internal report **334**

Population variability in the response of Moinodaphnia macleayi to uranium and cadmium

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April 2000

supervising scientist

Population Variability in the Response of *Moinodaphnia macleayi* to Uranium and Cadmium

Research thesis submitted in partial fulfilment of

Bachelor of Applied Science (Honours) (Applied Biology and Biotechnology)

1999

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Declaration

This work described in this report was performed while I was an enrolled student for the Bachelor of Applied Science (Honours) (Applied Biology and Biotechnology) in the Department of Applied Biology and Biotechnology at the Royal Melbourne Institute of Technology.

To the best of my knowledge, all work performed by others, published or unpublished, has been duly acknowledged in the report.

This report has not been submitted, in whole or in part, for any reward.

Signed: .....

Date: .....

Acknowledgments

Firstly, I would like to thank my project supervisors: Professor Doug Holdway, for his guidance, support and endless encouragement throughout the year, Dr. Rick van Dam, for his extreme dedication and invaluable advice and encouragement.

A special thankyou to Catriona Turley and Caroline Camilleri for their technical advice and valuable assistance. I would also like to thank all the staff at *eriss* and my friends in Jabiru for making my stay a welcomed and enjoyable one who I will never forget.

Thanks to all the honours students and PhD students for making this year an enjoyable one.

I would like to thank my friends Henry, Elias, Fred, Norman and Claud for their encouragement and support.

Finally, I would like to thank my family (Dad, Mum, Vera, Mary and George), who without I could not have achieved all that I have.

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List of Abbreviations

ANOVA - analysis of variance

ARR - Alligator River Region

ASTM - American Society of Testing and Materials

BB - Bowerbird Billabong or Bowerbird Billabong stock population of *M. macleayi*

CI - 95 percent confidence interval

DjB - Djalkmara Billabong

DjB1 - Djalkmara Billabong stock population of *M. macleayi* cultured in Djalkmara Billabong water

DjB2 - Djalkmara Billabong stock population of *M. macleayi* cultured in Bowerbird Billabong water

DO - dissolved oxygen

EC20 or EC50 - concentration causing a 20 or 50% effect in test organisms relative to the control in a given time

ERA - Energy Resources of Australia

eriss - Environmental Research Institute of the Supervising Scientist

FFV - fermented fish food and vitamins

GB - Georgetown Billabong

Lab - Laboratory stock population of *M. macleayi*

LC50 - concentrations lethal to 50% of the test organisms relative to the control in a given time

LOEC - lowest observed effect concentration

MTs - metallothioneins

NOEC - no observed effect concentration

NT - Northern Territory

OECD - Organisation for Economic Cooperation and Development

SE - standard error

^{o}C - degrees Celcius

cm - centimetres

cm^2 - centimetres squared

d - day(s)

g - grams

g/cm^3 - grams per centremetre cubed

$g\ mol^{-1}$ - grams per mol (molecular weight)

h - hour(s)

km - kilometre

km^2 - kilometres squared

L - litre

$\mu g/L$ or $\mu g\ L^{-1}$ - micro-gram per litre

μL - micro-litre

μm - micro-metre

$\mu S/cm$ or $\mu S\ cm^{-1}$ - micro-siemen per centimetre

min - minute(s)

mg/L or $mg\ L^{-1}$ - milligram per litre

mm - millimetre

$t_{0/24/48}$ - at time = 0 / 24 / 48 hours

y - year(s)

Ca - calcium

Cd - cadmium

U - uranium

< - less than

≤ - less than or equal to

> - more than

≥ - more than or equal to

% - percentage

± - plus or minus

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1. Introduction

1.1 General Introduction

Ecotoxicology is the study of the effects of pollutants on the environment and its biota (Butler, 1978; Baudo, 1987). It is a relatively new and continually changing science, always seeking the best assessment of potential and present chemical hazards (Baudo, 1987). Aquatic toxicology, a component of ecotoxicology that focuses on aquatic ecosystems, has received increasing attention over the last few decades as the problems of water pollution are faced in both industrialized and developing countries (Baudo, 1987).

Toxicity testing is an approach developed to measure the response of living organisms to various pollutants (Baudo, 1987). The major reason for carrying out toxicity tests with aquatic organisms is to determine which concentrations of a substance are harmful to the organism and which have no apparent effect (Sprague, 1990). From the results, an ecotoxicologist can recommend maximum concentrations for the well being of aquatic organisms. All the data provided by toxicity tests may be assembled to derive water quality criteria, which should be scientifically sound numbers relating concentration to effect (Sprague, 1990). These in turn can be used to create water quality standards. A second major use of toxicity tests is to monitor the toxicity of effluents or to evaluate the quality of surface waters. This approach offers an alternative to monitoring chemicals and trying to interpret the measurements (Sprague, 1990), and is known as whole effluent toxicity (WET) testing, or direct toxicity assessment (DTA) (Grothe *et al.*, 1996; van Dam and Chapman, 1999).

1.2 Cladocerans as toxicity testing organisms

The cladocerans, commonly known as water fleas are regularly used as freshwater toxicity testing organisms. They belong to the phylum Arthropoda, the subphylum Crustacea, and the class Branchiopoda (Smirnov and Timms, 1983; Rupert and Barnes, 1994). Branchiopods are characterized by their trunk appendages that have a flattened life-like structure (Rupert and Barnes, 1994). The water fleas constitute half of the branchiopods and include many widespread and common species, such as those belonging to the genus *Daphnia* (Rupert and Barnes, 1994). Six families are represented in inland waters of Australia: Sididae, Chydoridea, Macrothricidea, Moinidea, Bosminidea and Daphniidae (Smirnov and Timms, 1983). The majority of toxicity tests involving cladocerans have used the genus *Daphnia* (Baird *et al.*, 1989; Koivisto, 1995). However, other genera also used for toxicity testing purposes include *Ceriodaphnia*, *Moinodaphnia* and *Moina* (Julli *et al.*, 1990).

An important distinguishing feature of water fleas (cladocerans) is that the carapace encloses the trunk and not the head (Figure 1.1). Most cladocerans are suspension feeders and collect food particles with fine setae on the trunk appendages. The cladocerans swim by means of powerful secondary antennae (Rupert and Barnes, 1994). They are parthenogenetic organisms that reproduce asexually producing mainly females (Smirnov and Timms, 1983; Rupert and Barnes, 1994). Males are usually produced only in times of stress (Rupert and Barnes, 1994).

The ideal criteria for aquatic organisms for toxicity tests have been proposed as follows (Buikema Jr. *et al.*, 1982):

- the organism is representative of an ecologically important group (in terms of taxonomy, trophic level or realised niche);

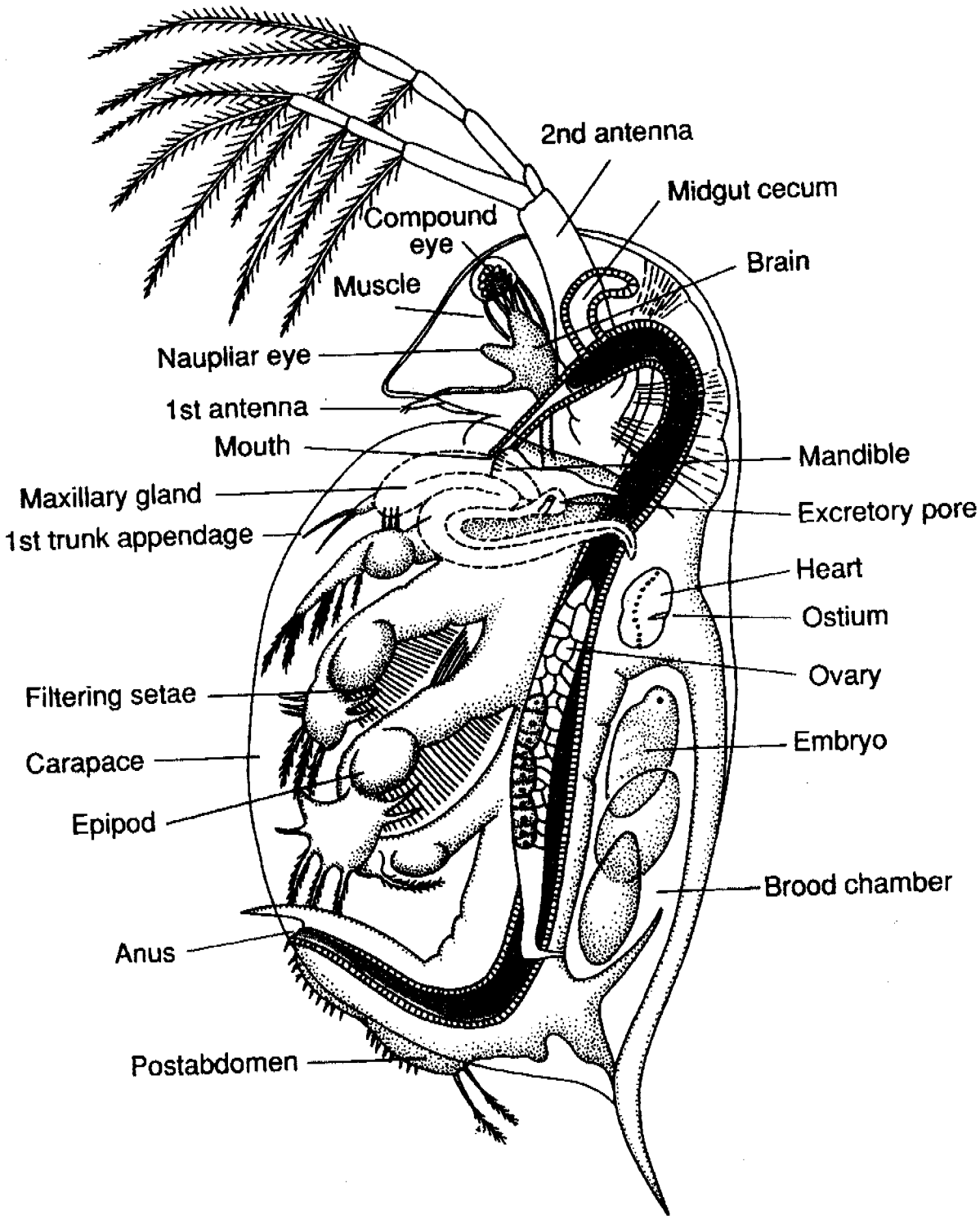

Figure 1.1: Anatomy of a female *Daphnia* sp. (Modified from Rupert and Barnes, 1994)

- the organism occupies a position within a food chain leading to man or other important species;
- the organism is widely available, is amenable to laboratory testing, easily maintained, and genetically stable so uniform populations can be tested; and
- there is adequate background data on the organism (ie. its physiology, genetics, taxonomy, role in natural environment are well understood.

Cladocerans as test organisms cover all these criteria, as they are ubiquitous in nature, they play an important role in freshwater trophic chains, as they are among the dominant consumers of primary producers (Herbert, 1978), and are also an important food for both invertebrate and vertebrate predators. They also have many properties that make them suitable for laboratory testing, such as their small size, short lifecycle, high fecundity, parthenogenetic reproduction, and ease of laboratory handling (Koivisto, 1995). For these reason, cladocerans are one of the oldest test organisms in aquatic toxicology, with literally thousands of papers having been published involving toxicological studies using cladocerans, in particular *Daphnia* sp.

However, there are some disadvantages in using cladocerans as toxicity testing organisms that need to be mentioned. These include the following (Baudo, 1987):

- Sensitivity to toxicants is age-dependent in *Daphnia*;
- Unfed *Daphnia* should not be used in toxicity tests lasting more than 48 h.
- When fed, a variable amount of toxicant is taken up through the ingestion of contaminated particles instead of direct adsorption from the water.
- A lack of reproducibility in both short-term and long-term toxicity tests (Adema, 1978).

It should be noted that most of these problems also occur in all toxicity testing organisms, not just in cladocerans. It is also important to recognise that cladocerans are not used alone for toxicity testing, and that the best and most accurate prediction of potential pollutant impacts can only be achieved by using a variety of approaches, including the use of several toxicity test species (Sprague, 1990).

1.3 Standard Cladoceran toxicity tests

Chronic life-cycle tests using survival and reproduction as endpoints are the most commonly used bioassays for freshwater cladocerans. The cladoceran reproduction test aims to measure the effect of toxic chemicals on adult reproduction, in terms of the number of viable offspring produced (Baird *et al.*, 1991). Chronic survival tests are less common, and as their name suggests, only measure the effect of chemicals on adult survival. The methods for chronic toxicity tests are quite variable because they must specifically suit the life history of the individual test organism. Thus, the length of a chronic test can range from days to weeks depending on the test species. Other aspects of test design such as containers, food and water supply, and the number of animals exposed are equally variable (Buikema Jr. *et al.*, 1982).

Some of the chronic toxicity tests that have been developed for different cladoceran species are as follows:

- The 21-day chronic test with *Daphnia magna* Straus, which aims to detect sublethal effects on adult female reproduction in terms of fecundity, by assessing impact on the number of viable offspring that are produced over a 21-day period (Baird *et al.*, 1991).

- The 7-day toxicity test to evaluate the effects of effluents on *Ceriodaphnia dubia* survival and reproduction, developed by The U.S. Environmental Protection Agency (USEPA) (Cooney *et al.*, 1992).
- The 3 brood (~ 6 day) reproduction and 5-day survival tests to detect effects on adult females in terms of the number of viable offspring produced and mortality, respectively for the tropical Australian cladoceran, *Moinodaphnia macleayi* (Hyne *et al.*, 1996).

Acute toxicity tests are also commonly used to provide information about the relative lethality of waste material. These tests are designed to determine the concentration of a toxicant that is sufficient to affect some percentage (eg. 50%) of a limited number of test organisms (Buikema Jr. *et al.*, 1982). This critical concentration is estimated by exposing organisms to a graded, often logarithmic or geometric series of concentrations of a toxicant or effluent and then observing their responses.

A standard acute toxicity test that has been developed for cladocerans involves exposing test organisms (e.g. *D. magna*) to a particular toxicant (e.g. copper) for periods of 24 or 48 hours and monitoring mortality, or more often, immobility as an endpoint (Mark and Solbe, 1998). It is important to note that an "immobile" animal may not be dead. If the cladoceran was transferred to another solution containing no toxicant it may be able to revive itself; however, if it remained in the test solution it would eventually die. An organism is determined as "immobile" if no signs of movement are seen after agitating the solution with a pipette for several seconds (OECD, 1999).

1.4 *Moindaphnia macleayi* as a toxicity testing organism in the Australian wet-dry tropics

Biological toxicity tests play an important role in the regulation of discharge of waste waters into the aquatic environment of the Alligator Rivers Region (ARR) in tropical northern Australia (Hyne *et al.*, 1993). The ARR is of great significance as it includes catchment areas and associated wetlands of two major river systems, the South and East Alligator Rivers. The South Alligator River (and a significant part of the East Alligator River) is protected by its inclusion within Kakadu National Park, an important conservation area cited on the World Heritage List and the Convention on Wetlands of International Importance (Hyne *et al.*, 1993). Microcrustacean zooplanktons (Cladocerans) are important components of the aquatic ecosystem in the ARR (Julli, 1986), as they feed upon detritus, bacteria, and phytoplankton, and grow to a size that makes them a suitable diet for numerous fish species (Bishop *et al.*, 1980).

Over 19 local freshwater species have been assessed for their potential use as toxicity testing organisms (Holdway, 1992a). On the basis of its high fecundity and successful rearing, *M. macleayi* was selected as one of eight species (from five different phyla) to have excellent potential as a toxicity test species (Figure 1.2). *M. macleayi* is normally found in weedbed habitats of the Northern Territory, Queensland and New South Wales (Julli, 1986). *M. macleayi* has a very short life-cycle, making it very suitable for toxicity testing (Holdway, 1992a). However, it has also been established that the organism requires careful maintenance of the main culture (Holdway, 1992a). Historically, culturing of *M. macleayi* in incubators had proved unreliable (Julli *et al.* 1990). Parthenogenetic females had high variable fecundity when fed a synthetic diet as well as high mortality that exceeded

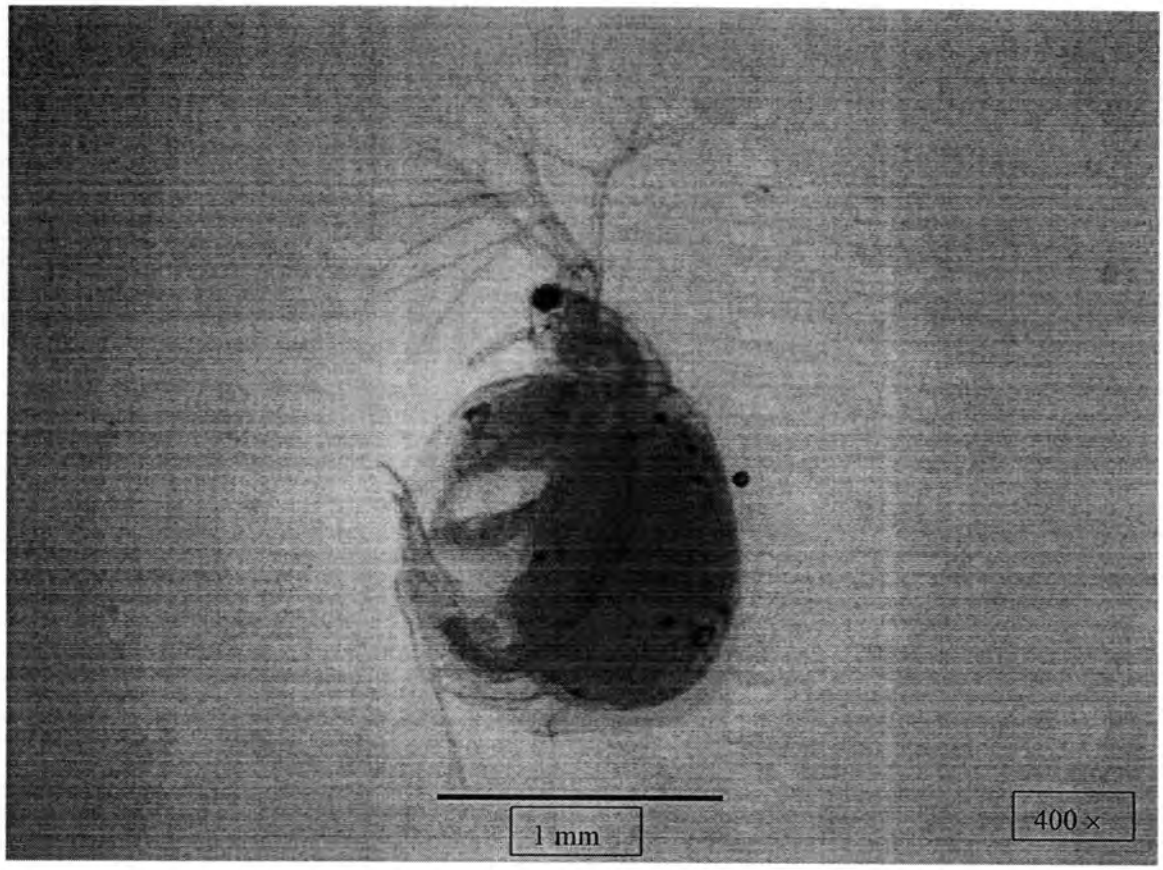

Figure 1.2: Photomicrograph of *M. macleayi*

acceptable requirements set by the OECD (1987), where at least 20 young are produced in third broods and at least 80% of the adults survive. Hyne *et al.* (1993) found that an improved diet of *Chlorella* sp. with fermented fish food and vitamns (FFV) caused an increase in fecundity and survival of *M. macleayi* culture stocks. Two protocols have now been developed for toxicity testing on *M. macleayi* using reproduction and survival as endpoints (Hyne *et al.*, 1996).

1.5 Metals of interest in the ARR

The ARR lies within an ancient basin called the Pine Creek Geosyncline that covers an area of approximately 66 000 km^2 (Figure 1.3). The Geosyncline has a history of mineral production dating back as early as 1865 (Hyne *et al.*, 1996). Throughout this time up to 16 metals have been extracted, some of which include arsenic, gold, copper, cadmium and lead (Hyne *et al.*, 1996). The ARR today is heavily mined for uranium, where the East Alligator River uranium field, which covers a large area of 22 500 km^2 in the north-east of the Pine Creek Geosyncline (east of the South Alligator River), has been developed. This has resulted in uranium being the metal of most interest to ecotoxicologists and the local mining industries. As uranium is extracted other metals of concern are also removed during the mining process. The metals aluminium, cadmium, cobalt, copper, nickel, manganese, lead, uranium, vanadium and zinc were identified as priority metals of potential ecotoxicological in aquatic ecosystems of tropical Australia, mainly as a consequence of mining activities, but also from urban impacts (Markich and Camilleri, 1997).

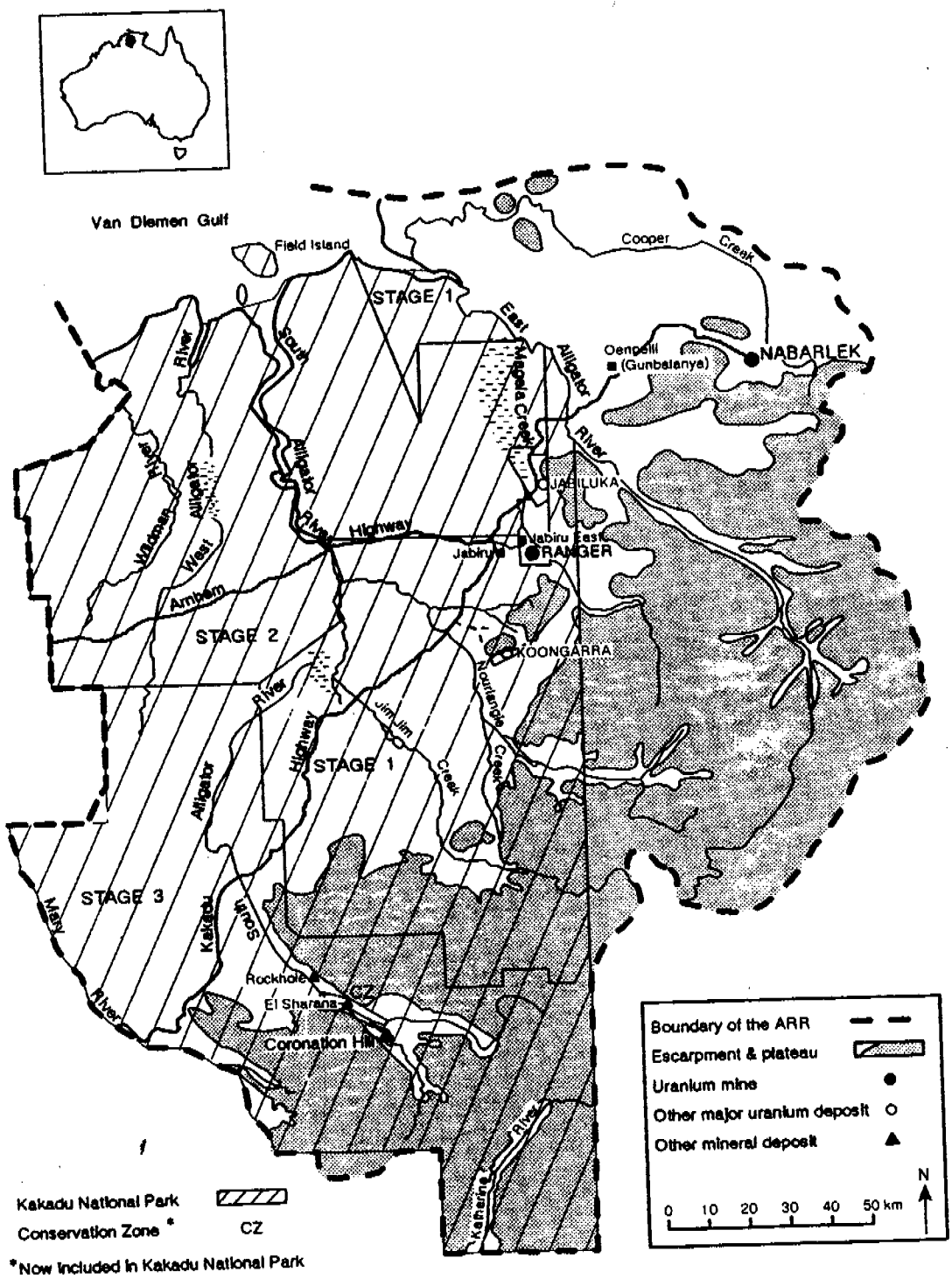

Figure 1.3: The Alligator River Region (from Hyne *et al.*, 1996).

1.5.1 Selection of metals for toxicity testing

The metals selected for toxicity testing in this research were uranium and cadmium. Uranium was chosen as the main metal for investigation, as it is the metal that has generated most interest in the Northern Territory. Cadmium was chosen, as it is another commonly found element throughout the Top End, and is extremely toxic to aquatic organisms at low concentrations (Griffiths, 1980).

1.6 Uranium

Uranium is a non-essential metal (with no known biological purpose) that is generally toxic to aquatic organisms at elevated concentrations (Hyne *et al.*, 1992). Uranium never occurs naturally in the free state but is found as an oxide or complex salt in minerals such as pitchblend (U_3O_8) (Environment Canada, 1983). Uranium ores are distributed throughout the world, usually in the form of pitchblend deposits which are the richest source of uranium ore (Environment Canada, 1983). The amount of uranium in the earth's crust is relatively low having been estimated at between 2.7 $\times 10^{-4}$ to 4.0 $\times 10^{-4}$ percent of the earth's crust (Environment Canada, 1983).

Uranium is being increasingly used in the nuclear industry for the production of nuclear weapons and for power generation, while it is also present in wastes from the phosphate industry and coal-fired power stations (Ahsanullah and Williams, 1986). Concentrations of less than 0.1 mg/L have been classified as posing "minimal" risk to the marine environment (Ahsanullah and Williams, 1986). The average uranium concentration for groundwater in Canada is 0.2 µg/L (Environment Canada, 1983). Concentrations of uranium in surface waters associated with uranium-bearing rocks will vary from place to place due to site-specific factors such as substrate, water quality and other environmental conditions (Environment Canada, 1983). Background

uranium concentrations in surface waters of streams and rivers within Kakadu National Park are usually less than 1 μg/L (Hyne *et al.*, 1992). In waters of the Walker Basin, Nevada, which flow through areas of known uranium deposits uranium concentrations of 1.3 to 1.9 μg/L have been reported (Environment Canada, 1983).

Mine-sites have had a large impact on the distribution of uranium into the environment. Through design faults arising from over-estimation of evaporation and under-estimation of rainfall, uranium mines in tropical Australia, have had ongoing problems with the disposal of contaminated water (Krockenberger, 1997). Radionuclides and heavy metals find their way into the wetlands of Kakadu National Park through disposal, leaching and erosion (Krockenberger, 1997). Poor design and management of waste at another mine in the arid zones of South Australia saw up to five billion litres of highly toxic and acidic water leak into groundwater (Krockenberger, 1997). Over the years, mining techniques have improved as community expectations of environmental protection have increased and action is demanded to maintain the integrity of national parks, world heritage areas and the wider environment.

1.6.1 Speciation and bioavailability of *uranium*

The speciation of uranium (U) is relatively complex in natural surface waters (Markich and Camilleri, 1997). Uranium may occur in natural waters in three oxidation states: U^{4+}, UO_2^{+} and UO_2^{2+}. The free uranyl ion (UO_2^{2+}) constitutes a minor proportion of the total U concentration (ie. 8% at 0.1 μg L^{-1} declining to 2% at 4000 μg L^{-1}) at pH 6. In contrast, polymeric uranyl species, such as $(UO_2)_2(OH)_3CO_3^{-}$, $(UO_2)_2(OH)_5^{+}$ and $(UO_2)_2(OH)_7^{-}$ increase in significance with total U concentration. It is well recognised that the speciation of the uranyl ion in natural waters is influenced

by factors such as pH and the concentration of inorganic and organic ligands (Markich *et al.* 1996).

Markich *et al.* (1996) provided evidence that UO_2^{2+} and UO_2OH^+ are the dissolved U species primarily responsible for causing adverse behavioural responses in the freshwater bivalve *Velesunio angasi*, between pH 5 and 6, where UO_2^{2+} is assigned twice the toxic effect of UO_2OH^+. These results provided the first evidence that the toxicity of U to biota is governed by the uranyl ion (UO_2^{2+}), rather than the sum total of inorganic uranyl species or uranyl-organic species.

1.6.2 The toxicity of uranium to cladocerans and other organisms

At elevated levels uranium is toxic to cladocerans (Table 1.1). Uranium's high capacity for solubilisation and transport, causes it to pose a potential toxic hazard to aquatic organisms exposed to effluents from uranium mining and milling processes (Poston *et al.*, 1984). However, information on the toxicity of uranium to aquatic organisms is very limited, especially when looking at the effects and mode of action of uranium on cladocerans. Exposure to uranium has been reported to suppress reproduction in the cladoceran *D. magna* (Poston *et al.*, 1984). Ahsanullah and Williams (1986) reported of a reduction in respiration rates in the marine amphipod, *Allorchestes compressa*, after monitoring the exposure of uranium over three generations.

Intracellular accumulation of uranium has been documented in the lysosomes, macrophages, gill epithelia, hindgut epithelia and hepatopancreas cells of molluscs and crustacea exposed to uranium contaminated seawater or food (Chassard-Bouchard, 1983; Hyne *et al.*, 1992). It is well known that substantial amounts of uranium may be accumulated inside some types of cells. The accumulation of

Table 1.1. The acute toxicity of uranium to selected cladoceran species.

Organism	Duration (h)	LC50 (mg/L)	Total hardness (mg/L $CaCO_3$)	Reference
D. magna	48	5.3-7.6	66-73	Poston *et al.*, 1984
D. magna	48	44.6-30.4	126-140	Poston *et al.*, 1984
D. magna	48	30.6-74.3	188-205	Poston *et al.*, 1984
M. macleayi	24	1.3	NR	Bywater *et al.*, 1991
Diaphanosoma Excisum	24	1.0	NR	Bywater *et al.*, 1991
Latonopsis Fasciculata	24	0.4	NR	Bywater *et al.*, 1991
Dadaya macrops	24	1.1	NR	Bywater *et al.*, 1991

NR – not reported

uranium may or may not have a toxicological effect on the cell. Development of toxicity will depend on whether the uranium binds to a protein and interferes in its function or whether it is detoxified by forming a uranium phosphate microgranule as observed in the crab (*Carcinus maenas*) (Chassard-Bouchard, 1983; Hyne *et al.*, 1992). Uranium has also been shown to be able to inhibit the activity of ATPase, an enzyme located on the plasma membrane of the epidermal cell layer of hydra (Hyne *et al.*, 1992). ATPase is involved in osmoregulation and other active transport processes (Hyne *et al.*, 1992).

In a study conducted by Bywater e*t al.*, (1991), symptoms of uranium intoxication appeared in species of different fishes from tropical freshwaters in Northern Australia. Symptoms involved the progression from increased respiration rates to erratic swimming, loss of equilibrium, darkening in colour, haemorrhaging of blood vessels in fins, and eventually death. At 5.8 mg/L, respiration rate was elevated in all species within 30 mins of initial exposure. Death occurred over a range of between 2 to 6 h.

The toxicity of uranium in humans and terrestrial animals has been well documented by Environment Canada (1983), and is summarised below. Uranium enters the body either by ingestion through drinking water or contaminated food, inhaled during respiration, or absorbed through contact with skin. Uranium is absorbed into human and other organisms almost always in the uranyl ion form. Sixty percent of the absorbed uranyl ion is carried as a soluble bicarbonate complex, while the rest is bound to plasma proteins. The main target tissue types and organs for uranyl ion deposition in mammalian bodies are the kidneys, bones and to a lesser extent, the liver. A possible mechanism of toxic action of the uranyl ion in the kidneys would involve the circulation of the uranyl ion in the blood plasma as a relatively inert but acid labile bicarbonate-uranyl complex. It then may be filtered into the

kidney tubules and the uranyl ion is set free by the action of hydrogen ions. The uranyl ion is then liberated and concentrated in the tubular lumen as a result of normal tubular action. The ions, however, tend to cause damage to kidney structure, which if severe enough cause kidney failure. Renal toxicity, with classical signs of impairment such as albuminuria and elevated blood urea nitrogen as well as loss of weight are brought about by necrosis of the kidney tubules. Other effects of uranium include cellular necrosis brought about by alterations in the transport of organic compounds and ions across tubule cell membranes along with changes in intracellular protein binding. Severe kidney impairment may result in death.

Although the mechanism of toxicity of uranium to aquatic organisms is lacking in literature, information on how uranium effects humans and mammals gives us a basic understanding of the mode of action of uranium.

1.7 Cadmium

Cadmium is regarded by many as one of the most toxic trace elements in the environment (Foulkes, 1986). Increased emissions from, production, use, and waste disposal combined with long term persistence in the environment, and its relatively rapid uptake and accumulation by food chain crops contribute to its potentially hazardous nature (Foulkes, 1986).

Cadmium is a non-essential metal, however, it is closely related to the essential metal zinc and is found wherever zinc is found (Friberg *et al.*, 1985). Cadmium is found in most of the poly-metallic ores (Tsuchiya, 1978). Most of the cadmium in crude ore is recovered as a by-product in the refining of other metals, in particular zinc (Tsuchiya, 1978). Uses of cadmium include electroplating, pigments for paint production, as colouring agents, stabilisers in plastic materials, alloys in production of

bearings for aircraft and other internal combustion engines, as well as in batteries in the manufacture of nickel-cadmium batteries (Foulkes, 1986).

Cadmium enters naturally into environmental waterways through the air by the emission of cadmium from cadmium industries, from contaminated soil by sewage sludge or phosphate fertilisers and through incorrect use and disposal of cadmium products (Friberg, 1985). The normal cadmium concentration in seawater is about 0.01 to 0.10 μg/L (Friberg, 1985). Concentrations in rainwater collected from areas without cadmium pollution range from 0.01 to 0.07 μg/L (Friberg, 1985). In fresh surface and ground waters cadmium concentrations are usually less than 1 μg/L (Friberg, 1985). However natural waters occasionally contain concentrations higher than 1 μg/L, particularly in areas where there are zinc bearing mineral formations, where concentrations may reach up to 10 μg/L (Friberg, 1985). In natural, non-contaminated water and as well as in contaminated water systems, cadmium is found mainly in bottom sediments and suspended particles (Friberg, 1985).

1.7.1 Speciation and bioavailability of cadmium

In fresh surface waters (pH ≤ 8.5) the predominant species of dissolved cadmium is the free hydrated ion (Cd^{2+}) (French, 1986). Cadmium begins to hydrolyse at about pH 8, forming $CdOH^+$, which occurs only as a very minor species (< 2%) (French, 1986; Markich *et al.*, 1997). In freshwater systems from pH 8-9, the percentage of Cd^{2+} declines about three- to four-fold; this decline is caused by an increase in the percentage of $CdCO_3$ and $CdHCO_3^+$ (French, 1986; Markich *et al.*, 1997). $CdCO_3$ and $CdHCO_3^+$ are governed by the alkalinity of the water, where the higher the alkalinity and pH in fresh surface waters, the greater the percentage of $CdCO_3$.

It is normally considered that the free cadmium ion (Cd^{2+}) is the form of cadmium that is primarily responsible for causing a toxic response in aquatic organisms (Campbell, 1995; Markich *et al.*, 1997). Cadmium complexes with inorganic and/or organic ligands/agents, however, this normally reduces the uptake and toxicity of the metal by reducing the concentration of Cd^{2+}.

1.7.2 The toxicity of cadmium to cladocerans and other organisms

Cadmium is very toxic to cladocerans, having no known biological function (Table 1.2). Cadmium toxicity in cladocerans (eg. *D. magna*) has been known to induce feeding inhibition (Allen *et al.*, 1995; Taylor *et al.*, 1998), reduce growth and response of average swimming velocity (Baillieul and Blust, 1999). It also has a dramatic effect on reproduction by reducing brood sizes, and at extremely high levels ($\geq$ 150 µg/L in *D. carinata*) will completely inhibit reproduction and cause mortality in all daphnids within a few days (Chandini, 1989).

Griffiths (1980) investigated the morphological and ultra-structural effects of cadmium poisoning on *D. magna*. Cadmium caused rapid changes in the appearance of the gut diverticulum (shrinkage of the organ) of *Daphnia* sp. (in 2 h). Evidence of the mode of action by which cadmium poisons living organisms was determined. Cadmium poisoning interferes with calcium metabolism and results in the formation of abnormal calcium deposits on cell membranes (Griffiths 1980). Shrinkage of the gut diverticulum may be due to an osmotic effect resulting from a change in calcium metabolism. Cadmium may also affect the diverticular muscles and can cause them to become paralysed in extreme circumstances. This effect would most likely be caused by an interference in calcium metabolism in view of the known importance of calcium in muscle function (Griffiths, 1980). The toxicity of cadmium has thus been attributed

Table 1.2. The 48-h acute toxicity of cadmium to selected cladoceran species.

Organism	LC50 (μg/L)	Reference
D. magna	80	Allen *et al.*, 1995
D. magna	118	Mount and Norberg, 1984
D. pulex	68	Mount and Norberg, 1984
C. dubia	54	Bitton *et al.*, 1996

to its ability to bind with biochemical systems that normally interact with divalent ions such as calcium (Griffiths, 1980).

Forshaw (1977) showed that cadmium inhibited the calcium function, of releasing the neurotransmitter at presynaptic nerve terminals of rat diaphragm and suggested that the cadmium ions may reduce calcium influx by binding onto Ca^{2+} receptor sites on the nerve terminal membrane.

De Coen and Janssen (1997) assessed the effect of sublethal exposure of $CdCl_2$ on the activity of five digestive enzymes of *D. magna*. The five enzymes were chosen as each are partially responsible for the digestion of one of the three major food classes: carbohydrates (amylase, cellulase and β-galactosidase), proteins (trypsin) and lipids (esterase). A reduction of β-galactosidase and trypsin activity to as low as 10% of the control value was observed in organisms exposed for 48 h to 570 μg/L $CdCl_2$. After a further 48 h (at 96 h) all daphnids exposed to 570 μg/L were dead. In general, prolonged exposure (96 h) caused an increase in the overall digestive enzyme activity of organisms exposed to concentrations ranging from 0.8 to 80 μg/L. The increase in digestive enzyme activity was thought to occur as a result of the test organisms altered food assimilation efficiency in order to cope with the reduced food uptake.

Three possible primary sites for metal uptake are the body surface, gills and the alimentary canal (De Coen and Janssen, 1997). In daphnids, the midgut is lined with highly differentiated columnar epithelial cells that are responsible for both the enzyme secretion and the absorption of digested food (Bodar *et al.*, 1990; De Coen and Janssen, 1997). The alimentary canal is an important route of uptake for heavy metals entering aquatic animals (De Coen and Janssen, 1997). The adverse effects of these chemicals could be attributed to impaired transport of nutrients across the epithelial

cells (De Coen and Janssen, 1997). Effects on energy metabolism of an organism are highly possible since digestive cells are tightly connected to the storage cells that are responsible for production of lipid- and carbohydrate reserves (Bodar *et al.*, 1990; De Coen and Janssen, 1997).

Tolerance to sublethal concentrations of cadmium and other heavy metals has been observed in cladocerans and other aquatic organisms (Brown, 1976; Fraser *et al.*, 1978; Moraitou-Apostolopoulou, 1978; Le Blanc, 1982; Bodar *et al.*, 1990). Free cadmium ions (Cd^{2+}) at the cellular level enter a cell through transport proteins or ion channels. They then interact with proteins including metallothioneins (MTs). MTs are ubiquitous low molecular weight proteins that are involved in the biochemical detoxification of heavy metals (Bodar *et al.*, 1990). They are characterised by an unusually high cysteine content and a selective capacity to bind with heavy metal ions such as mercury, zinc, cadmium, and copper (Cherian and Goyer, 1978; Karin, 1985). MTs are naturally present in animal liver and kidney where they serve as the major storage for the essential trace elements, zinc and copper (Karin 1985). Cadmium tends to accumulate in the kidney and liver and is not usually distributed among different organs and tissues of an organism (Kotsonis and Klaassen, 1978; Tsuchiya, 1978; Karin, 1985). The free cadmium ion binds to MT transcription factors, which increases the transcription of the MT gene, inturn initiating MT synthesis (Hamer, 1986). The subsequent binding of the heavy metal to the MT prevents the toxic effect of the metal (Cherian and Goyer, 1978; Karin, 1985).

Some other toxic effects of cadmium to other organisms include its ability to cause vertebral deformities in fishes (Bengtsson *et al.*, 1975). High cadmium concentrations in molluscs cause considerable changes in the phospholipid composition of their organs (Evtushenko *et al.*, 1986). Cadmium is also known to inhibit the activity of the

ATPase enzyme (Bodar *et al.*, 1988; Evtushenko *et al.*, 1986). A lack of ATP-energy will then cause a decrease in muscle cell activities. The ability of cadmium to inhibit lipid synthesis in liver and kidney of rats (Rana *et al.*, 1980). Cadmium also causes renal dysfunction, testicular atrophy, hepatic injury, and anemia in humans and animals (Kotsonis and Klaassen, 1978).

1.8 Population variation and implications for cladoceran toxicity testing

The ultimate aim of ecotoxicological studies is to predict how natural populations respond to contaminant exposure (Barata *et al.*, 1998). Toxic effects of metals in *Daphnia* have been considered by many investigators (in particular *D. magna*) (Stuhlbacher *et al.*, 1992). All of these studies show considerable variability in the response of the studied species. There are two main factors that contribute to population variation within a particular species: genetic and environmental variability. Variability in toxic responses may also be related to the ability of cladocerans to develop tolerance or resistance to environmental pollutants through physiological adaptation (Stuhlbacher *et al.*, 1992). Thus, it is crucial to understand how genetic and environmental factors in the field modify responses measured in the laboratory.

1.8.1 Genetic and Environmental Variability

Genetic variability between laboratory stock populations appears to arise due to two reasons. Firstly, a number of laboratories obtain stocks directly from local wild populations, and have proven to be genetically unique. Secondly, some genetic heterogeneity has been due to clonal divergence. Here, a number of laboratories receive their stocks from a central source that at some point give rise to two sub-clones, either due to mutation or sexual recombination (Baird *et al.*, 1989).

Two separate components of environmental variability have been recognised: the conditions experienced prenatally in the mother ('maternal effects'), and the conditions experienced by individuals after birth (Baird *et al.*, 1989).

The existence of a true genetic basis for trace metal tolerance can only be properly assessed by separating and quantifying the genetic, environmental and genetic × environmental components of variability in tolerance (Barata *et al.*, 1998). Environmental factors, such as differences in water chemistry, can influence how genotypes respond to trace metals. For example, if genetic variability in tolerance to pollutants is inherited by single major genes, as has been suggested for trace metals, then it should remain constant across environments (Barata *et al.*, 1998). In contrast, genetic variability in tolerance to pollutants with polygenic inheritance will vary across environments.

1.8.2 Problems with Inconsistencies in Cladoceran Laboratory Bioassays

One of the essential properties of a laboratory bioassay is that it should be repeatable (Baird *et al.*, 1989). Recently, studies have evaluated problems that cause inconsistencies among single-species bioassays for freshwater cladocerans. Despite the widespread use of the freshwater cladoceran, *D. magna*, in standard ecotoxicity tests, results from several 'ring tests' have identified significant variation between testing laboratories involving the same toxicant (Soares *et al.*, 1992). An example of this was seen in a ring test conducted by the European Commission (EC), using the toxicant sodium bromide. The criterion used was the no-observed-effect-concentration (NOEC), defined as the concentration immediately below that which a statistically significant effect on reproduction occurred. Of 37 participating laboratories, 22 returned results that satisfied validity criteria specified in the protocol

(Baird *et al.*, 1989). Most tests that failed validity did so because of low fecundity in the control treatments. The values obtained ranged from 3 ppm to 117 ppm (Baird *et al.*, 1989). Baird *et al.* (1989) concluded that, in order to improve consistency in the *D. magna* bioassay among testing labs, both genotype and culture conditions must be specified. Test protocols must be apparent; if tests are carried out under conditions that deviate from the protocol the results must be discarded (Baird *et al.*, 1989).

1.8.3 Population variation to toxicants

Baird *et al.* (1990) conducted a study, comparing and contrasting responses of different genotypes of *D. magna* to cadmium and 3,4-dichloroaniline (DCA), under acute and chronic conditions. Results indicated that there were large interclonal differences in acute tolerances to cadmium, with LC50 values ranging from 0.06 - > 100 ppb. Differences in interclonal variation in tests using DCA were not as significant. Interclonal variation in chronic stress tolerance was also observed, although in this case differences (though significant) were relatively small (effect concentrations 25-50 ppb for DCA and 0.6-6.0 ppb for cadmium). The conclusions were that specific mechanisms dominated differences in acute responses, whereas general mechanisms dominated chronic responses.

Barata *et al.* (1998) carried out a study to determine the genetic and environmental components of variability in acute responses among four *D. magna* populations exposed to both essential (zinc and copper) and non-essential (cadmium and uranium) metals. *D. magna* populations were exposed to single-metal solutions of varying concentration at two or three levels of water hardness (soft, moderate-hard, and hard) for periods ranging from 12-96 h. The results indicated that genetic and environmental variability were evident in the acute tolerance of *D. magna* exposed to

zinc, copper, cadmium and uranium. The results for cadmium and zinc supported the hypothesis that the genetic variability in tolerance to non-essential metals is greater than essential metals, whereas, copper and uranium did not. Thus, genetic variability in toxicity has to be assessed for each metal in order to predict the response of natural populations to trace metal contamination.

1.9 Aims

The aims of this research were:

1. To assess variability in the responses of different populations of *M. macleayi* to uranium and cadmium.

2. To assess tolerance in a population of *M. macleayi* exposed to a contaminated environment containing elevated levels of uranium.

1.10 Null Hypotheses

This research involved testing the following null hypotheses:

1. There is no difference in the responses of different populations of *M. macleayi* to uranium and cadmium.

2. There will be no tolerance in a population of *M. macleayi* exposed to a contaminated environment containing elevated levels of uranium.

2. Materials and Methods

2.1 Description of site

All research was carried out at the Environmental Research Institute of the Supervising Scientist (*eriss*) Wetland Research Laboratory from April to September 1999. The laboratory was situated in Jabiru East, Northern Territory, near the eastern border of Kakadu National Park on the lease of Ranger Uranium Mines Pty. Ltd. The Magela Creek flows adjacent to the mine and runs into the Magela Floodplain, a comprehensive wetland area of significant ecological importance (Figure 2.1).

2.2 *M. macleayi* populations

Three populations of *M. macleayi* were chosen for testing based on their considerably different past environmental conditions. All were originally from local waterbodies. One population had been collected from Magela Creek floodplain (Figure 2.1; Hyne *et al.*, 1996) and has been cultured in the *eriss* Wetland Research laboratory for over 10 y. A 'wild' population was collected from Bowerbird Billabong (Arnhem Land) on the 15 October 1998, and has been maintained in the laboratory since. Bowerbird Billabong is a permanent water body in the headwaters of Magela Creek, approximately 20 km south-east (upstream) of Jabiru East (Figure 2.2). The third population was collected from Djalkmara Billabong on the 30 June 1999. Djalkmara Billabong is a black-flow billabong of Magela Creek, located adjacent to the Ranger uranium mine-site. Road construction through the middle of the billabong has led to the enclosed portion being used as a storage pond to hold water pumped from Retention Pond 4, which is also located on the Ranger uranium mine-site (Figure 2.3). Water from Djalkmara Billabong is either

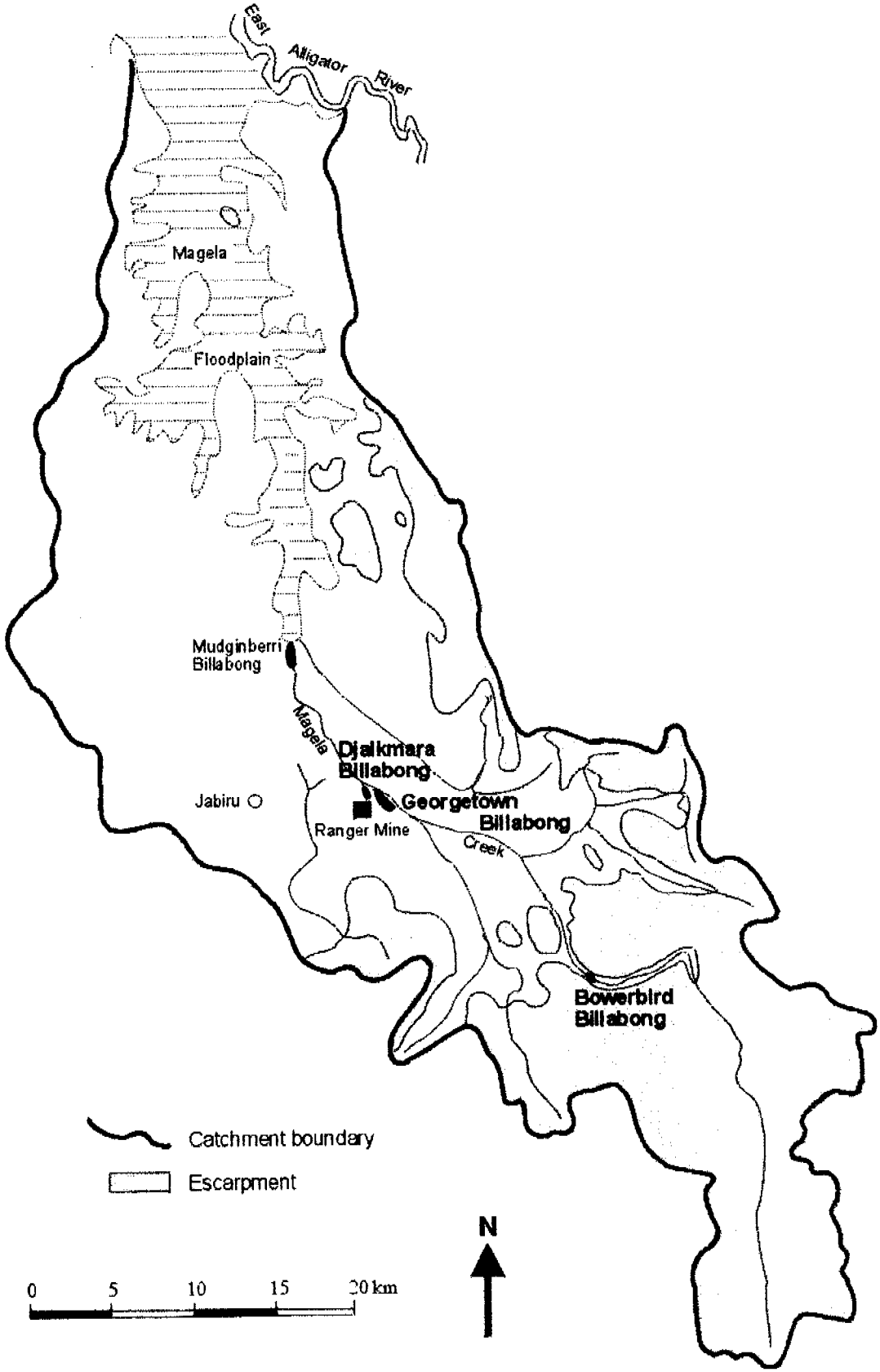

Figure 2.1: A map of the Magela floodplains showing Magela Creek, Georgetown Billabong, Bowerbird Billabong and Djalkmara Billabong.

Figure 2.2: Aerial photograph of Bowerbird Billabong

Figure 2.3: Aerial photograph of Djalkmara Billabong.

pumped into the Magela Creek, during the wet season, or used for spray irrigation purposes during the dry season (ERA, 1997).

2.3 Culturing of *M.macleayi* Populations

In total, 3 populations of *M. macleayi* were cultured and assessed: the long established laboratory stock (Lab), Bowerbird Billabong stock (BB) and Djalkmara Billabong stock (DjB (Figure 2.4). Each stock was maintained on a separate culture tray consisting of 8-12 *M. macleayi*. Each *M. macleayi* was placed into a separate 45 mL screw-capped vial with two 2 mm diameter ventilation holes per cap (Duranol type 12). Each vial contained 30mL of filtered creek water, 2×10^5 cells/mL of *Chlorella* sp., and 1μL/mL of fermented fish food and vitamins (FFV) (for details of food ingredients and protocols for how food was made see Appendix 2). These combined food sources were established from research previously conducted to improve fecundity and survival of *M. macleayi* culture stocks (Padovan, 1992; Rippon, 1993; Hyne *et al.*, 1993). Water and food were renewed daily and cultures were restarted using 2nd brood neonates, (~ every 3-4 d). The DjB stock was cultured in both DjB water and BB water (see Table 2.1, for water chemistry details), as the animals were generally stressed and unhealthy in DjB water, evidenced by the fact that they often were only surviving to have first broods. Thus, the DjB stock that was cultured in DjB water was restarted using 1st brood neonates, following their release (~ every 2-3 d) (details of the collection of the DjB population and its acclimatisation to BB water are in Appendix 1). Populations were kept in a constant temperature incubator (Labec) at 27 ± 1°C, with a photoperiod of 12 h light: 12 h dark.

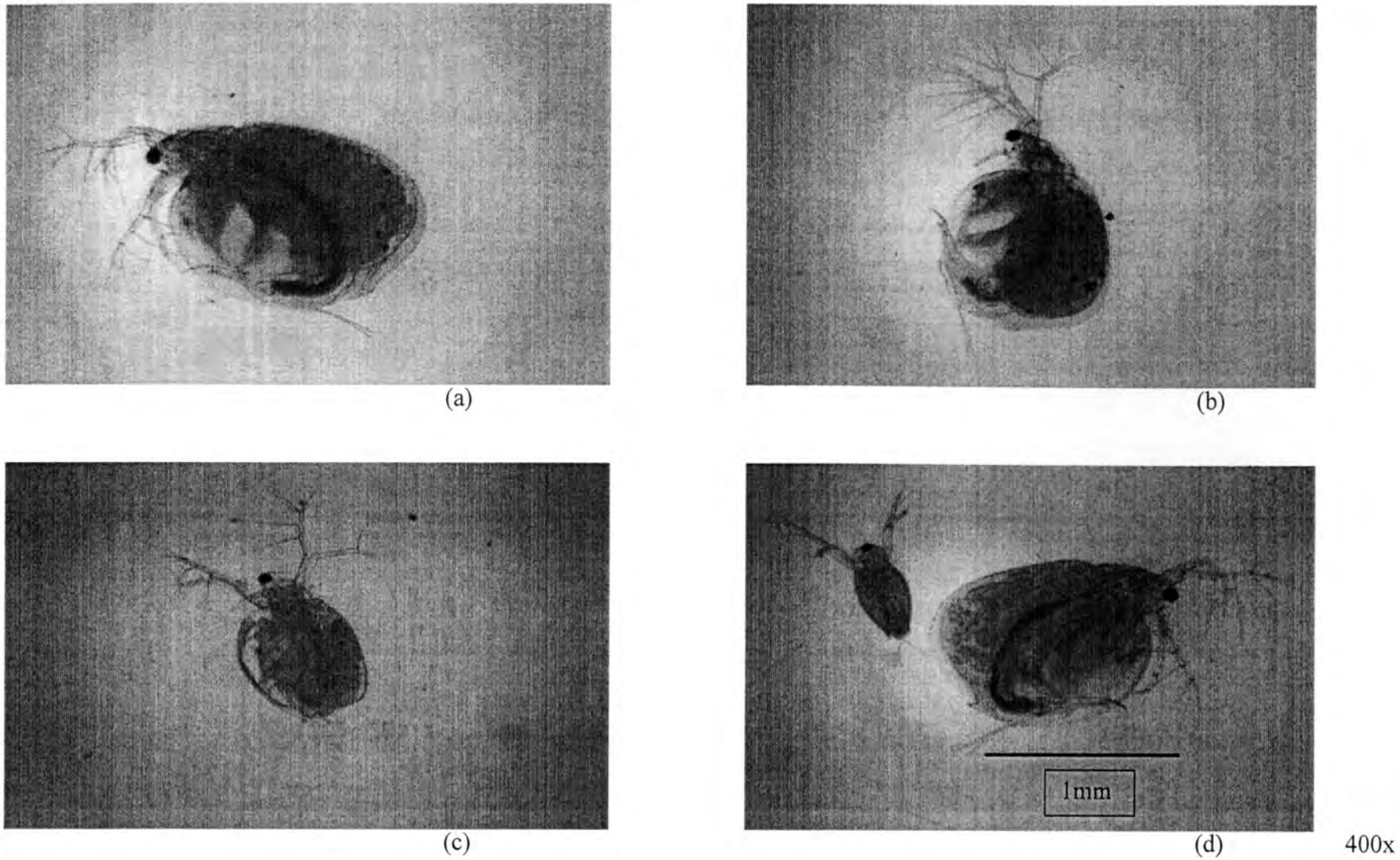

Figure 2.4: Photographs of all four *M. macleayi* populations (a) Lab, (b) BB, (c) DjB1 and (d) DjB2 & neonate (~6-10 h old).

Table 2.1 Summary of Magela Creek (MC), Bowerbird Billabong (BB) and Djalkmara Billabong (DjB) water chemistry.

Chemical	MC[a]	BB[b]	DjB[a]
Aluminium, Al	NTF	17 µg/L	NTF
Calcium, Ca	0.6 mg/L	0.2 mg/L	10 mg/L
Cadmium, Cd	NTF	< 0.02 µg/L	NTF
Cobolt, Co	NTF	0.07 µg/L	NTF
Chromium, Cr	NTF	0.20 µg/L	NTF
Copper, Cu	< 2 µg/L	1.20 µg/L	< 2 µg/L
Iron, Fe	NTF	110 µg/L	NTF
Magnesium, Mg	0.9 mg/L	1.0 mg/L	85-130 mg/L
Manganese, Mn	3.5 µg/L	2.70 µg/L	1.5 µg/L
Nickel, Ni	NTF	0.30 µg/L	NTF
Lead, Pb	< 1 µg/L	0.06 µg/L	NTF
Sulphate, SO_4	0.2-0.4 mg/L	0.3 mg/L	250-510 mg/L
Selenium, Se	NTF	< 0.2 µg/L	NTF
Uranium, U	< 0.1 µg/L	0.13 µg/L	25-170 µg/L
Zinc, Zn	< 2 µg/L	NTF	< 2 µg/L

NTF – not tested for
a - Data obtained from ERA (1999)
b – Data supplied by analysis performed by CHEMNORTH laboratory, in Darwin, NT.

During daily water and food renewals, or culture restarts, trays were removed from the incubator and placed on warming trays that were set at 27 °C.

2.4 Collection of control/diluent water

Natural (control/diluent) water was collected from two sites along Magela Creek, depending on seasonal availability. During the Wet season (April-June), water was collected from Magela Creek (MC), just downstream of Georgetown Billabong (GB). During the Dry season (July- September), when creek flow had ceased and water was unable to be collected from the creek channel, water was collected from Bowerbird Billabong (BB), approximately 18 km upstream of GB. Water was collected in 25-L polyethylene containers. Each container was pre-cleaned using detergent (2% Neutracon) and nitric acid (5% HNO_3 BDH Aristar®), and rinsed thoroughly with deionised water (Milli Q, <1 $\mu S\ cm^{-1}$) before being used to collect water. In the field, containers were thoroughly rinsed (three times) with surface water before being filled. The water was then transported to the laboratory (*eriss*) where pH, electrical conductivity (EC) and dissolved oxygen (DO) were measured on unfiltered and filtered sub-samples. EC and pH were measured using a WTW Multiline P4 Universal meter, and DO was measured using an Activon Model 401 oxygen meter. All control/diluent water was filtered using a Whatman No. 91 (10μm) filter paper to remove any wild zooplankton and reduce particulate matter. The mean (SE) water parameters for water collected from MC were: pH: 6.55 (0.02); EC: 17 (0.50) μS/cm; and DO: 97 (5.00) %. The mean (SE) water parameters for water collected from BB were: pH: 6.45 (0.03); EC: 17 (0.75) μS/cm; and

DO: 95 (3.90) %. Collection dates and water parameters on filtered and unfiltered water are listed in Appendix 4.

A continuous flow of water during the main Wet season effectively washes out the creek channel and forms consistent and common water chemistry in all billabongs (Camilleri *et al.*, 1998). Thus, the surface water from BB during the Dry season is typical of Wet season Magela creek water (Camilleri *et al.*, 1998). Therefore, relatively constant water chemistry was used for all experiments (see Table 2.1 for water chemistry details), although slight differences did exist, as discussed later.

2.5 Preparation of stock and test solutions

A stock solution of uranium was prepared using Uranyl Sulphate ($UO_2SO_4.3H_2O$) (supplied by AJAX Chemicals) where 1.25g of $UO_2SO_4.3H_2O$ was diluted into a litre of deionised water (Milli Q 18 MΩ cm^{-1} resistivity), resulting in a stock uranium concentration of 0.708g U/L.

A stock solution of cadmium was prepared using Cadmium Sulphate ($3CdSO_4.8H_2O$) (supplied by BDH Chemicals Ltd.) where 0.571g of $3CdSO_4.8H_2O$ was diluted into a litre of deionised water (Milli Q 18 MΩ cm^{-1} resistivity), resulting in a stock cadmium concentration of 0.250g Cd/L.

Stock solutions were prepared in pre-cleaned 1-L plastic containers and refrigerated at 4°C. Prior to use, the stock solution was allowed to equilibrate to room temperature. Test solutions were prepared by serially diluting the stock solution with filtered (<10μm) Magela Creek water (ie. from the downstream creek channel or Bowerbird billabong), which was collected as close as practicable to the commencement of each toxicity test.

Test concentrations were determined from preliminary results obtained by Hyne *et al.* (1993) for uranium and Orchard (1999) for cadmium. Test solutions were prepared in pre-cleaned 5-L polyethylene screw-topped containers immediately prior to test commencement. Throughout the test, the test solutions were kept at 4°C until required for daily solution renewals, when they were allowed to equilibrate to 27 ± 1°C inside a constant temperature incubator (Labec) for several hours.

2.6 Toxicity testing procedures

Both chronic and acute toxicity of uranium to the three populations of *M. macleayi* (Lab, BB, DjB) were assessed using standard protocols. Chronic toxicity was assessed using the Cladoceran (*M. macleayi*) 3 brood/5-6 d reproduction test (protocol BTT-D; Hyne *et al.*, 1996). Acute toxicity was assessed using a 48-h Acute Immobilisation/lethality test (based on a modified version from the protocol BTT-A (Hyne *et al.*, 1996) and the *Daphnia* sp., Acute Immobilisation Test (OECD, 1999)). The standard protocols (BTT-A and BTT-D) were initially designed to assess the toxicity of pre-release waste waters from the Ranger uranium mine (Hyne *et al.*, 1996). A total of seven uranium chronic tests were carried out. Three tests were performed on the Lab population, two tests were performed on the BB population and two tests were performed on the DjB population (that was cultured in BB water; DjB2). A total of eight uranium acute tests were carried out. Two tests were performed on each of the populations (ie. Lab, BB, DjB population cultured in DjB water (DjB1) and the DjB population cultured from BB water (DjB2)).

Due to a shortage of time, the chronic toxicity of cadmium was only assessed for two populations of *M. macleayi* (Lab, BB), again using the Cladoceran (*M. macleayi*) 3 brood/5-6 d reproduction test (protocol BTT-D; Hyne *et al.* (1996). A total of four cadmium chronic tests were carried out, two tests on each of the Lab and BB populations.

2.6.1 Cladoceran (M. macleayi) 3 brood/ 5-6 day reproduction test

Female *M. macleayi* (asexually-reproducing, parthenogenetic) neonates (<6 h-old) were exposed to nominal concentrations of uranium ranging from 0 (control) to 75 μg L^{-1} (Table 2.2) and nominal concentrations of cadmium ranging from 0 (control) to 20 μg L^{-1} (Table 2.3) until control cladocerans released their third brood offspring (ie. ~ 5-6 d). For each population the results of the initial tests helped determine the concentration ranges for the following tests. Cladocerans were exposed to 30 mL of each test concentration in 45 mL screw-capped vials with two 2 mm diameter ventilation holes per cap (Duranol type 12). Each vial initially contained one neonate. Ten replicates were used for each test concentration; therefore a test with six test concentrations including the control, a total of 60 test vials and 60 neonates were required to start the test. Test vials were placed in random order in a constant temperature incubator at 27 ± 1°C, with a photoperiod of 12 h light: 12 h dark (Figure 2.5). Tests solutions were renewed every 24 h, following observation of the number of neonates in each vial. Cladocerans were fed daily with the unicellular green alga, *Chlorella* sp. (at a cell density of 2×10^5 cells/mL), as well as 1 μL of FFV per mL of test solution (Hyne *et al.*, 1993).

Observations were recorded every 24 h on the survival of each female, the number of neonates produced, and the number of surviving neonates. The numbers of neonates from

all broods were summed for each adult cladoceran, resulting in a count of the total number of offspring per adult. The test was considered valid if mortality in the controls did not exceed 20%, and reproduction in the controls averaged 30 or more neonates per surviving female over the test period. The water parameters pH, EC, and DO in the test waters were measured daily for fresh (t_0) and 24 h-old (t_{24}) test water (parameters were measured as described in section 2.4). The mean (SE) water parameters for all fresh (t_0) test treatments were: pH: 6.85 (0.01); EC: 19 (0.18) µS/cm; and DO: 112 (0.61) %. The mean (SE) water parameters for all 24 h-old (t_{24}) test treatments were: pH: 7.14 (0.02); EC: 19 (0.21) µS/cm; and DO: 97 (0.16) %. Water parameters for each test are listed in Appendix 4.

Table 2.2. Nominal concentrations of uranium (μg/L) used in the Cladoceran (*M. macleayi*) 3 brood / 5-6 day reproduction tests.

Exp. No.	Population	Nominal Concentration (μg/L)
U-Lab-1	Lab	0, 5, 10, 25, 50
U-Lab-2	Lab	0, 5, 10, 15, 25, 50
U-Lab-3	Lab	0, 5, 10, 15, 25, 50
U-BB-1	BB	0, 5, 10, 15, 25, 50
U-BB-2	BB	0, 10, 20, 30, 40, 50
U-DjB2-1	DjB2	0, 5, 10, 15, 25, 50
U-DjB2-2	DjB2	0, 10, 20, 30, 40, 50

Table 2.3. Nominal concentrations of cadmium (μg/L) used in the Cladoceran (*M. macleayi*) 3 brood / 5-6 day reproduction tests.

Exp. No.	Population	Nominal Concentration (μg/L)
Cd-BB-1	BB	0, 1, 2, 5, 10, 15, 20
Cd-BB-2	BB	0, 0.5, 1, 2, 3.5, 5, 7
Cd-Lab-1	Lab	0, 0.5, 1, 2, 5, 10, 20
Cd-Lab-2	Lab	0, 0.5, 1, 2, 3.5, 5, 7

Figure 2.5: Photograph showing the set up of a reproduction test in a constant temperature incubator.

2.6.2 48-h Acute Immobilisation/lethality test

M. macleayi neonates (<6 h-old) were exposed to nominal concentrations of uranium ranging from 0 (control) to 700 μg L^{-1} (Table 2.4) for 48 h. Cladocerans were exposed to 150 mL of each test concentration in 200 mL screw-capped vials with two 5 mm diameter ventilation holes per cap. Each vial initially contained 10 neonates. Three replicates were used for each test concentration; therefore a test with seven test concentrations including the control, a total of 21 test vials and 210 neonates were required to start the test. Test vials were placed in random order in a constant temperature incubator at 27 ± 1°C, with a photoperiod of 12 h light: 12 h dark (Figure 2.6). Test solutions were not renewed. Observations were recorded at the end of the test (i.e. after 48 h) on the number of surviving *M. macleayi*. The test was considered valid if mortality in the controls did not exceed 20%. The water parameters pH, EC, and DO in the test waters were measured on fresh (t_0) and 48 h-old (t_{48}) test water (parameters were measured as described in section 2.4). The mean (SE) water parameters for all fresh (t_0) test treatments were: pH: 6.63 (0.01); EC: 16 (0.12) μS/cm; and DO: 109 (0.48) %. The mean (SE) water parameters for all 48 h-old (t_{48}) test treatments were: pH: 6.92 (0.01); EC: 19 (0.61) μS/cm; and DO: 98 (0.29) %. Water parameters for each test are listed in Appendix 4.

Table 2.4. Nominal concentrations of uranium (μg/L) used in the 48-h Acute Immobilisation/lethality tests.

Exp. No.	Population	Nominal Concentration (μg/L)
U-DjB1-1	DjB1	0, 50, 100, 200, 300, 400, 600
U-BB-1	BB	0, 50, 100, 200, 300, 400, 600
U-DjB1-2	DjB1	0, 200, 300, 400, 500, 600, 700
U-BB-2	BB	0, 100, 200, 300, 400, 500, 600
U-DjB2-1	DjB2	0, 100, 200, 300, 400, 500, 600
U-Lab-1	Lab	0, 100, 200, 300, 400, 500, 600
U-DjB2-2	DjB2	0, 100, 200, 300, 400, 500, 600
U-Lab-2	Lab	0, 50, 100, 150, 200, 300, 400

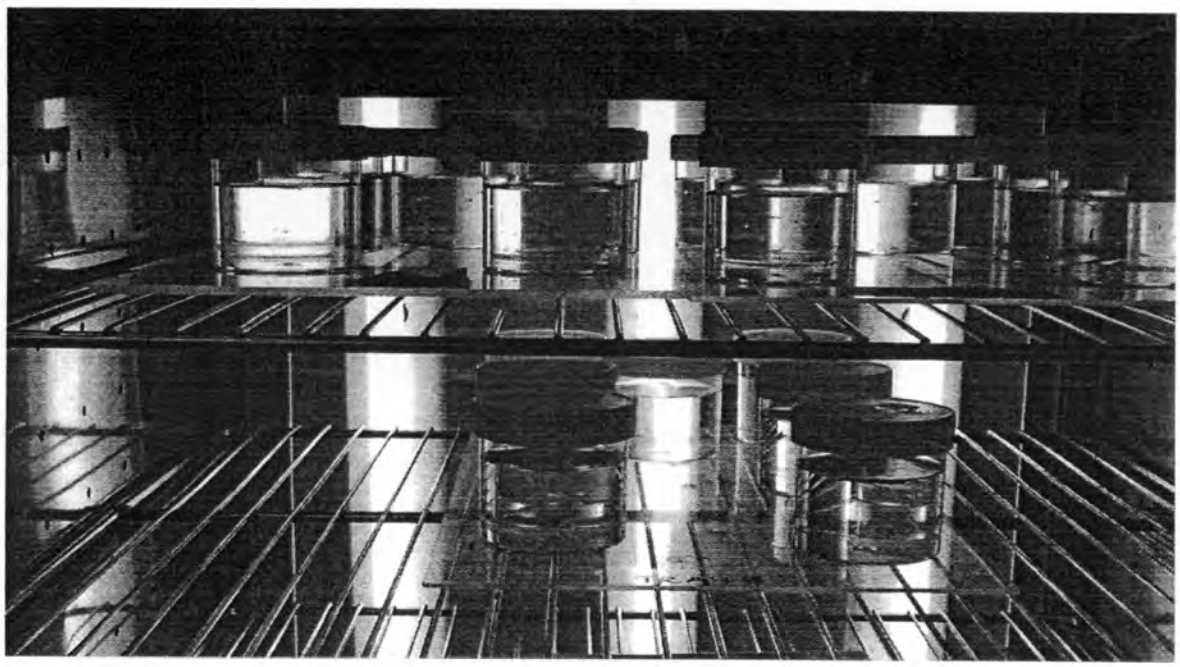

Figure 2.6: Photograph showing the set up of the 48-h acute immobilisation/lethality test in a constant temperature incubator.

2.7 Chemical Analysis

50 mL samples from each test concentration were taken at the beginning of each test to be analysed to determine actual concentrations. All analyses were performed at the CHEMNORTH laboratory, in Darwin, NT, using Inductively coupled plasma-mass spectrometry (ICP-MS). Nominal and actual measured concentrations for all treatments for all tests are listed in Appendix 3, and all statistical analysis was based on measured concentrations. Results were reported based on measured concentrations.

2.8 Statistical Analysis

2.8.1 Cladoceran (M. macleayi) 3 brood/ 5-6 day reproduction test

Total number of live offspring data were analysed using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test using the software programs Minitab or Statistica. When the ANOVA or Kruskal-Wallis test was significant ($P \leq 0.05$), a Tukey's multiple comparison test was used to determine the values that were significantly different and enable the lowest-observed-effect-concentrations (LOECs) and no-observed-effect-concentrations (NOECs) to be determined for each test. Data were tested for equality of variance and normality. If either of these tests returned a significant result ($P \leq 0.05$), the data were transformed. When transformations of data failed to meet the assumption of normality for ANOVA (Zar, 1999), a non-parametric test was conducted on test data (ie. The Kruskal-Wallis test was used to analyse data).

The Kruskal-Wallis test, often called an 'analysis of vaiance' by ranks, is employed in situations where the parametric single-factor ANOVA is not applicable (Zar, 1999). The non-parametric analysis is especially desirable when the samples do not come from

normal populations and also when population variances are somewhat heterogeneous (Zar, 1999).

Mean brood sizes (ie. first, second, and third broodsizes) were also determined and analysed by one-way ANOVA. Tukey's multiple comparison test was used to determine where any differences existed ($P \leq 0.05$).

M. macleayi adults that died without having offspring were not included when calculating the mean total number of offspring. In addition, *M. macleayi* adults that did not have a particular brood (ie. first, second or third brood) were not included when calculating the means for that particular brood.

2.82 48-h Acute Immobilisation/lethality test

Data on the number of surviving *M. macleayi* were analysed using ToxCalc™ software. This involved firstly checking assumptions with Shapiro-Wilk's Test (for normal distribution) as well as Bartlett's Test (for homogeneity of variance). If data conformed to assumptions, Dunnett's Test was performed to determine NOECs and LOECs. The Trimmed Spearman-Karber method was then used to determine the concentrations causing a 50% effect in test organisms relative to the control in a given time (EC50s) and 95% confidence intervals (CI). Standard error of difference was used to determine significant differences between EC50s ($P \leq 0.05$) (Zar, 1999). The use of standard error of difference was first recommended by Sprague and Fogels (1977).

3. Results

3.1 Chronic toxicity of uranium to *M. macleayi* populations

The effect of uranium on reproduction in the Lab population of *M. macleayi* is shown in Table 3.1. In the first uranium experiment conducted on the Lab population of *M. macleayi*, the effect of uranium on individual brood sizes in all reproductive instars was not significant. However, there was a significant reduction in the total number of offspring per adult at the higher U concentrations of 20 and 39 μg/L, compared to controls, ($P \leq 0.05$; Table 3.1; Figure 3.1). However, higher U concentrations also resulted in increased mortality, with 70 and 50% of animals dying at 20 and 39 μg/L, respectively (Figure 3.1). The NOEC and LOEC obtained in this experiment were 7.8 and 20 μg/L, respectively. In the second experiment, the effect of uranium on individual brood sizes in all reproductive instars was significant at the higher U concentrations of 22 and 42 μg/L (Table 3.1). All adults exposed to the highest U concentration of 42 μg/L died without having third brood offspring (Table 3.1). There was a significant reduction in the total number of offspring per adult at the highest U concentration of 42 μg/L, compared to controls, ($P \leq 0.05$; Table 3.1; Figure 3.2). However, most U concentrations also resulted in increased mortality, with 50, 60, 40 and 100% of animals dying at 3.8, 7.2, 22 and 42 μg/L, respectively (Figure 3.2). The NOEC and LOEC obtained in this experiment were 22 and 42 μg/L, respectively. In the third experiment, the effect of uranium on third brood was significant at the higher U concentrations of 13, 28 and 46 μg/L (Table 3.1). However, there was no significant reduction in the total number of offspring per adult at all U concentrations tested, compared to controls, ($P \leq 0.05$; Table

Table 3.1. Individual brood sizes and total number of offspring per adult of the Lab population of *M. macleayi* exposed in three separate experiments to uranium until the fourth reproductive instar (3 reproductive broods). Results are expressed as the mean (SE, n*).

Exp. No.	Measured U conc. (µg/L)	1st Brood	2nd Brood	3rd Brood	Total number of offspring per adult
U-Lab-1	0.04	9.5 [a] (0.4, 10)	13.5 [a] (0.3, 10)	16.5 [a] (0.3, 10)	39.1 [a] (0.6, 10)
	3.50	9.7 [a] (0.2, 10)	13.2 [a] (0.4, 9)	14.6 [a] (1.4, 8)	32.3 [a] (3.3, 10)
	7.80	10.1 [a] (0.2, 10)	13.4 [a] (0.6, 7)	14.9 [a] (0.6, 7)	29.8 [a] (4.5, 10)
	20.0	9.4 [a] (0.3, 7)	13.0 [a] (0.6, 5)	15.3 [a] (0.3, 3)	25.3 [b] (4.9, 7)
	39.0	9.6 [a] (0.3, 10)	13.4 [a] (0.5, 7)	13.0 [a] (1.5, 3)	22.1 [b] (3.4, 10)
U-Lab-2	0.04	9.9 [a] (0.38, 10)	13.4 [a] (0.3, 9)	15.8 [a] (0.3, 8)	34.6 [a] (3.2, 10)
	3.80	9.6 [ab] (0.3, 10)	14.3 [a] (0.3, 7)	15.6 [ab] (0.6, 5)	27.4 [a] (4.4, 10)
	7.20	8.5 [ab] (1.4, 6)	13.0 [a] (0.3, 5)	13.6 [ab] (2.2, 5)	32.3 [a] (6.1, 6)
	14.0	9.4 [ab] (0.2, 9)	13.3 [a] (0.5, 9)	15.3 [a] (0.3, 9)	38.1 [a] (0.8, 9)
	22.0	7.5 [b] (1.2, 8)	12.5 [b] (0.9, 6)	13.0 [b] (0.7, 6)	26.6 [a] (5.5, 8)
	42.0	7.0 [b] (1.1, 5)	1.0 [b] (0.0, 2)	AD	7.4 [b] (1.1, 5)
U-Lab-3	0.10	9.9 [a] (0.2, 10)	15.5 [a] (0.3, 10)	20.3 [a] (0.4, 8)	41.6 [a] (2.8, 10)
	3.90	10.0 [a] (0.3, 10)	16.1 [a] (0.2, 10)	20.1 [a] (0.4, 9)	44.2 [a] (2.2, 10)
	8.70	9.8 [a] (0.3, 10)	15.2 [a] (0.4, 9)	18.8 [ab] (0.6, 8)	38.5 [a] (3.8, 10)
	13.0	9.7 [a] (0.3, 9)	16.0 [a] (0.3, 9)	17.0 [c] (0.8, 7)	38.9 [a] (2.3, 9)
	28.0	9.3 [a] (0.3, 9)	15.7 [a] (0.3, 9)	18.3 [b] (0.4, 8)	41.2 [a] (2.1, 9)
	46.0	10.0 [a] (0.3, 10)	15.1 [a] (0.3, 9)	15.8 [c] (0.7, 8)	36.2 [a] (3.4, 10)

Values within a column block (eg 1st brood) with a superscript letter in common are not significantly different ($P \geq 0.05$)

*n - represents the number of *M. macleayi* that had live offspring out of a maximum of ten *M. macleayi*.

AD - all adults died without having offspring

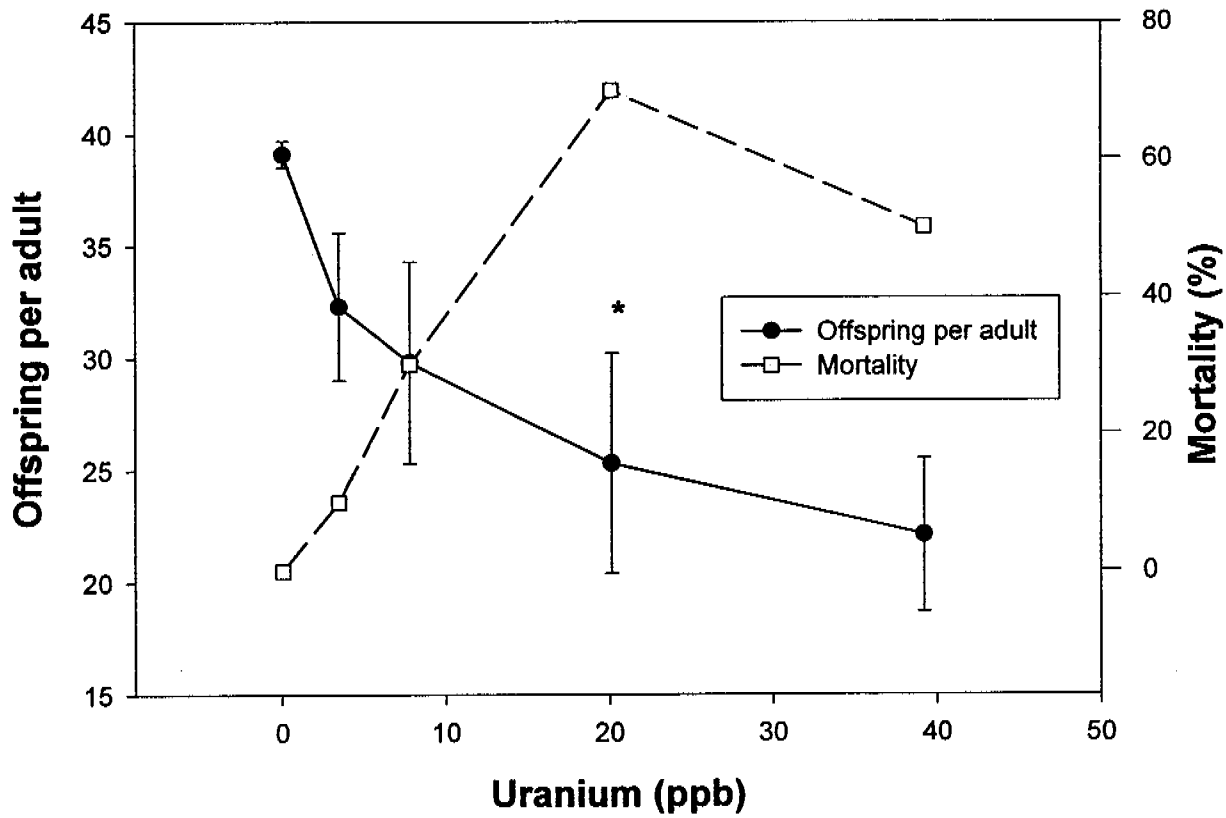

Figure 3.1: Offspring per adult (mean ± SE) and Mortality (%) of experiment 1-Lab population *M. macleayi* exposed to uranium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

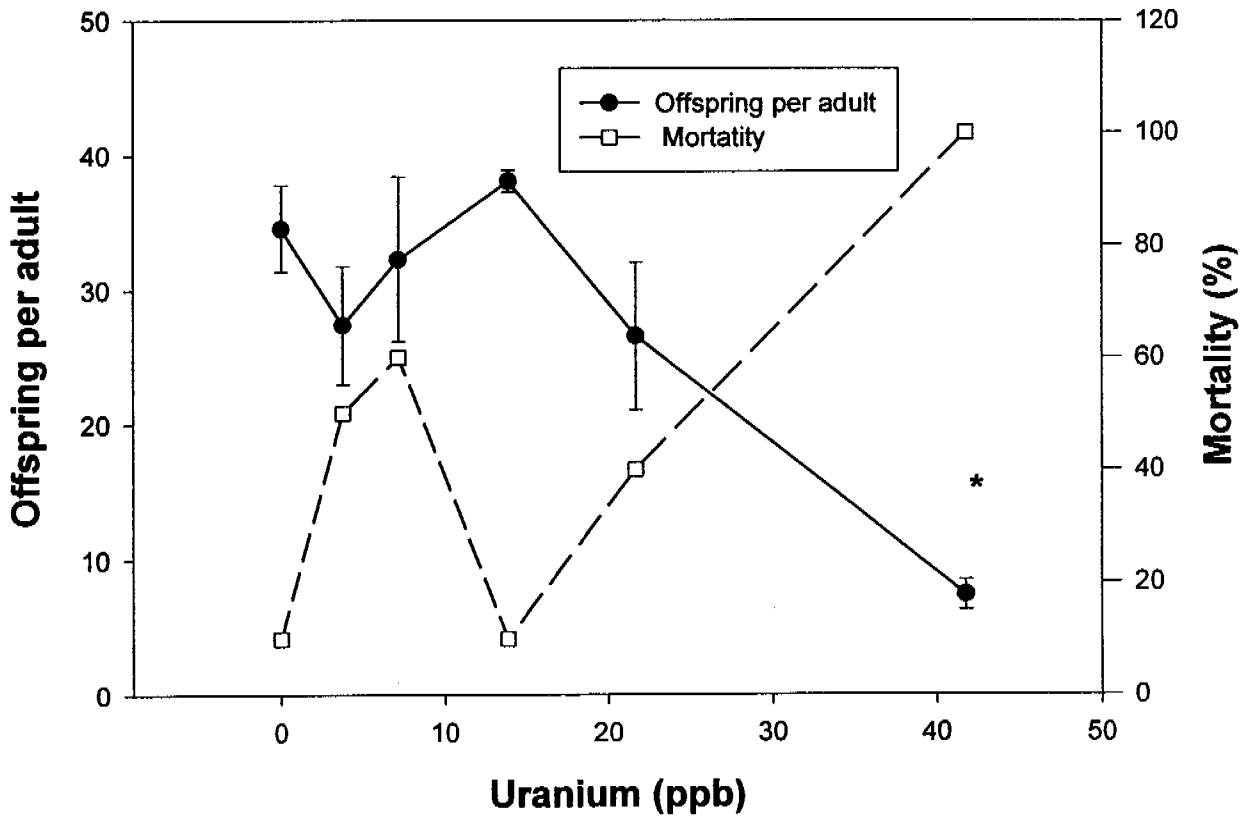

Figure 3.2: Offspring per adult (mean ± SE) and Mortality (%) of experiment 2-Lab population *M. macleayi* exposed to uranium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

3.1; Figure 3.3). Although mortality was quite low it still had an effect on mean number of offspring per adult at U concentrations of 13 and 46 μg/L, with 30 and 40% of animals dying, respectively (Figure 3.3). The NOEC and LOEC for this experiment were unable to be determined as there were no concentrations that were significantly different from the control ($P \leq 0.05$).

The effect of uranium on reproduction in the BB population of *M. macleayi* is shown in Table 5. In the first uranium experiment conducted on the BB population of *M. macleayi*, the effect of uranium on individual brood sizes in all reproductive instars was significant at the highest U concentration of 49 μg/L (Table 3.2). Third brood was also significantly reduced at the U concentration of 25 μg/L (Table 3.2). All adults exposed to the highest U concentration of 49 μg/L died without having third brood offspring (Table 3.2). There was only a significant reduction in the total number of offspring per adult at the highest U concentration of 49 μg/L, compared to controls ($P \leq 0.05$; Table 3.2; Figure 3.4). However, at the highest U concentration of 49 μg/L, there also was an increase in mortality with 80% of the animals dying (Figure 3.4). The NOEC and LOEC obtained in this experiment were 25 and 49 μg/L, respectively. In the second experiment, the effect of uranium on individual brood sizes in all reproductive instars was significant at the higher U concentrations of 36 and 49 μg/L (Table 3.2). Second brood was also significantly reduced at the U concentrations of 20 and 29 μg/L (Table 3.2). All adults exposed to the highest U concentration of 49 μg/L died without having third brood offspring (Table 3.2). There was a significant reduction in the total number of offspring per adult at the higher U concentrations of 36 and 49 μg/L ($P \leq 0.05$; Table 3.2; Figure

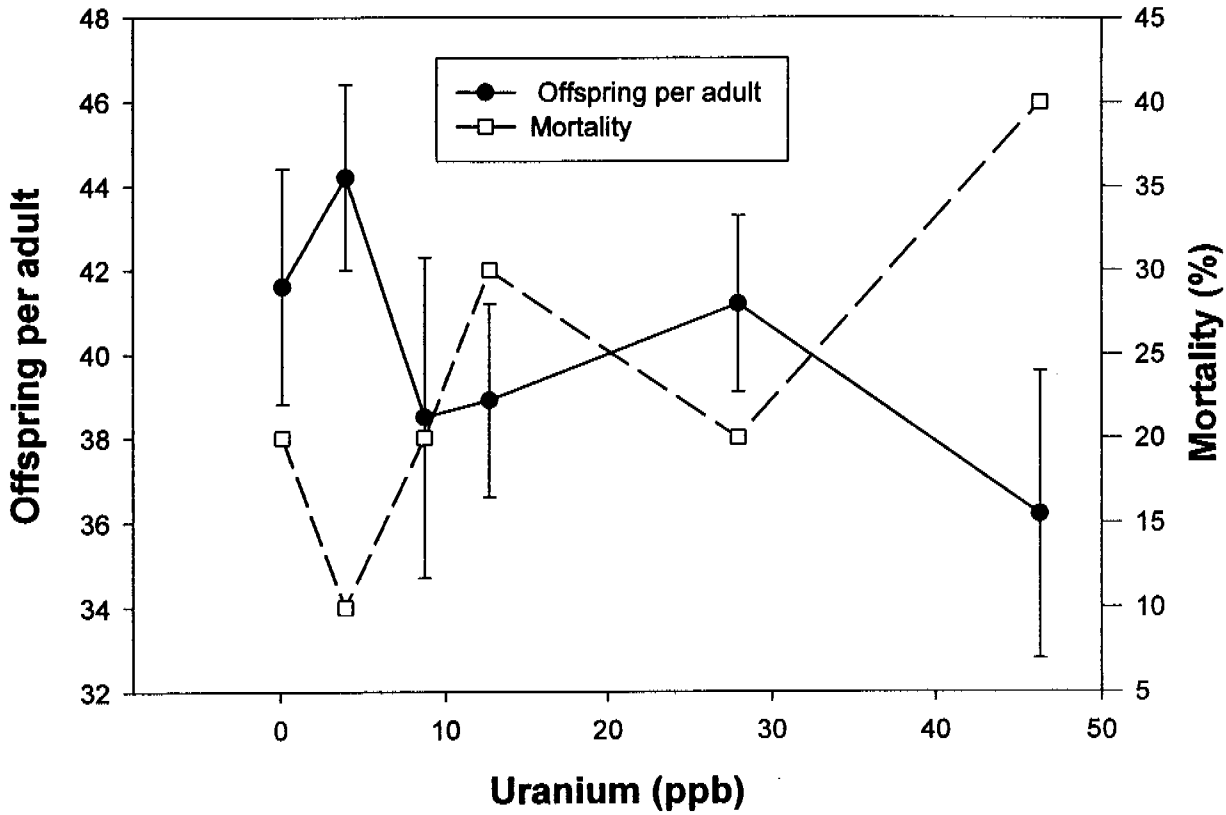

Figure 3.3: Offspring per adult (mean ± SE) and Mortality (%) of experiment 3-Lab population *M. macleayi* exposed to uranium. There was no concentration that was significantly different from controls in this experiment.

Table 3.2. Individual brood sizes and total number of offspring per adult of the BB population of *M. macleayi* exposed in separate experiments to uranium until the fourth reproductive instar (3 reproductive broods). Results are expressed as the mean (SE, n*).

Exp. No.	Measured U conc. (µg/L)	1st Brood	2nd Brood	3rd Brood	Total number of offspring per adult
U-BB-1	0.03	9.3 [a] (0.2, 10)	14.8 [a] (0.3, 9)	16.6 [a] (0.2, 9)	37.5 [a] (3.1, 10)
	5.40	9.5 [a] (0.3, 10)	14.9 [a] (0.4, 9)	16.8 [a] (0.2, 9)	38.0 [a] (3.2, 10)
	9.60	9.2 [a] (0.3, 10)	14.0 [a] (0.3, 10)	15.4 [b] (0.2, 10)	38.6 [a] (0.5, 10)
	13.0	9.2 [a] (0.2, 9)	14.1 [a] (0.3, 8)	17.0 [a] (0.4, 8)	37.0 [a] (3.4, 9)
	25.0	8.7 [a] (0.3, 10)	14.0 [a] (0.4, 10)	15.1 [b] (0.1, 8)	34.9 [a] (2.4, 10)
	49.0	3.4 [b] (1.0, 5)	2.0 [b] (0.0, 1)	AD	3.8 [b] (1.2, 5)
U-BB-2	0.01	9.6 [a] (0.2, 10)	15.0 [a] (0.4, 9)	16.3 [a] (0.5, 8)	36.1 [a] (3.3, 10)
	9.10	9.9 [a] (0.4, 10)	14.8 [ab] (0.4, 9)	15.4 [a] (0.2, 8)	35.5 [a] (3.1, 10)
	20.0	9.7 [a] (0.2, 10)	13.7 [b] (0.2, 10)	15.3 [a] (0.2, 7)	34.1 [a] (2.4, 10)
	29.0	9.8 [a] (0.3, 10)	13.8 [b] (0.2, 10)	15.0 [a] (0.5, 6)	32.8 [a] (2.3, 10)
	36.0	8.9 [b] (0.4, 10)	11.0 [c] (1.1, 10)	13.3 [b] (0.9, 3)	24.1 [b] (2.5, 10)
	49.0	8.9 [b] (0.3, 10)	8.8 [c] (0.8, 10)	AD	17.7 [c] (0.8, 10)

Values within a column block (eg 1st brood) with a superscript letter in common are not significantly different ($P \geq 0.05$)

*n - represents the number of *M. macleayi* that had live offspring out of a maximum of ten *M. macleayi*.

AD - all adults died without having offspring

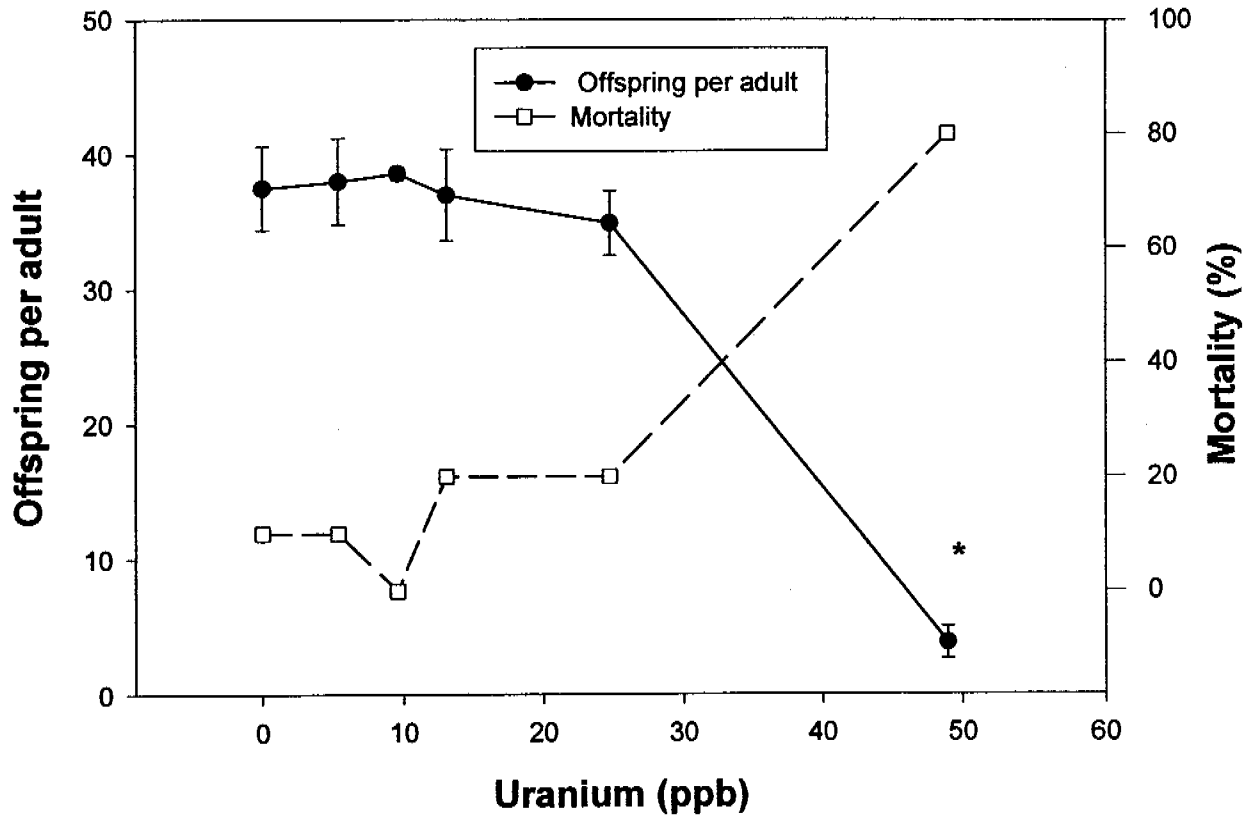

Figure 3.4: Offspring per adult (mean ± SE) and Mortality (%) of experiment 1 BB population *M. macleayi* exposed to uranium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

3.5). However, at the U concentration of 36 μg/L, there also was an increase in mortality with 60% of the animals dying (Figure 3.5). The NOEC and LOEC obtained in this experiment were 29 and 36 μg/L, respectively.

The effect of uranium on reproduction in the DjB2 population of *M. macleayi* is shown in Table 3.3. In the first uranium experiment conducted on the DjB2 population of *M. macleayi*, the effect of uranium on individual brood sizes in all reproductive instars was significant at the higher concentrations of 44 and 65 μg/L. There was also a significant reduction in total number of offspring per adult at the higher U concentrations of 44 and 65 μg/L, compared to controls, ($P \leq 0.05$; Table 3.3; Figure 3.6). All adults exposed to the highest U concentration of 65 μg/L died without having second and third brood offspring (Table 3.3). All adults exposed to 44 μg/L of U died without having third brood offspring (Table 3.3). Most U concentrations resulted in increased mortality, with 40, 40, 60 and 100% of animals dying at 4.4, 14, 44 and 65 μg/L, respectively (Figure 3.6). The NOEC and LOEC obtained in this experiment were 22 and 44 μg/L, respectively. In the second experiment, the effect of uranium on individual brood sizes in all reproductive instars was significant at the higher U concentrations of 24, 31 and 41 μg/L (Table 3.3). All adults exposed to the highest U concentration of 41 μg/L died without having third brood offspring (Table 3.3). There was also a significant reduction in the total number of offspring per adult at the higher U concentrations of 31 and 41 μg/L, compared to controls ($P \leq 0.05$; Table 3.3; Figure 3.7). Higher U concentrations also resulted in increased mortality, with 30% and 70% of animals dying at 31 and 41 μg/L, respectively (Figure 3.7). The NOEC and LOEC obtained in this experiment were 31 and 41 μg/L,

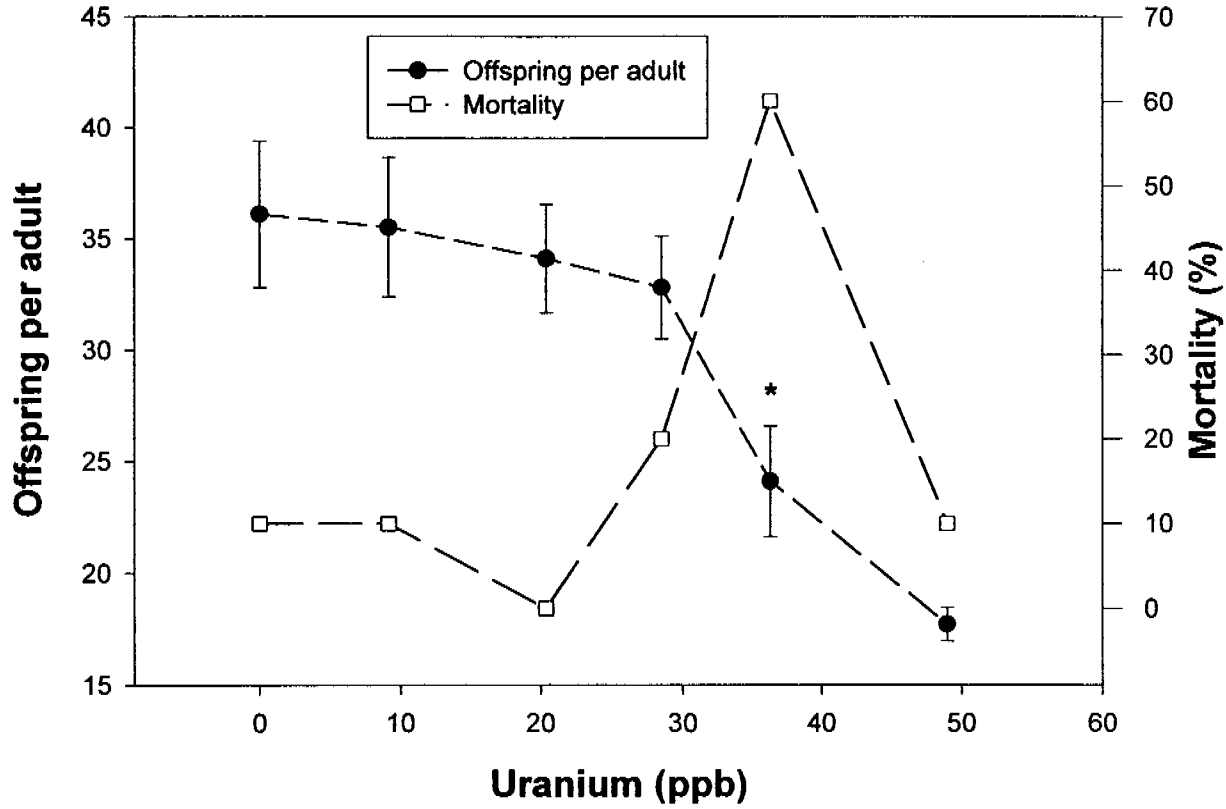

Figure 3.5: Offspring per adult (mean ± SE) and Mortality (%) of experiment 2-BB population *M. macleayi* exposed to uranium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

Table 3.3. Individual brood sizes and total number of offspring per adult of the DjB2 population of *M. macleayi* exposed in separate experiments to uranium until the fourth reproductive instar (3 reproductive broods). Results are expressed as the mean (SE, n*).

Exp. No.	Measured U conc. (µg/L)	1st Brood	2nd Brood	3rd Brood	Total number of offspring per adult
U-DjB2-1	0	9.2 [a] (0.1, 10)	13.6 [a] (0.2, 9)	16.7 [a] (0.2, 9)	36.4 [a] (3.1, 10)
	4.40	7.8 [a] (0.5, 10)	13.7 [a] (0.4, 6)	17.2 [a] (0.2, 6)	26.3 [a] (5.6, 10)
	8.70	9.0 [a] (0.7, 9)#	13.4 [a] (0.2, 9)#	16.8 [a] (0.7, 8)#	37.3 [a] (2.0, 9)#
	14.0	8.6 [a] (0.7, 8)	14.0 [a] (0.2, 7)	17.0 [a] (0.3, 6)	33.6 [a] (4.8, 8)
	22.0	8.7 [a] (0.2, 10)	12.9 [a] (0.3, 10)	16.4 [a] (0.2, 10)	38.0 [a] (0.5, 10)
	44.0	4.0 [b] (0.6, 7)	5.3 [b] (1.2, 3)	AD	6.3 [b] (1.5, 7)
	65.0	6.0 [c] (0.0, 1)	AD	AD	6.0 [c] (0.0, 1)
U-DjB2-2	0	9.4 [a] (0.2, 10)	12.5 [a] (1.1, 10)	15.8 [a] (0.3, 8)	34.6 [a] (2.9, 10)
	7.60	8.9 [a] (0.2, 10)	13.5 [a] (0.2, 8)	15.6 [a] (0.2, 8)	32.2 [a] (4.0, 10)
	16.0	8.3 [a] (0.2, 10)	12.5 [a] (0.2, 10)	15.3 [ab] (0.8, 7)	31.5 [a] (2.5, 10)
	24.0	6.2 [b] (0.5, 10)	9.0 [b] (0.5, 10)	14.0 [b] (0.5, 8)	26.4 [a] (2.0, 10)
	31.0	5.9 [b] (0.4, 10)	6.3 [c] (1.0, 7)	12.0 [b] (0.0, 2)	12.7 [b] (2.6, 10)
	41.0	4.5 [c] (0.3, 4)	3.7 [d] (0.7, 3)	AD	7.3 [c] (1.1, 4)

Values within a column block (eg 1st brood) with a superscript letter in common are not significantly different ($P \geq 0.05$)

*n - represents the number of *M. macleayi* that had live offspring out of a maximum of ten *M. macleayi*.

AD - all adults died without having offspring

#1 adult *M. macleayi* was accidently killed while being transferred in to fresh water.

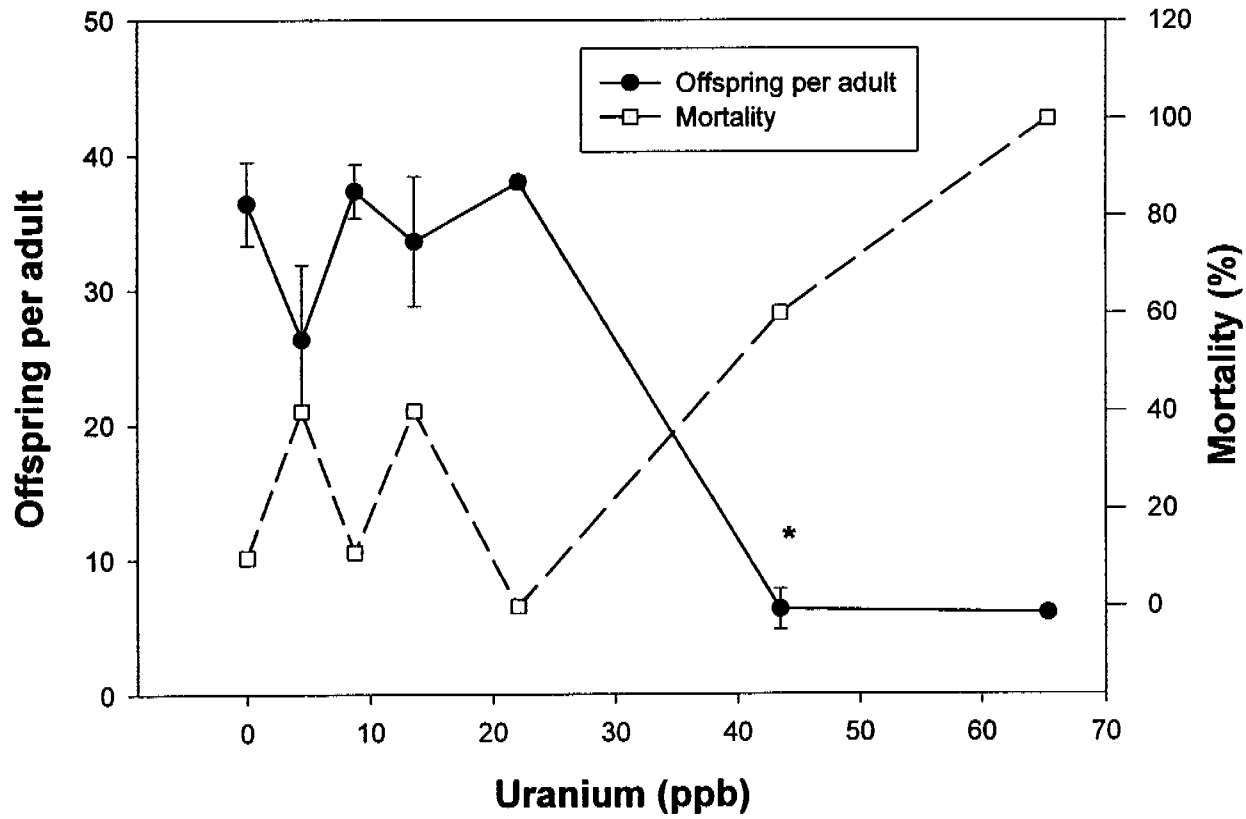

Figure 3.6: Offspring per adult (mean ± SE) and Mortality (%) of experiment 1-DjB2 population *M. macleayi* exposed to uranium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

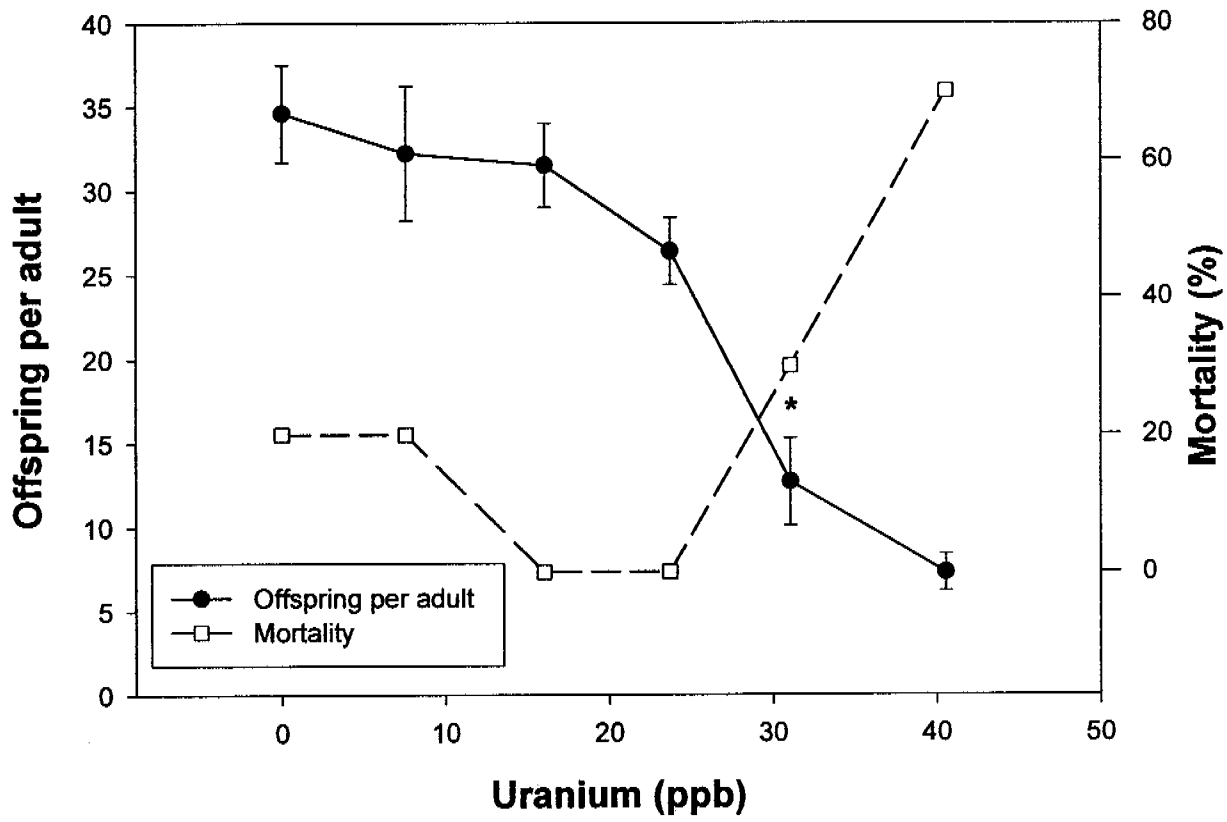

Figure 3.7: Offspring per adult (mean ± SE) and Mortality (%) of experiment 2-DjB2 population *M. macleayi* exposed to uranium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

respectively.

When comparing the range of NOECs and LOECs obtained for uranium, there appears to be no major difference between the different *M. macleayi* populations (Table 3.4).

Table 3.4. Toxicity of uranium to *M. macleayi* populations. Results are expressed as the ranges of LOECs and NOECs obtained from separate experiments.

Population	LOEC (µg/L)	NOEC (µg/L)
Lab (n=3)	20-42	7.8-22
BB (n=2)	36-49	25-29
DjB2 (n=2)	31-44	22-24

n - represents the number of experiments conducted on *M. macleayi* populations

3.2 Acute toxicity of uranium to *M. macleayi* populations

The acute toxicity of uranium to the Lab, BB, DjB1 and DjB2 populations is shown in Table 3.5. In the first uranium experiment conducted on the Lab population of *M. macleayi* the NOEC and LOEC obtained were 100 and 190 μg/L, respectively. The EC50 (95% CI) was determined to be 160 (110-220) μg/L. In the second experiment, the NOEC and LOEC obtained were 140 and 180 μg/L, respectively. The EC50 (95% CI) was determined to be 240 (150-400 μg/L). In the first uranium experiment conducted on the BB population of *M. macleayi* the NOEC and LOEC obtained were 270 and 370 μg/L, respectively. The EC50 (95% CI) was determined to be 360 (270-460 μg/L). In the second experiment, the NOEC and LOEC obtained were 180 and 280 μg/L, respectively. The EC50 (95% CI) was determined to be 260 (210-330) μg/L. In the first uranium experiment conducted on the DjB1 population of *M. macleayi* the NOEC and LOEC obtained were 270 and 370 μg/L, respectively. The EC50 (95% CI) was determined to be 390 (300-500) μg/L. In the second experiment, the NOEC and LOEC obtained were <180 and 180 μg/L, respectively. The EC50 (95% CI) was determined to be 210 (160-270) μg/L. In the first experiment conducted on the DjB2 population of *M. macleayi* no result was obtained, as the test was determined to be invalid, as greater than 20% of death occurred in the controls. In the second experiment, the NOEC and LOEC obtained were <100 and 100 μg/L, respectively. The EC50 (95% CI) was determined to be 90 μg/L (60-110) μg/L.

Overall, the LOEC and NOEC concentrations from the acute immobilisation/lethality experiments showed no major difference between the Lab, BB, and DjB1 *M. macleayi*

Table 3.5. Acute toxicity of uranium to *M. macleayi* populations. Results are expressed as LOECs, NOECs, and EC50s (95% CI) (n=2).

Exp. No.	LOEC (µg/L)	NOEC (µg/L)	EC50 (µg/L)
U-Lab-1	190	100	160 (110-220) [a]
U-Lab-2	180	140	240 (150-400) [ab]
U-BB-1	370	270	360 (270-460) [bc]
U-BB-2	280	180	260 (210-330) [bc]
U-DjB1-1	370	270	390 (300-500) [bc]
U-DjB1-2	180	<180	210 (160-270) [ab]
U-DjB2-1	NR	NR	NR
U-DjB2-2	100	<100	90 (60-110) [d]

Values written in last column with a superscript letter in common are not significantly different ($P \geq 0.05$)

NR - no result as test was invalid due to > 20% death in controls

populations (Table 3.5). However, there was a major difference between the DjB2 *M. macleayi* population in comparison with the other *M. macleayi* populations (Table 3.5).

From Table 3.5, U-Lab-1 has a significantly different EC50 than U-BB-1, U-BB-2, and U-DjB-1. However, in the second test on Lab stock (U-lab-2), the EC50 was not significantly different to all three tests (ie. than U-BB-1, U-BB-2, and U-DjB-1) (Table 3.5). Overall, the EC50 concentrations obtained between the Lab, BB, and DjB1 *M. macleayi* populations were not significantly different (Table 3.5). However, the EC50 concentration obtained for the DjB2 *M. macleayi* population was significantly different to the EC50 concentrations obtained for the other *M. macleayi* populations (Table 3.5).

3.3 Chronic toxicity of cadmium to *M. macleayi* populations

The effect of cadmium on reproduction in the BB population of *M. macleayi* is shown in Table 3.6. In the first cadmium experiment conducted on the BB population of *M. macleayi*, the effect of cadmium on individual brood sizes in the first reproductive instar was significant at the Cd concentration of 4.7 μg/L (Table 3.6). The second and third brood sizes were significantly different for animals exposed to 1.9 μg/L Cd (Table 3.6). Third brood size was also significantly different for animals exposed to 1.0 μg/L Cd (Table 3.6). All adults exposed to the Cd concentration of 4.7 μg/L died without having second and third brood offspring (Table 3.6). All adults exposed to the Cd concentrations of 10, 15, and 20 μg/L died without having any offspring (Table 3.6). There was a significant reduction in the total number of offspring per adult at the Cd concentrations of

Table 3.6. Individual brood sizes and total number of offspring per adult of the BB population of *M. macleayi* exposed in separate experiments to cadmium until the fourth reproductive instar (3 reproductive broods). Results are expressed as the mean (SE, n*).

Exp. No.	Measured Cd conc. (µg/L)	1st Brood	2nd Brood	3rd Brood	Total number of offspring per adult
Cd-BB-1	0	8.2 [a] (0.2, 9)	13.9 [a] (0.4, 8)	16.4 [a] (0.3, 8)	35.1 [a] (3.4, 9)
	1.00	8.1 [a] (0.1, 10)	12.8 [a] (0.6, 10)	8.7 [b] (2.7, 6)	26.4 [a] (1.9, 10)
	1.90	7.8 [a] (0.2, 10)	7.9 [b] (1.6, 7)	14.0 [c] (0.0, 1)	14.7 [b] (2.7, 10)
	4.70	6.7 [b] (0.5, 9)	AD	AD	6.7 [c] (0.5, 9)
	10.0	AD	AD	AD	AD
	15.0	AD	AD	AD	AD
	20.0	AD	AD	AD	AD
Cd-BB-2	0	8.6 [a] (0.2, 10)	12.6 [a] (1.3, 10)	14.3 [a] (1.7, 9)	34.1 [a] (3.0, 10)
	0.45	8.3 [a] (0.2, 10)	12.6 [a] (1.0, 9)	16.0 [a] (0.7, 5)	27.6 [a] (3.7, 10)
	1.90	7.4 [b] (0.2, 10)	11.3 [a] (0.5, 10)	AD	18.7 [c] (0.6, 10)
	2.30	7.6 [b] (0.2, 10)	6.2 [b] (1.9, 6)	AD	11.4 [b] (1.5, 10)
	3.30	6.4 [c] (0.2, 9)	AD	AD	6.4 [d] (0.2, 9)
	4.90	5.6 [d] (0.6, 10)	AD	AD	5.6 [d] (0.6, 10)
	7.50	5.0 [d] (0.3, 5)	AD	AD	5.0 [e] (0.3, 5)

Values within a parameter block (eg 1st brood) with a superscript letter in common are not significantly different ($P \geq 0.05$)

*n - represents the number of *M. macleayi* that had live offspring out of a maximum of ten *M. macleayi*.

AD - all adults died without having offspring

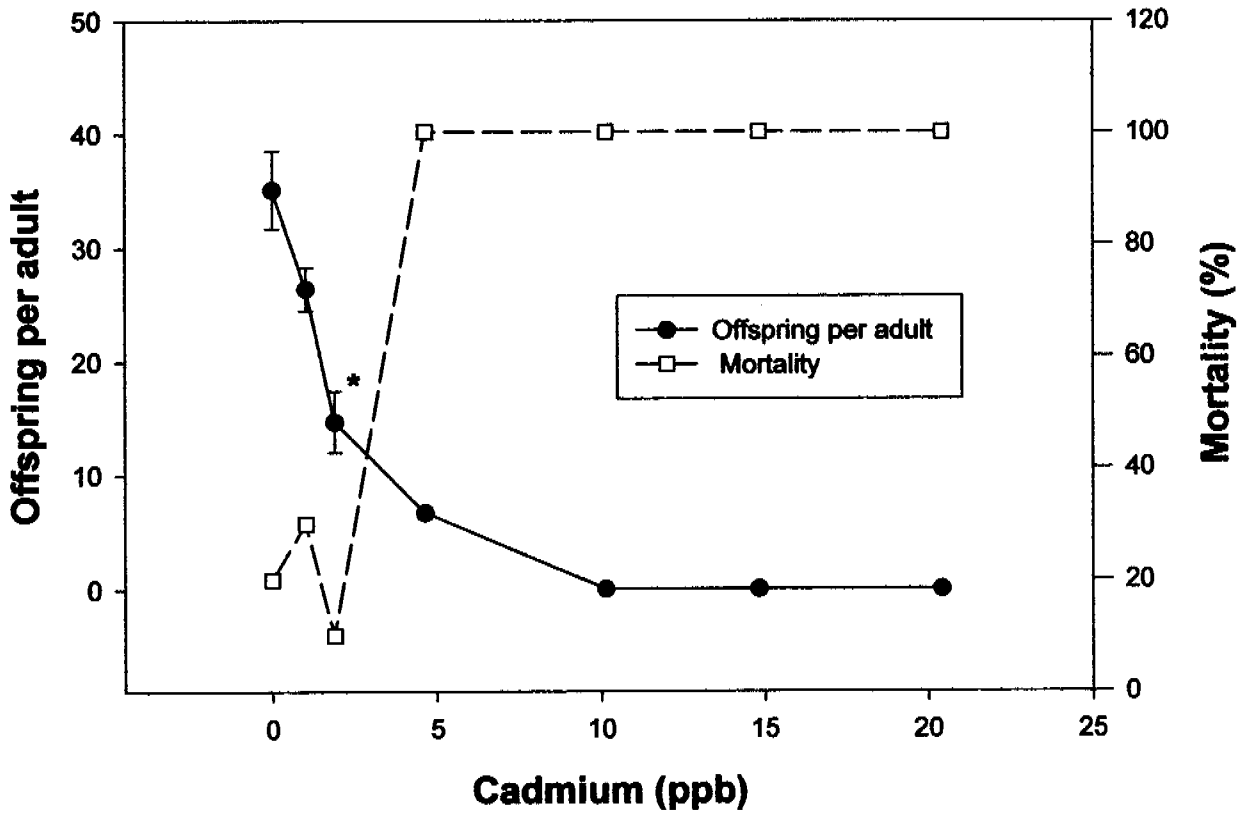

Figure 3.8: Offspring per adult (mean ± SE) and Mortality (%) of experiment 1-BB population *M. macleayi* exposed to cadmium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

1.9 and 4.7 µg/L ($P \leq 0.05$; Table 3.6; Figure 3.8). Mortality at the higher Cd concentration of 4.7 µg/L did have an effect on mean number of offspring (Figure 3.8). The NOEC and LOEC obtained in this experiment were 1.0 and 1.9 µg/L, respectively. In the second experiment, the effect of cadmium on first brood size was significant at the Cd concentrations of 1.9, 2.3, 3.3, 4.9 and 7.5 µg/L (Table 3.6). The second brood size was significantly reduced at the Cd concentration of 1.9 µg/L (Table 3.6). All adults exposed to the Cd concentrations of 3.3, 4.9 and 7.5 µg/L died without having second and third brood offspring (Table 3.6). All adults exposed to the Cd concentrations of 1.9 and 2.3 µg/L died without having third brood offspring (Table 3.6). There was a significant reduction in the total number of offspring per adult at the Cd concentrations of 1.9, 2.3, 3.3, 4.9 and 7.5 µg/L ($P \leq 0.05$; Table 3.6; Figure 3.9). Mean number of offspring per adult was affected by mortality at all Cd concentrations tested (Figure 3.9). The NOEC and LOEC obtained in this experiment were 0.45 and 1.9 µg/L, respectively.

The effect of cadmium on reproduction in the Lab population of *M. macleayi* is shown in Table 3.7. In the first cadmium experiment conducted on the Lab population of *M. macleayi*, the effect of cadmium on first brood size was significant at the Cd concentration of 5.0 µg/L (Table 3.7). All adults exposed to the Cd concentration of 2.0 µg/L died without having third brood offspring (Table 3.7). All adults exposed to the Cd concentration of 5.0 µg/L died without having second and third brood offspring (Table 3.7). All adults exposed to the Cd concentrations of 9.8 and 20 µg/L died without having any offspring (Table 3.7). There was a significant reduction in the total number of offspring per adult at the Cd concentrations of 2.0 and 5.0 µg/L ($P \leq 0.05$; Table 3.7;

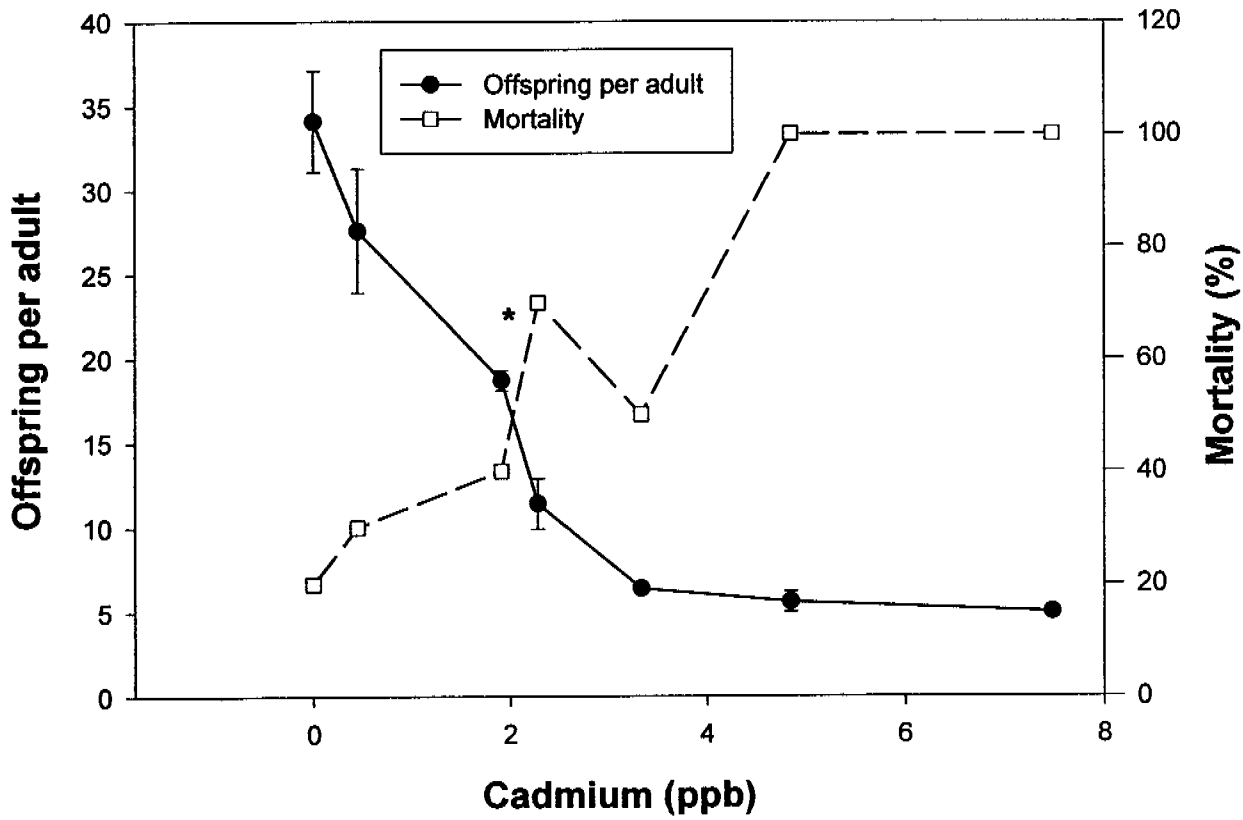

Figure 3.9: Offspring per adult (mean ± SE) and Mortality (%) of experiment 2-BB population *M. macleayi* exposed to cadmium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

Table 3.7. Individual brood sizes and total number of offspring per adult of the Lab population of *M. macleayi* exposed in separate experiments to cadmium until the fourth reproductive instar (3 reproductive broods). Results are expressed as the mean (SE, n*).

Exp. No.	Measured Cd conc. (µg/L)	1st Brood	2nd Brood	3rd Brood	Total number of offspring per adult
Cd-Lab-1	0.03	8.7 [a] (0.2, 9)	12.6 [a] (0.3, 8)	15.3 [a] (0.3, 8)	33.4 [a] (3.1, 9)
	0.60	8.4 [ab] (0.8, 8)	12.6 [a] (0.5, 7)	15.5 [a] (0.8, 6)	31.0 [a] (4.6, 8)
	1.00	7.2 [ab] (1.0, 10)	11.6 [a] (1.2, 8)	15.0 [a] (0.0, 2)	19.5 [b] (3.8, 10)
	2.00	6.9 [ab] (1.0, 9)	11.8 [a] (0.5, 5)	AD	13.4 [b] (2.7, 9)
	5.00	5.9 [b] (1.1, 8)	AD	AD	5.9 [c] (1.1, 8)
	9.80	AD	AD	AD	AD
	20.0	AD	AD	AD	AD
Cd-Lab-2	0	9.2 [a] (0.3, 10)	14.3 [a] (0.4, 9)	16.8 [a] (0.4, 9)	37.2 [a] (3.2, 10)
	0.50	9.3 [a] (0.3, 10)	13.6 [a] (0.2, 9)	16.0 [a] (0.4, 4)	27.9 [b] (3.3, 10)
	1.10	8.7 [a] (0.2, 9)**	12.8 [b] (0.2, 9)**	15.0 [b] (0.0, 1)**	23.1 [b] (1.8, 9)**
	1.80	7.4 [b] (0.3, 9)	10.3 [c] (1.1, 8)	AD	16.6 [c] (1.7, 9)
	3.30	7.1 [b] (0.2, 9)	AD	AD	7.1 [d] (0.2, 9)
	4.80	7.1 [b] (0.3, 9)	AD	AD	7.1 [d] (0.2, 9)
	7.00	6.00 [c] (0.2, 9)	AD	AD	6.00 [e] (0.2, 9)

Values within a parameter block (eg 1st brood) with a superscript letter in common are not significantly different ($P \geq 0.05$)

*n - represents the number of *M. macleayi* that had live offspring out of a maximum of ten *M. macleayi*.

AD - all adults died without having offspring

** 1 adult *M. macleayi* was misplaced while being transferred in to fresh water.

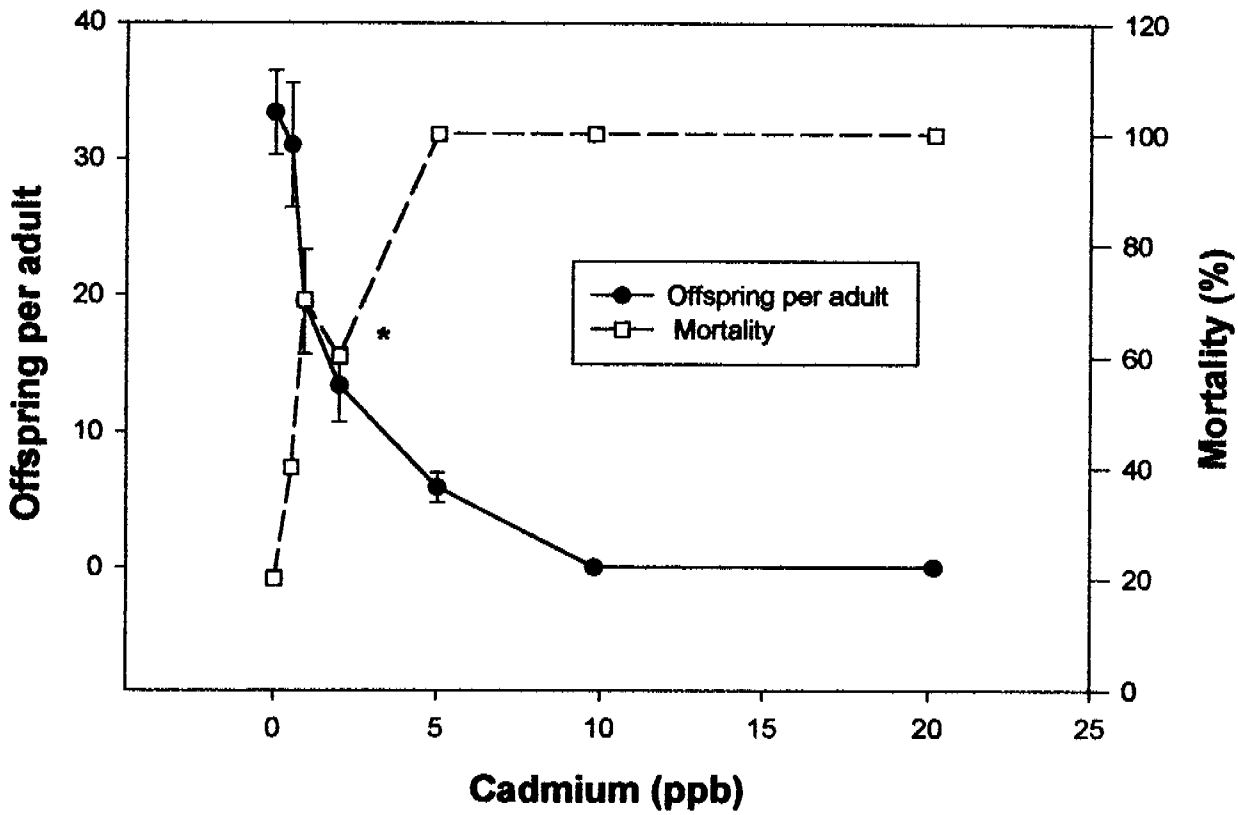

Figure 3.10: Offspring per adult (mean ± SE) and Mortality (%) of experiment 1-Lab population *M. macleayi* exposed to cadmium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

Figure 3.10). Mortality at higher Cd concentrations of 2.0 and 5.0 μg/L did have an effect on mean number of offspring (Figure 3.10). The NOEC and LOEC obtained in this experiment were 0.6 and 1.0 μg/L, respectively. In the second cadmium experiment, the effect of cadmium on first brood size was significant at the Cd concentrations of 1.8, 3.3, 4.8 and 7.0 μg/L (Table 3.7). Second brood size was significantly reduced in animals exposed to Cd concentrations of 1.1 and 1.8 μg/L (Table 3.7). Third brood size was significantly reduced in animals exposed to the Cd concentration of 1.1 μg/L (Table 3.7). All adults exposed to Cd concentrations of 3.3, 4.8, and 7.0 μg/L died without having second and third brood offspring (Table 3.7). All adults exposed to the Cd concentration of 1.8 μg/L died without having third brood offspring (Table 3.7). There was a significant reduction in the total number of offspring per adult at the Cd concentrations of 0.45, 1.1, 1.8, 3.3, 4.8 and 7.0 μg/L ($P \leq 0.05$; Table 3.7; Figure 3.11). Mortality at Cd concentrations of 0.45, 3.3, 4.8 and 7.0 μg/L did have an effect on mean number of offspring (Figure 3.11). The NOEC and LOEC obtained in this experiment were 0.45 and 1.1 μg/L, respectively.

When comparing the range of NOECs and LOECs obtained for each of the *M. macleayi* populations there appears to be no major difference between the different *M. macleayi* populations (Table 3.8).

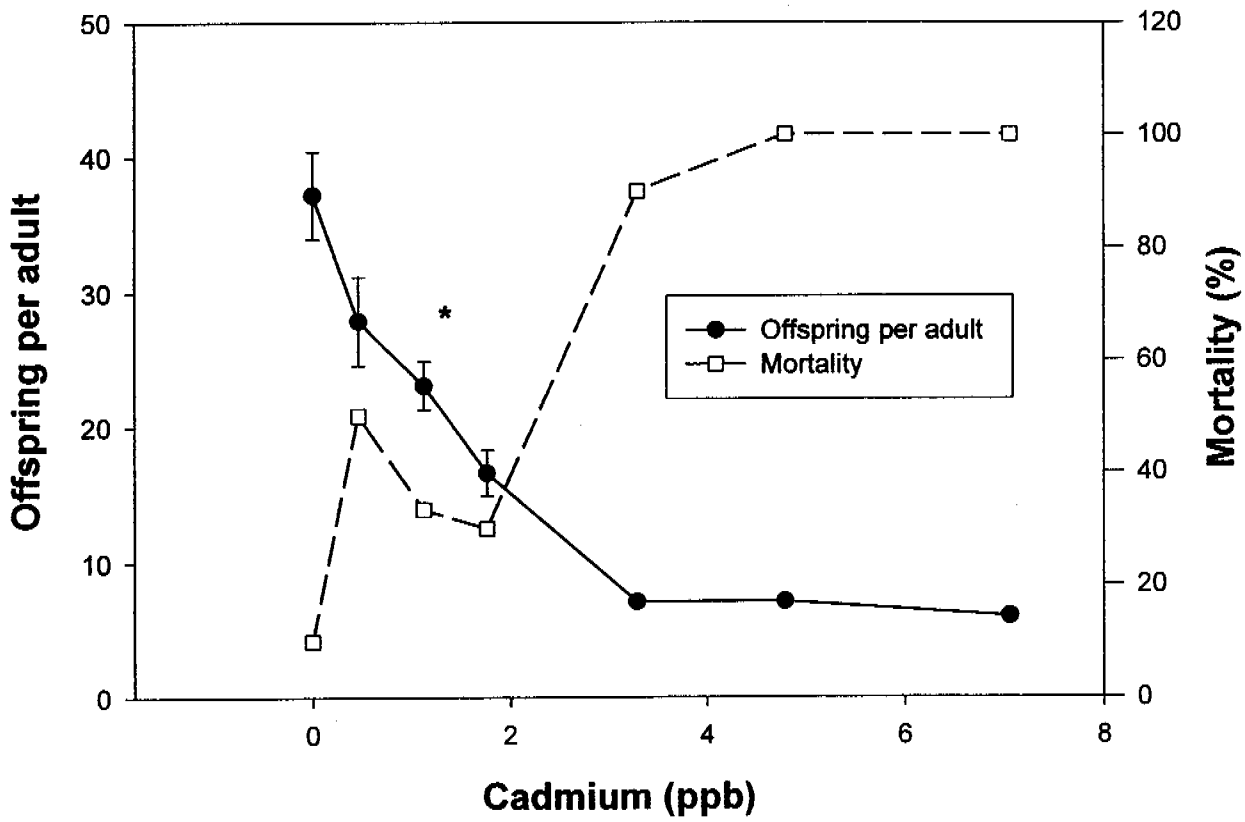

Figure 3.11: Offspring per adult (mean ± SE) and Mortality (%) of experiment 2-Lab population *M. macleayi* exposed to cadmium. Asterisk (*) indicates lowest concentration that was significantly different from controls (LOEC).

Table 3.8. Toxicity of cadmium to *M. macleayi* populations. Results are expressed as ranges of LOECs and NOECs obtained from separate experiments.

Population	LOEC (µg/L)	NOEC (µg/L)
Lab (n=2)	0.5-1.0	<0.5-0.6
BB (n=2)	1.9	0.45-1.0

n - represents the number of experiments conducted on *M. macleayi* populations

4. Discussion

4.1 Assessment of variability between different populations of *M. macleayi*

Natural populations in polluted areas are likely to be subjected to selective pressures for an increased tolerance to toxicants (Klerks and Weis, 1987). This can result in the evolution of resistance or tolerance, in turn this may play an important role in decisions regarding safe ambient toxicant levels. However, results of experiments conducted in this study found that there was little variability between the three different populations of *M. macleayi*. This is further discussed, below.

4.1.1 Reproduction tests using uranium

There was no major difference between the three populations (Lab, BB and DjB2) that were tested for uranium toxicity using the reproduction test. The LOECs obtained in the uranium experiments indicate that uranium was toxic to all the different populations at concentrations ranging between 30 to 50 µg/L of uranium (Table 3.4). There was no evidence to suggest that the DjB2 population was more tolerant to uranium. The *M. macleayi* population that was collected from DjB was not healthy when cultured in water from DjB and usually only survived to have first brood offspring. This was a clear indication that *M. macleayi* populations had not acclimatised or physiologically adapted to the elevated levels of uranium that were present in DjB. The fact that no major difference in the sensitivity of uranium was obtained between the different populations of *M. macleayi* means that the Lab population which has been cultured for over 10 years in the laboratory can still be considered as representative of natural populations in the environment.

The objective of the reproduction test is to determine the maximum concentration at which pollutants (in this case uranium) have no statistically significant effect on

cladoceran fecundity over a five day period of exposure (Hyne *et al.*, 1996). Poston *et al.* (1984) found that uranium toxicity caused suppression in reproduction of *D. magna* between 0.5 and 3.5 mg/L of uranium in Columbia River water. However, results of this study showed that mortality of adults and not reproductive impairment in some experiments was a major contributing factor to the endpoint measured, where uranium would cause mortality to adults before causing reproductive impairment.

There has been a limited amount of information published on the toxicity of uranium to aquatic organisms. However, it is has been established that at elevated levels uranium is toxic to cladocerans and other organisms, where it has the ability to accumulate in some types of cells and targets particular tissue types and organs, such as the kidneys (Environment Canada, 1983). Accumulation of uranium may or may not cause a toxic effect depending on whether it binds to a protein and disrupts its function or whether it is detoxified by forming a uranium phosphate microgranule which has been reported for the crab (*Carcinus maenas*) (Chassard-Bouchard, 1983; Hyne *et al.*, 1992). Poston *et al.* (1984) reported a significant reduction in the survival of *D. magna* after 5 d at a uranium concentration of 520 µg/L, *D. magna* is therefore extremely less sensitive to uranium when compared with *M. macleayi*. Ahsanullah and Williams (1986) obtained a reduction in growth of the marine amphipod (*Allorchestes compressa*) at 2 µg/L of uranium after 4 weeks. Hyne *et al.* (1992) found that the LOEC of exposure to uranium for *Hydra viridissima* after 4 d was 150 µg/L in the dry season, or 200 µg/L in the wet season. For *Hydra vulgaris* the LOECs for the dry and wet season were 400 and 550 µg/L, respectively. The seasonal changes in water quality were determined to be the main cause of the difference in the toxicity of uranium.

4.1.2 Acute immobilisation/lethality tests using uranium

The three populations (Lab, BB and DjB1) that were tested for uranium toxicity using the acute immobilisation/lethaity test, overall, were not significantly different. The EC50s obtained in the acute test for the three populations indicate that uranium was severely toxic to three populations at concentrations ranging between 160 to 390 µg/L of uranium (Table 3.5). However the EC50 obtained for the DjB2 population was significantly different to all three populations. Uranium caused severe short-term toxicity to the DjB2 population at 90 µg/L of uranium (Table 3.5). A possible explanation for this effect would be that at the time the acute test was performed on the DjB2 population, the adults were not healthy resulting in adverse maternal effects on their offspring. Due to shortage of time only two tests were performed on the DjB2 population. The other test performed on the DjB2 population was invalid, as there was greater than 20% death in the control, again indicating that the culture was not performing well at the time. . This problem was not apparent in the reproduction tests previously performed on the DjB2 population.

Previous studies performed on cladocerans, including *M. macleayi* using Magela Creek water, reported 24-h and 48-h LC50s (concentrations lethal to 50% of the test organisms relative to the control in a given time) of uranium as being greater than 400 µg/L for animals that were either not fed or fed bacteria-based diets (Poston *et al.*, 1984; Baywater *et al.*, 1991). Hyne *et al.* (1993) showed that dietary uranium (ie. using food in acute tests) causes higher toxicity to *M. macleayi* survival than waterborne uranium, with a 48 h LOEC of 200 µg/L algal-fed animals and a 5 d LOEC of 25 µg/L of uranium. The results obtained by Hyne *et al.* (1993), are similar to that obtained in this study, where the 48-h LOEC for uranium ranged between 180-370 µg/L (for the Lab, BB and DjB1 populations, see Table 3.5) and the LOEC

obtained from the reproduction tests, ranged between 20-50 μg/L (Table 3.4). Therefore, the higher toxicity of dietary uranium compared with waterborne uranium needs to considered when performing future tests on cladocerans and other toxicity testing organisms.

The main purpose in performing the acute immobilisation/lethality tests was to determine if the DjB1 would be more tolerant to higher levels of uranium in comparison to the other *M. macleayi* populations. The reproduction test could not be performed on the DjB1 population; The adult *M. macleayi* that were cultured in DjB water would not survive to have third brood offspring and would die shortly after having first or second brood offspring. The DjB1 population was retained and cultured in DjB water (and not transferred BB water) so that any potential physiological adaptation to elevated levels of uranium would also be retained. However, the results of the acute immobilisation/lethality tests indicated that the DjB1 population was not more tolerant to short-term toxic effects of uranium and was not significantly different to the Lab and BB populations (Table 3.5).

Previous studies have shown that populations collected from contaminated environments were able to tolerate higher levels of toxicants when tested and compared with populations that were collected from a non-contaminated site (Brown, 1976; Bryan, 1976; Fraser *et al.*, 1978; Moriatou-Apostolopoulou, 1978; Le Blanc, 1982; Wright, 1986; Boder *et al.*, 1990). For example, Bryan (1976) showed that the calanoid copepod, *Acartia clausi*, collected from a polluted site had a significantly higher 48-h LC50 value for copper than the copepods that were collected from an uncontaminated site. However, there was no tolerance to higher uranium concentrations in the DjB1 population when compared with the Lab and BB population. An explanation for why there was no tolerance in the DjB1 population to

higher levels of uranium concentrations is not quite clear. The Lab and BB populations have never been exposed to high levels of uranium prior to the acute tests that were carried out on them. The DjB1 population, on the other hand, was collected from an environment that contains elevated levels of uranium where it was able to survive and sustain a population. However, there have been studies also reported where no tolerance to high levels of toxicants were found in populations collected from a contaminated environment when compared with populations which were collected from a uncontaminated area. Moraitou-Apostolopoulou *et al.* (1982) compared the tolerance to cadmium and chromium of the decapod, *Palaemon elegans*, collected from a polluted area to that of a population collected from an unpolluted environment. The polluted area had higher levels of cadmium, whereas the levels of chromium were similar to that of the clean area. However, no significant difference between the different populations was demonstrated for the 48-h LC50 values for either cadmium or chromium. The fact that some studies have reported tolerance in different populations when exposed to polluted environments and other studies have not, suggests that it is necessary to assess the effects of metals and other toxicants to different species individually, and that each metal may affect different organisms differently. However, there have been no previous studies that have demonstrated tolerance to elevated levels of uranium in aquatic or other organisms.

An organisms ability to tolerate higher levels of toxicants has been linked to a group of low molecular weight proteins known as metallothioneins (MTs) (Karin, 1985; Bodar *et al.*, 1990). MTs are naturally present in the animals' liver and kidney and have a selective capacity to bind to heavy metal ions such as mercury, zinc, cadmium and copper (Cherian and Goyer, 1978; Karin, 1985). The free metal ion normally responsible for causing toxicity, binds to MT transcription factors causing

an increase in transcription of the MT gene (Hamer, 1986). The MT gene initiates MT synthesis, inturn increasing the levels of MT in the selected parts of the organism (Hamer, 1986). The binding of the heavy metal to MTs eliminates the effect of the metal (Cherian and Goyer, 1978; Karin, 1985). Tolerance in the DjB1 population to uranium did not occur, meaning that uranium metal ion present in test waters (ie. pH ~ 6.5-6.6), may not bind to the MT transcription factors and therefore does not initiate an increase in the transcription of the MT gene. Therefore, the uranium metal ion present was not detoxified, causing no apparent increase in tolerance to high concentrations of uranium.

4.1.3 Reproduction tests using cadmium

There was no major difference between the two populations (Lab and BB) that were tested for cadmium toxicity using the reproduction test. The LOECs obtained in the cadmium experiments indicated that cadmium was toxic to the different populations at concentrations ranging between 1.0 to 2.0 µg/L of cadmium (Table 3.8).

Cadmium at concentrations ranging between 1-7 µg/L caused severe reproductive impairment with a reduction in brood sizes. Some neonates were not alive when released due to the adults being affected by cadmium. At higher concentrations of cadmium (≥ 10 µg/L) mortality of all test organisms occurred within two or three days of exposure. The results of this study clearly indicate that cadmium is extremely toxic to *M. macleayi*. Orchard (1999) reported that the NOEC-LOEC for cadmium toxicity using a feeding inhibition test on *M. macleayi* to range from 0.5-1.0 µg/L of cadmium, these results were similar to that obtained in this study using the reproduction test.

Previous studies have also shown that cadmium is highly toxic to other cladocerans at relatively low concentrations. Van Leeuwin *et al.* (1985) reported that the NOEC-LOEC for *D. magna* ranged from 1.0-1.8 μg/L of cadmium in synthetic water. Roux *et al.* (1993), using a reproduction test, reported that the NOEC-LOEC for *D. pulex* ranged from 0.003- 3 μg/L of cadmium. Other toxic responses to cadmium observed in aquatic organisms include feeding inhibition (Allen *et al.*, 1995; Taylor *et al.*, 1998), vertebral deformities in fish (Bengtsson *et al.*, 1975) and the ability to inhibit the activity of ATPase enzyme which causes a decrease in muscle cell activities (Evtushenko *et al.*, 1986).

4.1.4 Comparison between uranium and cadmium toxicity

The toxicity of metals to aquatic biota has been studied extensively and has often been observed to vary considerably (Borgmann, 1983). Variability in the toxicity of metals usually occurs due to differences in their rate of uptake, physical and chemical properties and mechanism of toxicity. Metals can occur in a variety of physico-chemical forms in aquatic ecosystems; either as the free hydrated metal ion (M^{n+}) or as metals complexed with a range of naturally occurring organic and inorganic compounds in soluble, colloidal or particulate forms (Markich and Camilleri, 1997). It is generally considered that metal toxicity is primarily controlled by the activity of the free hydrated metal ion (Campbell, 1995).

Both uranium and cadmium are both non-essential metals. However, cadmium is much more toxic to *M. macleayi* than uranium. The reproduction experiments showed that there is about a ten-fold difference in toxicity between the two metals. One explanation for cadmium being more toxic than uranium would be that the free cadmium ion (Cd^{2+}) which is primarily responsible for eliciting a toxic response in

aquatic organisms is more readily available in the creek test water than the free uranyl ion (UO_2^{2+}). UO_2^{2+} only constitutes a minor proportion of the total uranium concentration, and therefore higher concentrations of uranium are required to elicit a toxic response to aquatic organisms (Markich and Camilleri, 1997).

Cadmium and uranium possess very different physical and chemical properties (Table 4.1), which may also be a reason for why cadmium is a highly more toxic metal than uranium.

The mechanisms involved in causing toxicity in uranium are also quite different from that of cadmium. The cadmium free ion (Cd^{2+}) tends to reduce calcium influx by binding onto Ca^{2+} receptors. This inturn causes problems such as inhibiting the activity of ATPase enzymes (Evtushenko *et al.*, 1986), which then decreases muscle cell activities in organisms. The absorbed uranyl ion (UO_2^{2+}) on the other hand, is either carried as a soluble bicarbonate complex (60%) or is bound to plasma proteins (40%) (Environment Canada, 1983). The uranyl ions then tend to cause damage in kidney structure, and if severe enough can cause kidney failure.

Table 4.1. Summary of physical and chemical properties of cadmium and uranium[a].

Properties	Cadmium	Uranium
Symbol	Cd	U
Group in periodic table	Transition elements	Actinide series
Molecular weight (g mol^{-1})	112.411	238.029
Electron configuration	[Kr], $5s^2$, $4d^{10}$	[Rn], $5f^3$, $6d^1$, $7s^2$
Crystal structure	Hexagonal	Orthorhombic
Melting point (°C)	321	1132
Boiling point (°C)	767	3927
Density (g/cm^3)	18.90	8.65

a - Data from McMurry (1992) and Winter (1999)

4.2 Variability in results between experiments

A lack of reproducibility in both short and long-term toxicity tests occurred, resulting in significant variation between results obtained for both acute and chronic tests when experiments were repeated. There were inconsistencies in survival and mortality data between different experiments that utilised the same *M. macleayi* population. This occurred mainly in tests using uranium. Factors that may have contributed to variability in results included the following: the health of cultured organisms, use of creek water and not synthetic water for testing, the binding agents available in test water, as well as the pH of the creek water. These are elaborated upon, below.

4.2.1 Health of cultured stocks

A plausible explanation for inconsistencies between repeated tests is the condition of the cultured population stocks of *M. macleayi* that produced the test offspring. A healthy population of test organisms is needed for toxicity testing, however the health or quality of a test population can vary significantly within 'acceptable' limits (Poston *et al.*, 1983). An indication of a healthy population would be that there was < 20 % mortality in adults, the lack of ephippia in the parent stock and that brood sizes were normal (ie. for *M. macleayi*: first brood 8-10 neonates, second brood 10-12 neonates) (Hyne *et al.*, 1996). Although parent stocks appeared healthy and prolific prior to both chronic and acute tests, with no production of ephippia, variability and inconsistencies were observed. Variation in experiments conducted on the DjB2 population compared to other populations of *M. macleayi* were likely as a result of stock cultures being unhealthy during the acute immobilisation/lethality tests. It is reasonable to assume that the stock cultures were unhealthy at the time of testing as

previously, reproduction tests had been carried out on the DjB2 population where no major difference was seen in comparison to the other populations of *M. macleayi* that were tested. Laboratory cultures of *M. macleayi* are known to experience short periods of reduced fecundity and higher than normal mortality (van Dam, pers. comm.).

4.2.2 Use of creek water

The use of natural creek water in experiments is a major factor that contributes to variability and inconsistency. Previous studies have found that stock cultures of *M. macleayi* were not able to be maintained in synthetic water (Orchard, 1999) and that cultures were only able to maintained in a healthy state when cultured in creek water. Using natural water meant that although similar, the control/diluent water was not the same for every experiment. Therefore, factors such as binding agents/ligands vary in creek water, and are not exactly the same for each experiment. Chemical analysis of water samples from test solutions only gave an indication of total uranium present. The actual organic or inorganic binding agents/ligands were not determined, which may have been useful in explaining possible causes in variation between experiments. However, a more comprehensive analysis of two different water types from BB indicated there were few differences in the inorganic composition of the water. A similar comparison with Magela Creek water (from downstream of Georgetown Billabong) would have provided useful information.

Toxicity of metals, including uranium, depends on solubility and complexation with organic and inorganic ligands (Poston *et al.*, 1984). Reported decreases in the uptake and toxicity of the free hydrated uranyl ion (UO_2^{2+}) were attributed to: (a) a reduction in the concentration of UO_2^{2+}, resulting mainly from an increase in the

formation of uranyl carbonate complexes (Poston *et al.*, 1984); and or (b) an increase in Ca^{2+} and/or Mg^{2+} that may compete with UO_2^{2+} for binding and transport sites at the cell surface of organisms (Markich *et al.*, 1997).

Changes in pH can cause a considerable amount of variation when trying to obtain reproducible results in separate experiments. In the present study an increase in pH (eg. from 6.5 to 7.0) was associated with a reduction in the toxicity of uranium in experiments. Several studies have also shown that the speciation of uranium is almost directly related to the pH of the test water. Markich *et al.* (1996) showed that the sublethal toxicity of uranium to a freshwater bivalve *Velesunio angasi* in synthetic water was about five times greater at pH 5 (48 h EC50 = 117 μg/L) than at pH 6 (48 h EC50 = 634 μg/L). Speciation modelling of the test solutions predicted that, as the pH increased from 5-6, the relative proportions of UO_2^{2+} and UO_2OH^+ declined, while the proportions of several uranyl-hydroxides and mixed uranyl-hydroxide-carbonate increased. Hyne *et al.* (1992) carried out a study on the freshwater hydra *Hydra viridissima* to determine whether the toxicity of uranium was pH-dependent. At a pH of 8.5 and uranium concentration of 1000 ppb in Magela Creek water there was no significant effect on hydra survival or population growth. In contrast, at a pH of 6.5, uranium concentrations ≥ 200 ppb in Magela Creek water did affect hydra survival and population growth.

An ideal experiment that would help reduce variability, due to modifying factors such as differences in pH and binding agents, would involve conducting three separate tests at the same time, therefore you could use the same water and test solutions for each test. However, this experiment was not able to be carried out due to factors such insufficient equipment needed to run tests all at once, and the excessive work load for an individual person required to complete three experiments at once.

4.3 Sensitivity of *M. macleayi* in comparsion with other organisms

When comparing the results of the uranium tests conducted on *M. macleayi* Lab population with other tests previously performed on other freshwater organisms, it is clear that *M. macleayi* is more sensitive to uranium than the other organisms studied (Table 4.2). The NOEC and LOEC obtained for the Lab population of *M. macleayi* using the 3 brood (5-6 d) reproduction test ranged between 10-20 µg/L and 20-40 µg/L respectively. Comparing these results with that obtained in the 21-day chronic test conducted on the cladoceran *D. magna*, the LOEC ranged between 500-3500 µg/L (Poston *et al.*, 1984) Thus, there is approximately 100-700 fold difference, between the sensitivity of the two species. The NOEC and LOEC obtained in the 96 h population growth test conducted on *Hydra viridissima* were 160 and 190 µg/L, respectively (Supervising Scientist, 1988). Thus, there is approximately an 8-fold and 4-fold difference respectively, between the sensitivity of the two species. The NOEC and LOEC obtained in the 14 d exposure test carried out on *Mogurnda mogurnda* were 880 and 1790 µg/L, respectively (Holdway, 1992b). Thus, there is approximately a 40-fold and 45-fold difference respectively, between the sensitivity of *M. macleayi* when compared with *Mogurnda mogurnda.*

The sensitivity of the *M. macleayi* Lab population to cadmium when compared with other tests previously performed on other freshwater aquatic organisms, shows that *M. macleayi* is either more sensitive to cadmium compared with other organisms studied (eg. *H. vulgaris, D. magna* and *Pimephales promelas*) or has a similar sensitivity to cadmium compared with other organisms studied (eg. *H. viridissima*) (Table 4.3).

The fact that *M. macleayi* is very sensitive to toxicant exposure makes it an excellent toxicity test organism. It is important that toxicity test organisms are relatively sensitive to toxicant exposure so that safety levels of toxicants in natural environments are not underestimated.

Table 4.2. A summary and comparison of uranium toxicity to freshwater species with that of the *M. macleayi* Lab population.

Species	Life stage	Water type (pH)	Endpoint	NOEC; LOEC (µg/L)	EC50 (CI) (µg/L)	Reference
Moinodaphnia macleayi	< 6 h neonate	Magela Creek (6.6)	3 brood (5-6 d); 48 h survival	NOEC: 10-20 LOEC: 20-40	160 (110-220)	This study
Daphnia magna	first instar	Columbia River (7.6-8.1)	21-day chronic test; 48 h survival	LOEC: between 500-3500	6000 (5720-6700)	Poston *et al.* (1984)
Hydra viridissima	Adult	Buffalo Billabong (6.5)	96 h population growth	NOEC: 160 LOEC: 190	NR	Supervising Scientist (1988)
Morgurnda mogurnda	7-d larvae	Magela Creek (6.6)	96 h survival	NR	1110 (830-1450)	Bywater *et al.* (1991)
Morgurnda mogurnda	1-d larvae	Buffalo Billabong (6.4)	14 d exposure	NOEC: 880 LOEC: 1790	NR	Holdway (1992b)

NR – not reported

Table 4.3. A summary and comparison of cadmium toxicity to freshwater species with that of the *M. macleayi* Lab population.

Species	Life stage	Water type (pH)	Endpoint	NOEC; LOEC (µg/L)	EC20/50 or LC50 (CI) (µg/L)	Reference
Moinodaphnia macleayi	< 6 h neonate	Bowerbird Billabong (6.5)	3 brood (5-6 d);	NOEC: 0.5-1.0 LOEC: 1.0-2.0	NR	This study
Moinodaphnia macleayi	adults	Bowerbird Billabong (6.2-6.5)	Feeding inhibition	NOEC: 0.5-1.0 LOEC: 1.0-2.0	5.7 (0.0-7.5)	Orchard (1999)
Daphnia magna	< 24 h neonate	ASTM (NR)	21 d reproduction test; 48 h LC50	NOEC-LOEC: 0.6-6.0	LC50: 0.06-100 (NR)	Baird *et al.*(1990)
Hygra vulgaris	adult	Autoclaved carbon-filtered main (7.2-7.5)	7-d population growth; 96 h survival	NOEC: < 13 LOEC: 13	82.5 (NR)	Lok (1998)
Hydra viridissima	adult	Autoclaved carbon-filtered tap (7.2-7.5)	7-d population growth; 96 h survival	NOEC: 0.4 LOEC: 0.8	3.0 (NR)	Lok (1998)
Pimephales promelas (flathead minnow)	adult	Aerated and chlorinated tap water (7.1)	ATPase activity	NOEC: 1.0 LOEC: 10	NR	Watson and Benson (1987)

NR – not reported

5. Conclusion

This study set out to assess whether variability between different populations of *M. macleayi* would occur in response to uranium and cadmium toxicity. It also aimed at determining if tolerance to elevated levels of uranium would occur in a population collected from a slightly more contaminated site. The major conclusions of the research can be summarised as follows:

5.1 Overview

1. Population variability needs to be considered in ecotoxicological studies when attempting to predict how natural populations respond to contaminant exposure.

2. There was no difference in the response of different populations of *M. macleayi* when compared to the Lab population to uranium and cadmium.

3. There was no apparent tolerance to water-borne uranium in the populations of *M. macleayi* obtained from DjB that were exposed to a contaminated environment containing elevated levels of uranium.

4. The Lab population of *M. macleayi* is still representative of how toxicants (in this case uranium and cadmium) impact natural populations even after being cultured in the laboratory for over 10 years.

5. *M. macleayi* is more sensitive to uranium compared to other aquatic organisms. *M. macleayi* is either more sensitive or has similar sensitivity to cadmium compared to other aquatic organisms.

5.2 Future Research

While this research achieved its objectives, there were a number of questions and issues that arose during its course. These could be addressed by further experiments, as described below.

1. Experiments looking at the toxicity of cadmium on the DjB population. This was not performed due to shortage of time. Also a comparison of how other toxicants (eg. copper, mine wastewater) affect different populations of *M. macleayi*.

2. Perform acute immobilisation/lethality tests where food is applied and water is renewed every 24 h over the 48-h period. Food in cladoceran tests usually increases the toxicity of metals.

3. Conduct tests on cladocerans that will investigate the mechanism of toxicity of uranium, as there is a lack of information in literature of uranium's mode of action.

4. Development of synthetic water that enables *M. macleayi* to survive in and maintain healthy stock cultures. This will reduce the amount of variability in results of repeated experiments.

6. References

Adema, D.M.M., 1978. *Daphnia magna* as a test animal in acute and chronic toxicity tests. Hydrobiologia 59, 125-134.

Ahsanullah, M., Williams, A.R., 1986. Effect of uranium on growth and reproduction of the marine amphipod *Allorchestes compressa*. Mar. Biol. 93, 459-464.

Allen, Y., Calow, P., Baird, D.J., 1995. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. Environ. Toxicol. Chem. 14, 1625-1630.

Baillieul, M., Blust, R., 1999. Analysis of the swimming velocity of cadmium-stressed *Daphnia magna*. Aquatic Toxicol. 44, 245-254.

Baird, D.J., Barber, I., Bradley, M., Calow, P., Soares, A.M.V.M., 1989. The *Daphnia* Bioassay. Hydrobiologia 188/189, 403-406.

Baird, D.J., Barber, I., Calow, P., 1990. Clonal variation in general responses of *Daphnia magna Straus* to toxic stress. I. Chronic life-history effects. Funct. Ecology 4, 399-407.

Baird, D.J., Barber, I., Soares, A.M.V.M., Calow, P., 1991. An early life-state test with *Daphnia magna* Straus: An alternative to the 21-day chronic test. Ecotoxicol. Environ. Safety 22, 1-7.

Barata, C., Baird, D.J., Markich, S.J., 1998. Influence of genetic and environmental factors on the tolerance of *Daphnia magna* Straus to essential and non-essential metals. Aquatic Toxicol. 30, 249-258.

Baudo, R., 1987. Ecotoxicological testing with *Daphnia magna*. Mem. Ist. Ital. Idrobiol. 45, 461-482.

Bengtsson, B., Carlin, C.H., Larsson, A., Svanberg, O., 1975. Vertebral damage in minnows, *Phoxinus phoxinus* L., exposed to cadmium. Ambio 4, 701-708.

Bishop, K.A., Allen, S.A., Pollard, D.A., Cook, M.J., 1980. Ecological studies on the fishes of the Alligator Rivers Region, Northern Territory. Open File Record 23. Supervising Scientist for the Alligator Rivers Region, Canberra, Australia.

Bitton, G., Rhodes, K., Koopman, B., 1996. CerioFAST™: an acute toxicity test based on *Ceriodaphnia dubia* feeding behaviour. Environ. Toxicol. Chem. 15, 123-125.

Bodar, C.W.M., van der Sluis, I., Voogt, P.A., 1988. Effects of cadmium consumption, assimilation and biochemical parameters of *Daphnia magna*: possible implications for reproduction. Comp. Biochem. Physiol. 90C, 341-346.

Bodar, C.W.M., van der Sluis, I., van Montfort, J.C.P., Voogt, P.A., Zandee, D.I., 1990. Cadmium resistance in *Daphnia magna*. Aquatic Toxicol. 16, 33-40.

Borgmann, U., 1983. Aquatic Toxicology - Metal speciation and toxicity of free metal ions to aquatic biota. Chapter 2. John Wiley & Sons, New York, 47-72.

Brown, B.E., 1976. Observations on the tolerance of the isopod *Asellus meridianus* Rac. to copper and lead. Water Res. 10, 555-559.

Bryan, G.W., 1976. Some aspects of heavy metal tolerance in aquatic organisms. In: Effects of pollutants on aquatic organisms. Cambridge, Cambridge University Press. p. 7-34.

Buikema Jr., A.L., Niederlehner, B.R., Cairns Jr., J., 1982. Biological Monitoring – Part IV- Toxicity testing. Water Res. 16, 239-262.

Butler, G.C., 1978. Principles of ecotoxicology. SCOPE Report 12, Wiley New York. In: Baudo, R., 1987. Ecotoxicological testing with *Daphnia magna.* Mem. Ist. Ital. Idrobiol. 45, 461-482.

Bywater, J.F., Banaczkowski, R., Bailey, M., 1991. Sensitivity to uranium of six species of tropical freshwater fishes and four species of cladocerans from Northern Australia. Environ. Toxicol. Chem. 10, 1449-1458.

Camilleri, C., Markich, S., van Dam, R.A., Pfeifle, V., 1998. Toxicity of the herbicide Tebuthiuron to Australia tropical freshwater organisms. Supervising Scientist Report 131, Supervising Scientist, Canberra, Australia.

Campbell, P.G.C., 1995. Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. In Metal speciation and bioavailability in aquatic systems, John Wiley & Sons, Chichester, 45-102.

Chandini, T., 1989. Survival, growth and reproduction of *Daphnia carinata* (crustacea: cladocera) exposed to chronic cadmium stress at different food (*Chlorella*) levels. Environ. Pollut. 60, 29-45.

Chassard-Bouchard, C., 1983. Cellular and subcellular localisation of uranium in the crab *Carcinus maenas*: a microanalytical study. Mar. Pollut. Bull. 14, 133-136.

Cherian, M.G., Goyer, R.A., 1978. Metallothioneins and their role in the metabolism and toxicity of metals. Life Sci. 23, 1-10.

Cooney, J.D., Degraeve, E.L., Moore, B.J., Pollock, T.L., Smith, G.J., 1992. Effects of environmental and experimental design factors on culturing and toxicity of *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 11, 839-850.

De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna* toxicity testing II. Digestive enzyme activity in *Daphnia magna* exposed to sublethal concentrations of cadmium, chromium and mercury. Chemophere 35, 1053-1067.

Environment Canada, 1983. Guidelines for surface water quality. Vol. 1 Inorganic Chemical Substances. Uranium. Inlands Waters Directorate, Water Quality Branch Ottawa, Canada.

ERA., 1997. Water Management System Operation Manual, September 1997, Revision 19/2002-0003. Energy Resources of Australia Ltd. Ranger Mine, Jabiru, NT, Australia.

ERA., 1999. Environmental Monitoring Program, Environmental Annual Report 1999. Energy Resources of Australia Ltd. Ranger Mine, Jabiru, NT, Australia.

Evtushenko, Z.S., Belcheva, N.N., Lukyanova, O.N., 1986. Cadmium accumulation in organs of the scallop *Mizuhopecten yessoensis*-I. Activities of phosphatases and composition and amounts of lipids. Comp. Biochem. Physiol. 83C, 371-376.

Forshaw, P.J., 1977. The inhibitory effect of cadmium on neuromuscular transmission in the rat. European J. Pharmacol. 42, 371-377. In: Griffiths, P.R.E., 1980. Morphological and ultrastructural effects of sublethal cadmium poisoning on *Daphnia*. Environ. Res. 22, 277-284.

Foulkes, E.C., 1986. Cadmium, Handbook of Experimental Pharmacology, Vol. 80. Springer-Verlag,, Berlin, Heidelberg, New York, Tokyo.

Fraser, J., Parkin, D.T., Verspoor, E., 1978. Tolerance to lead in the freshwater isopod *Asellus aquaticus*. Water Res. 12, 637-641.

French, P., 1986. The speciation and solubility of copper and cadmium in natural waters. Environmental Report 1226-M. Water Research Centre-Environment. Medmedham, UK. In: Markich, S.J., Brown, P.L., Batley, G.E., Apte, S.C., Stauber, J.L., 1997. Incorporation of metal speciation and bioavailability into guidelines for fresh and marine water quality in Australia and New Zealand. ANSTO C/503. Australian Nuclear Science and Technology Organisation, Sydney.

Friberg, L., Elinder, C., Kjellstrom, T., Nordberg, G.F., 1985. Cadmium and Health: A toxicological and epidemiological appraisal, Volume I Exposure, Dose, and Metabolism. CRC Press, Inc., Boca Raton, Florida.

Griffiths, P.R.E., 1980. Morphological and ultrastructural effects of sublethal cadmium poisoning on *Daphnia*. Environ. Res. 22, 277-284.

Grothe, D.R., Dickson, K.L., Reed-Judkins, D.K., 1996. Whole Effluent Toxicity Testing: An evaluation of methods and prediction of receiving system impacts. Society of Environmental Toxicology and Chemistry, United States of America.

Hamer, D.H., 1986. Metallothionein. Ann. Rev. Biochem. 55, 913-951.

Hebert, P.D.N., 1978. The population biology of *Daphnia*. Biol. Rev. 53, 387-426.

Holdway, D.A., 1992a. Control of metal pollution in tropical rivers in Australia. In Connell, D.W. and Hawker, D.W., editors. Pollution in Tropical Aquatic Systems. CRC Press, Boca Raton, Florida, FL, USA. P 231-246.

Holdway, D.A., 1992b. Uranium toxicity to two species of Australian tropical fish. The Sci. Total Environ. 125, 137-158.

Hyne, R.V., Rippon, G.D., Ellender, G., 1992. pH-Dependent uranium toxicity to freshwater hydra. The Sci. Total.Environ. 125, 159-173.

Hyne, R.V., Padovan, A., Parry, D.L., Renaud, S.M., 1993. Increased fecundity of the cladoceran *Moinodaphnia macleayi* on a diet supplemented with green alga, and its use in uranium toxicity tests. Aust. J. Freshwater Res. 44, 389-399.

Hyne, R.V., Rippon, G.D., Hunt, S.M., Brown, G.H., 1996. Procedures for the biological toxicity testing of mine waste waters using freshwater organisms. Supervising Scientist Report 110, Supervising Scientist for the Alligator Rivers Region, Australian Government Publishing Service, Canberra, Australia.

Julli, M.E., 1986. The taxonomy and seasonal population dynamics of some Megela Creek flood plain microcrustaceans (Cladocera and Copepoda). Technical Memorandum 18 SS. Supervising Scientist for the Alligators Rivers Region, Canberra, Australia.

Julli, M.E., Chapman, J.C., Thompson, G.B., 1990. Use of Australian cladocerans to generate life-cycle toxicity data. Environ. Mon. Assess. 14, 353-362.

Karin, M., 1985. Metallothioneins: Proteins in search of function. Cell 41, 9-10.

Klerks, P.L., Weis, J.S., 1987. Genetic adaptation to heavy metals in aquatic organisms: A review. Environ. Pollut. 45, 173-205.

Koivisto, S., 1995. Is *Daphnia magna* an ecologically representative zooplankton species in toxicity tests? Environ. Pollut. 90, 263-267.

Kotsonis, F.N., Klaassen, C.D., 1978. The relationship of metallothionein to the toxicity of cadmium after prolonged oral administration to rats. Toxicol. Appl. Pharmacol. 46, 39-54.

Krockenberger, M., 1997. Environmental Effects of uranium mining in Australia. SEA-US. http://www.sea-us.org.au/uran-res.htmt

Le Blanc, G.A., 1982. Laboratory investigation into the development of resistance of *Daphnia magna* (Straus) to environmental pollutants. Environ. Pollut. Ser. A. 27, 309-322.

Lok, K., 1998. The acute and sub-chronic toxicity of cadmium and zinc to two hydra species. Honours thesis, Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, Melbourne, Victoria.

Mark, U., Solbe, J., 1998. Analysis of the Ecetoc Aquatic Toxicity (EAT) database V- the relevance of *Daphnia magna* as a representative test species. Chemosphere 36, 155-166.

Markich, S.J., Brown, P.L., Jeffree, R.A., 1996. The use of geochemical 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., Stauber, J.L., 1997. Incorporation of metal speciation and bioavailability into guidelines for fresh and marine water quality in Australia and New Zealand. ANSTO C/503. Australian Nuclear Science and Technology Organisation, Sydney.

Markich, S.J., 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, Australia.

McMurry, J., 1992. Organic Chemistry. 3rd Ed. Brooks/Cole Publishing Company, United States of America.

Mount, D.T., Norberg, T.J., 1984. A seven-day lifecycle cladoceran toxicity test. Environ. Toxicol. Chem. 3, 425-434

Moraitou-Apostolopoulou, M., 1978. Acute toxicity of copper to a copepod. Mar. Pollut. Bull. 9, 278-280.

Moraitou-Apostolopoulou, M., Verriopoulos, G., Rogdakis, I., 1982. Evaluation of the stress exerted by a polluted environment to a marine organism by comparative toxicity tests. Bull. Environ. Contam. Toxicol. 28, 416-423.

Orchard, S.J., 1999. Development of a rapid response toxicity bioassay based on feeding rate of the tropical cladoceran, *Moinodaphnia macleayi.* Honours thesis, Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, Melbourne, Victoria.

Oraganisation for Economic Co-operation and Development, 1987. OECD Guidelines for testing of Chemicals, Second Addendum. Oraganisation for Economic Co-operation and Development, Paris.

Oraganisation for Economic Co-operation and Development, 1999. OECD Guidelines for testing of chemicals. Draft proposal for updating Guideline 202. *Daphnia* sp., Acute Immobilisation Test. p. 1-8.

Padovan, A., 1992. Isolation and culture of five species of freshwater algae from the Alligator Rivers Region, Northern Territory, Technical memorandum 37, Supervising Scientist for the Alligator Rivers Region, AGPS, Canberra.

Poston, T.M., Hanf Jr., R.W., Simmons, M.A., 1984. Toxicity of uranium to *Daphnia magna*. Water, Air, Soil Pollut. 22, 289-298.

Rana, S., Kumar, A., Bhardway, N., Kumer, A., 1980. Lipid in the liver and kidney of rats, fed various heavy metals. Acta anat. 108, 402-412.

Rippon, G., 1993. Effects of diet on uranium toxicity to water fleas, Proceedings of a workshop on water flea diet and its effect on toxicity testing and uranium toxicity. Internal Report No. 103. Supervising Scientist for Alligator Rivers Region, Canberra, Australia. p. 27-34.

Roux, D.J., Kempster, P.L., Truter, E., van der Merwe, L., 1993. Effect of cadmium and copper on survival and reproduction of *Daphnia pulex*. Water S.A. 19, 169-274.

Rupert, E.E., Barnes, R.B., 1994. Invertebrate Zoology. 6th Edition. Saunders College Publishing, United States of America.

Smirnov, N.N., Timms, B.V., 1983. Records of the Australian Museum. A Revision of the Australian Cladocera (Crutacea). Supplement 1. The Australian Museum, Sydney.

Soares, A.M.V.M., Baird, D.J., Calow, P., 1992. Interclonal variation in the performance of *Daphnia magna* Straus in chronic bioassays. Environ. Toxicol. Chem. 11, 1477-1483.

Sprague, J.B., Fogels, A., 1977. Watch the Y in bioassay. Proc. 3rd Aquatic Toxicity Workshop, Halifax, N.S., Nov. 2-3, 1976. Environmental Protection Service Technical Report No. EPS-%-AR-77-1, Halifax, Canada. p. 107-118.

Sprague, J.B., 1990. Methods from Fish Biology, Aquatic Toxicology – Chapter 15. American Fisheries Society Bethesda, Maryland. 491-527.

Stuhlbacher, A., Bradley, M.C., Naylor, C., Calow, P., 1992. Induction of cadmium tolerance in two clones of *Daphnia magna* Straus. Comp. Biochem. Physiol. 101C, 571-577.

Supervising Scientist, 1988. Alligators River Region Research Institute Annual Research Summary 1987-88. Supervising Scientist for the Alligator Rivers Region, Canberra, Australia.

Taylor, G., Baird, D.J., Soares, A.M.V.M., 1998. Surface binding of contaminants by algae: consequences for lethal toxicity and feeding to *Daphnia magna* Straus. Environ. Toxicol. Chem. 17, 412-419.

Tsuchiya, K., 1978. Cadmium studies in Japan: A Review. Kodansha Scientific Books, Kodansha Ltd, Tokyo, Japan.

van Dam, R.A., Chapman, J.C., 1999. Direct toxicity assessment (DTA) for water quality guidelines in Australia and New Zealand. Australasian J. Ecotoxicol. In press.

Van Leeuwin, C.J., Luttmer, W.J., Griffioen, P.S., 1985. The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: a cadmium example. Ecotoxicol. Environ. Safety 9, 26-39.

Watson, C.F., Benson, W.H., 1987. Comparitive exposure of gill ATPase in three freshwater teleosts exposed to cadmium. Ecotoxicol. Environ. Safety 14, 252-259.

Winter, M., 1999. Wed Elements^{2000}, the periodic table on the www, URL. http://www.webelements.com

Wright, D.A., 1986. Trace metal uptake and sodium regulation in *Gammarus marinus* from metal polluted estuaries in England. J. Mar. Biol. Assn. U.K. 66, 83-92.

Zar, J.H., 1999. Biostatistical analysis. 4th Ed. Prentice Hall Intenational, Inc., New Jersey.

(Reference format style from Aquatic Toxicology)

7. Appendices

Appendix 1

A1.1 Collection of Djalkmara Billabong population

The collection of the Djalkmara Billabong (DjB) population involved using a plankton sweep net. Sweep net samples were collected from the littoral zone of the billabong. Water was collected and then placed into a 20L barrel and then taken back to the laboratory to be sorted underneath a binocular microscope to identify *M. macleayi*. *M. macleayi* was identified from Julli (1986). Once identified, *M. macleayi* was transferred and cultured in 45mL screw-capped vials containing DjB water with food (algae & FFV). After monitoring the survival of the DjB population for a few weeks it was obvious that the cladocerans were stressed and not surviving beyond release of their first brood. At this point in time it was decided to start a culture of the DjB population in BB water.

A1.2 Acclimatisation of DjB population to BB water

Approximately 10-15 first brood neonates were firstly transferred to separate 30mL plastic vials with perforated lids containing 1:1 filtered Bowerbird Billabong (BB) water - Djalkmara Billabong (DjB) water with food (*Chlorella* sp. - 2×10^5 cells/mL and FFV - 1μL/mL). Water and food was renewed daily for at least 3 d at the same ratio (ie. 1:1 - BB/DjB). DjB populations were then placed in 3:1 filtered BB water - DjB water for another 3 d with daily water and food renewal; then finally DjB populations were transferred to 100% filtered BB water. The DjB population was kept in a constant temperature incubator at 27 ± 1°C, with a photoperiod of 12 h light: 12 h dark.

Appendix 2

A2.1 Fermented pellet fish food with added vitamins (FFV):

5g cichlid pellets (Wardley brand)

0.25g dried powdered alfalfa

250 mL Milli-Q water

0.3g calcium pantothenate

1.5 mL Vitamin B_{12} stock solution (100μg/L). Make this Vitamin up in 100mL amounts, and dispense smaller amounts as required.

Method (modified from Hyne *et al.*, 1996):

Five grams of a commercially available-available proprietary pelleted cichlid food (Wardley Products, New Jersey) and 0.25 g of dried and powdered alfalfa are homogenised in 250 mL of Milli-Q water for 5 minutes using a domestic food-blender. The mixture is then placed this on a oscillating digital shaker at 25-28^{o}C for no longer than 3 days, in a 1L sediment cone covered with a fine mesh (to keep out dust, flies etc, and to allow gases to be released). At the end of this 3-day period, the mixture is collected into a beaker and placed at 4^{o}C for 1 hour and allowed to settle. The supernatant (150 mL) is then decanted carefully into another beaker and 0.3 g of calcium pantothenate and 1.5 mL of a stock Vitamin B_{12} solution (100 mg/L) is added to this beaker. This is then divided into aliquots of about 1 mL in glass vials, capped and frozen. An aliquot is thawed when needed, and resuspended by gentle shaking before use. The aliquot can then be refrigerated (4°C) but must be discarded one week after thawing. The addition of 1 μL/mL was added to *M. macleayi* culturing and testing solutions.

A2.2 Protocol for mass culture of algae (Chlorella sp.)

Two types of algal cultures are maintained:

(i) stock or 'starter' cultures

(ii) mass or 'food' cultures

All steps outlined below should be carried out in the Laminar Flow Cabinet using sterile media and glassware. Starter cultures are placed in the front of the growth cabinets (approximately 30μE/m^2/s) set on a 12:12 light/dark cycle at 27 ± 1°C and are mixed daily by gently swirling the flask. Culture flasks for mass culture of algae for food are placed on an oscillating digital shaker (that ensures cells don't pool on the bottom of the flask) in the middle of an incubator (40-50 μE/m^2/s), set on a 12:12 light/dark cycle at 27 ± 1°C. A mass culture should be prepared at least monthly.

1 Approximately 7 to 10 days before commencing a mass culture, transfer ~ 1 mL of algae from the older starter culture into 250 mL Erlenmeyer flask to which 100 mL of half-strength MBL medium (MBL/2) has been added. Cells in this new starter culture should be exponentially growing by the time they are used to inoculate the food culture.

2 To prepare medium for the food culture, make up 2 L of MBL/2 in a 2 L Erlenmeyer flask and adjust pH to between 7.1 to 7.3. Stopper with non-absorbent cotton wool wrapped into the top of the pipette to serve as an air filter. Autoclave for 40 minutes, and complete medium on cooling. Allow medium to sit for a least 1 day before use (refer to Appendix 2.3)

3 Before inoculating the food culture medium, aseptically sample the new starter culture for a cell count. Count both chambers on the haemocytometer, wash, reload, then count both again (a total of 4 counts). Count at least 100 cells per grid. Average the cell counts and calculate the number of cells per mL.

4 Calculate the volume of new starter culture (step 1) needed to add to 2 L of food culture to get a cell density of 10^4 cells/mL. Using either a sterile pipette or a measuring cylinder aseptically inoculate the food culture medium. Place the flask in an incubator set to 27± 1°C on an oscillating digital shaker that ensures cells don't pool on the bottom of the flask, culture for 10-14 days (until cells reach a density of 2×10^6 cells/mL) and then harvest the algae (refer to Appendix 2.4).

A2.3 Growth Media for Algae

Growth media is prepared each month for the maintenance of starter cultures, algal slopes, and the mass culturing and harvesting of *Chlorella sp.* It is sterilised by autoclaving and can be stored in sealed glass bottles for prolonged use. Always check media prior to use for contamination by bacteria. This will appear as foreign particles in what should be a clear liquid.

MBL Media:Stock Solutions

Tris Buffer	100g/L	- Add 10mL / L
$NaNO_3$	85.24g/L	
$CaCl_2$	27.71g/L	
$MgSO_4$	36.97g/L	
$NaHCO_3$	12.6g/L	
K_2HPO_4	8.72g/L	
Na_2EDTA	4.36g/L	

$FeCl_3$	0.436g/L

Trace Metals: Make up 1L solution containing the following compounds:

$CoCl_2.6H_2O$	0.010g/L
$CuSO_4.5H_2O$	0.009g/L
$Na_2SiO_3.5H_2O$	0.007g/L
$MnCl_2.4H_2O$	0.180g/L
$ZnSO_4.7H_2O$	0.022g/L

Working Solution Ratios:

Add 1ml of each stock solution per litre Milli-Q water, except Tris Buffer - add 10mL / L. Prepare MBL media in 6 × 2L flasks (using a 1L volumetric flask to measure 1L per flask) and adjust to pH 7.1 - 7.3 using 10% HCl or 1M NaOH. Autoclave at 121°c for 20 min.

Agar Media-Solid

1. Prepare MBL media as above. Just prior to autoclaving add 1.5% (15g/L) agar powder to liquid media.
2. Autoclave as normal depending on volume being prepared.
3. Allow agar media to cool on a bench to just being able to touch the glass.
4. Pour the agar media into pre-sterilised Macartney bottles, or sterilised plastic bottles inside a laminar flow hood. Angle the bottles to about 30 degrees to optimise surface area for inoculation.
5. Allow agar to cool in laminar flow hood. Seal firmly and store excess bottles at room temperature in main laboratory.

A2.4 Protocol for harvesting algal cells (from Hyne *et al.*, 1996).

1 Aseptic technique is not essential when harvesting cells, however work area should be as clean as possible. All dilution water (eg Magela Creek water) used in the following steps is sterilised by filtration through a 0.2 μm filter.

2 12 × 80 mL plastic tubes are filled with 60 mL of the food culture. The tubes are placed in a refrigerator centrifuge fitted with a swing-out head. Tubes placed opposite each other are balanced or matched to the nearest 0.1 g, both for this step and all other steps. Another12 tubes are prepared while these tubes are spun (step3). Any remaining food culture can be spun in used tubes after the supernatant has been removed and the resuspended pellet transferred (step 5).

3 The centrifuge is set to a rotor speed of 2800 rpm (2000 × g) and a temperature of 15 °C. A spinning time of 20 minutes is adequate. While one set of 12 tubes is spinning, another set of 12 can be prepared for the next run.

4 The supernatant is removed through suction, using a glass Pasteur pipette directly attached to a venturi suction via flexible plastic tubing. A sufficient quantity of water (~ 5 mL) is left behind to allow resuspension of the pellet.

5 The resuspended pellets are combined and transferred into the minimum number of tubes possible. The empty tubes are rinsed by adding 20-30 mL of sterile dilution water, capped and vigorously shaken. To keep the volume of rinsing water used a minimum, the same aliquot is transferred from one tube to another, eventually to find a final tube to be re-spun. This rinsing procedure is repeated twice, or until the final aliquot appears clear.

6 The above procedure is repeated until all algal cells are concentrated into one tube. These cells are washed twice with sterile dilution to remove any trace metals (from the culture media) by spinning, removing the supernatant, adding approximately 50 mL sterile dilution water and shaking vigorously to resuspend the pellet.

7 The pellet from step 6 is finally resuspended in approximately 50 mL of sterile dilution water.

8 The algal density of the suspension must be measured to calculate the required volume needed to be added to *M. macleayi* treatment vials to attain the pre-determined density known density of 2×10^5 algae/mL. To measure algal density, transfer 1 mL of the suspension to a 100 mL volumetric flask and make up to the mark using sterile dilution water. Seal with Parafilm and mix well by inverting 5 times. Quickly break the seal and load the haemocytometer for a cell count. If the algal density is too high, try a greater dilution (eg. add 0.5 mL concentrate to a 250 mL measuring cylinder). Count at least 100 cells in each of 4 chambers (re-seal and invert 5 times before loading the haemocytometer again), average the values and back-calculate to get the algal density of the concentrate. A typical 2-L culture should produce 8.2×10^9 algal cells.

9 The algal suspension (step 7) should be divided into 1mL aliquots and stored in the dark at 4°C. A typical algal food culture that produces 8.2×10^9 algal cells in a 50 mL suspension would provide approximately 270 feedings at 185µL/feeding to give 30×10^6 algae/150 mL cladoceran culture. The algal feeding aliquot of 30 ×

10^6 cells should always have a volume less than 500 μL. If not, the algal concentrate should be re-centrifuged and volume adjusted.

Appendix 3

A3.1 Nominal and Measured concentrations of test solutions

Table A3.1 Nominal and measured concentrations of test solutions for uranium reproduction tests for the Lab population of *M. macleayi* .

Exp. No.	Date	Nominal Conc. U (µg/L)	Measured Conc U (µg/L)	Blank corrected U (µg/L)
U-Lab-1	5.5.99	Blank	0.01	0
		Control	0.05	0.04
		5	3.49	3.48
		10	7.79	7.79
		25	20.1	20.1
		50	39.2	39.2
U-Lab-2	21.5.99	Blank	0.25	0
		Control	0.29	0.04
		5	4.04	3.79
		10	7.43	7.18
		15	14.2	13.9
		25	21.9	21.7
		50	42.0	41.8
U-Lab-3	2.6.99	Blank	0.43	0
		Control	0.54	0.12
		5	4.35	3.92
		10	9.14	8.72
		15	13.1	12.7
		25	28.4	27.9
		50	46.7	46.3

Table A3.2 Nominal and measured concentrations of test solutions for uranium reproduction tests for the BB and DjB2 populations of *M. macleayi* .

Exp. No.	Date	Nominal Conc. U (µg/L)	Measured Conc U (µg/L)	Blank corrected U (µg/L)
U-BB-1	12.6.99	Blank	0.04	0
		Control	0.07	0.03
		5	5.41	5.37
		10	9.61	9.57
		15	13.1	13.1
		25	24.8	24.7
		50	49.0	48.9
U-BB-2	19.6.99	Blank	0.05	0
		Control	0.06	0.01
		10	9.19	9.14
		20	20.4	20.3
		30	28.6	28.5
		40	36.3	36.3
		50	49.0	49.0
U-DjB2-1	2.8.99	Blank	0.13	0
		Control	0.12	0
		5	4.57	4.44
		10	8.89	8.74
		15	13.7	13.6
		25	22.2	22.1
		50	43.6	43.5
		75	65.4	65.3
U-DjB2-2	9.8.99	Blank	0.19	0
		Control	0.13	0
		10	7.75	7.56
		20	16.2	16.0
		30	23.9	23.7
		40	31.3	31.1
		50	40.7	40.6

Table A3.3 Nominal and measured concentrations of test solutions for cadmium reproduction tests for the BB and Lab populations of *M. macleayi*.

Exp. No.	Date	Nominal Conc. Cd (µg/L)	Measured Conc Cd (µg/L)	Blank corrected Cd (µg/L)
Cd-BB-1	3.7.99	Blank	<0.02	0
		Control	<0.02	0
		1	1.04	1.02
		2	1.91	1.89
		5	4.67	4.65
		10	10.2	10.1
		15	14.9	14.8
		20	20.4	20.4
Cd-BB-2	11.7.99	Blank	<0.02	0
		Control	<0.02	0
		0.5	0.47	0.45
		1	2.30	2.28
		2	1.93	1.91
		3.5	3.35	3.33
		5	4.87	4.85
		7	7.50	7.48
Cd-Lab-1	18.7.99	Blank	<0.02	0
		Control	0.05	0.03
		0.5	0.58	0.56
		1	0.98	0.96
		2	2.06	2.04
		5	5.05	5.03
		10	9.85	9.83
		20	20.2	20.2
Cd-Lab-2	25.7.99	Blank	<0.02	0
		Control	<0.02	0
		0.5	0.48	0.46
		1	1.14	1.12
		2	1.78	1.76
		3.5	3.31	3.29
		5	4.82	4.80
		7	7.08	7.06

Table A3.4 Nominal and measured concentrations of test solutions for the uranium 48-h acute immobilisation/lethality tests for the DjB1 and BB populations of *M. macleayi*.

Exp. No.	Date	Nominal Conc. U (µg/l)	Measured Conc. U (µg/l)	Blank corrected U (µg/l)
U-DjB1-1	18.8.99	Blank	0.13	0
		Control	0.12	0
		50	44.1	44.0
		100	92.9	92.8
		200	182	182
		300	268	268
		400	367	367
		600	561	561
U-BB-1	18.8.99	Blank	0.13	0
		Control	0.12	0
		50	44.1	44.0
		100	92.9	92.8
		200	182	182
		300	268	268
		400	367	367
		600	561	561
U-DjB1-2	23.8.99	Blank	0.15	0
		Control	4.01	3.86
		200	180	180
		300	279	278
		400	376	376
		500	518	517
		600	617	617
		700	718	717
U-BB-2	23.8.99	Blank	0.15	0
		Control	4.01	3.86
		100	95.0	94.5
		200	180	180
		300	279	278
		400	376	376
		500	518	517
		600	617	617

Table A3.5 Nominal and measured concentrations of test solutions for the uranium 48-h acute immobilisation/lethality tests for the DjB2 and Lab populations of *M. macleayi*.

Exp. No.	Date	Nominal Conc. U (µg/l)	Measured Conc. U (µg/l)	Blank corrected U (µg/l)
U-DjB2-1	26.8.99	Blank	0.24	0
		Control	2.64	2.40
		100	96.0	95.8
		200	190	190
		300	280	279
		400	382	382
		500	470	470
		600	569	569
U-Lab-1	29.8.99	Blank	0.20	0
		Control	3.01	2.81
		100	96.0	96.0
		200	191	191
		300	283	282
		400	436	436
		500	523	522
		600	581	581
U-DjB2-2	29.8.99	Blank	0.20	0
		Control	3.01	2.81
		100	96.0	96.0
		200	191	191
		300	283	282
		400	436	436
		500	523	522
		600	581	581
U-Lab-2	1.9.99	Blank	0.49	0
		Control	1.51	1.02
		50	40.0	39.5
		100	90.4	89.9
		150	138	137
		200	183	183
		300	278	278
		400	372	372

Appendix 4

A.4.1 Water Collection Dates and Test water Parameters

Table A4.1 Water collection dates and water parameters

Billabong	Date	pH		Conductivity (µS/cm)		Dissolved Oxygen (%)	
		unfiltered	filtered	unfiltered	filtered	unfiltered	filtered
Georgetown	4.5.99	6.40	6.57	20	17	98	102
Georgetown	24.5.99	6.30	6.53	18	18	94	92
Bowerbird	11.6.99	6.40	6.50	14	16	94	98
Bowerbird	1.7.99	6.41	6.42	16	18	89	88
Bowerbird	22.7.99	6.44	6.51	15	19	103	104
Bowerbird	23.8.99	6.34	6.38	16	16	86	88
Djalkmara	30.6.99	7.35	7.46	910	900	83	90
Djalkmara	9.7.99	7.78	7.85	980	990	95	89
Djalkmara	5.8.99	7.90	7.88	1485	1486	95	93

Table A4.2 Mean (SE) values of tested water parameters in uranium chronic toxicity (reproduction) tests to Lab *M. macleayi* populations.

Population (Exp. No.)	U conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
U-Lab-1	0	0	6.55 (0.05)	21 (0.60)	98 (0.93)
	5		6.73 (0.06)	20 (0.40)	98 (0.84)
	10		6.75 (0.06)	20 (0.24)	98 (0.98)
	25		6.85 (0.05)	21 (0.24)	98 (1.14)
	50		6.91 (0.05)	21 (0.37)	98 (1.14)
	0	24	6.79 (0.03)	21 (0.49)	97 (1.82)
	5		6.95 (0.03)	21 (0.37)	97 (1.24)
	10		6.93 (0.05)	21 (0.58)	96 (1.92)
	25		7.01 (0.04)	20 (0.51)	96 (1.36)
	50		6.97 (0.04)	21 (0.40)	95 (1.66)
U-Lab-2	0	0	6.59 (0.02)	22 (0.63)	111 (1.69)
	5		6.72 (0.02)	23 (0.22)	110 (1.48)
	10		6.76 (0.02)	23 (0.21)	110 (1.76)
	15		6.81 (0.01)	23 (0.17)	111 (1.41)
	25		6.81 (0.01)	22 (0.31)	110 (1.19)
	50		6.78 (0.01)	20 (0.33)	110 (1.43)
	0	24	6.83 (0.02)	23 (0.37)	94 (0.73)
	5		6.88 (0.03)	23 (0.32)	95 (0.75)
	10		6.92 (0.01)	23 (0.37)	94 (0.55)
	15		6.96 (0.02)	23 (0.20)	95 (0.68)
	25		6.92 (0.02)	23 (0.80)	95 (0.24)
	50		6.90 (0.02)	21 (0.51)	94 (0.71)
U-Lab-3	0	0	6.79 (0.01)	22 (0.98)	104 (2.33)
	5		6.93 (0.02)	22 (1.52)	105 (2.20)
	10		6.99 (0.03)	23 (1.67)	105 (2.67)
	15		7.00 (0.02)	22 (1.03)	105 (1.76)
	25		7.00 (0.03)	22 (0.69)	104 (2.10)
	50		7.02 (0.03)	22 (0.87)	104 (2.14)
	0	24	7.12 (0.07)	23 (1.27)	97 (0.82)
	5		7.22 (0.09)	22 (1.23)	97 (1.06)
	10		7.34 (0.16)	22 (1.11)	98 (1.60)
	15		7.22 (0.10)	22 (1.08)	97 (0.79)
	25		7.12 (0.06)	22 (0.88)	97 (1.03)
	50		7.31 (0.11)	22 (0.71)	97 (0.71)

Table A4.3 Mean (SE) values of tested water parameters in uranium chronic toxicity (reproduction) tests to BB *M. macleayi* populations.

Population (Exp. No.)	U conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
U-BB-1	0	0	6.80 (0.04)	18 (0.31)	111 (2.54)
	5		6.88 (0.03)	18 (0.21)	110 (2.54)
	10		6.90 (0.04)	18 (0.33)	109 (2.64)
	15		6.91 (0.04)	18 (0.33)	109 (1.87)
	25		6.93 (0.04)	19 (0.22)	109 (2.32)
	50		6.93 (0.03)	19 (0.21)	109 (2.29)
	0	24	6.98 (0.03)	18 (0.37)	97 (0.50)
	5		7.03 (0.04)	18 (0.52)	97 (0.62)
	10		7.08 (0.09)	17 (0.40)	96 (0.84)
	15		7.10 (0.07)	18 (0.60)	96 (1.23)
	25		7.07 (0.07)	18 (0.33)	96 (1.23)
	50		7.15 (0.08)	18 (0.31)	97 (0.73)
U-BB-2	0	0	6.76 (0.07)	17 (0.24)	117 (2.70)
	10		6.92 (0.08)	18 (0.93)	117 (2.89)
	20		6.98 (0.08)	18 (0.40)	116 (2.87)
	30		6.99 (0.08)	17 (0.24)	115 (3.03)
	40		6.97 (0.07)	18 (0.20)	113 (2.67)
	50		6.98 (0.05)	18 (0.20)	113 (2.25)
	0	24	7.30 (0.19)	17 (0.24)	100 (1.70)
	10		7.49 (0.29)	17 (0.20)	99 (2.58)
	20		7.47 (0.26)	17 (0.24)	99 (2.43)
	30		7.54 (0.26)	17 (0.20)	98 (1.95)
	40		7.56 (0.27)	17 (0.24)	98 (1.30)
	50		7.41(0.19)	17 (0.20)	99 (1.78)

Table A4.4 Mean (SE) values of tested water parameters in uranium chronic toxicity (reproduction) tests to DjB2 *M. macleayi* populations.

Population (Exp. No.)	U conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
U-DjB2-1	0	0	6.76 (0.03)	19 (0.00)	114 (0.60)
	5		6.82 (0.02)	19 (0.20)	116 (0.77)
	10		6.86 (0.02)	19 (0.20)	116 (0.87)
	15		6.86 (0.04)	19 (0.20)	117 (0.86)
	25		6.90 (0.03)	19 (0.00)	117 (0.73)
	50		6.91 (0.04)	19 (0.24)	117 (1.05)
	75		6.94 (0.03)	20 (0.24)	116 (1.03)
	0	24	7.21 (0.07)	19 (0.32)	98 (0.49)
	5		7.30 (0.11)	19 (0.00)	98 (0.73)
	10		7.33 (0.11)	19 (0.20)	98 (0.68)
	15		7.29 (0.12)	19 (0.00)	97 (0.68)
	25		7.28 (0.14)	19 (0.24)	96 (0.92)
	50		7.33 (0.13)	19 (0.20)	96 (0.66)
	75		7.33 (0.13)	19 (0.24)	97 (0.75)
U-DjB2-2	0	0	6.71 (0.02)	19 (0.20)	114 (0.97)
	10		6.82 (0.02)	19 (0.32)	116 (1.25)
	20		6.85 (0.02)	19 (0.32)	113 (1.29)
	30		6.86 (0.03)	20 (0.20)	113 (1.64)
	40		6.85 (0.02)	20 (0.20)	113 (1.33)
	50		6.90 (0.02)	20 (0.20)	114 (1.29)
	0	24	7.11 (0.05)	19 (0.24)	99 (0.37)
	10		7.35 (0.18)	19 (0.20)	99 (0.68)
	20		7.29 (0.13)	19 (0.00)	98 (0.80)
	30		7.21 (0.10)	19 (0.37)	97 (1.05)
	40		7.34 (0.14)	19 (0.20)	97 (1.03)
	50		7.42 (0.13)	19 (0.00)	98 (1.38)

Table A4.5 Mean (SE) values of tested water parameters in cadmium chronic toxicity (reproduction) tests to BB *M. macleayi* populations.

Population (Exp. No.)	Cd conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
Cd-BB-1	0	0	6.76 (0.04)	18 (0.24)	117 (1.28)
	1		6.87 (0.02)	19 (0.24)	117 (1.60)
	2		6.89 (0.03)	19 (0.24)	116 (1.29)
	5		6.89 (0.02)	19 (0.24)	116 (1.12)
	10		6.93 (0.03)	19 (0.25)	117 (1.80)
	15		6.93 (0.02)	19 (0.00)	116 (3.28)
	20		6.94 (0.02)	19 (0.00)	117 (3.06)
	0	24	7.07 (0.08)	17 (0.24)	99 (1.82)
	1		7.14 (0.06)	17 (0.24)	98 (1.58)
	2		7.16 (0.06)	17 (0.24)	97 (1.76)
	5		7.17 (0.08)	18 (0.37)	98 (1.46)
	10		7.20 (0.16)	18 (0.58)	99 (2.52)
	15		7.29 (0.06)	18 (1.00)	98 (2.50)
	20		7.35 (0.11)	17 (0.00)	100 (1.00)
Cd-BB-2	0	0	6.62 (0.04)	19 (0.32)	115 (0.51)
	0.5		6.71 (0.02)	19 (0.24)	114 (0.49)
	1		6.75 (0.03)	19 (0.24)	114 (0.58)
	2		6.78 (0.03)	19 (0.24)	114 (0.68)
	3.5		6.79 (0.02)	20 (0.24)	113 (1.58)
	5		6.82 (0.02)	20 (0.24)	114 (0.55)
	7		6.81 (0.02)	20 (0.20)	114 (0.68)
	0	24	6.89 (0.05)	20 (0.20)	97 (0.98)
	0.5		6.91 (0.04)	20 (0.20)	95 (1.12)
	1		6.96 (0.06)	19 (0.24)	96 (0.73)
	2		6.97 (0.08)	19 (0.40)	96 (0.97)
	3.5		6.98 (0.07)	19 (0.40)	97 (1.08)
	5		7.04 (0.06)	20 (0.24)	97 (0.92)
	7		7.04 (0.06)	19 (0.49)	98 (0.97)

Table A4.6 Mean (SE) values of tested water parameters in cadmium chronic toxicity (reproduction) tests to Lab *M. macleayi* populations.

Population (Exp. No.)	Cd conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
Cd-Lab-1	0	0	6.70 (0.03)	18 (0.32)	113 (2.25)
	0.5		6.75 (0.02)	18 (0.20)	113 (2.16)
	1		6.80 (0.01)	18 (0.20)	113 (2.08)
	2		6.77 (0.04)	18 (0.24)	113 (1.78)
	5		6.79 (0.02)	18 (0.20)	112 (1.52)
	10		6.79 (0.03)	18 (0.25)	113 (1.55)
	20		6.83 (0.06)	18 (0.33)	113 (0.88)
	0	24	6.92 (0.03)	19 (0.75)	97 (0.68)
	0.5		7.01 (0.02)	19 (0.37)	97 (0.55)
	1		7.06 (0.02)	19 (0.37)	97 (0.71)
	2		7.06 (0.03)	18 (0.24)	96 (0.40)
	5		7.06 (0.05)	19 (0.40)	97 (0.73)
	10		7.13 (0.04)	18 (0.33)	97 (0.88)
	20		7.13 (0.03)	19 (1.00)	98 (0.50)
Cd-Lab-2	0	0	6.84 (0.06)	19 (0.00)	113 (0.60)
	0.5		6.96 (0.06)	19 (0.00)	114 (0.68)
	1		6.99 (0.05)	19 (0.00)	114 (0.58)
	2		7.06 (0.06)	19 (0.00)	114 (0.58)
	3.5		7.04 (0.06)	19 (0.20)	114 (0.63)
	5		7.02 (0.07)	19 (0.20)	114 (0.45)
	7		7.02 (0.05)	19 (0.24)	114 (0.37)
	0	24	7.04 (0.05)	20 (0.58)	98 (0.49)
	0.5		7.09 (0.07)	19 (0.00)	97 (0.75)
	1		7.14 (0.06)	20 (0.97)	98 (0.84)
	2		7.18 (0.08)	19 (0.32)	96 (0.86)
	3.5		7.11 (0.06)	21 (0.97)	96 (1.03)
	5		7.11 (0.02)	19 (0.00)	96 (1.14)
	7		7.15 (0.03)	19 (0.20)	96 (1.20)

Table A4.7 Values of tested water parameters in the uranium 48-h acute immobilisation/lethality tests to DjB1 *M. macleayi* populations.

Population (Exp. No.)	U conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
U-DjB1-1	0	0	6.39	16	106
	50		6.57	16	105
	100		6.59	16	105
	200		6.63	16	105
	300		6.63	16	103
	400		6.69	16	104
	600		6.58	16	104
	0	48	6.62	26	99
	50		6.76	26	97
	100		6.81	26	97
	200		6.95	27	97
	300		6.98	26	97
	400		6.98	26	97
	600		7.00	30	99
U-DjB1-2	0	0	6.49	16	107
	200		6.63	15	109
	300		6.63	15	107
	400		6.65	15	106
	500		6.69	16	106
	600		6.73	16	104
	700		6.77	16	106
	0	48	6.92	26	96
	200		6.95	26	96
	300		6.97	27	96
	400		6.99	30	96
	500		7.02	27	96
	600		7.01	27	96
	700		6.98	29	95

Table A4.8 Values of tested water parameters in the uranium 48-h acute immobilisation/lethality tests to BB *M. macleayi* populations.

Population (Exp. No.)	U conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
U-BB-1	0	0	6.45	16	108
	50		6.49	16	108
	100		6.56	16	107
	200		6.66	16	108
	300		6.56	16	108
	400		6.74	16	107
	600		6.74	16	107
	0	48	6.67	16	96
	50		6.74	16	95
	100		6.82	16	95
	200		6.87	17	96
	300		6.90	16	96
	400		6.92	16	97
	600		6.91	16	96
U-BB-2	0	0	6.49	16	107
	100		6.57	15	107
	200		6.63	15	109
	300		6.63	15	107
	400		6.65	15	106
	500		6.69	16	106
	600		6.73	15	104
	0	48	6.74	16	98
	100		6.88	16	98
	200		6.91	16	98
	300		6.89	16	98
	400		6.96	16	96
	500		6.90	16	97
	600		6.99	16	97

Table A4.9 Values of tested water parameters in the uranium 48-h acute immobilisation/lethality tests to DjB2 *M. macleayi* populations.

Population (Exp. No.)	U conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
U-DjB2-1	0	0	6.49	16	114
	100		6.61	17	114
	200		6.66	17	116
	300		6.71	17	116
	400		6.73	17	114
	500		6.72	17	114
	600		6.70	17	112
	0	48	6.93	17	100
	100		6.97	17	103
	200		7.01	17	103
	300		7.04	17	103
	400		7.06	17	103
	500		7.06	17	103
	600		7.12	17	103
U-DjB2-2	0	0	6.49	17	108
	100		6.63	17	109
	200		6.64	17	110
	300		6.62	17	110
	400		6.59	17	110
	500		6.62	17	109
	600		6.70	17	109
	0	48	6.74	17	98
	100		6.86	17	99
	200		6.91	17	99
	300		6.94	17	98
	400		6.97	17	98
	500		6.99	17	99
	600		6.99	17	98

Table A4.10 Values of tested water parameters in the uranium 48-h acute immobilisation/lethality tests to Lab *M. macleayi* populations.

Population (Exp. No.)	U conc. (µg/l)	Time (hours)	pH	Conductivity (µS/cm)	Dissolved Oxygen (%)
U-Lab-1	0	0	6.49	17	108
	100		6.63	17	109
	200		6.64	17	110
	300		6.62	17	110
	400		6.59	17	110
	500		6.62	17	109
	600		6.70	17	109
	0	48	6.84	17	100
	100		6.94	17	100
	200		6.96	17	100
	300		6.97	17	99
	400		6.73	17	99
	500		6.81	17	98
	600		6.88	17	99
U-Lab-2	0	0	6.57	17	115
	50		6.73	19	115
	100		6.74	17	117
	150		6.73	18	115
	200		6.74	18	114
	300		6.77	18	112
	400		6.71	19	113
	0	48	6.80	17	98
	50		6.91	19	97
	100		6.95	18	98
	150		6.98	19	98
	200		6.99	19	97
	300		7.06	19	97
	400		7.08	19	97