980)				Giesy et al. (1986)	
	Measured (%)[a]		Predicted (%)[b]		Measured (%)[c]	Predicted (%)[b]
Inorganic U	21[d]	8[e]	23[d]	5[e]	68	62
U-Fulvate	79[d]	92[e]	77[d]	95[e]	–	–
U-Humate	–	–	–	–	32	38

[a] 20 mg l^{-1} FA.
[b] Predicted using geochemical speciation modelling in this study (see text).
[c] pH 5.5 and 3.5 mg l^{-1} HA.
[d] pH 5.0.
[e] pH 6.0.

Table 4
Sensitivity of *V. angasi* exposed to Mn or U in SMCW under conditions of varying pH and/or model FA concentration[a]

Treatment		Duration of valve opening (DVO)					
pH	Model FA (mg l^{-1})	Mn			U		
		BEC_{10}	MDEC	EC_{50} (95% CL)	BEC_{10}	MDEC	EC_{50} (95% CL)
5.0	–	17.2	18.5	30.0 (1.5)	92.1	95.0	117 (3)
5.0	3.15	16.6	17.4	28.5 (1.4)	113	118	144 (5)
5.0	7.91	17.1	18.3	29.4 (1.4)	197	208	247 (9)
5.3	–	16.9	17.8	29.5 (1.5)	108	114	141 (5)
5.5	–	16.7	17.6	28.9 (1.5)	125	132	163 (6)
5.5	3.15	16.1	16.8	27.5 (1.4)	192	228	242 (9)
5.5	7.91	16.8	17.6	28.8 (1.4)	399	417	497 (19)
5.8	–	16.5	17.3	28.2 (1.4)	214	224	290 (14)
6.0	–	16.2	17.3	27.7 (1.4)	416	440	634 (28)
6.0	3.15	16.2	16.9	27.9 (1.4)	558	598	824 (39)
6.0	7.91	16.3	17.1	27.9 (1.4)	913	958	1228 (48)

[a] Values are reported in mg l^{-1} for Mn and μg l^{-1} for U (as uranyl) for 48 h.

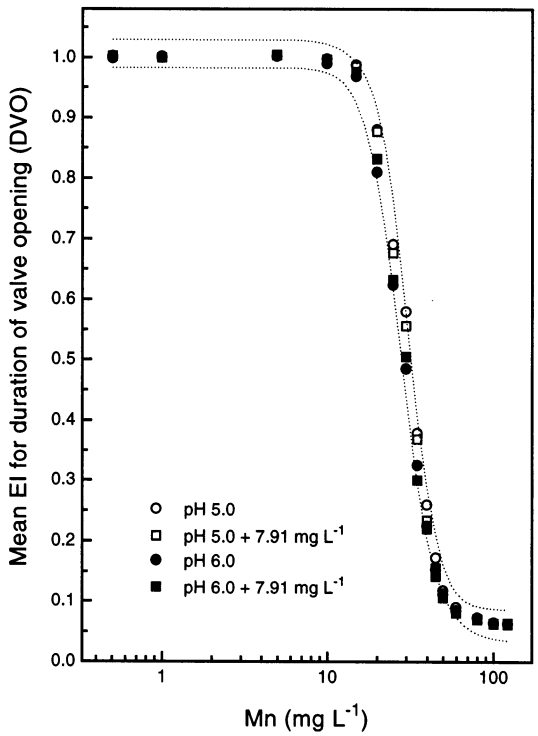

Fig. 3. Concentration-response relationships of the duration of valve opening (DVO) for *V. angasi* exposed to Mn in SMCW at pH 5.0 and 6.0 without model FA and pH 5.0 and 6.0 with model FA (7.91 mg l^{-1}). Each plotted point represents the mean response of six individuals. The dotted lines represent the upper and lower 95% confidence limits around the sigmoidal curve. Error bars and curve fits are excluded for clarity.

to three species of freshwater fungi (*Aspergillus niger*, *Gliocladium* sp. and *Penicillium vermiculatum*) was not significantly ($P > 0.05$) different at pH 5.5, 6.5 or 7.5. Similarly, Witters et al. (1990) reported that the toxicity (96 h LC_{50}) of Mn to the rainbow trout, *Oncorhynchus mykiss*, was not significantly ($P > 0.05$) different at pH 6.0, 7.0 or 8.0. Both studies varied pH independently of other related water quality parameters, such as hardness and alkalinity. However, some studies (France and Stokes, 1987; Schenck et al., 1988; Rouleau et al., 1996) have shown that Mn toxicity to, and/or uptake by, freshwater organisms may increase or decrease with increasing pH between pH 3.5 and 8.5. In the only reported study on the effects of fulvic and/or humic acids on Mn uptake by, or toxicity to, aquatic organisms, Rouleau et al. (1994) showed that the uptake of Mn by brown trout (*Salmo trutta*) in the presence of 5.0 mg l^{-1} humic acid, was not significantly ($P > 0.05$) different to Mn uptake in the absence of humic acid.

In contrast to the results for Mn, the valve movement responses of *V. angasi* to U were highly dependent ($P \leq 0.001$) on pH and/or the concentration of model FA used (Fig. 4). For a given model FA concentration, the sensitivity of *V. angasi* to U decreased exponentially as pH increased from 5.0 to 6.0. Similarly, for a given pH, the sensitivity of *V. angasi* to U decreased exponentially as the model FA concentration increased from 0 to 7.91 mg l^{-1}. Based on Table 4

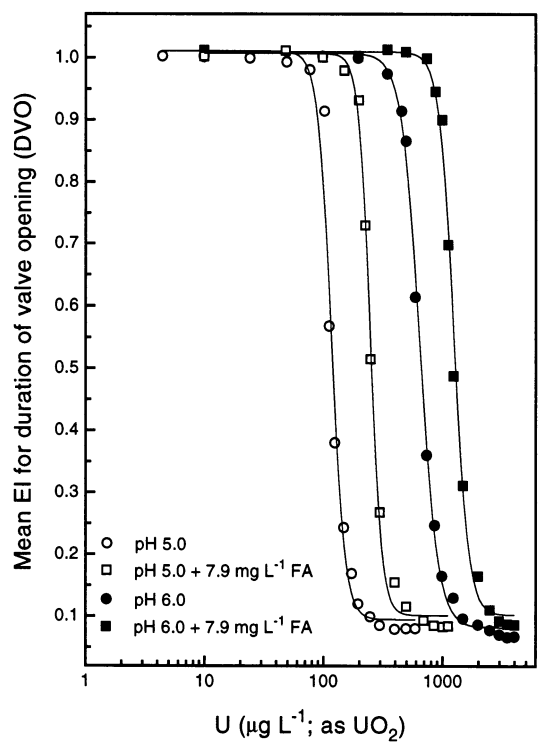

Fig. 4. Concentration-response relationships of the duration of valve opening (DVO) for *V. angasi* exposed to U in SMCW at pH 5.0 and 6.0 without model FA and pH 5.0 and 6.0 with model FA (7.91 mg l^{-1}). Each plotted point represents the mean response of six individuals. Error bars are excluded for clarity.

and Fig. 4, it is evident that the sensitivity of *V. angasi* to U decreases by a factor of about five, with an increase in pH from 5.0 to 6.0 (e.g. the EC_{50} decreases from 634 μg l^{-1} at pH 6.0 to 117 μg l^{-1} at pH 5.0). Additionally, in the presence of the maximum concentration of model FA (7.91 mg l^{-1}), the sensitivity of *V. angasi* to U at pH 5.0 and 6.0 is reduced by about a factor of two. For example, the EC_{50} for the DVO increases from 634 μg l^{-1} at pH 6.0 to 1228 μg l^{-1} at pH 6.0 + 7.91 mg l^{-1} FA (Table 4). The sensitivity of *V. angasi* at a given total U concentration was greatest at pH 5.0 without model FA, and least at pH 6.0 with the maximum concentration (7.91 mg l^{-1}) of model FA (Table 4 and Fig. 4). *V. angasi* is more sensitive to U at pH 5.0 with the maximum concentration of model FA (i.e. pH 5.0 + 7.91 mg l^{-1}), than at pH 6.0 without model FA.

The effects of pH on the valve movement response of *V. angasi* to U apparently differ with the results of Franklin et al. (2000), who showed that the toxicity (cell division rate) of U to the tropical unicellular alga, *Chlorella* sp., increased by a factor of 1.8 (i.e. from a 72 h EC_{50} of 89 to 44 μg l^{-1} as UO_2) when pH increased from 5.7 to 6.5 in SMCW. The predicted speciation of U in this range is dominated (70–93%) by the polymeric uranyl hydroxy carbonate species, $UO_2(OH)_3CO_3^-$, with only a very small (< 4%) proportion occurring as the free uranyl ion (UO_2^{2+}). Given the small proportion of UO_2^{2+} at both pH values, the effect of H^+ itself may have been responsible for ameliorating the toxicity of U to *Chlorella* sp. at pH 5.7. Indeed, Franklin et al. (2000) found that intracellular U was two-fold lower at pH 5.7 than at 6.5. Nakajima et al. (1979) and Greene et al. (1986) also determined the effect of pH on the uptake of U by unicellular green algae (*Chlorella regularis* and *C. vulgaris*, respectively). However, the two latter studies determined the effect of pH over a broad pH range (3.0–9.0), and simply concluded that uptake was highest at pH 5–6. Unlike the work of Franklin et al. (2000), the results were not sufficient to discern the true effects of pH on U uptake by the two algal species between pH 5.0 and 6.5.

No previous studies have determined the effects of fulvic and/or humic acids on U uptake by, or toxicity to, aquatic organisms. However, with respect to the effect of citrate, one of the organic acids used in the model FA, the results of this study appear to be consistent with those of Yong and Macaskie (1995), who found that the formation of uranyl-citrate reduced the toxicity of U to the bacterium, Citrobactor, at pH 7.0.

3.4. Relationship between valve movement response and metal speciation

3.4.1. Exposure of *V. angasi* to Mn

The speciation of Mn and the valve movement response (DVO) of *V. angasi* to Mn are both independent of pH and/or the model FA concentration (Sections 3.2.1 and 3.3). Based on the concentration–response curves for each experimental treatment, individual BR endpoints (i.e. EC_{10}, EC_{15}, EC_{20}, through to EC_{90}) were calculated. Given that there was no significant ($P > 0.05$) difference (generalised F test) between the concentration–response curves for each treatment (Fig. 3), the individual BR endpoints were pooled (i.e. a mean value was calculated). The pooled values of total Mn concentration at each selected BR endpoint are given in Table 5. These are expressed in terms of the activity of both Mn^{2+} and $MnSO_{4(aq)}$, as predicted using HARPHRQ. Therefore, the valve movement response of *V. angasi* to total Mn can potentially be explained in terms of Mn^{2+} and/or $MnSO_{4(aq)}$ (Table 5).

The selected BR endpoints (i.e. EC_{10}–EC_{90}) were regressed against Mn^{2+} or $MnSO_{4(aq)}$. Highly significant ($P \leq 0.001$) simple linear regressions were fitted for both Mn^{2+} ($r^2 = 0.973$) and $MnSO_{4(aq)}$ ($r^2 = 0.927$). Although simple linear regressions were fitted, the plotted points noticeably deviated from linearity (as might be expected from a sigmoidal concentration–response curve) and violated the assumptions of linear regression analysis (Sokal and Rohlf, 1995). Based on residual and outlier (Cooks Distances) analyses (Helsel and Hirsch, 1992), data points on the upper tail of the linear regressions (i.e. EC_{80}, EC_{85} and EC_{90}) were iteratively removed, until the assumptions of linear regression analysis were satisfied. The resulting linear fits were highly significant ($P \leq 0.001$) for Mn^{2+} ($r^2 = 0.994$) and $MnSO_{4(aq)}$

Table 5
Concentrations of total Mn, expressed in terms of the activity of Mn^{2+} and $MnSO_{4(aq)}$, causing a fixed response (DVO) in *V. angasi*[a]

Biological response endpoint	Total Mn	Mn^{2+}	$MnSO_4$(aq)
EC_{10}	17.8 (1.8)	17.1 (1.8)	0.68 (0.05)
EC_{15}	19.6 (1.7)	18.8 (1.7)	0.78 (0.04)
EC_{20}	21.2 (1.7)	20.3 (1.7)	0.91 (0.04)
EC_{25}	22.5 (1.6)	21.5 (1.6)	1.01 (0.04)
EC_{30}	23.8 (1.6)	22.7 (1.6)	1.12 (0.03)
EC_{35}	25.0 (1.6)	23.7 (1.6)	1.22 (0.03)
EC_{40}	26.1 (1.5)	24.8 (1.5)	1.33 (0.03)
EC_{45}	27.3 (1.5)	25.9 (1.5)	1.43 (0.02)
EC_{50}	28.6 (1.4)	27.0 (1.4)	1.55 (0.02)
EC_{55}	28.8 (1.5)	28.1 (1.5)	1.68 (0.02)
EC_{60}	31.1 (1.5)	29.3 (1.5)	1.81 (0.03)
EC_{65}	32.6 (1.6)	30.7 (1.6)	1.94 (0.03)
EC_{70}	34.3 (1.6)	32.1 (1.6)	2.15 (0.03)
EC_{75}	36.2 (1.7)	33.8 (1.7)	2.33 (0.04)
EC_{80}	38.5 (1.7)	35.8 (1.7)	2.64 (0.04)
EC_{85}	41.5 (1.8)	38.4 (1.8)	3.03 (0.05)
EC_{90}	45.9 (1.9)	42.3 (1.9)	3.58 (0.05)

[a] Response is based on the DVO for 11 experimental treatments [i.e. pH 5.0–6.0, both with and without the addition of the model FA (3.15 and 7.91 mg l^{-1})]. Values are reported as the mean (and 95% CL) of 11 treatments in mg l^{-1}.

($r^2 = 0.990$) between the EC_{10} and EC_{75}. Thus, from a statistical standpoint, both Mn^{2+} and $MnSO_{4(aq)}$ are equally capable (i.e. similar r^2 values) of explaining the valve movement response (BR) of *V. angasi*. Furthermore, Mn^{2+} and $MnSO_{4(aq)}$ were highly correlated ($r = 0.993$). These features precluded the effective use of multiple linear regression analyses.

The high correlation between Mn^{2+} and $MnSO_{4(aq)}$ was not surprising given that total Mn was added to the synthetic water in the form of sulfate, and the activity of $MnSO_{4(aq)}$ is directly proportional to the activity of Mn^{2+} Eq. (3).

$$[MnSO_{4(aq)}] = K[Mn^{2+}][SO_4^{2-}] \quad (3)$$

where K is the conditional equilibrium constant for $MnSO_{4(aq)}$.

To determine whether Mn^{2+} was governing the decrease in the DVO in *V. angasi*, a supplementary experiment was designed to uncouple the effects of Mn^{2+} and $MnSO_{4(aq)}$. This was achieved by adding Mn to the synthetic water in the form of nitrate, to remove the contribution of sulfate, and hence, $MnSO_{4(aq)}$. Furthermore, Mn was added in the form of nitrate because it forms very weak complexes with nitrate and is predicted to occur solely as Mn^{2+} (99.6%). This was confirmed using EPRS. With respect to the experimental design, six individuals were exposed to Mn (as nitrate) at 27.7 mg l^{-1}, the EC_{50} value for the DVO calculated from the logistic regression at pH 6.0 without model FA (Table 4), following the procedure described in Section 2.4. If Mn^{2+} was governing the valve movement response of *V. angasi*, then the mean EI should be similar to that reported for the experiment where Mn was added in the form of sulfate [$EI = 0.532 \pm 0.039$ (95% CL)].

The mean EI for this supplementary experiment was 0.519 ± 0.038 (95% CL). This was not significantly ($P > 0.05$) different (i.e. overlapping 95% CL) to that reported for the experiment in which Mn was added as sulfate. These results indicate that the activity of $MnSO_{4(aq)}$ does not govern the valve movement response of *V. angasi* under the conditions prescribed in the experimental design. Therefore, Mn^{2+} governs the valve movement response of *V. angasi*. An alternative test of the FIAM would be to add sulfate (e.g. as $NaSO_4$) to increase the activity of $MnSO_{4(aq)}$, and hence, decrease Mn^{2+}. The EI for the DVO could then be evaluated and related to Mn^{2+}.

In summary, the results for Mn support the FIAM, where the valve movement response (DVO) of *V. angasi*, from the EC_{10} to the EC_{75}, was directly proportional to the activity of Mn^{2+} in SMCW. The results of this study are consistent with those of Schenck et al. (1988), who showed that the uptake of Mn by the freshwater green microalga, *Chlamydomonas variabilis*, under conditions of varying EDTA concentrations, but at a fixed pH, was directly proportional to the activity of Mn^{2+}.

3.4.2. Exposure of *V. angasi* to U

In contrast to Mn, the speciation of U and the valve movement response (DVO) of *V. angasi* to U are both highly dependent on the pH and/or model FA concentrations used in this study (Fig.

2 and 4. The concentrations of total U that elicit a given BR (e.g. EC_{20}, EC_{50} and EC_{80}) in *V. angasi* under conditions of varying pH and/or model FA concentration, are given in Table 6. The total U concentration causing a fixed BR varied considerably. For example, the U concentration differed by a factor of 10.5 for the EC_{50} between pH 5.0 without model FA and pH 6.0 with the maximum concentration (7.91 mg l^{-1}) of model FA (Table 6). However, between pH 5.0 and 6.0, without the addition of model FA, the U concentration causing a 50% decline in BR differed by a factor of 5.4.

Conversely, experimental evidence indicates that the valve movement behaviour of *V. angasi* under control conditions (i.e. background U concentration) does not significantly ($P > 0.05$) differ between pH 5.0 and 6.0. This finding is supported by previous studies (Mackie, 1989; Dimmock and Wright, 1993) which have shown that freshwater unionid bivalves are highly tolerant to changes in the pH (2–3 pH units) of their aquatic medium. Furthermore, the valve movement behaviour of *V. angasi* to Mn was shown to be independent of pH. Overall, there is no evidence to suggest that a change in the concentration of protons (H^+) alone affects BR. However, the change in pH between 5.0 and 6.0 has a marked effect on U speciation (Fig. 2), with the activity of UO_2^{2+} being highly dependent on both the pH and the model FA concentration.

The activity of UO_2^{2+}, based on speciation calculations, at the EC_{20}, EC_{50} and EC_{80} is given in Table 6. The activity of UO_2^{2+} causing a given BR (e.g. EC_{50}) is virtually identical for all model FA concentrations (0, 3.15 and 7.91 mg l^{-1}) at a given pH (e.g. pH 5.0). However, the activity of UO_2^{2+} increased markedly as the pH decreased from 6.0 to 5.0 for a given BR. For example, in the absence of model FA, the activity of UO_2^{2+} increased by a factor of 3.2 (from 21.6 to 69.0 μg l^{-1}), but the EC_{50}, expressed in terms of total U, decreased by a factor of 5.4 (from 634 to 117 μg l^{-1}). In contrast to Mn, BR is not directly proportional to the activity of UO_2^{2+}.

Markich et al. (1996) postulated that UO_2^{2+} and UO_2OH^+ were the primary uranyl species that induced a BR in *V. angasi*. To test this postulate, a stepwise multiple linear regression analysis was performed using data from the EC_{10} to the EC_{80} (sample data for the EC_{20}, EC_{50} and EC_{80} are given in Table 6), since the assumptions of multiple linear regression were satisfied between these BR endpoints only. The results showed that, of the 14 uranyl species predicted to exist under the experimental conditions, only UO_2^{2+} and UO_2OH^+ were found to be significant ($P \leq 0.001$) predictors of valve movement response. Both uranyl species collectively explain 97.5% ($r^2 = 0.975$) of the variability in the valve movement response [EC_{10} (BR_{10})–EC_{80} (BR_{80})] of *V. angasi* to U. However, UO_2^{2+} and UO_2OH^+ individually explain only 7.6% ($r^2 = 0.076$) and 7.4% ($r^2 = 0.074$), respectively, of the variability in valve movement response. These two uranyl species were inversely related ($P \leq 0.001$; $r^2 = 0.714$) to one another across the range of pH and model FA concentrations used in the study. Collectively, both species were highly complementary. This is evident by adding the activities of ($1.86 \times$) UO_2^{2+} and UO_2OH^+ (see below) given in Table 6 for a given biological endpoint (EC_{20}, EC_{50} or EC_{80}); a constant value can be calculated, regardless of the pH and/or model FA concentration.

With respect to the reaction of the complex $MOH^{(z-1)+}$ with a surface site on the cell membrane, an analogous equation to M^{z+} [Eq. (1)] can be derived, namely:

$$MOH + X\text{-cell} \overset{K_2}{\leftrightarrow} HOM - X\text{-cell} \quad (4)$$

and therefore, the activity of MOH on the surface is given by

$$\{HOM - X\text{-cell}\} = K_2\{X\text{-cell}\}[MOH] \quad (5)$$

If both $[M^{z+}]$ and $[MOH^{(z-1)+}]$ induce a BR, then the overall BR will be directly proportional to a weighted function of the activities of the two aqueous species, as given in Eq. (6).

$$BR \propto \{X\text{-cell}\}(K_1[M] + K_2[MOH]) \quad (6)$$

The multiple linear regression equation that describes the valve movement response of *V. angasi* in terms of U species, is given in Eq. (7).

$$BR = 2.165[UO_2^{2+1}] + 1.163[UO_2OH^+] - 148.7 \quad (7)$$

Table 6
Values of selected biological response (DVO) endpoints for *V. angasi* exposed to U in SMCW under conditions of varying pH and/or model FA concentration

Treatment		Biological response endpoint	Total U (μg l^{-1} as UO_2)	Predicted speciation[a]			
PH	Model FA (mg l^{-1})			UO_2^{2+} (μg l^{-1} as UO_2)	UO_2OH^+(μg l^{-1} as UO_2)	H+(X 10^6 mol l^{-1})	OH ($\times 10^9$ mol l^{-1})
5.0	–	EC_{20}	103	60.8	36.2	10.2	1.03
5.0	3.15	EC_{20}	121	57.5	34.3	10.2	1.03
5.0	7.91	EC_{20}	213	58.7	35.0	10.2	1.03
5.3	–	EC_{20}	120	49.0	58.3	5.10	2.05
5.5	–	EC_{20}	137	38.8	73.1	3.22	3.24
5.5	3.15	EC_{20}	210	39.3	74.0	3.22	3.24
5.5	7.91	EC_{20}	425	38.1	71.8	3.22	3.24
5.8	–	EC_{20}	238	26.6	100	1.61	6.47
6.0	–	EC_{20}	501	19.1	114	1.02	10.3
6.0	3.15	EC_{20}	657	18.9	112	1.02	10.3
6.0	7.91	EC_{20}	1025	18.9	112	1.02	10.3
5.0	–	EC_{50}	117	69.0	41.1	10.2	1.03
5.0	3.15	EC_{50}	144	68.6	40.9	10.2	1.03
5.0	7.91	EC_{50}	247	68.4	40.8	10.2	1.03
5.3	–	EC_{50}	141	57.4	68.2	5.10	2.05
5.5	–	EC_{50}	163	45.6	85.9	3.22	3.24
5.5	3.15	EC_{50}	242	45.2	85.6	3.22	3.24
5.5	7.91	EC_{50}	497	45.7	86.0	3.22	3.24
5.8	–	EC_{50}	290	30.6	115	1.61	6.47
6.0	–	EC_{50}	634	21.6	129	1.02	10.3
6.0	3.15	EC_{50}	824	21.8	130	1.02	10.3
6.0	7.91	EC_{50}	1228	21.7	130	1.02	10.3
5.0	–	EC_{80}	133	78.4	46.7	10.2	1.03
5.0	3.15	EC_{80}	171	81.6	48.6	10.2	1.03
5.0	7.91	EC_{80}	286	80.1	47.1	10.2	1.03
5.3	–	EC_{80}	165	66.8	79.4	5.10	2.05
5.5	–	EC_{80}	194	54.3	101	3.22	3.24
5.5	3.15	EC_{80}	278	51.8	98	3.22	3.24
5.5	7.91	EC_{80}	578	54.4	103	3.22	3.24
5.8	–	EC_{80}	354	34.9	131	1.61	6.47
6.0	–	EC_{80}	806	24.2	145	1.02	10.3
6.0	3.15	EC_{80}	1033	24.8	148	1.02	10.3

[a] Calculated using the HARPHRQ geochemical speciation code.

The negative y-intercept value (-148.7) given in Eq. (7) suggests that *V. angasi* can tolerate concentrations of U up to a certain toxicity threshold, beyond which the bivalve will exhibit a significant ($P \leq 0.05$) decline in valve movement response (DVO), relative to 'control' behaviour. From Eqs. (6) and (7)

$$K_1\{\text{X-cell}\} = 2.165 \quad (8)$$

and

$$K_2\{\text{X-cell}\} = 1.163 \quad (9)$$

Dividing Eq. (8) by Eq. (9), gives

$$\frac{K_1}{K_2} = 1.86 \quad (10)$$

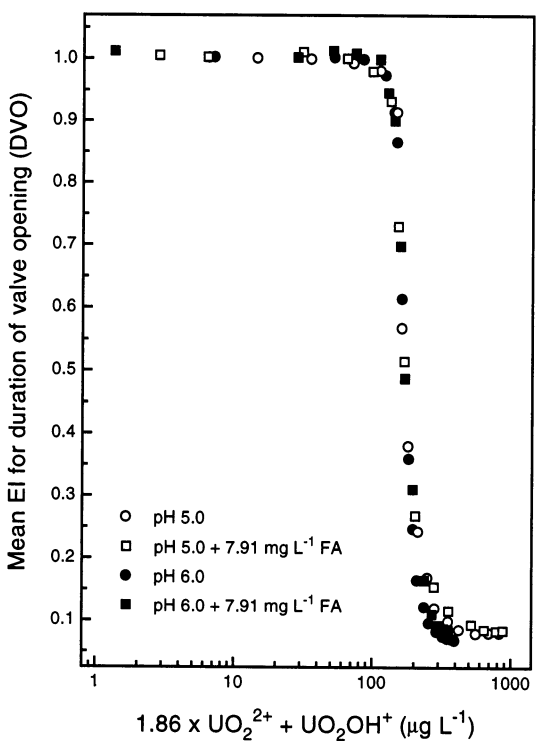

Fig. 5. Concentration-response relationships of the DVO for *V. angasi* expressed in terms of the activities of UO_2^{2+} and UO_2OH^+ at pH 5.0 and 6.0 without model FA and pH 5.0 and 6.0 with model FA (7.91 mg l^{-1}). Each plotted point represents the mean response of six individuals. Error bars and curve fits are excluded for clarity.

That is, the ratio of the binding constants of UO_2^{2+} [K_1, Eq. (1)] and UO_2OH^+ [K_2, Eq. (4)] at the cell membrane surface is 1.86; UO_2^{2+} has nearly a two-fold higher binding affinity than UO_2OH^+. Therefore, the valve movement response (DVO) of *V. angasi*, between the EC_{10} (BR_{10}) and EC_{80} (BR_{80}), is proportional to $1.86 \times UO_2^{2+} + UO_2OH^+$. Consequently, if the concentration–response relationships given in Fig. 4 (expressed as total U), are replotted in terms of $1.86 \times UO_2^{2+} + UO_2OH^+$, the mean responses of *V. angasi* for all four treatments should fall on one general concentration–response curve. Indeed, this is confirmed in Fig. 5. Therefore, the BR from the EC_{10} to the EC_{80} is directly proportional to the activity of $1.86 \times UO_2^{2+} + UO_2OH^+$.

In contrast to Mn, the U results are an apparent exception to the FIAM. That is, UO_2^{2+} and UO_2OH^+ cause a BR. These results, however, are consistent with those of previous studies with aquatic animals (e.g. Wilkinson et al., 1990; Vercauteren and Blust, 1996), where inorganic (AlF^{2+}) or organic (Zn–histidine) metal species, in addition to the free metal ion (i.e. Al^{3+} or Zn^{2+}, respectively), have been shown to cause a BR. A more detailed discussion is given by Brown and Markich (2000).

3.5. Comparative sensitivity of *V. angasi* to Mn or U in natural and synthetic Magela Creek water

The suitability of the selected FA models as analogues of natural DOM was tested by comparing the valve movement behaviour (DVO) of *V. angasi* exposed to Mn or U in SMCW (including the FA models) with filtered natural Magela Creek water (NMCW), both matched in terms of major and trace element concentrations, pH (5.5) and COOH concentration (30 µmol l^{-1}; 5.7 mg l^{-1} model FA; 6.4 mg l^{-1} DOC). The mean measured concentrations of Mn or U used to derive the concentration–response relationships were similar to the nominal concentrations (i.e. $<4\%$ difference for all concentrations of both metals). The mean valve movement response (DVO) of *V. angasi* exposed to either Mn or U in

Table 7
Comparative sensitivity of the DVO of *V. angasi* exposed to Mn or U in customised synthetic and natural Magela Creek water at pH 5.5 and 30 μ mol l^{-1} COOH (ca. 6.4 mg l^{-1} DOC; 5.7 mg l^{-1} FA)[a]

Endpoint	Synthetic water		Natural water	
	Mn	U	Mn	U
BEC_{10}	16.9	275	17.1	285
MDEC	17.8	292	18.1	301
EC_{50}[b]	28.9 ± 1.5	358 ± 23	29.6 ± 1.6	367 ± 24

[a] Values are reported in mg l^{-1} for Mn and μg l^{-1} for U (as uranyl).
[b] Values are reported as the mean ± 95% CL.

customised SMCW, was not significantly ($P > 0.05$) different (generalised F test) to that in NMCW. The BEC_{10}, MDEC and EC_{50} values calculated from the concentration–response relationships are shown in Table 7. The percentage difference in the BEC_{10}, MDEC and EC_{50} values for Mn and U were 1.2–2.4% and 2.5–3.6%, respectively. There were no significant ($P > 0.05$) differences (overlapping 95% CL) in the mean EC_{50} values for Mn and U between experiments conducted in customised SMCW and NMCW.

Electron paramagnetic resonance spectroscopy measurements showed that the concentrations of Mn^{2+} in customised SMCW were not significantly ($P > 0.05$) different (i.e. overlapping 95% CL) to those measured in NMCW. The majority (90–99%) of total Mn in both waters consisted of Mn^{2+}. The measured concentrations of Mn^{2+} were consistent with results from the geochemical speciation modelling of SMCW. Reliable measurements of $UO_2{}^{2+}$ and UO_2OH^+ using time-resolved laser-induced fluorescence spectroscopy (Moulin et al., 1995) could not be achieved in either natural or synthetic Magela Creek water (pH 5.5), due to the low fluorescence of these species in the presence of other uranyl species, including uranyl–FA complexes, that predominate in solution.

The above results strongly indicate that the valve movement response of *V. angasi* to Mn or U in filtered surface water from Magela Creek can be reliably estimated using customised synthetic water. The use of synthetic water has the advantage over natural water in that it has a simple, standard and well-defined composition, which confers a sound knowledge of metal speciation. This permits a more detailed understanding of the mechanisms of metal–organism interactions, and thus, greatly improves the capability of predicting the biological effects of metals in fresh surface waters of varying chemistry. This may ultimately improve risk assessment models for the protection of aquatic ecosystems. The use of synthetic customised water requires further verification with other organisms (and metals), including the use of alternative endpoints (e.g. survival and uptake) and longer exposures, to determine its true value in predicting the biological effects of metals in natural waters. It is perhaps premature to vigorously embrace such a relatively 'simple' approach until these issues have been addressed, and any shortcomings exposed.

4. Conclusions

The veracity of the FIAM was tested by examining the valve movement response (DVO) of the freshwater bivalve, *V. angasi*, to Mn or U exposures, under conditions of varying pH and/or model FA concentration. The BR of *V. angasi* exposed to Mn was consistent with the FIAM; that is, BR was directly proportional to the activity of Mn^{2+}. Conversely, the valve movement response of *V. angasi* to U was an exception to the FIAM, since BR was found to be proportional to a weighted function of the activities of (1.86) $UO_2{}^{2+}$ and UO_2OH^+. Additionally, the effect of U on *V. angasi* demonstrates the importance of examining BR at more than one pH. At a fixed pH (e.g. pH 5.0), the results for U from the present study were consistent with the FIAM (i.e. BR was directly proportional to $UO_2{}^{2+}$); only when pH was altered were the results inconsistent with the FIAM. This inconsistency of the valve movement response of *V. angasi* to U exposures, together with similar examples from other studies using different metals (e.g. Al or Zn), raises questions regarding the veracity of the FIAM. There is a requirement, therefore, to ex-

amine in detail, the conceptual development of the FIAM, to ascertain if the thermochemical basis of the model is sound. Deficiencies in the basis of the model may explain the apparent failure of the FIAM to describe several metal–organism interactions.

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