Use of the freshwater Mussel, *Velesunio angasi*, in the Monitoring and Assessment of Mining Impact in Top End Streams

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Since the early 1980s, a large number of studies have been conducted on the freshwater mussel, Velesunio angasi, in coastal streams of the Northern Territory, particularly populations from Magela Creek (East Alligator River catchment) and the Finniss River. Ecological, toxicological, chemical and radiological studies have been carried out by the Environmental Research Institute of the Supervising Scientist and its consultants, as well as ANSTO, to assess the indicator potential of *V. angasi* for detecting and assessing mining impact, and for ongoing monitoring of mining activities.

A number of features of freshwater mussels make them well suited as indicators of past environments and present-day environmental health, including their sedentary habit, low position in food chains, large size (for procuring flesh and for ease of sampling), long life-spans, ecological importance (large biomass and significant filtering capacity), shell size and shape very responsive to the surrounding environment, good preservation of shells, and in some cases importance as a resource for humans (Humphrey and Simpson, 1985).

The specific categories of studies that have been investigated under the broad areas of environmental and human health are summarised in **Table 1**. Under environmental health, particularly water quality, responses of mussels may be used for the early detection of changes and for the wider assessment of ecological importance of change through field population studies (sensu ANZECC and ARMCANZ, 2000). For these assessments, a number of attributes listed above make freshwater mussels particularly valuable as archival monitors. In general, *V. angasi* has limited value as a laboratory organism for ecotoxicological assessment, due to the inability to culture stocks, high filtering rates and food demands, and/or ability to close the valves at threshold contaminant concentrations (e.g. ARRRI, 1987b, 1988).

Proven or potential value of measured responses from the field for past (archival) and present-day monitoring has been demonstrated for presence/absence data, reproduction, shell form, size and growth rates, chemistry of shell laminations, and chemical and radionuclide content of soft tissues (Humphrey and Simpson, 1985; ARRRI, 1984, 1985, 1987a, 1987b, 1988, 1991, 1992a, 1992b; Jeffree, 1985, 1988; Allison and Simpson, 1989; Humphrey, 1995; Markich et al., 2002). Survey and age data from field populations may be particularly useful for assessing the success of rehabilitation efforts in streams over the long-term. However, correct interpretation of these data and responses requires a good understanding of the ecology of *V. angasi* across a broad geographical range (**Table 1**).

Table 1. The indicator potential of *Velesunio angasi* for detecting and assessing mining impact. (Superscripts refer to numbered specific studies listed in column 2)

General attribute	Specific study	Indicator potential ADVANTAGES	DISADVANTAGES (or caveats)
ECOSYSTEM CONDITION OR HEALTH Early detection			
Laboratory -sublethal	1. Glochidial snap rate (mortality, behavioural) 2. Valve response (behavioural) 3. Growth and survival of metamorphosed juveniles	• Sensitive [1,2] (up to threshold concentrations [3]) • Multiple life stage test possible (larvae ⇒ encysted larvae ⇒ juvenile) [3] • Husbandry requirements elucidated for young juveniles [3]	• Dependence on field-collected material [1,2,3] • Behavioural responses not recognised as ecologically relevant [1,2] • Husbandry requirements not well understood and may be demanding for post-juveniles [1,2,3] • Valve closure (⇒ lack of exposure) at threshold concentrations [2]
Field -sublethal	1. Growth and survival of metamorphosed juveniles 2. Reproduction: larval production in females 3. Bioaccumulation (bioavailability)	• Sensitive [1,2] • Enhanced natural survival and growth under lotic conditions [1] • When breeding synchronised, possible to track timing of impact and recovery [2] • Possible to determine sex and larval brooding stages without injury to mussels [2] • May be enhanced contaminant signal (cf water) [3] • Flux of some elements well understood [3]	• Dependence on field-collected larvae[1] • Response not correlated with population or community-level response [1,2,3] • Studies limited where natural densities are low (destructive sampling) [3] • Flux of elements in mussels should be determined [3]
Population study and archival monitoring	1. Presence/absence: sedentary and long-lived ⇒ assessing long-term rehabilitation 2. Reproduction: larval production in females (field) 3. Shell form, size and growth rates 4. Chemistry of shell laminations 5. Chemical and radionuclide content of soft tissues	• Integrative: short to long-term historical record available of past environments, exposures and/or insults [1,2,3,4,5] • Biology and ecology of *V. angasi* well understood [1-5] • Ecologically relevant [1, others?] • See superscript 2 comments in cell above, relevant to reproduction • Superscript 3 comments in cell above also relevant to chemical and radionuclide content of soft tissues	• Requires good understanding of ecology across geographical range, including habitat preferences, causes of growth checks, and environmental correlates of shell form and life history parameters generally • Flux of elements in mussels should be determined [4,5]
HUMAN HEALTH Body burdens	Chemical and radionuclide content of soft tissues	• Significant food source for some aboriginal groups • May concentrate contaminants to high levels • Non-destructive, live-counting approaches available for isotopes of radium and their progeny	• Bioaccumulation studies involving non-radionuclides may be limited where natural densities are low (destructive sampling)

The burdens and flux of some metals and radionuclides in tissues of *V. angasi* have been well studied and this information has been used in ongoing bioaccumulation studies in Magela Creek. These studies are used for the early detection of changes in water quality and to ensure elemental concentrations are within limits safe for human consumption. Known half-lives of different elements in mussels, including uranium (Allison and Simpson, 1989) and various radionuclides (ARRRI, 1984, 1985, 1987a, 1987b), infers a short- to long-term monitoring potential of tissue burdens, depending upon the element. Radium and thorium isotope loads, and activity ratios such as Ra-228/Ra-226 and Th-228/Ra-228, are strongly age-dependent (ARRRI, 1985, 1987a). Determination of this dependency for a site can give information on pollution events even several years later. For this type of investigation, radioisotope activity ratios are more sensitive and reliable indicators than concentrations or loads of individual radionuclides.

An example of the archival potential of the Ra-228/Ra-226 ratio in mussels for demonstrating downstream effects in Magela Creek of hypothetical releases of process water from the Ranger uranium mine is shown in **Figure 1**. The Ra-228/Ra-226 activity ratio decreases with age due to the disproportionate decay of the two isotopes: 6 year half-life for Ra-228 vs 1600 year half-life for Ra-226. Hence, the ratio is lower in older mussels as the Ra-228 activity incorporated early in life has partially decayed (**Figure 1**). If there had been an "event" several years ago, this would be observed in the age-dependence of the ratio. For example, if radium was released from Ranger eight years ago resulting in a permanent increase in Ra-226 in the creek system then (dashed line in **Figure 1**):

- The increase in Ra-226 would result in a lowered Ra-228/Ra-226 ratio. In **Figure 1** it has been assumed that the Ra-226 concentration in the creek water has doubled, resulting in a decrease in the y-intercept (i.e. ratio at age = 0) from 0.75 down to the observed value of 0.37.
- Assuming that the 'new', hypothetical equilibrium ratio is 0.37, then the ratio in mussels younger than eight years, exposed only to the new, post-event ratio, would decline because of the disproportionate decay of the two isotopes (as prior to the 'event').
- Mussels older than eight years would have a higher ratio than expected from the curve fit because they had experienced the higher ratio (0.75) in the water prior to the event. This is indicated by the dashed line in the figure.

In the Australian context, most metal bioaccumulation work has been performed on the species, *V. angasi, V. ambiguus* and *Hyridella depressa* (e.g., Allison and Simpson, 1989; Jeffree and Brown, 1992; Jeffree et al., 1993; Markich and Jeffree, 1994; Brown et al., 1996; Markich et al., 2001). Many of these papers have attempted to construct models leading to mechanistic insights into bioaccumulation. Uranium provides an unusual example of metal absorption and bioaccumulation. In one field study (Allison and Simpson, 1989) mussels were placed in water containing an elevated concentration of U for a number of weeks, then removed to a billabong with a background U concentration. These experiments showed that U is rapidly lost from mussel tissue, with a half-life of a few days. This observation superficially disagrees with the findings of Markich et al. (2001), who showed that about 90% of the soft-tissue U burden of *H. depressa* and *V. ambiguus* resided in the extracellular granules. The solubility of UO_2PO_4 is very low, which is inconsistent with rapid mobilisation and loss of U from tissues. However, the tissue-water concentration ratio for U, as measured by Markich et al. (2001), is also very low compared with the presumed slow rate of exchange from the $CaHPO_4$ matrix.

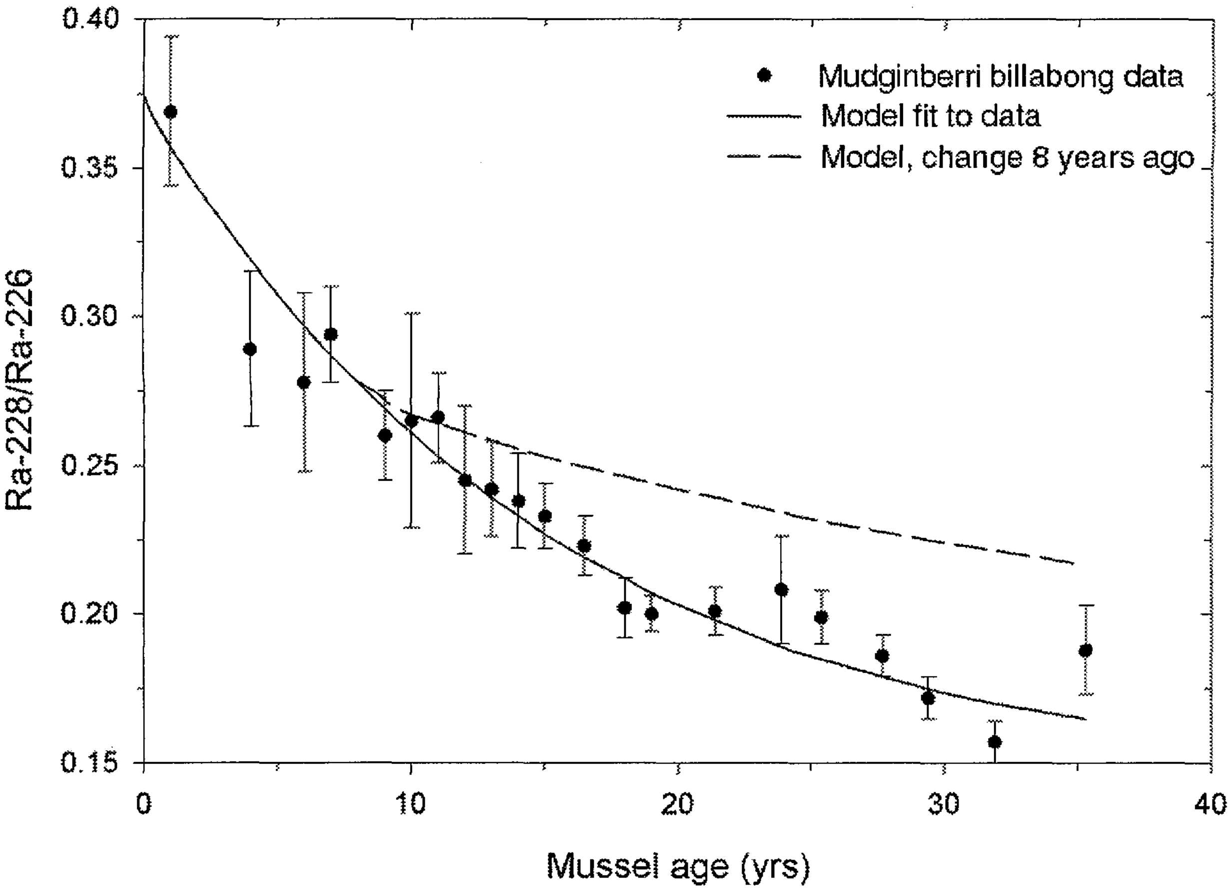

Figure 1. Dependence of the radium activity ratio on age for mussels from Magela Creek billabongs.

The overall evidence suggests that, although U has a short half-life in mussel protoplasm, the transport mechanism to extracellular granules is impeded in a way that does not occur with many other divalent ions. This may be because UO_2^{2+} is mainly only nominally divalent at physiological and most naturally-occurring pH values. The transport of metals from an intracellular environment to an extracellular phosphate granule probably involves a decomplexation step. For most divalent metals, extracellular pH is unlikely to be sufficiently high to cause significant hydrolysis.
For U, however, extensive hydrolysis occurs at pH > 4 (Moulin et al., 1995), lowering the effective charge on the U species, and restricting its incorporation (as an unhydrolysed UO_2^{2+} ion) into the phosphate crystal structure. At the physiological pH of mussel body fluid (~7.8; Brown et al., 1996), the fraction of U present as UO_2^{2+} is ~0.3% (calculated from Moulin et al., 1995).
The low percentage of the metal readily fixed in the granules, coupled with (in most cases) a low ambient concentration of total soluble U, may provide a high kinetic barrier to retention in the granules.

This may explain the observation of Markich et al. (2001) that the concentration ratio (tissue: water) for U was comparatively low, despite the very low solubility of UO_2PO_4. For Cd (pK_a ~10.3), Co (pK_a ~10.5), Cu (pK_a ~7.5), Mn (pK_a ~10.6), Ni (pK_a ~9.9), Pb (pK_a ~7.8) and Zn (pK_a ~9.0), the fraction of metals present in divalent form at pH 7.8 is about 100%, 100%, 33%, 100%, 99%, 50% and 94%, respectively (Aylward and Findlay, 1974; Hogfeldt, 1982). For these elements, there should be little kinetic barrier to incorporation of the ion into phosphate granules, as sufficient divalent form is available. After incorporation of metals into the granules, the equilibrium shifts to produce more divalent ion in the aqueous phase, by mass action. It may be worthwhile to study the incorporation of U, and other easily hydrolysed divalent metal ions, into phosphate granules as a function of ambient pH.

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