Ecotoxicological Studies of the Effects of Heavy Metals and Hydrocarbons on Antarctic and Temperate Echinoderms

by

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Submitted in fulfilment of the requirements
for the Degree of
Doctor of Philosophy

University of Tasmania

March, 2005

DEDICATION

For Dave Gardner, who was killed in an avalanche while guiding on Mount Tasman, New Zealand on 31 December 2003.

Declaration

This thesis contains no material that has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis. To the best of my knowledge and belief no material previously published or written by another person has been used except where due acknowledgement of authorship is make in the text of the thesis.

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Abstract

Contamination of Antarctic marine environments with heavy metals and hydrocarbons has occurred as a result of human habitation and activities over the past 100 years. Despite a commitment by Antarctic Treaty Nations to minimise environmental harm and remediate existing contaminated sites, there is insufficient data on the sensitivities of Antarctic marine species to set priorities or targets for clean-up efforts or to establish guidelines for water and sediment quality. As a pre-requisite to collecting these data there is need for relevant and practical toxicity testing protocols for Antarctic species.

Morphological deformities of *Abatus* spp. heart urchins from contaminated sites were quantitatively investigated. Urchins from the contaminated sites were found to be smaller, flatter and wider, particularly those from the inshore areas closest to the contamination source. Obvious deformities occurred in nearly 50% of the inshore urchins. Metal concentrations in carbonate tests of urchins from these sites appear to correlate with the observed morphological differences.

Techniques for the larval culture of echinoids with planktonic larvae are well developed, however culture of brooding species has not previously been described. Methods were developed for the collection, maintenance and transport of juvenile *Abatus* spp. with juveniles that were removed from the brood pouch and then reared for one year. This work has potential application to a range of biological and toxiciological studies of brooding echinoids.

Juvenile *Abatus ingens* and *A. nimrodi* urchins were exposed to metals in seawater for 10 days. Copper and zinc caused mortality at concentrations affecting larval development in other echinoid species. In particular copper was toxic within concentration ranges that may occur in contaminated Antarctic marine environments.

To assess accumulation of metals by heart urchins, the temperate species *Echinocardium cordatum* were exposed to sub-lethal concentrations of lead and copper in sediments for 60 days. Chemical analysis of the carbonate shell at the end of this period showed increased concentrations of copper in exposed animals,

although results varied between and within individuals. Results of high resolution elemental analysis suggest that the incorporation of metals occurs throughout the carbonate test and not only in newly deposited shell material.

The toxicity of diesel in sediments to the Antarctic ophiuroid *Ophiura crassa* and to the temperate urchin *Echinocardium cordatum* was examined over 10 days. Undispersed diesel impacted on ophiuroid movement within 24 hours although the effects were reduced over a longer period. Dispersed diesel was more toxic than undispersed diesel to ophiuroids, causing high rates of mortality. *E. cordatum* was not tested with dispersed diesel, but was far more sensitive to undispersed diesel than *O. crassa*, with mortality of all exposed animals at concentrations $\geq$ 42 mg diesel kg dry sediment^{-1}.

The methods for culture and toxicological testing described in this thesis have potential application to other related echinoderm species and toxicants. The results of the studies have relevance to the development of water and sediment quality guidelines for Antarctica.

Acknowledgements

To Chris, thank you so much for encouraging me to follow my dream and begin this mad venture in the first place, and for your support and friendship throughout all that followed.

To Martin Riddle, a huge thank you for giving me the opportunity to do this project and for the privilege of working in Antarctica that went with it. Thank you also for your support, encouragement and friendship throughout the time we have worked together.

Many thank are also due to Ian Snape for support, advice and regular reality checks.

To Paul Goldsworthy, Andrew Tabor, Letitia Bright and the other members of the Casey Dive Team, this project would not have happened without your support.

To Andrew McMinn and the staff at IASOS, thank you for your support to do this PhD and assistance throughout the time we have worked together.

Both seasons spent in Antarctica were times of forming special friendships and sharing incredible experiences. To my fellow expeditioners at Casey over the Summer seasons of 2001/2002 and 2002/2003, I will always remember those Summers as being among the most extraordinary times of my life.

Finally, to all my good friends within the Australian Antarctic Division and outside it, thank you for all the patience, understanding, encouragement, proof-reading, and most of all for the parts you all played in making the past three and a half years such an adventure.

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1 General Introduction

1.1 Why study ecotoxicology in Antarctica?

Although remote from large-scale human development, Antarctica is not free from anthropogenic contamination. The past 100 years of exploration and human habitation have left their trace on the land and the seas around the Antarctic and the sub-Antarctic. Although practices have dramatically improved over the past 20 years, there remains thousands of tonnes of refuse that have been dumped on the land and into the ocean (Cameron 1972, Deprez et al. 1999, Poland et al. 2003). Large quantities of fuel and oil products are stockpiled at research stations and along traverse routes, some of which has been there for decades. There have been numerous documented cases of fuel or oil spills in Antarctica of more than 20,000 litres and up to 260,000 litres (Hansom and Gordon 1998). Human sewage and wastewater are still disposed of by burial in the ice or ocean discharge, generally with not more than primary treatment (Lenihan et al. 1990, Waterhouse 2001). It is estimated that there is between 1 and 10 million m^3 of abandoned, unconfined refuse and a similar volume of hydrocarbon contaminated soil in Antarctica (Snape et al. 2001).

In most cases pollution is localised around the 74 (42 year round and 32 summer only (CIA 2003) research stations that currently operate in the Antarctic. The majority of these research stations are located on the ice-free coastal land that makes up only 0.05% of the continent and provides vital habitat for breeding seabirds (Poland et al. 2003). Such ice-free coastal areas are also the sites of the majority of Antarctica's few terrestrial vegetation communities (Hansom and Gordon 1998, Poland et al. 2003). Terrestrial pollution can enter marine waters and sediments by the direct run-off of liquids such as fuel or be transported by melt-waters, glacial movement or wind (Warren 1981, Hansom and Gordon 1998, Cole et al. 2000). Concentrations of hydrocarbons and metals in soils in waste disposal sites or areas used for fuel storage, refuelling areas and workshops may be high. The mobilisation of soil particles from these areas has been identified as a major source of contamination in near-shore marine environments (Cole et al 2000, Scouller 2000, Snape et al 2001). As a result of these historic and present waste disposal and storage practices there has been contamination of water, soils

and sediments by a range of metals and organic pollutants, and elevated levels of various contaminants have been measured in Antarctic wildlife. In places there is now substantial evidence of impacts on marine benthic communities resulting from the contamination of seawater and sediments (Lenihan and Oliver 1995, Kennicutt II and McDonald 1996, Stark 2000, Waterhouse 2001).

The presence of anthropogenic pollution in environments worldwide is increasingly being viewed as unacceptable. The general public is more aware of environmental protection issues generally since the "green revolution" of the 1960's and there is increasing pressure on governments and industry to clean up past mistakes and to prevent future pollution. In the Antarctic context, the Protocol on Environmental Protection to the Antarctic Treaty (Madrid Protocol) entered into force in 1998 after being ratified by all 26 Antarctic Treaty consultative parties (Joyner 2000). In addition to legislating for the prevention of future pollution, the Madrid Protocol clearly identifies the responsibility of treaty nations to remove existing waste except where this will result in greater environmental damage than leaving the site undisturbed (Annex III to the Protocol on Environmental Protection to the Antarctic Treaty: Waste Disposal and Waste Management, Article 1, Para. 5). Although philosophically admirable, cleaning up contaminated sites in Antarctica is not easily accomplished. Aside from the considerable cost and logistic difficulty involved in site remediation in such a remote and extreme environment (Poland et al. 2003), there are large gaps in our knowledge and understanding of what levels and types of pollution will in fact cause "harm". Without baseline, pre-pollution data it is difficult to know where existing contamination is causing environmental damage, or to set priorities for its control or remediation. This information is also required to set targets for clean-up of contaminated sites that will ensure environmental protection.

A great deal of research has been done into the toxicity of a wide range of substances on many of northern hemisphere temperate species. For example, the USEPA ECOTOX database includes data dating from 1915, with the results of tests of over 4,000 aquatic species and more than 7,000 chemicals (EPA 2004). The culturing and exposure methods and threshold tolerances of many of these organisms are now well established, and both government and private organisations have standardised protocols for environmental hazard identification

using key species and tests (Traunspurger and Drews 1996). Toxicity testing is required by law for many substances, particularly in cases where disposal into the environment is being considered. A range of commercially available tests are used to compare the toxicity of these substances to reference toxicants such as copper.

It may be argued that existing data can readily be applied to any other geographic location. In Australia and New Zealand, water and sediment quality guidelines are largely based on northern hemisphere data because there exists so little data on local species. However, it is well recognised that the blanket application of data from other geographic regions is not adequate, and there is an ongoing effort to gather more information regarding the sensitivities of endemic species (Batley and Maher 2001). At present the minimum toxicological data requirements considered necessary for deriving sediment quality guideline values include the need for at least 20 tests for each chemical, with studies to use two or more invertebrate species including life-cycle tests and standardised protocols (CCME *cited in* Batley and Maher 2001). It has been shown that species' sensitivities may vary substantially even within a single genus, and clearly environmental conditions and ecological interactions are not the same throughout the world. This is particularly evident in polar regions where fundamental environmental factors such as light and temperature, and subsequently the biology of the endemic species, differ greatly from temperate or tropical environments (Berkman 1992). These factors combine to create unique ecological communities and biological and chemical interactions, and the application of environmental management guidelines from elsewhere is clearly unsatisfactory.

Antarctica's remoteness and climate make collection of data on local species a challenging and expensive undertaking. During their limited time on the continent researchers face the risk of weather conditions making field work impossible or even at times restricting access to laboratory facilities. Sites and facilities may only be accessible during the summer field season, further restricting the amount of work that can be achieved. Because of these restrictions, studies of closely related or analogue species from other regions are also needed. Although region and species specific information is still essential, understanding

the relative sensitivities of Antarctic and temperate species would be valuable in developing interim guidelines and targeting Antarctic research.

The remote and undeveloped nature of Antarctic and its uniquely protected status mean that there is little demand for the type of standardised commercial toxicity tests that are used in developed regions. In the present political climate it is unlikely that anyone will be seeking permission to dispose of dredge spoils or mine tailings into the Antarctic marine environment. There is a need for testing methods to answer specific questions relevant to the protection of the Antarctic environment.

1.2 Why Study Echinoderms?

Echinoderms represent a dominant faunal group in many soft sediment and hard substrate marine habitats around Antarctica (Dell 1972, Dayton et al. 1974, Dearborn and Fell 1974, Brey and Dahm 1994). Aside from their abundance, certain echinoderm groups appear to be keystone species in Antarctic environments, shaping the benthic habitat through reworking of sediments, scavenging detritus and as predators (Dayton et al. 1974, Dearborn 1977, Brey and Dahm 1994, McClintock 1994).

With a phylogeny dating back at least 600 million years (Fell and Pawson 1966) related echinoderm species may be found throughout the world, although many Antarctic species have particular adaptations common to polar regions such as increased longevity, size and the predominance of lecithotrophic young that feed on the remnants of the egg yolk remaining in their gut, and brood protection (Thomson 1876, Dearborn and Fell 1974, Brey et al. 1995, Schatt and Féral 1996). It is thought that between 70 and 80% of Antarctic echinoderm species are endemic (Dell 1972). Despite the high level of endemism of Antarctic echinoderm species, they represent many genera that are also found in temperate and tropical regions.

In temperate and tropical regions toxicity testing using echinoderms, particularly reproductive studies of fertilisation success and embryo and larval development, is common (Hyman 1955, Fell and Pawson 1966, Binyon 1972). Well established protocols exist for a range of echinoid species to be tested against contaminants in

water and sediments. The larval development of a variety of urchin species is used as a sensitive endpoint for a range of contaminant types (Nacci et al. 1986, Traunspurger and Drews 1996, Geffard et al. 2001).

In addition to their ecological importance, echinoderms have several practical advantages for Antarctic researchers. Many species are relatively easy to collect, either by divers or in traps. A number of these species have been found to be amenable to being kept for prolonged periods in aquaria (Pearse et al. 1986b, Schatt 1988, Schatt and Féral 1991, Lenihan et al. 1995, King and Riddle 2001). Aside from the obvious advantages of using animals that are tolerant to laboratory conditions, the ability to easily maintain large numbers of animals at a time reduces the number of field collection trips needed.

As with temperate and tropical species, some Antarctic echinoderms are able to be induced to spawn gametes that can be used in toxicity testing (Bosch and Pearse 1990, Pearse and McClintock 1990, King and Riddle 2001). Urchin species with brooded young may easily provide 20 or more juveniles (Bosch and Pearse 1990, King and Riddle 2001, personal observation) suitable for testing work from a single adult female, reducing the number of animals needing to be collected and the potential impact of large-scale collection on the existing population.

The two echinoderm groups that have been used in the studies described in the following chapters are spatangoid urchins and ophiuroids. Both groups are amongst the most common representatives of Antarctic benthic fauna (Dell 1972). In addition, these organisms were selected because of their relative abundances in areas subject to contamination around the study location. The Antarctic heart urchins, *Abatus nimrodi* and *A. ingens*, are very common around Casey Station, and both have circumpolar distributions. Spatangoids are burrowing species that directly ingest sediment while maintaining contact with the overlying seawater (Binyon 1972, de Ridder and Lawrence 1982). This behavioural pattern means that they are potentially exposed to contaminants through a number of pathways including seawater, marine sediments and sediment pore waters.

The genus *Abatus* contains a total of 30 described species, all of which have marsupiate brood protection of developing young in invaginations on the adult

females' body (David et al. 2001). Some species are believed to be seasonal breeders (Magniez 1983) although both *A. ingens* and *A nimrodi* appear to have non-seasonal reproduction, with juveniles at all stages found in the females' brood pouches (Pearse and McClintock 1990, Anderson 1998). The brooding period for *Abatus cordatus* is thought to be approximately 250 days but brooding period has not been described for any other species. The young develop directly from embryos to juveniles without an intervening larval phase and do not feed during the brooding period but are nourished by yolk reserves from the large egg. Long juvenile development periods are a common feature of Antarctic marine invertebrates (Picken 1980, Pearse et al. 1991), providing an extended period during which this relatively sensitive life stage may be exposed to contamination or other impacts. *Abatus nimrodi* and *A. ingens* are both present in Brown Bay near Casey Station, which is known to be subject to contamination from historic marine dumping and a nearby abandoned waste disposal site (Deprez et al. 1999, Cole et al. 2000, Scouller et al. 2000). However, these urchins are far less abundant in Brown Bay than in nearby uncontaminated areas (Stark 2000, Stark and Riddle 2000). There is also anecdotal evidence that some of these urchins are morphologically different from those in other sites (Anderson 1998, M. Riddle & C. King, pers. comm.) suggesting some previously unreported effect of long-term pollution on the species.

When this project was first conceived it was thought that a useful temperate analogue for *Abatus* spp. would be found in the cosmopolitan spantangoid urchin *Echinocardium cordatum*, which is abundant around southern Tasmania. *Echinocardium* also directly ingest the sediment through which it burrows, although in common with most temperate echinoderms, this species spawns gametes that develop through a planktotrophic larval stage before settlement and metamorphosis (Moore 1936, Buchanan 1966, de Ridder et al. 1984). However, it was found that differences between *Echinocardium* and the *Abatus* spp. most common around Casey precluded the direct application of testing methods from one species to another. *Echinocardium* are quite an active species, burrowing rapidly and completely into the sediment. It is also possible to readily identify unhealthy urchins from discolouration and loss of rigidity of the spines. In contrast, *Abatus ingens* and *A. nimrodi* burrow only partially, move very slowly and do not show obvious signs of poor health. It was also found to be more

difficult to maintain *Abatus* in aquaria except at very low densities. Adult *E. cordatum* were used partially to develop and trial testing protocols that may be useful for Antarctic species, as well as to compare the sensitivities of the species. *E. cordatum* was also used in long-term experiments of metal uptake from spiked sediments in order to assess the biomonitoring potential of burrowing urchins in polluted sediments and to help explain morphological effects seen in *Abatus* from contaminated sites.

The Antarctic ophiuroid, *Ophiura crassa*, is found in a range of benthic habitats and is common around the Casey area. Compared to other ophiuroids in the area this species is sufficiently abundant that collection of large numbers for toxicity testing was not considered to be likely to impact on the natural populations or communities. With an average disk size of approximately 1.5 cm, *O. crassa* is also considerably smaller than many Antarctic ophiuroids (Madsen 1955), better suiting it to maintenance in aquaria and in smaller test vessels. These ophiuroids were also found to be resilient to long-term aquaria culture and transport. *Ophiura crassa* is often found on sediments where it is believed to scavenge detrital particles from the sediment surface, ingesting some sediment in the process (Dearborn 1977). These ophiuroids may therefore have an intimate association with any contaminants that may be present in the sediment matrix or the pore waters.

1.3 So what is this thesis about?

The following chapters describe some of the first steps in understanding the effects of anthropogenic contaminants on Antarctic echinoderms. In each case the development of sensible and relevant testing protocols involved learning more of the behaviour and characteristics of the species than had previously been published. This thesis details new information regarding the behaviour and maintenance of these organisms under laboratory conditions, testing protocols for these species against a range of metals and of hydrocarbons, and the sensitivities of the test organisms to these contaminants.

The main objectives of this project are to develop ecotoxicological testing methods that are relevant and applicable to:

- Antarctic benthic species that may be exposed to anthropogenic contaminants;
- the ecological role of these species in the community;
- the likely route of exposure of these species to contamination; and
- a variety of contaminant types and related benthic species, including those from other geographical regions.

Although we are gradually increasing our knowledge about Antarctic species and ecosystems, there is still a great deal more to be leaned to allow for the effective management and protection of Antarctic marine environments. It is my hope that the broad and exploratory nature of this study will provide a useful foundation for other Antarctic researchers, and that this work will contribute to the further understanding and protection of this beautiful place.

2 Morphology and Growth of *Abatus nimrodi* in Contaminated and Uncontaminated Sites around Casey Station, East Antarctica

Abstract

In an examination of potential impacts of contamination on benthic fauna, *Abatus* spp. heart urchins from sites around Casey Station were studied to determine if significant morphological differences were evident in animals from contaminated and uncontaminated locations. *Abatus nimrodi* were collected from an uncontaminated bay and from three sites within a contaminated bay during the summers of 2001 and 2002. Brooding status was assessed for female urchins and a series of measurements was made of the calcium carbonate test of each animal, with any apparent abnormalities in morphology described in detail. Measurements of the intervals between growth lines in individual shell plates were made to compare growth rates of animals from different sites. The results show that urchins from the contaminated sites were generally smaller, flatter and wider than those from the clean site, with this trend most evident in urchins from the inshore areas of the contaminated bay nearest to the contamination source. Morphological deformities in the form of deep depressions of various parts of the test were present only in animals from the contaminated bay and occurred in nearly 50% of the urchins from the inshore site. Although direct relationship of growth band patterns to annual growth rates is not certain, urchins from the contaminated site had significantly narrower growth bands than those from the uncontaminated site. Correlations were identified between measured metal concentrations in the urchin tests and the morphology and growth of animals from different sites, suggesting possible impacts on the urchins as a result of sediment contamination with metals.

2.1 Introduction

Burrowing spatangoid heart urchins of the genus *Abatus* are widespread and abundant throughout Antarctic waters. Eleven species of *Abatus* have been reported from depths of between 2 and 860 metres (David et al. 2001). In near-shore areas around Casey Station, East Antarctica the most common *Abatus*

species are *Abatus nimrodi* and *Abatus ingens*. These urchins have been observed at densities of up to 10 per metre^2 and constitute the dominant benthic macrofaunal species in soft-sediment marine environments around Casey.

Burrowing urchins are important components of the Antarctic benthos, not only because of their relative abundances but also for their role in reworking sediments. Spatangoid urchins are non-selective deposit feeders, ingesting both surface and sub-surface sediments for organic content as they burrow through the substrate (de Ridder and Lawrence 1982, de Ridder et al. 1984). In these relatively low energy environments, *Abatus* urchins have been found to turn over very large volumes of sediment each year (B. Thompson personal communication). This reworking has the effect of oxygenating the sediments and may influence the availability and partitioning of sediment bound contaminants (Widdicombe and Austen 1999, Sandnes et al. 2000).

In common with many other Antarctic echinoderm species, *Abatus* spp. have a brooding mode of reproduction (Thomson 1876). Large, yolky eggs that develop directly into lecithotrophic juveniles are brooded in invaginations, known as brood pouches, in the female urchins' bodies (Pearse and McClintock 1990, Anderson 1998). The development period is believed to be up to 250 days for *Abatus cordatus* (Schatt and Féral 1996) but has not been determined for other species. *Abatus* spp. including *A. nimrodi* appear to have a non-seasonal reproduction pattern with juveniles at all stages of development commonly found in the females' brood pouches at any one time (Pearse et al. 1986a, Pearse and McClintock 1990, Anderson 1998).

Despite the considerable ecological importance of these species there is still much about their biology and ecology that has not been investigated. Ageing techniques for sea urchins are not well established. A number of researchers have developed methods for viewing concentric growth rings within individual shell plates. These growth rings occur in cycles of opaque and more translucent test material that correspond with cycles of faster and slower growth (Jensen 1969, Pearse and Pearse 1975, Duineveld and Jenness 1984). In some species a single cycle of growth rings has been shown to be formed annually, however this is not the case for all species. Accurate ageing of urchins using growth rings would require field

studies to calibrate the patterns of growth rings in a particular species to a range of environmental variables (Russell and Meredith 2000). Longevity combined with slow growth rates compared to related temperate or tropical species is a common feature in Antarctic fauna. There are no reported studies on the growth rates or life spans of Antarctic irregular echinoids, although a study of one species of Antarctic regular urchin *Sterechinus numayeri* suggests that these urchins live in excess of 40 years (Brey et al. 1995). The very long brooding period reported for *Abatus cordatus* suggests that *Abatus* spp. urchins may also be very long lived.

Previous studies of macrofauna from sites around Casey Station include anecdotal reports of unusual morphology of *Abatus* urchins collected from Brown Bay. Brown Bay is known to be contaminated by both heavy metals and organic pollutants (Cole et al. 2000, Scouller et al. 2000), and both the faunal and floral communities of the bay are significantly different from other sites where anthropogenic contaminants are not present (Stark 2000). While both *A. nimrodi* and *A. ingens* are present in Brown Bay, they are less abundant than at other uncontaminated locations with similar physical and sedimentary characteristics. Within Brown Bay, the density of urchins increases with distance from the disused waste disposal site in Thala Valley, which is the major source of contaminants entering the bay.

Morphological abnormalities and reduced growth rates have been reported in regular urchins from the vicinity of a power and desalination plant in the Red Sea (Dafni 1980). Moore (1973) also described test abnormalities in a number of species of regular urchins collected from around the Florida Keys. In these reported cases the observed deformities are the result of abnormal skeletal growth, including absence of plates or series of plates, inclusion of additional series of plates or abnormal plate shape (Moore 1973, Dafni 1980). A direct link to the presence of contaminants has not been confirmed, however it is thought that the abnormalities may be the result of metabolic upset (Moore 1973).

As part of research aimed at developing an understanding of the impacts of sediment contamination on Antarctic marine benthos, *Abatus* spp. urchins were identified as potentially useful candidates for toxicity testing and biomonitoring. However, an essential step in developing such protocols is gaining a better

understanding of the growth and longevity of the organisms to be used. This chapter presents the results of preliminary studies on differences in the morphology, brooding status and growth of *Abatus nimrodi* collected from sites within Brown Bay and O'Brien Bay near Casey Station, East Antarctica.

2.2 Materials and Methods

2.2.1 Morphological Analysis

Specimens of *Abatus nimrodi* were collected by divers from O'Brien Bay (n=73) and three sites within Brown Bay (n=56) in the vicinity of Casey Station, Wilkes Land, East Antarctica (Figure 2.1) in December 2001 and December 2002. O'Brien Bay is unaffected by anthropogenic contaminants (Scouller et al. 2000), and urchins were collected at a range of sites within the bay from depths of between 10 and 16 metres.

Urchins were collected randomly, with all animals in each of a number of $1m^2$ plots gathered to ensure that a range of sizes and both male and female animals were collected. Brown Bay is contaminated with a range of metals and hydrocarbons as a result of historical rubbish disposal directly into the bay and from run-off of contaminated water and soil from the adjacent abandoned refuse site at Thala Valley (Deprez et al. 1999, Cole et al. 2000). The three sites in Brown Bay from which urchins were collected were approximately 20 m (Brown Bay Inner), 100 m (Brown Bay Middle) and 200 m (Brown Bay Outer) from the shore of Thala Valley. *A. nimrodi* were collected from approximately 4 m, 8 m and 10 m depth at the inner, middle and outer sites respectively. The collection sites in both bays are covered by sea-ice for approximately 8 months each year and are subject to minimal water currents or wave action. Table 2.1 details sediment characteristics and metal concentrations at each of the collection sites.

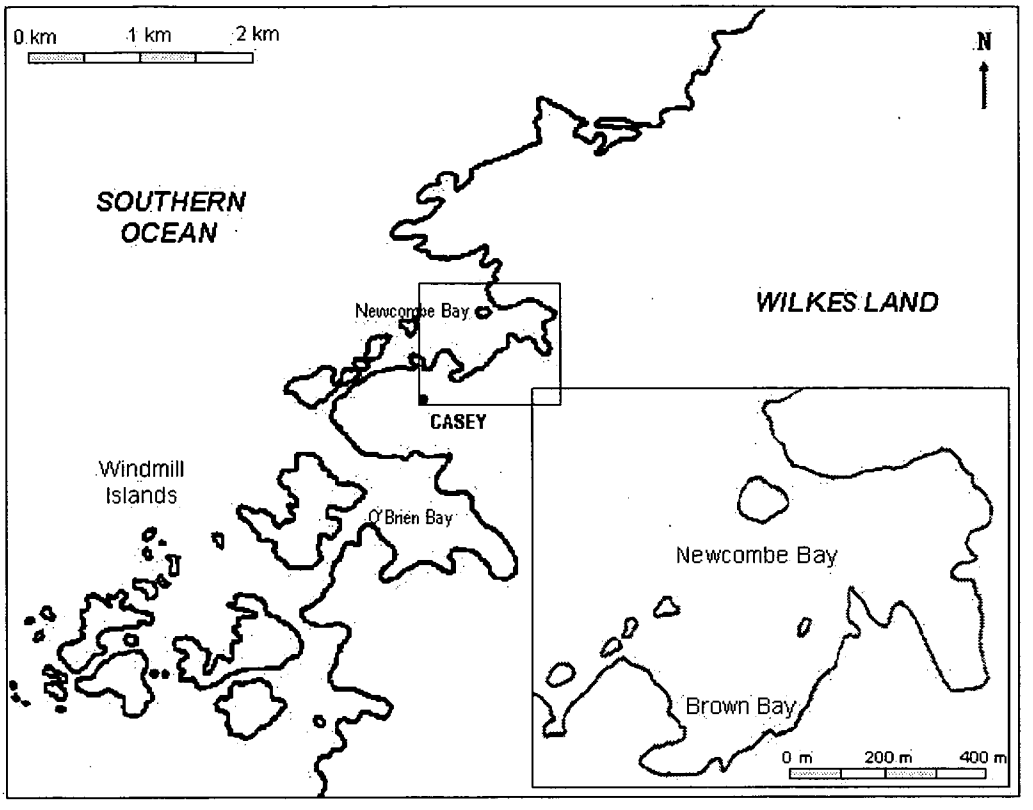

Figure 2.1 Map of Study Location in East Antarctica

Table 2.1 Sediment Characteristics and Metal Concentrations from 1M HCl Extraction (mg kg^{-1})

Characteristic	O'Brien Bay	Brown Bay Inner	Brown Bay Middle	Brown Bay Outer
Grain Size				
< 63 µm	19.7%	25.0%	40.5%	Not available
63 µm – 2 mm	79.9%	68.5%	59.0%	Not available
> 2mm	0.45%	6.5%	0.5%	Not available
Arsenic	1.30	13.42	23.76	16.29
Cadmium	0.10	0.63	.1.74	1.71
Chromium	0.84	2.48	3.44	1.40
Copper	0.62	11.09	7.16	4.51
Iron	290.1	2559.12	1055.89	422.62
Lead	0.39	43.55	20.35	6.58
Nickel	0.62	0.60	3.02	2.27
Silver	0.04	0.24	0.27	0.17
Tin	0.01	5.56	1.95	0.98
Zinc	4.77	30.73	40.70	25.44

Source: Snape et al. (2004).

Abatus nimrodi only burrow partially into the sediment and are therefore easily collected by divers with little risk of physical damage to the urchins. At each site urchins were collected from within the area without regard to size or morphology. Immediately on being brought to the surface the urchins were placed in buckets of seawater on the ice and shaded to prevent light stress. It had previously been found that when adult females were stressed due to low salinity or rough handling there was some expulsion of juvenile urchins from the brood pouches (personal observation), although whether this process is a response initiated by the adult or by the juvenile urchins is not known. Urchins were therefore handled as gently as possible throughout the collection and transport process. Urchins were kept in buckets of seawater during transport and on return to Casey Station were transferred to temperature-controlled aquaria at -1° C and shaded.

The gender of the urchins was determined by the presence or absence of brood pouches, as evidenced by the different morphology and arrangement of spines covering the brood pouches of females. This allowed female urchins to be processed first to further reduce the likelihood of juvenile expulsion. The number of juveniles was determined by gently pushing back the spines covering the brood pouch with a mounted needle and levering the juveniles out of the pouch. Although eggs and pre-hatched embryos were also present in the pouches of all females together with the more advanced, spined juveniles it was not possible to remove them intact without risking damage to the test of the female urchin. For this reason only the number of juvenile urchins with spines was counted in this study.

Detailed measurements were made of the carbonate test of each urchin using callipers. Test measurements included the length, the width at the widest point, the height from the base of the test to the apex (Figure 2) and, in females the depth of the brood pouches. At this time any obvious morphological peculiarities were noted and described. Urchins were preserved by freezing for later analysis of metal concentrations in the test.

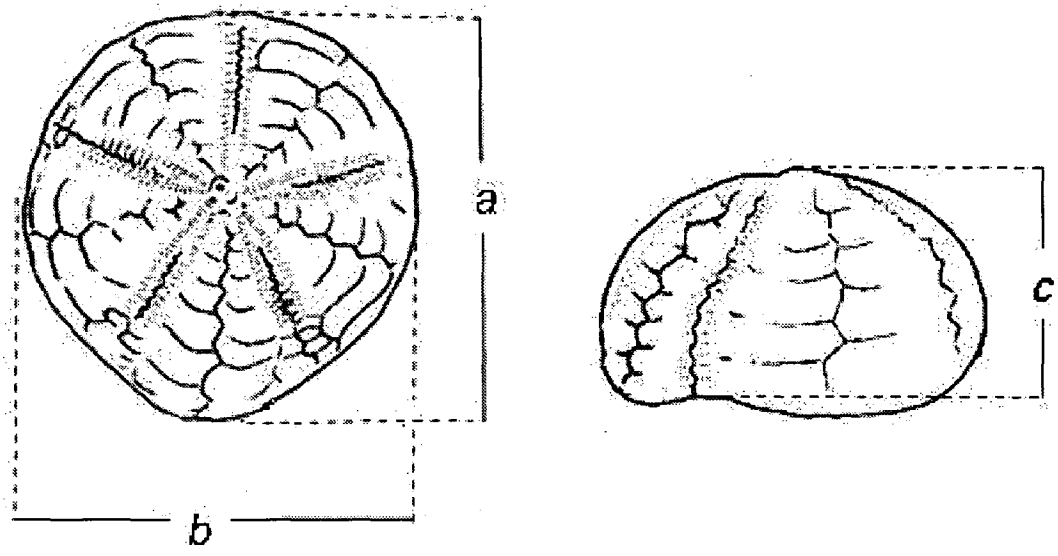

Figure 2.2 Location of *Abatus* Test Measurements: *a* - length; *b* – width; *c* – height

2.2.2 Growth Line Measurement

A sub-sample of 10 urchins from each of the sites was used for analysis of growth rings. Animals selected for growth ring analysis ranged across the span of sizes collected from each site, and equal numbers of males and females were selected. For each urchin the left front quarter of the test was dissected out and treated for 4 hours in 1:1 sodium hypochlorite solution to remove all tissue from the carbonate matrix of the test before being rinsed in distilled water and air dried. Individual plates from the middle portion of the test, above and below the marginal fasciole, were then prepared using a modification of the methods described by Jensen (1969), Pearse and Pearse (1975), and Russell (2000). These test plates were selected as they are among those formed in the initial development phase of the juvenile urchin (Gordon 1926), as well as being large enough to enable handling. Test plates were charred by repeatedly passing them through a 70% alcohol flame for approximately 40 seconds. The charred plates were then gently wet-sanded on both sides with 600 grit sandpaper to remove the outer callus and spine bosses. The sanded plates were washed in distilled water and dried before being mounted onto glass slides using a xylene-based mounting medium (DPX mountant).

Mounted slides were viewed on a dissecting microscope with transmitted light and digitally photographed. Between 5 and 15 growth cycles, each consisting of an opaque (*o*) and a translucent (*t*) band, were generally visible on each prepared plate using this technique (Figure 3). Clearly defined sequences (*s*) of growth

lines were measured using digital callipers. These measurements were then calibrated to a known distance and converted to millimetres for analysis.

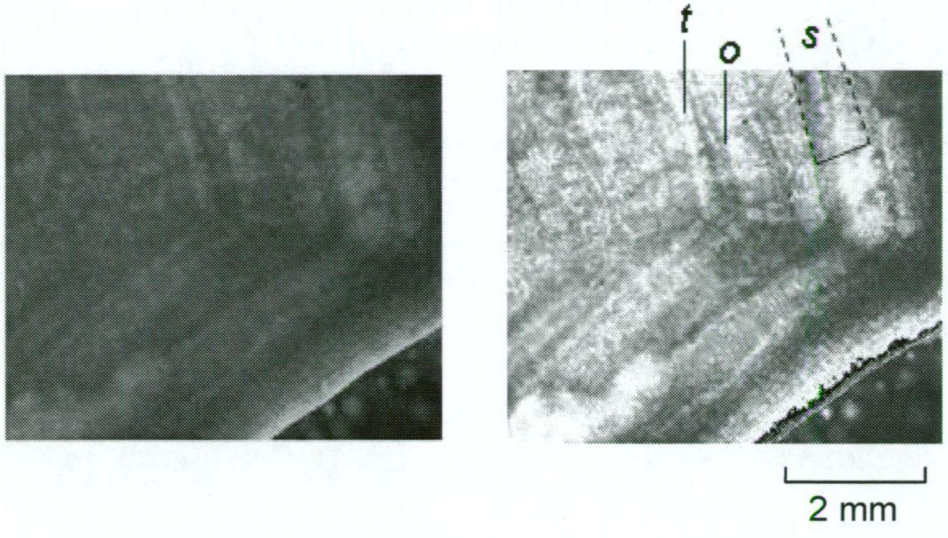

Figure 2.3 Digital Microphotograph of Prepared Shell Plate and Features Measured for Growth Analysis *t* - Translucent Band, *o* - Opaque Band, *s* - Complete Growth Series

2.2.3 Metal analysis

Four plates were dissected from the tests of each of three urchins from the inner, middle and outer Brown Bay sites and from O'Brien Bay. Plates were cleared of tissue by soaking in 30% analytical grade hydrogen peroxide at 40°C for 24 hours. Cleared plates were rinsed thoroughly in Mili-Q deionised water and dried for 48 hours at 50°C to a constant weight. Fragments of the test plates were weighed on a high precision analytical balance to allow calculation of the dilution for each sample, and ~20mg of the test material was digested in 1 ml high purity nitric acid. The weight to Digests were further diluted to 20 ml with Mili-Q deionised water and analysed for a suite of metals by ELEMENT High Resolution ICP-MS (Finnigan-MAT, Bremen, Germany). Indium was added to urchin samples at a concentration of 100 ppb as an internal standard. External calibration used multi-element mixed standards from QCD Analysts, USA, and calibration accuracy was checked by running a NIST 1640 Standard Reference Water for each calibration made. The final results were normalised to a constant percentage of calcium (33.7%) based on the average calcium for all samples to reduce the effects of any weighing errors resulting from the extremely low density of the shell material or other analytical errors.

2.2.4 Statistical analysis

One-way ANOVA was used to determine whether there were significant differences in brooding status and shell measurements, with all results calculated to 0.01 significance level. Regression statistics were used to examine the relationship between shell measurements and metal concentrations. All analysis was done on untransformed data.

2.3 Results

2.3.1 Brooding Status

There was not a significant difference in the proportion of females with brooded juveniles between O'Brien Bay and Brown Bay or between different sites in Brown Bay. Of the female urchins collected from O'Brien Bay in 2001, 40% were brooding spined juveniles. Within Brown Bay the lowest proportion of females with spined juveniles occurred at the inner and middle sites, both with 40% of the females brooding spined juveniles. At the outer Brown Bay site 75% of the females were brooding at the time of collection. The average number of spined juveniles in the female urchins' brood pouches was 12.2 for Brown Bay urchins and 15.1 for O'Brien Bay urchins, but was not significantly different between the two bays or between sites within Brown Bay. There was some variation in the depth of the brood pouches of animals collected from Brown Bay, which is described below. No juvenile urchins were found in brood pouches less than 4 mm in depth.

2.3.2 Test Measurements

The average length of *Abatus nimrodi* collected was significantly different between O'Brien Bay and Brown Bay ($P = 4.37 \times 10^{-10}$), with the Brown Bay urchins being shorter on average by >10 mm. The average width and height of the urchins from the two sites were not significantly different. However, the ratios of height:length and width:length were both significantly different between the two sites. On average, Brown Bay urchins were significantly wider relative to their length ($P = 1.04 \times 10^{-5}$) but flatter relative to length ($F = 21.89$) than O'Brien Bay urchins.

Further assessment of the differences between the three sites within Brown Bay also showed differences in morphology. There were no significant differences in the average length or width of urchins collected from any of the Brown Bay sites, however the height of urchins from the outer site was significantly greater than for either the middle or the inner site ($P = 0.009$). This also corresponded to a significantly greater height of the urchins relative to their length ($P = 0.027$). The ratio of width to length of the urchins also varied between Brown Bay sites, with the urchins from the inner site being significantly wider relative to their length than the urchins from the inner or middle sites ($P = 0.006$).

Pouch depth of females was not significantly different between O'Brien Bay and Brown Bay animals. Pouch depth in females from O'Brien Bay was generally very consistent across all of the four pouches in a single urchin. In contrast, some of the Brown Bay urchins showed large differences in depth of up to 5 mm between the pouches of a single female. Table 2.2 summarises all urchin test measurement data.

Table 2.2 Averages and Range of Test Measurements of *Abatus nimrodi* from O'Brien Bay and Brown Bay

Average Measurement	O'Brien Bay	Brown Bay Outer	Brown Bay Middle	Brown Bay Inner
Length (mm)	48.3 (31.0 – 63.0)	37.4 (28.0 – 44.0)	33.9 (23.0 – 44.0)	35.9 (26.0 – 46.0)
Width (mm)	42.6 (28.0 – 60.0)	39.7 (26.0 – 42.0)	35.9 (21.0 – 42.0)	37.4 (25.5 – 43.0)
Height (mm)	27.8 (13.5 – 38.5)	24.2 (15.0 – 29.5)	19.2 (8.0 – 29)	18.9 (12.0 – 25.0)
Pouch Depth (mm)	6.8 (3.0 – 12.0)	7.7 (<1.0 – 12)	not measured	not measured
Width : Length	0.89 (0.84 – 0.97)	0.94 (0.86 – 1.00)	0.94 (0.89 – 1.00)	0.98 (0.91 – 1.03)
Height : Length	0.56 (0.42 – 0.67)	0.61 (0.50 – 0.89)	0.53 (0.32 – 0.66)	0.51 (0.32 – 0.64)

2.3.3 Abnormalities in Morphology

No cases of visible asymmetry were found in any *Abatus nimrodi* collected from O'Brien Bay during the two years of the study and the morphology of these

specimens was consistent with images and descriptions of this species in the literature (Anderson 1998, David et al. 2001). Of the urchins collected from Brown Bay almost half of the animals from both the inner and the middle sites displayed obvious deformities. Of the 20 urchins collected from the outer site only one showed abnormal morphology. The nature of test abnormalities observed and their frequency are described in Table 2.3 and illustrated in Figures 2.4 and 2.5, and can be classified into four general patterns including: depression of the interambulacral petals (Fig. 2.4, 10.) or ambulacral grooves (Fig. 2.4, 7); bilateral asymmetry (Fig. 2.4, 6); deep depressions on the dorsal or oral surface (Fig 2.4, 8 and 11); and generally flattened appearance of the test (Fig. 2.4, 9). Several of the urchins, particularly those collected from Brown Bay Inner, displayed more than one deformity type.

2.3.4 Growth Bands

Differences in the width of growth bands were evident in urchins from different bays and sites within Brown Bay. The narrowest growth bands were observed in urchins from the inner Brown Bay site with an average width of 0.12 mm. Urchins from both the middle and outer Brown Bay site had an average of 0.16 mm between growth bands, although this was not significantly greater than at the inner site. Urchins from O'Brien Bay had significantly wider growth bands than urchins from all Brown Bay sites, with an average width of 0.22 mm.

Table 2.3 Occurrence of Irregularities in *Abatus nimrodi* Tests from Brown Bay

Nature of Irregularity	Brown Bay Inner	Brown Bay Middle	Brown Bay Outer
Bilateral asymmetry	7	0	0
Depression of front interambulacral petal	5	5	1
Deep frontal ambulacral groove	5	0	0
Depression in oral surface	4	0	0
Depression in dorsal surface	2	0	0
Flattened appearance	1	3	0
Depression in both dorsal and oral surface	1	1	0
Multiple irregularities	6	2	0

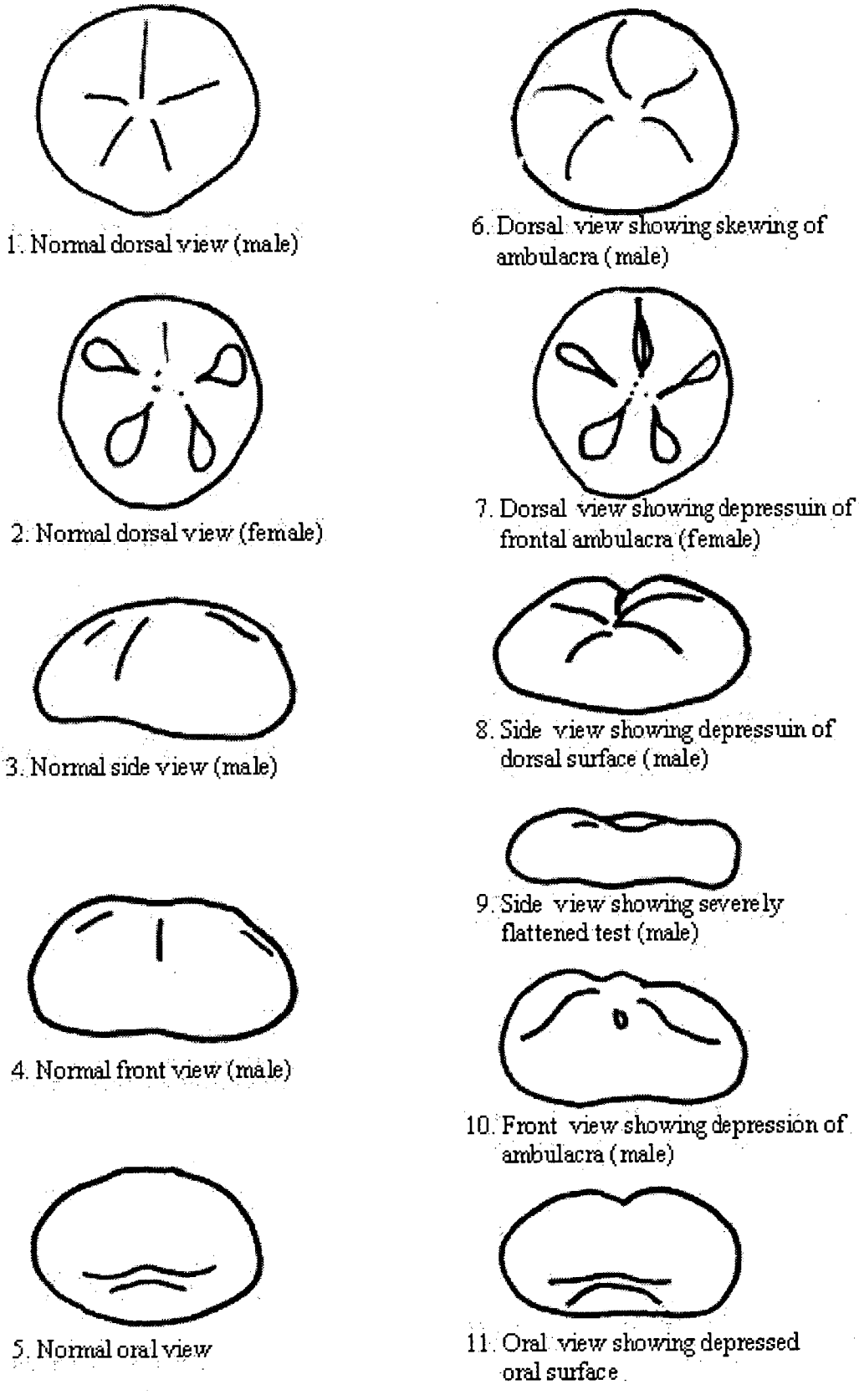

Figure 2.4 Morphological Irregularities in *Abatus nimrodi* from Brown Bay

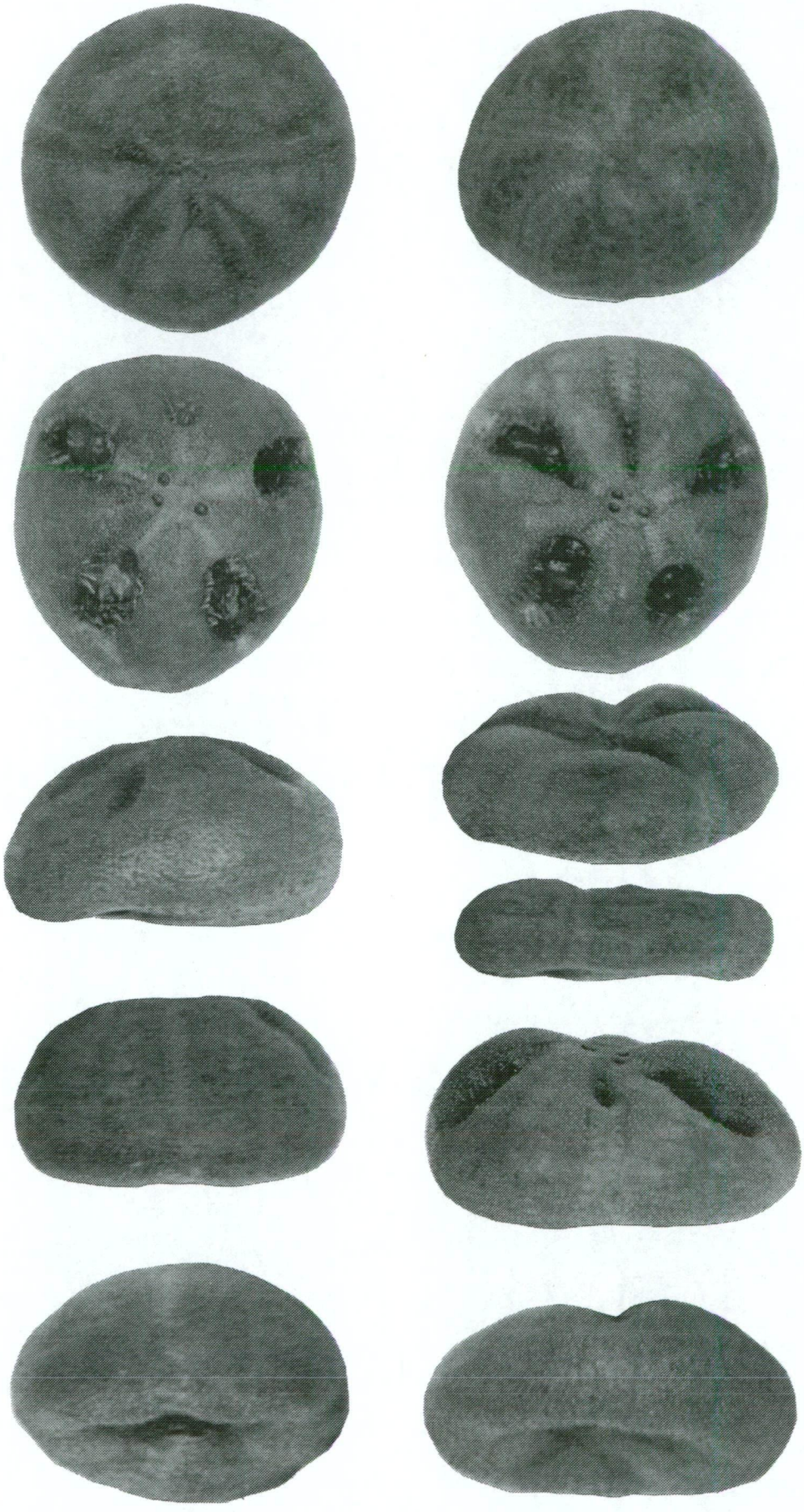

Figure 2.5 Photographs of Morphological Irregularities in *Abatus nimrodi* from Brown Bay (Images correspond to position of illustrations in Figure 2.4.

2.3.5 Metal Concentrations

The average concentrations of metals measured in urchin tests from each site are shown in Table 2.4. The metals for which differences between sites were most evident include barium, cadmium, copper, iron, lead and zinc. In particular copper, iron, lead and zinc occurred in the highest concentrations in urchins from the Brown Bay innermost site. Barium and cadmium concentrations were also consistently higher in Brown Bay urchins than those from O'Brien Bay although with little difference between urchins from the different the Brown Bay sites.

Table 2.4 Average Metal Concentration in Urchin Tests (mg kg^{-1})

Metal	O'Brien Bay	Brown Bay Inner	Brown Bay Middle	Brown Bay Outer
Ba	2.08	33.05	44.67	48.35
Cd	0.02	0.13	0.17	0.14
Cu	0.02	0.79	0.21	0.01
Fe	6.89	172.48	15.95	16.21
Pb	0.01	1.60	1.06	0.63
Zn	0.75	3.74	0.43	0.63

2.4 Discussion and Conclusions

This work reveals some clear differences in *Abatus nimrodi* collected from different sites around Casey Station. The brooding status of female urchins, as indicated by the presence and numbers of brooded, spined juveniles, did not vary significantly at different sites although on average there were fewer juveniles in the pouches of Brown Bay urchins. This may be partly accounted for by the differences in morphology of the brood pouches, with several animals from Brown Bay having one or more very shallow pouches in which there were no juvenile urchins. Adult urchins are less abundant in Brown Bay than at other locations, and this may be a consequence of reduced breeding success, although adult mortality may also be a factor.

The most pronounced differences between locations were in the size and morphology of adult urchins. *Abatus nimrodi* collected from O'Brien Bay were significantly longer than those from any of the sites in Brown Bay. The width and height of the urchins relative to their length also varied between sites, with Brown

Bay urchins on average being wider and flatter in profile than those from O'Brien Bay. This morphology was most pronounced in urchins from the innermost site at Brown Bay and expressed to a lesser extent in the animals from the site furthest from the shore. Although the data are limited for confident regression analysis, the highest observed correlation between metal concentration in the tests and height:length of the urchins was found for copper ($R^2 = 0.62$). The width:length of the urchins correlated most strongly with test concentrations of lead ($R^2 = 0.93$).

The functional consequences of altered morphology were not assessed in this study. However, it is possible that the burrowing and movement of the urchins through the sediment would be affected by alterations to the test shape. Sediment ingestion by the urchin is directly linked to its movement through the substrate and if this is less than optimal the growth, reproductive success and survival of the urchin may all be reduced.

Pronounced abnormalities in the shape of the test were entirely restricted to animals from Brown Bay and were most common in the innermost site. Deep depressions in either the dorsal or oral surface of the test or of an interambulacral plate series were seen in more than 20% the urchins collected from the inner and middle Brown Bay sites. Other abnormalities included bilateral asymmetry, an apparent skewing of the ambulacral plate series and very low height to length ratios. In common with the other observed differences in size and shape it is not known whether these irregularities have any consequences for the health and survival of the urchin, although burrowing and movement may well be affected to some degree.

There were significant differences in the patterns of growth as measured by growth lines within the shell plates of individual urchins. *Abatus* from O'Brien Bay had the widest growth bands, and the urchins from the innermost site at Brown Bay had the narrowest. There was a negative correlation between growth band width and lead ($R^2 = 0.92$) with lesser negative correlations between growth band width and cadmium ($R^2 = 0.63$), copper ($R^2 = 0.60$) and iron ($R^2 = 0.58$). The timing of growth line formation in this species is not known, and as a consequence we cannot with certainty interpret differences in band width as indicating differences in growth rates. A variety of factors may influence the

cyclical pattern of growth lines in urchin tests including seasonal pulses of food availability due to summer plankton blooms, sea-ice cover affecting light penetration to the benthic surface, summer/winter light differences, and slight seasonal changes in water temperature. Some of these factors such as the diurnal light cycle, seasonal plankton blooms and water temperature will not vary substantially between different sites. Other factors such as sea-ice cover and sedimentation from terrestrial sources may vary between sites and also from year to year.

Despite the lack of certainty about the direct relationship of the growth lines to annual growth, it seems likely that animals from Brown Bay are slower growing than those in O'Brien Bay. This is supported by the significantly smaller size of the Brown Bay urchins and differences in development demonstrated by the observed deformity of many of these animals. Slower growth and reduced size may have a number of ecological consequences. Animals will be vulnerable to predation for a more extended period. Particularly in the case of females that require a certain test and pouch size to accommodate brooded juveniles, they will take longer to become reproductively mature.

Although a variety of natural factors have the potential to influence carbonate shell growth, the results of this study suggest that contamination in the form of heavy metals pollutants from the Thala Valley tip site may be responsible for the observed differences in growth and morphology of urchins between Brown Bay and O'Brien Bay. This is supported by the observed correlations between metal concentrations and the height and width of the animals relative to their length and to the width of the growth bands of urchins from different sites. Heavy metals and hydrocarbons have been demonstrated to affect the development of regular sea urchin embryos and larvae in a number of studies (Pagano et al. 1982, Dinnel et al. 1987, Vashchenko and Zhadan 1993, King and Riddle 2001). Copper and zinc in seawater are toxic to juveniles of *Abatus nimrodi* and *A. ingens* at low concentrations (Chapter 4), and diesel contaminated sediments are highly toxic to the temperate heart urchin *Echinocardium cordatum* (Chapter 6). Additionally, the benthic fauna and flora of Brown Bay are significantly depauperate compared to uncontaminated sites around Casey, with densities of *Abatus* lower in Brown Bay and particularly at the inner Brown Bay site.

Several of the observed differences in Brown Bay urchins may have negative effects on the fitness and survival of individual urchins and potentially on population numbers. Because of its contribution to sediment bioturbation, *Abatus* is likely to be a keystone species in Antarctic sediment communities, and any significant impact on urchin populations may have substantial ecological consequences. Smaller size or any impairment of the movement and burrowing of animals will directly impact on the degree of oxygenation and movement of sediments by urchins in these locations, with potential impacts on chemical cycling and community structure.

This work demonstrates smaller size, with a high likelihood of reduced growth rates, and abnormal development of *A. nimrodi* in Brown Bay that are consistent with anthropogenic pollution. The combination of sensitivity to long-term contamination together with its important role as a keystone species indicates that further toxicity investigations of *A. nimrodi* and its potential for bio-monitoring of contaminants in Antarctica is warranted.

3 Methods for Collecting and Rearing Juveniles of the Antarctic Urchins *Abatus nimrodi* and *A. ingens* and Applications to Toxicity and Development Studies

Abstract

Techniques for the induced spawning and larval culture of sea urchins with planktonic larval stages are well developed (Tyler 1949, Heslinga 1976, Pagano et al. 1982), however no published studies describe the captive culture of any echinoderm species with brooding mode of reproduction. In common with many Antarctic echinoderm species, irregular sea urchins of the genus *Abatus* brood lecithotrophic young for an extended period of several months. *Abatus* spp. heart urchins are potentially keystone species in Antarctic soft-sediment environments, yet very little is known about the juvenile life stages of these animals. This paper describes techniques for the collection, maintenance and transport of juvenile *Abatus nimrodi* and *A. ingens*, that have been used to successfully rear juvenile urchins outside the adult brood pouch for periods in excess of one year. During this period the juvenile urchins made the transition from the lecithotrophic phase to the stage of sediment burrowing and feeding.

3.1 Introduction

Abatus spp. heart urchins are widespread and abundant in Antarctic waters, with a total of 11 species collected from depths between 2 and 3800 metres. More than 30 species of Antarctic echinoid that have brood protection of developing young have been identified, including all known *Abatus* spp. (David et al. 2001). In these urchins fertilised eggs are retained in invaginations in the body wall of the female urchin and develop directly from embryos to juvenile urchins without an intermediate larval stage (Thomson 1876, Pearse et al. 1985).

Little is known of the brooding ecology of irregular urchins or of the advantages of brood protection in these species, although the brooded young are clearly protected to some extent from predation. The temperate echinoid *Echinocardium cordatum* has been shown to circulate water from above the sediment around it's body while burrowing (de Ridder and Lawrence 1982), and although this has not

been demonstrated for other irregular echinoids, it is possible that female *Abatus* may similarly flush oxygenated water through the brood pouch. There appears never to be any sediment in the brood pouches of *Abatus nimrodi* or *A. ingens* (personal observation). The exclusion of sediment from the pouches may result in higher levels of dissolved oxygen and reduced bacterial activity in the water surrounding the juveniles than in the local sediment environments. Where adult *Abatus* are exposed to temperature or salinity stress they produced large quantities of thick mucus that covers the outer surface of the animal. Healthy juveniles have been recovered from the pouches of adults that have been exposed to salinities as low as 10 g l^{-1}, from which the adults subsequently died (personal observation). It may be that the mucus layer prevents or limits water exchange into the brood pouch and protects the juveniles from salinity shock or other adverse water quality conditions.

There are well established methods for the artificial spawning and culture of many species of sea urchin and other echinoderms with planktonic larval phases, but not for species with brooded young. Aquarium culture is used in commercial sea urchin fisheries and is also used in standardised toxicity testing protocols and biological research. In the present study of the juvenile development and potential use in toxicity testing of *Abatus nimrodi* and *A. ingens*, juvenile urchins were removed from the brood pouches of adult females and reared in aquaria for more than one year. As these species are not seasonal reproducers, immature urchins at all stages of development, from unhatched eggs to long-spined juveniles are commonly found in the pouches of adult females (Anderson 1998, Pearse & McClintock 1990, personal observation). The eggs of these urchins are large and yolky and the developing young are lecithotrophic (Pearse and McClintock 1990, Schatt and Féral 1996, Anderson 1998). Although not all females have young at all times, it was found that approximately 50% of the females collected from November 2002 to January 2003 were brooding. Anderson (1998) reported a clutch size of up to 54 embryos per female for *A. nimrodi* from around Casey Station, and Pearse and McClintock (1990) found a mean clutch size of 30 for this species collected in McMurdo Sound. In the present study there was an average of 16 spined juveniles for each female, although eggs and unhatched embryos were not counted.

It is not known how long the juveniles of these species take to develop to the point of leaving the brood pouch. Spined *Abatus nimrodi* and *A. ingens* juveniles in the pouch range from 2.6 mm to 4.9 mm in diameter (Anderson 1996, personal observation). Schatt (1988) suggests that juveniles of *A. cordatus* spend approximately 250 days in the brood pouch, and larvae of the lecithotrophic regular Antarctic urchin *Sterechinus numereyi* are believed to spend up to 3-4 months in the plankton before settling as juvenile urchins (Bosch et al. 1987). The extended period of juvenile development is characteristic of Antarctic marine species, and is far longer than the approximately 4 weeks of planktonic development before metamorphosis and settlement of the temperate heart urchin *Echinocardium cordatum* (Gordon 1926)

This paper describes techniques developed for the collection and rearing of the juvenile urchins through the lecithotrophic stage of development to the stage where they are feeding and burrowing in sediments. Methods for transport of the juveniles are also described and potential applications for the laboratory culture of these species are discussed.

3.2 Culture Techniques

3.2.1 Collection

Adult *Abatus ingens* and *A. nimrodi* were hand collected by divers from depths of between 10 and 18 m from O'Brien Bay, near Casey Station, Wilkes Land, East Antarctica and immediately placed into buckets of seawater placed on the sea-ice. The densities of urchins were kept down to ≤ 10 urchins in 15 litres of seawater. Shading was provided to prevent light stress to the animals, which are usually protected from high light levels by sea-ice cover or by dense plankton blooms during the summer months. Within two hours of collecting the adult urchins any brooded juveniles present were collected by using a thick mounted needle to push back the spines covering the brood pouch and gently lever the juvenile urchins from the pouch. The juvenile urchins were maintained as described below and were examined under a dissecting microscope after four days. At this time approximately 9% of the juvenile urchins were either not moving or showed signs of physical damage sustained during collection and were discarded.

3.2.2 Maintenance

Immediately following removal from the brood pouch the juveniles urchins were placed into pre-soaked 1-litre glass beakers filled with clean, filtered (23 μm) seawater at -1°C ± 0.5°C. Seawater was collected from a control location in O'Brien Bay. Beakers were not aerated and were covered with watch glasses to minimise water movement and evaporation leading to elevated salinity. Approximately 500 ml of the seawater was replaced every third day. With a density of not more than 40 animals per beaker the pH levels remained stable at ~ pH 8 and dissolved oxygen remained above 12 mg l^{-1}.

Although unhatched eggs and pre-metamorphosed embryos were kept for up to 3 weeks it was difficult to determine the health of these young other than by signs of decomposition several days following mortality. However, the more advanced, spined juveniles (Plate 1) could be readily observed to move under a dissecting light microscope and also showed signs of distinctive discolouration, becoming very pale, if not healthy. The average test length of the spined *Abatus nimrodi* juveniles was 3.7 mm (n = 215). On their removal from the brood pouch the peristomial membrane covering the mouth of all the juveniles examined appeared to be intact, suggesting they had not developed to the point of sediment feeding. Dissected animals had no sign of internal gut structure but contained the remains of the yolky egg mass that apparently nourished them during development.

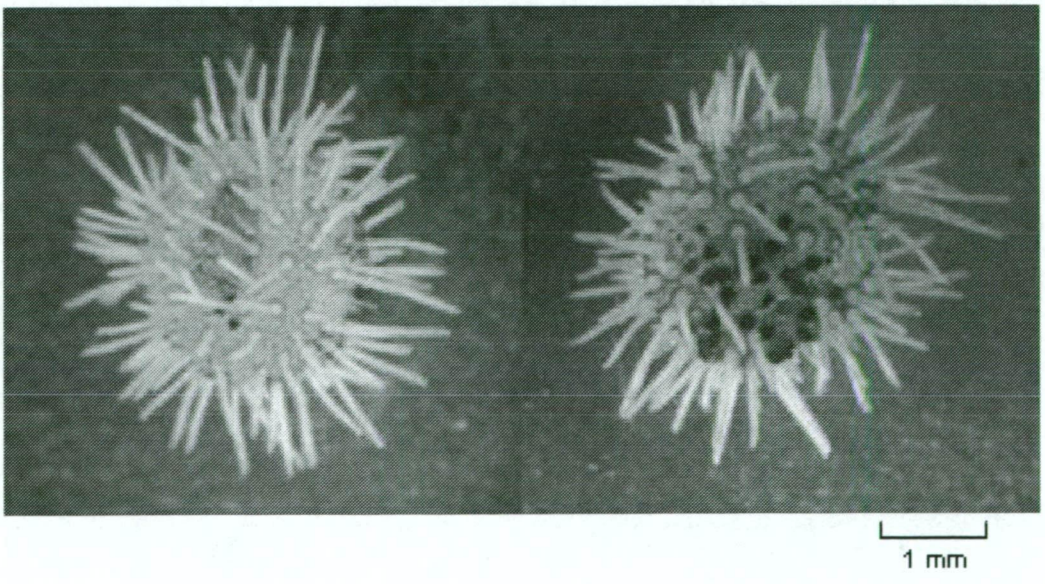

Figure 3.1 Microphotographs of *Abatus nimrodi* (left) and *A. ingens* (right) juveniles

The juvenile urchins were maintained for a period of 9 weeks using laboratory facilities at Casey Station. Every week the *Abatus nimrodi* juveniles were transferred into shallow dishes and photographed using a digital camera (Nikon Coolpix 995) mounted on a Leica dissecting microscope. These photographs were later viewed using Adobe Photoshop 6.0 and the length of the juvenile urchins measured along the longest visible axis of the test. The precision of this measurement technique was ≥95%. Growth rates over this 9 week period were extremely slow, with an average increase in length of only 0.17 mm (n = 120). Approximately 40% of the *A. nimrodi* juveniles died during this time. The *A. ingens* juveniles were not handled or measured during this same period, and suffered far lower mortality rates of approximately 10% over this period.

3.2.3 Transport

In March 2003 approximately 130 *Abatus nimrodi* juveniles and 110 *A. ingens* juveniles were transported by ship to Tasmania. Juvenile urchins were transferred into pre-soaked, one-litre lidded plastic pots containing clean, filtered seawater at a density of 20 juveniles per pot. The pots were floated in 60-litre drums filled with seawater to protect them as far as possible from the movement of the ship during the voyage. The drums were transported in a 1°C refrigerated shipping container throughout the voyage. Every third day approximately half the volume of seawater in each pot was replaced. During the 8-day voyage there were several days of very rough sea conditions and 15 % mortality of the juveniles occurred during the trip. The remaining juvenile urchins were transferred to 1-litre beakers of clean seawater and maintained in laboratory facilities at the Australian Antarctic Division at 0°C ± 0.5°C.

3.2.4 Transition to Sediment

One week after arrival in Australia it was observed that the peristomial membrane of many of the juveniles appeared to have opened, suggesting that they may have been ready to begin feeding. At this time 100 *Abatus nimrodi* juveniles were moved onto approximately 1 cm deep sediment in 1-litre beakers. The sediment used was a fine silty mud collected from O'Brien Bay near the collection site of the adult urchins. The sediment was sieved to 500 μm to remove resident macro-

fauna and stored at 4°C. The sediment was washed twice by mixing with clean seawater, drained and placed in beakers with seawater one week before the juveniles were introduced. Immediately prior to the introduction of the juvenile urchins to the sediment the overlying seawater was replaced. The density of juveniles was kept to approximately 20 per beaker and half of the overlying water was exchanged every 2-3 days. All of these urchins died within 14 days. The reason for this additional mortality is unknown, although the sudden change in water quality from the addition of sediment may have been responsible. It is also possible that the juveniles were not sufficiently developed to the stage where they would naturally have been exposed directly to sediment.

After a further 4 weeks less than 5% mortality had occurred among the *Abatus ingens* juveniles, which were being maintained in seawater without sediment. As these juveniles also appeared to be ready to begin feeding it was decided to move these animals onto sediment. To minimise adverse effects on dissolved oxygen and ammonia concentrations only a shallow (~ 5mm) layer of washed sediment was placed into 1-litre beakers and pre-conditioned in a 15-litre glass aquarium with constant biofiltration for one week. Ten juvenile *A. ingens* were then introduced into each of four beakers. A further ten urchins were placed in a beaker without sediment in the same aquarium. Survival of the juveniles on the sediment was 100% over the following 60 days, whereas all but two of the juveniles without sediment died within this period. Salinity in the aquarium was checked and adjusted to approximately 34 ppt twice a week. Every second week 5 litres of the aquarium seawater was replaced. Ammonia levels were measured weekly using a Hach DR/2000 Direct Reading Spectrophotometer and were always below detection limits.

For the first two weeks after transfer the juvenile urchins remained on the sediment surface but gradually were observed to burrow either partially or completely into the sediment. At this point fortnightly feeding was commenced to ensure that organic material in the sediment did not become depleted. Food provided was a mixture of live algae including *Gemingera cryophila*, *Phaeodactylum tricornatum* and *Pyraminonas gelidicola* at a rate of approximately 3.5×10^7 cells per litre of aquarium water. As the juvenile urchins have grown and burrowed more deeply into the sediment the depth of the

sediment has been gradually increased up to 2 cm. After 15 weeks the beakers were transferred into a 70-litre aquarium with constant biofiltration and aeration.

A total of 27 *Abatus ingens* juveniles have now been maintained for in excess of a year in the laboratory using the above techniques. During a one year period they have been observed to grow an average of 1.2 mm in length. Although this work has demonstrated the ability to grow juvenile *Abatus* for a prolonged period outside the brood pouch, further study is important to determine how the mortality and growth rates of aquarium reared urchins relate to animals in natural conditions.

3.3 Research Applications

3.3.1 Toxicity Testing

Adult *Abatus* urchins are not easily maintained in aquarium conditions, requiring a large volume of water and sediment, in addition to continuous high levels of biofiltration (personal observation, B. Thompson pers. comm.). Additionally, adult urchins move only slowly and observation of the state of health or mortality is quite difficult. Collection of sufficient urchins for a well replicated experiment would involve either removal of large numbers from a population in an area or many separate diving collection trips. Due to these factors, these adult urchins are not well suited to laboratory-based toxicity testing. In contrast, juveniles of these species are small and easily maintained in 1-litre beakers in sufficient numbers to allow for adequate experimental replication. Their small size also facilitates the transport of animals from the collection site to the research station or to other countries. The ability to obtain multiple juveniles from a single female urchin has the additional advantage of limiting the number of adults removed from the collection site.

Controlled toxicity experiments have been conducted using pre-feeding juveniles of both species exposed to heavy metals in seawater (Chapter 4). The juvenile urchins appear to be a suitable organism for short-term toxicity testing due to their high survival rates in control conditions and obvious response to toxic stress by ceasing movement. The juvenile urchins were particularly sensitive to copper in seawater, with concentrations of only 21 $\mu g\ l^{-1}$ causing significant mortality of *Abatus ingens*. Interestingly, the sensitivity of the juvenile *Abatus* to copper and

zinc is comparable to that of embryonic and larval stages of regular urchins. Juveniles that have reached the stage of feeding are likely to be suitable for sediment toxicity testing to determine threshold concentrations affecting survival, growth, morphological development and metal uptake.

3.3.2 Growth and Development Studies

Information on the growth and development of *Abatus* spp. and other brooding echinoderms is extremely limited. The importance of these species in Antarctic environments and their potential vulnerability to environmental disturbance or pollution warrants improved understanding of the biology and ecology of these urchins.

The ability to raise juveniles of brooding species in the laboratory provides a valuable research tool into improved understanding of these animals. The short Antarctic summer field season, and logistic and financial barriers to working in Antarctica, make research of these very slow growing species under natural conditions extremely difficult. By transporting juvenile urchins from Antarctica research can easily be extended to allow research into long-term growth and development.

Despite the advances in laboratory culture of these species described above, caution should be exercised in relating patterns of growth, development and survival of juveniles of brooding species raised in the laboratory to those living in the brood pouch. Effects may result from handling during removal from the pouch, and there needs to be further study into the possible nutritive and other functions of the female brood pouch. Further studies comparing the growth and development of juveniles maintained using these methods and those left in the females' brood pouches would help to resolve these questions.

3.4 Conclusions

This work demonstrates that it is possible to keep alive and grow juveniles of two species of spatangoid urchin outside the adult brood pouches, and is the first known description of the laboratory maintenance of juveniles of any brooding echinoderm species. The critical factor in maintaining the juvenile urchins

appears to be high water quality. Handling of the juveniles appears to increase mortality rates and should be kept to a minimum. Transport of juvenile urchins over long distances is possible as long as buffering from movement is provided and suitable water quality and temperature are maintained. The transfer of the urchins to sediment must be done when the juveniles are ready to begin feeding. In this study this stage was indicated by the disappearance of the peristomial membrane covering the juveniles' mouths and also by increased mortality in animals kept in seawater without sediment. It was found that washing and pre-soaking of the sediment, using a large volume of overlying seawater and the use of biofiltration improved survival during the transition to sediment. The use of flow-through seawater systems would likely be highly suitable for culturing these species, providing that water temperatures are well controlled.

It is not possible to relate the observed growth and development of juvenile urchins cultured in the laboratory to those developing in the adult brood pouches as there is no existing information on natural growth rates of these species. However, there has been obvious growth of the laboratory cultured juveniles, and successful development from the lecithotrophic phase to sediment ingestion and burrowing. This culturing technique would likely be suitable for other species of brooding echinoid and represents a valuable method for providing specimens for toxicological testing and for the study of growth and development of urchins over time.

4 The Toxicity of Heavy Metals to Juveniles of the Antarctic Heart Urchins *Abatus nimrodi* (Koehler) and *Abatus ingens* (Koehler)

Abstract

Although toxicity testing protocols using embryos and larvae of temperate echinoderms are well established, very limited toxicity testing data are available for Antarctic species or for juveniles of any echinoderm species. *Abatus* spp. are burrowing heart urchins that are an abundant and ecologically important species in Antarctic soft-sediment marine habitats, including nearshore environments where they are potentially exposed to a range of anthropogenic contaminants. These urchins brood developing young for several months, during which time the juveniles may be exposed to contaminants in seawater and in sediment pore waters. This chapter describes the development of a novel toxicity test using juvenile *Abatus nimrodi* and *Abatus ingens* that have been removed from the adult females' brood pouches. Juvenile urchins were exposed to a range of concentrations of copper, lead, zinc and cadmium in seawater for 4 and 10 days. Copper and zinc were toxic to juvenile *Abatus* at concentrations affecting embryonic and larval development in other echinoid species. In addition, the concentrations of copper that caused high levels of mortality in juvenile urchins were within the range that occurs in contaminated marine environments in Antarctica (Heslinga 1976, Nacci et al. 1986, Shcheglov et al. 1991, King and Riddle 2001). Cadmium and lead were not acutely toxic to *Abatus* at the concentrations tested over the 4-d test period.

4.1 Introduction

Anthropogenic contamination of soils, seawater and marine sediments has occurred in many areas of Antarctica over the past 150 years since humans started regular visits to the continent. Some areas around permanent research bases are heavily contaminated with metals and hydrocarbons as a result of historic waste dumping practices and ongoing human activities. Metal concentrations in seawater are elevated relative to background levels at a number of sites close to research stations in East Antarctica (Honda et al. 1987) and high concentrations of

metals have been reported in sediments from sites adjacent to areas of human activity around Antarctica. The sources of this contaminated include waste dumped in or near coastal marine areas and sewage discharges (Lenihan et al. 1990, Berkman and Nigro 1992, Cescon et al. 1994, Kennicutt II and McDonald 1996) including Brown Bay near Casey Station (Deprez et al. 1999, Scouller et al. 2000). Elevated levels of metals have been recorded in the tissues and shells of a range of Antarctic marine organisms including crustaceans (Duquesne and Riddle 2002), molluscs (Mauri et al. 1990, Berkman and Nigro 1992, Kennicutt II et al. 1995, Ahn et al. 1996, Bargagli et al. 1996, Nigro et al. 1996, Duquesne and Riddle 2002), echinoderms (Duquesne and Riddle 2002, Lane et al. in prep) and fish (Carginale et al. 1996).

Although there is a commitment by Antarctic Treaty nations to clean up existing contaminated sites, the Protocol on Environmental Protection to the Antarctic Treaty specifies that this should only be done if in doing so further environmental harm can be avoided. Excavation of waste material or contaminated soils may mobilise heavily contaminated particulates into adjacent marine habitats. This raises the questions of what environmental harm existing contaminated sites are presently causing, and what the potential impacts of disturbing these sites will be. In comparison to temperate regions, for which there is now a significant body of information on environmental effects of contamination, there are only two published data sets that directly measure the sensitivity of Antarctic species to specific contaminants (Hickey et al. 1999, King and Riddle 2001). As a consequence, in the few instances in which water and sediment quality guidelines have been applied in Antarctica they are largely based on toxicity data from northern hemisphere temperate species.

A feature of many Antarctic benthic ecosystems is the high abundance and diversity of echinoderms (Dearborn and Fell 1974, Brey and Dahm 1994, O'Loughlin et al. 1994). Thirty species of irregular echinoids have been reported from Antarctic waters from depths of 2 to 860 m (David et al. 2001). Heart urchins from the genus *Abatus* may well constitute keystone species in Antarctic soft sediment habitats, often being dominant in terms of both abundance and biomass. In what are otherwise very low energy environments, with long periods of sea-ice cover and minimal water currents, these animals play a major role in the

movement and recycling of sediments through bioturbation. Oxygenation of sediments by urchins may also cause reduced metals in anoxic sediments to be mobilised into pore waters and into the water column, potentially increasing their bioavailability (Reynoldson 1987, Campbell and Tessier 1996). Impacts on populations of irregular urchins resulting from contamination of seawater or sediments could therefore result both in substantial changes in geochemical conditions of sediments and waters and biological communities.

Abatus are gonochoric, marsupiate brooders with several species reproducing year round. Juveniles at various stages of development may therefore be present in the brood pouches of these species throughout the year. The juveniles develop directly from large, yolky eggs and do not appear to begin feeding until well after metamorphosis and the development of long, articulated spines once juveniles have left the brood pouch (Pearse and McClintock 1990, Schatt and Féral 1996, Anderson 1998). Schatt and Féral (1996) report that juveniles of *Abatus cordatus* spend approximately 250 days in the brood pouch. Brooding periods have not been determined for *Abatus* or other brooding species. Brooded juveniles of *Abatus nimrodi* have been recorded up to 4.9 mm in length (Anderson 1998). During the brooding period embryos and juveniles appear not to be directly exposed to sediment as no sediment particles have been found in the brood pouches (personal observation). However, brooded young are likely to be exposed to contaminants in the water column and in sediment pore water as the adult urchins burrow through the sediment.

There is very little published data on the concentrations of contaminants that cause toxic effects to Antarctic sea urchins. King and Riddle (2001) observed abnormal development and reduced survival of embryos and larvae of the regular urchin, *Sterechinus neumayeri*, in response to aqueous copper, cadmium and zinc. Lenihan et al. (1995), in field and laboratory tests near McMurdo Station, observed changes in the behaviour of adult *Abatus shackletoni* due to contaminated sediments. However, only undiluted sediments were used in this experiment and the actual concentrations of contaminants in the sediment were not reported. In a manipulative field experiment assessing the responses of soft-sediment invertebrate communities to multiple contaminants, decreased abundances of *Abatus shackletoni* and *S. numereyi* were found in response to

increasing copper concentrations in sediments (Lenihan et al. 2003). Although not conclusively linked to contamination, densities of *Abatus* spp. around Casey Station in East Antarctica are lower in areas affected by anthropogenic contamination than in nearby uncontaminated sites with similar sedimentary and geomorphological characteristics (Stark 2000, personal observation).

Studies on effects of various contaminants on sea urchin fertilisation, embryonic and larval development are common in temperate and tropical regions, however, only one account of toxicity testing using juvenile echinoids is published. The growth and survival of juveniles of the temperate sand-dollar *Dendraster excentricus* was found to be reduced in sediments contaminated with hydrocarbons and heavy metals (Casillas et al. 1992).

In the present study, methods were developed for toxicity testing using juveniles of the Antarctic irregular urchins *Abatus nimrodi* and *A. ingens.* This chapter describes these test methods and reports on the sensitivity of the juvenile urchins to heavy metals in seawater over a 10-day exposure period.

4.2 Materials and Methods

Adult *Abatus nimrodi* and *A. ingens* were hand-collected by divers from depths of 10 to 18 m in O'Brien Bay near Casey Station between December 2002 and February 2003. On their return to the laboratory, a thick mounted needle was used to push away the broad spines covering the females' brood pouches and gently lever out any juvenile urchins that were present. Juvenile urchins were immediately placed into 1-litre beakers containing 700 ml clean, filtered (23 μm) seawater (~34.0 ‰) and maintained at ambient seawater temperatures of $-1°C \pm 0.5°C$ in water baths. Approximately half of the water in the beakers was replaced every third day and the density of juveniles kept to < 40 animals per beaker to ensure high levels of water quality including salinity, pH, dissolved oxygen and ammonia levels were maintained. After four days the juvenile urchins were examined under a dissecting microscope to determine their health. Any that were not moving their spines or that showed signs of physical damage were discarded. Juveniles that were actively moving and had good colouration were considered to be in good health and were used in experiments within 10 days of collection.

Clean, filtered (23 μm) seawater was used to make up solutions of copper chloride ($CuCl_2.2H_2O$), zinc chloride ($ZnCl_2$), cadmium chloride ($CdCl_2.5H_2O$) and lead acetate ($(CH_3COO)_2$ $Pb.3H_2O$). Four concentrations were used in the exposures using lead due to the precipitation of the lead acetate at higher concentrations. For the other metals five concentrations of each were tested. A filtered seawater control was used for each experiment. Samples of the test solution at the beginning of each experiment were acidified to 2% by the addition of high purity HNO_3 and refrigerated at 4°C until analysed (on return to Australia). Metal analysis was performed using an ELEMENT High Resolution ICP-MS (Finnigan-MAT, Bremen, Germany) at the University of Tasmania's Central Science Laboratory. Indium was added to samples at a concentration of 100 ppb as an internal standard. External calibration used multi-element mixed standards from QCD Analysts, USA, and calibration accuracy was checked by running a NIST 1640 Standard Reference Water for each calibration made.

Table 4.1 Concentrations of Metal Solution Used in Juvenile *Abatus* Exposures (μg l^{-1})

	Copper		Lead		Cadmium	Zinc
	A. nimrodi	*A. ingens*	*A. nimrodi*	*A. ingens*	*A. nimrodi*	*A. nimrodi*
Control	< 2	< 2	< 2	< 2	< 2	< 2
Conc 1	23	4	687	687	53	18
Conc 2	24	8	2250	2250	129	42
Conc 3	42	21	5838	5838	315	136
Conc 4	101	64	11133	11133	780	390
Conc 5	315	214	N/A	N/A	1976	1162

Three replicate 1-litre beakers each containing 700 ml of test solution were used for each treatment. The juvenile urchins used in the experiments all had long, fully articulated spines. Although it is not known how long it takes this species to reach this stage of development, this represents the most advanced juvenile stage found in the brood pouches. Between 4 and 6 juvenile urchins were randomly added to the beakers, which were maintained in water baths at –1°C ± 0.5°C. *Abatus nimrodi* juveniles were tested using all four metals. *A. ingens* juveniles

were tested with copper and lead. At 4 days and 10 days the urchins were observed under a dissecting microscope for signs of movement, except for the *A. nimrodi* copper test in which the observation was made at 4 days only. Juveniles that were not moving were scored as dead. At each observation time the salinity, pH and dissolved oxygen in the beakers were measured. Average measured metal concentrations for each set of replicates were used in statistical analyses to determine point estimates. Dunnett's one-tailed hypothesis testing was used to determine the highest concentration not causing significant effects (NOEC) and the lowest concentration causing significant effects (LOEC). All values were calculated on a 0.05 level of significance. LC_{50} values were calculated using linear interpolation. All statistics were calculated on untransformed data. Due to the difficulty of retrieving healthy juveniles from adults and the logistic constraints of working in Antarctica, observations at the 4-d and at the 10-d exposure were made on the same juveniles, and tests could not be replicated so that point estimates are based on one test run only for each of the metals and species tested.

4.3 Results

Toxicity data obtained for *Abatus ingens* and *A. nimrodi* are summarised in Tables 3 and 4 below. The ranges of pH, dissolved oxygen and salinity for each experiment are provided in Table 4.2.

Table 4.2 Water quality ranges during experiments

Experiment	Salinity (ppt)	Dissolved Oxygen (mg l^{-1})	pH
A. ingens – Copper	**32.6 – 33.6**	**12.0 – 15.5**	**7.81 – 8.17**
A. ingens – Lead	**32.2 – 32.6**	**11.5 – 13.7**	**7.69 – 8.06**
A. nimrodi – Copper	**31.4 – 32.6**	**12.1 – 12.9**	**7.95 – 8.02**
A. nimrodi – Lead	**32.3 – 32.5**	**11.7 – 13.9**	**7.71 – 8.06**
A. nimrodi – Zinc	**32.0 – 33.4**	**12.1 – 12.7**	**7.90 – 8.10**
A. nimrodi - Cadmium	**30.7 – 33.3**	**12.5 – 13.3**	**7.89 – 8.03**

4.3.1 Abatus ingens

Copper

At 4 days all juveniles in the seawater controls and in the lowest tested concentration of 4 μg l^{-1} continued to move actively. Mortality of the juveniles increased with increasing copper concentration. On average 30% of the juvenile urchins ceased moving at 8 μg Cu l^{-1}, increasing to > 50% at 21 μg Cu l^{-1} and 73% at 64 μg Cu l^{-1}. At the highest concentration of 214 μg Cu l^{-1} an average of 80% of the urchins died during the 4 day exposure. Significant mortality relative to the controls occurred at copper concentrations ≥ 21 μg l^{-1}. The calculated 4-d LC_{50} was 19 μg Cu l^{-1}.

After 10 days exposure an average of 60% of the juveniles in the control treatment had died. In the copper treatments, mortality varied from 40% at 4 μg l^{-1} up to 100% at 65 μg Cu l^{-1} but this did not represent a significant result due to the low survival rates in the controls. The calculated 10-d LC_{50} was 17 μg l^{-1}.

Lead

At 4 days survival of juveniles was 100% in the controls and some mortality had occurred in all lead treatments, although not in order of increasing concentration. At the lowest concentration of 687 μg Pb l^{-1} an average of 4% mortality was observed, and this increased to between 25% and 45% at higher concentrations. Due to heterogeneity of the responses between replicate beakers, survival was not significantly reduced in any of the lead treatments relative to controls.

By 10 days mortality in the control had increased slightly to an average of 6%. The average mortality in all lead treatments was similar to that at the 4-d observation. Mortality rates were too low for an LC_{50} value to be calculated for either time period for lead, although LC_{20} values were able to be calculated on these data. The 96-h LC_{20} values was 1.5 μg Pb l^{-1} and the 240-h LC_{20} value 10.9

Table 4.3 Summary of toxicity data for *Abatus ingens*

	Lead (µg l^{-1})		Copper (µg l^{-1})	
	4-d	10-d	4-d	10-d
NOEC	11,130	11,130	8	21
LOEC	>11,130	>11,130	21	>21
LC_{20}	1.5	10.9		
LC_{50}			19	17
95% high			72	49
95% low			7	2
NOEC – No effect concentration LOEC – Lowest concentration causing significant effect				

4.3.2 Abatus nimrodi

Copper

No mortality in the control was observed over the 4-day exposure period. Over the same time period reduced survival in all copper treatments was observed in order of increasing concentration. At the lowest tested concentration (24 µg Cu l^{-1}) less than 10% mortality was observed. Survival was significantly reduced at concentrations ≥ 42 µg Cu l^{-1}, with mortality increasing to 67% at 101 µg Cu l^{-1}. None of the juvenile urchins survived exposure to the highest copper concentration of 315 µg l^{-1}. The 4-d LC_{50} value for *Abatus nimrodi* in this experiment was 40 µg Cu l^{-1}.

Lead

At 4 days the survival of juveniles in the control treatment was 100%, and less than 20% mortality was observed in all of the test solutions up to the highest concentration of 11,130 µg Pb l^{-1}. An average of 5% of the animals did not survive at concentrations of 690 µg Pb l^{-1}, 11% were dead at concentrations of 2,250 µg Pb l^{-1} and 17% at 11,130 µg Pb l^{-1}.

By 10 days there had been high mortality in two of the control replicates, with an average mortality of 60% across the controls. Due to this very high control mortality the tests are deemed invalid.

Zinc

After 4 days survival in controls and concentrations ≤ 42 µg Zn l^{-1} was 100%. At higher zinc concentrations mortality increased in order of concentration, with 7% of the juvenile urchins killed at 136 µg Zn l^{-1}, 53% at 390 µg Zn l^{-1}, and 89% at 1,162 µg Zn l^{-1}. Significantly increased mortality relative to controls occurred at concentrations ≥ 390 µg Zn l^{-1}. The calculated 4-d LC_{50} for zinc was 382.6 µg l^{-1}. At 10 days, survival in the controls was reduced to 67%. Due to this very high control mortality the tests are deemed invalid.

Cadmium

By 4 days substantial mortality of juvenile urchins had occurred in one replicate of the control treatment, with an average of 17% of mortality in the controls. Mortality at the other tested concentrations was < 25% and did not increase in order of increasing cadmium concentration. Average mortality in the highest concentration tested (1,980 µg l^{-1}) was 17%, and mortality was not significantly increased at any cadmium concentration relative to controls.

At 10 days average mortality in the controls had increased to 25%. Mortality in the other concentrations varied from 8% to 50%, although not in order of cadmium concentration. Mortality was not significantly greater in any concentration than in the controls. The high degree of heterogeneity between replicate meant that LC_{50} values could not be calculated for either time period.

Table 4.4 Summary of toxicity data for *Abatus nimrodi*

	Copper (µg l^{-1})	Zinc (µg l^{-1})	Cadmium (µg l^{-1})	
	4-d	4-d	4-d	10-d
NOEC	24	136	1,976	1,976
LOEC	42	390	>1,976	>1,976
LC50	40	383		
95% high	196	742		
95% low	35	296		

4.4 Discussion

In all but one experiment (cadmium with *A. nimrodi*) no mortality of the juvenile urchins was observed in the control treatments after 4 days of exposure. This high survival, along with the long-term survival and growth of juveniles in laboratory cultures, suggests that this life stage is suitable for laboratory toxicity testing. The increased mortality in control treatments by 10 days may be the result of the handling of the juveniles during the earlier observation. Handling in the laboratory has been shown to reduce survival of juvenile *Abatus nimrodi* in experiments on laboratory culture (Chapter 3), and mortality in the controls during longer toxicity experiments may be reduced by eliminating any interference with the juvenile urchins during the experiment. These organisms may also be sensitive to water quality, and static renewal of the test solution or use of a flow-through seawater system may further increase the survival of control animals. As these urchins will potentially be exposed to metals over a prolonged period during this life stage, refining protocols for longer-term experiments would be of value. This would include the need to account for the juveniles reaching a feeding stage of development, and the requirement for suitable material as a food supply. The reduced LC_{50} for copper at 10 days compared to 4 days for *A. ingens* suggests that toxicity may increase with exposure to contaminants over a longer period of time. Due to the increased mortality in controls and to the heterogeneity of the responses between replicates over 10 day exposure periods, which prevented the determination of point estimates, the following discussion of results focuses principally on data from the 4-d observation period.

Juveniles of both *Abatus nimrodi* and *A. ingens* were highly sensitive to copper in seawater. Low concentrations of this metal caused significant mortality in both species, with 100% mortality of *A. nimrodi* at 315 μg Cu l^{-1}. *Abatus ingens* was particularly sensitive to copper, with an average of 30% mortality at < 10 μg Cu l^{-1} and a 4-day LC_{50} value of only 24 μg Cu l^{-1}. The copper sensitivity of *Abatus* juveniles in this study is comparable to the sensitivity of embryos and larvae of regular urchins both in temperate and tropical regions of 20 - 42 μg Cu l^{-1} (Heslinga 1976, Nacci et al. 1986, Shcheglov et al. 1991). It is also comparable to the sensitivity of the Antarctica regular urchin *Sterechinus neumayeri*, which had EC_{50} of 11.4 μg Cu l^{-1} (King and Riddle 2001). The 10-d LC_{50} of bivalve larvae was similar to the 4-d LC_{50} for juvenile *Abatus* (Calabrese et al. 1977). The

reported concentrations causing toxicity to polychaete worms (Reish et al. 1976) or amphipods (Ahsanullah et al. 1988) are more than an order of magnitude higher than was found for juvenile *Abatus* in the present round of experiments.

Zinc was also toxic to *Abatus nimrodi* at concentrations similar to those affecting development of regular echinoids to the pluteus larval stage (Pagano et al. 1982, Nacci et al. 1986, Dinnel et al. 1987, King and Riddle 2001). In the present study juvenile mortality was close to 90% at the highest tested concentration of 1,200 μg Zn l^{-1} and mortality was significantly increased at concentrations of less than 400 μg Zn l^{-1}. King and Riddle (2001) report an EC_{50} of > 2,000 μg l^{-1} for zinc concentrations inhibiting the development of the Antarctic sea urchin *Sterechinus neumayeri* over 6-8 days, and an EC_{50} of 327 μg Zn l^{-1} over 23 days. Shcheglov et al. (1991) reported a 4-d LC_{50} of > 1,000 μg Zn l^{-1} for larvae of the tropical regular urchin *Strongylocentrotus intermedius*. For similar exposure times the 4-d LC_{50} (383 μg Zn l^{-1}) in the present study was considerably lower than those reported previously for regular urchins. However, the EC_{50} for zinc affecting larval development of the sand-dollar *Fellaster zelandiae* ranged from 40 and 130 μg l^{-1} (Nipper et al. 1997).

Some mortality occurred in all cadmium concentrations tested, although there was high variability between replicates and *Abatus nimrodi* mortality did not increase in order of concentration. Mortality also occurred in controls in this experiment, suggesting that factors other than exposure to the metal may have caused the reduction in survival that was observed. The highest reported cadmium concentration in Antarctic seawater is 10.2 μg l^{-1} (Honda et al. 1987), which is well below the concentrations that did not cause significant mortality of the juvenile urchins in the present study. *Abatus nimrodi* juveniles appeared to be less sensitive to cadmium by more than three orders of magnitude than has been found studies of temperate species including soft shell clams (Eisler 1977) and amphipods (Ahsanullah et al. 1988, Costa et al. 1998). Embryo growth of the temperate urchin species *Arbacia punctulata* was also far more sensitive to cadmium than *Abatus* juvenile survival, with a 2-h EC_{50} of 13,900 μg l^{-1} (Nacci et al. 1986). The high tolerances of *Abatus nimrodi.* to cadmium may have developed in response to the naturally high levels of cadmium that have been

reported in Antarctic seawater and sediments relative to other regions (Honda et al. 1987).

The concentrations of lead affecting the juvenile urchins were also high in terms of likely environmental concentrations in Antarctic marine waters. The limited solubility of lead in seawater would prevent concentrations greater than those used in the present test from being reached even in a severe pollution incident. However, increased mortality relative to controls was observed for both species in response to exposure to lead. Mortality of *Abatus nimrodi* increased in order of increasing lead concentration, but was less than 20% at the highest tested concentration (11,130 $\mu g\ l^{-1}$). The response of *A. ingens* to lead exposure varied and was up to 45% mortality in lead treatments compared to 100% survival in the controls. The LC_{50} for *Abatus* spp. juveniles was higher than concentrations found to affect embryonic development in the temperate urchins *Paracentrotus lividus* (Dinnel et al. 1987, Warnau and Pagano 1994) or *Arbacia punctulata* (Nacci et al. 1986). The *Abatus* juveniles also appeared to be less sensitive to lead in seawater than juvenile polychaetes (Reish et al. 1976) or soft shell clams (Eisler 1977). King and Riddle (2001) also found relatively low toxicity of lead (NOEC of 3,200 $\mu g\ l^{-1}$) to the development to the hatched blastula stage of the Antarctic urchin *Sterechinus neumayeri.*

The toxicity of copper and zinc to juvenile *Abatus* may help to account for the lower abundance of these urchins at contaminated locations around Casey Station compared to nearby uncontaminated sites (personal observation). This study demonstrated high toxicity of copper and zinc in short-term exposures, but natural exposure periods will be far longer than this due to the extended juvenile brooding period. The brooded juveniles have no escape mechanism from unsuitable environmental conditions, and the adults move only very slowly. Additionally, the brooding mode of development and therefore reduced dispersal of *Abatus* spp. means that the recolonisation of these species in impacted areas may be very slow.

Pore water concentrations of metals have not been reported for sediments from contaminated Antarctic sites however, in artificially spiked sediments from Antarctica ~ 0.1% of the total sediment metal concentration was measured in the pore water (Chapter 5). If this is the case for naturally contaminated Antarctic

sediments then copper concentrations in the pore water may far exceed those causing substantial mortality of juvenile urchins in this study. The bioturbation and subsequent oxygenation of sediments may also result in locally elevated metal concentrations in the pore water and overlying water surrounding the adult urchins.

Even where metal concentrations are below lethal limits, continued exposure throughout the early life history of the urchin may result in effects on growth and/or development. Metals found to be of low toxicity to the juvenile urchins, such as lead and cadmium, may also cause sub-lethal effects to juveniles and to adults over longer periods of time. Some evidence of such sub-lethal impacts on *Abatus* has been found in the relative sizes and morphologies of *Abatus nimrodi* adults from clean and contaminated sites. The average size of animals from a contaminated bay near Casey is significantly less than those collected from nearby uncontaminated sites, and a high proportion of morphological abnormalities are seen in animals from the contaminated site (Chapter 2). The observed differences in size and morphology of population of *Abatus* from clean and contaminated sites may represent a response to contamination at a particular life stage or the cumulative effect of long-term exposure to contamination.

4.5 Conclusions

The results of this study suggest that heavy metals, particularly copper and zinc, may be highly toxic to juveniles of both *Abatus nimrodi* and *A. ingens*. These relatively advanced juvenile urchins were sensitive to these metals at concentrations similar to those causing embryotoxicity in temperate and Antarctic regular echinoids.

This is the first reported study of toxicity testing using post-metamorphic juveniles of any urchin species. We are not recommending that testing with this life stage should replace fertilization or embryo development assays for rapid assessments of toxicity in other species. However, in terms of understanding the impacts of contaminants on populations and ultimately on ecosystems it is important to understand the relative sensitivity of all life history stages of a species. Additionally, the use of juveniles is applicable for testing of echinoderm

species that are unable to used in standardised reproductive level testing due to aseasonal gametogenesis or a brooding mode of reproduction. The results from these initial tests suggest the need for further testing, both to verify the sensitivity of these species to the metals tested, and also to determine their sensitivity over longer exposure periods. Long-term exposures in toxicological studies are highly relevant for Antarctic echinoderms and other species in the context of their very slow development times. Extended exposure periods may also help to explain the growth and morphological abnormalities observed in urchins from contaminated locations. These toxicity testing protocols should also be developed to include testing of other contaminants, and for application to other Antarctic brooding echinoids potentially exposed to anthropogenic contamination. The ability to laboratory-rear juvenile urchins to a stage in development in which they commence feeding on sediment also creates the potential for assessing impacts of contaminated sediments in laboratory-based whole sediment toxicity tests.

Antarctic heart urchins play an important role in the bioturbation of sediments and in the subsequent mobilisation of contaminants in the marine environment. In the normally very low energy environments in which these species are found, this movement and oxidation of sediments is potentially important in shaping soft-sediment marine ecosystems. The exclusion or reduced densities of these urchins due to contamination, or reduction in their size due to impaired growth, may result in significant ecosystem-level changes. It is essential that we understand the sensitivities of these and other Antarctic keystone species in order to allow for sensible decision making regarding how we deal with existing contamination as well as in setting guidelines for the future protection of the Antarctic marine environment.

5 Uptake of Metals in the Carbonate Test of the Temperate Heart Urchin *Echinocardium cordatum* (Pennant)

Abstract

To assess the metal accumulation by temperate heart urchins in response to environmental metal concentrations *Echinocardium cordatum* were exposed for 60 days to a range of sub-lethal concentrations of lead- and copper-spiked sediments. At the end of the exposure the metal concentrations in the carbonate tests of the urchins were measured by ICP-MS. Metal levels in the sediments and the pore waters were measured at the beginning and end of the experiment. *E. cordatum* appeared to accumulate copper from contaminated sediments, although there was differences in he accumulation by different urchins within treatments and also within individual urchins. Lead levels were high in the tests of all urchins and probably related to pre-experimental environmental exposure. Iron was also present in high concentrations in the urchins, with some evidence of depuration of this metal into the pore waters during the 60-d experiment. Elemental mapping using PIXE demonstrated that heavy metals are incorporated throughout the test plates, with no evidence of preservation of past metal exposures in the growth bands of the test, making these urchins unsuited for reconstruction of historic levels of metal contamination in the environment. However, due to its ability to accumulate and depurate certain metals, this species may have application in monitoring of bioavailable metals in soft-sediments and pore waters.

5.1 Introduction

Anthropogenic contamination of marine sediments by metals is widespread in intertidal and subtidal zones, especially in areas near large urban or industrial developments. Stormwater runoff, industrial and mining discharges and waste dumping may all contribute to elevated metal concentrations in seawater, sediments and pore waters. There have been many cases where the presence of contaminated sediment appears to have substantially impacted benthic communities (Cabioch et al. 1978, Warren 1981, Dodge et al. 1995, Lenihan and Oliver 1995, Morrisey et al. 1995, Stark 2000). With increasing concern about,

and study of, the impacts of both short- and long-term pollution in marine habitats a number of useful indicator species have been identified for monitoring contamination in seawater. However, less work has been done in developing methods for biomonitoring of contaminated sediments using infaunal species. Critical in establishing the usefulness of a particular organism for biological monitoring is an understanding of the mechanisms of uptake by the organism and how measured body burdens of a substance relate to exposure concentrations (Phillips 1977, Traunspurger and Drews 1996).

The use of calcareous material in biomonitoring has several potential advantages with results often being less variable than for soft tissues that may be affected by factors such as age, sex and tissue type. Skeletal samples are easier to preserve and homogenize, and are thought to reflect average exposure levels over long period of time (Phillips 1977, Auernheimer and Chinchón 1997). The tests of sea urchins are formed of multiple elements, or plates, which are principally made of calcium carbonate and contain varying amount of magnesium carbonate (Raup 1966). A distinctive feature of sea urchin tests is their very open structure, with pore spaces making up to more than half of the volume of the test. These pore spaces are filled with fibrous, connective tissue similarly to the internal bones of vertebrates. Unlike the shell of bivalves, the echinoderm test is of mesodermal origin and is thought to be susceptible to modification after it has been initially formed (Raup 1966, Swan 1966). Growth occurs on the outer surface and on the margins of the test plates, and is thought to continue throughout the life of the urchin although not at a constant rate (Swan 1966).

Although bivalve shells (Mauri et al. 1990, Berkman and Nigro 1992, Jeffree et al. 1995, Vander Putten et al. 2000) and coral skeletons (Howard and Brown 1987, Scott 1990, Bastidas and García 1999) have often been used for monitoring bioavailable metals in the environment, there have been few studies of metal concentrations in the calcite tests and tissues of sea urchins in response to environmental contamination (Bohn 1979, Auernheimer and Chinchón 1997, Augier et al. 1997). Bohn (1979) reported elevated zinc, iron and arsenic in urchins from near an ore deposit in the Arctic. Auernheimer and Chinchon (1997) also found high concentrations of manganese, iron, zinc and lead in *Paracentrotus*

lividus from a contaminated bay, with evidence of differences in the concentration of these metals by different species.

Regular sea urchins are commonly found in hard substratum communities, and as a consequence do not commonly have prolonged periods of direct contact with marine sediments. These urchins are therefore useful only in the assessment of bioavailable contaminants in seawater. Very few studies report accumulation of metals in irregular echinoids. Elevated concentrations of some metals have been measured in the tests and tissues of *Abatus nimrodi* and *Abatus ingens* collected from a contaminated site in Antarctica (Duquesne and Riddle 2002). Stronkhorst et al. (1999) also demonstrated accumulation of tributyl-tin in *Echinocardium cordatum* from contaminated sediments.

Echinocardium cordatum is a burrowing spatangoid urchin with a cosmopolitan distribution (Hyman 1955) often found in high abundances of 10 or more animals m^{-2} (Buchanan 1966, Bird 1999, personal observation). *E. cordatum* has been reported from a wide range of soft sediment marine habitats from intertidal sand flats to deep water up to 100 m (Moore 1936, Buchanan 1966, Rosenberg et al. 2002). These urchins are believed to live for approximately 15 years, with growth rates averaging 8 mm per year in test length depending on sediment type (Buchanan 1966). The species has been shown to be tolerant to a variety of sediment types (Moore 1936, Buchanan 1966), and is often found in areas vulnerable to anthropogenic contamination such as ports and in the vicinity of ocean bed drilling operations (Cabioch et al. 1978, Dulfer 1999, van het Groenewoud et al. 1999). In common with other irregular urchins, *E. cordatum* directly ingests sediment and so is directly exposed to contaminants bound to sediments and in pore waters. Heart urchins circulate seawater funnelled from the sediment surface around their bodies (Lawrence and Sammarco 1982, Hollertz 2002), potentially also exposing them to water-borne contaminants. Burrowing urchins are considered a major agent of bioturbation of marine sediments (Sandnes et al. 2000). Through the oxygenation of sediments these urchins may also directly influence the partitioning and bioavailability of heavy metals and other contaminants in marine sediments (Reynoldson 1987, Bird et al. 1999).

The cosmopolitan distribution of *Echinocardium cordatum*, along with the direct exposure of this species to whole sediments and pore waters suggests it has potential to be useful for monitoring contamination in these phases. Individual urchins may be up to 7 cm in length (Buchanan 1966) and therefore a large amount of material for analysis can readily be obtained. Previous ecotoxicological testing in which *Echinocardium cordatum* was exposed to sediments spiked with hydrocarbons and contaminated soil, along with this species' tolerances to salinity and dissolved oxygen variation (Chapter 5) suggest that *E. cordatum* is suited to extended laboratory exposures. *E. cordatum* has been recommended as a toxicological test species in the past by researchers in The Netherlands (Bjørnestad et al. 1993, Crane et al. 1996, Stronkhorst et al. 1999), and is considered to be a sensitive benthic indicator species (Dulfer 1999, van het Groenewoud et al. 1999). The present study aimed to quantify the rates of uptake of copper and lead into the tests and tissues of adult *E. cordatum* that had been exposed to sub-lethal concentrations of metal-spiked sediments.

5.2 Materials and Methods

5.2.1 Sediment Spiking

Fine silty marine sediments collected from a clean site in O'Brien Bay near Casey Station, Wilkes Land, Antarctica were wet-sieved through 500 μm mesh to remove large native infauna and settled for 48 hours before having excess seawater drained off. The sediments were separately spiked with solutions of lead acetate ($(CH_3COO)_2$ $Pb.3H_2O$) and copper chloride ($CuCl_2.2H_2O$) to a nominal concentration of 2,000 mg kg dry sediment $^{-1}$ and 700 mg kg dry sediment $^{-1}$ respectively. The metal salts were completely dissolved in 700 ml of clean, filtered (23 μm) de-oxygenated seawater and then mixed into 1,200 grams of wet sediment. The pH of the sediment slurry was adjusted to ~ pH 8 by the addition of analytical grade NaOH. The spiked sediments were mixed in sealed containers on a shaker table at low speed for three weeks. Nitrogen gas was continuously fed into the containers, which were covered by plastic film, to reduce the oxygen concentrations in sediments that would lead to oxidation of the sediments and subsequent changes in pH. After settling the sediments for 4 days any excess seawater was drained off and the spiked sediments were stored at 4°C in sealed containers for a period of 5 months. The spiked sediments were diluted for use in

experiments by the addition of fine silty sand from an uncontaminated site on the south-east coast of Tasmania. Granularity data for the spiked sediments are provided in Table 5.1 in the results.

5.2.2 Analysis of Sediment and Pore Water Metal Concentrations

Triplicate sediment samples from each replicate container were collected at the beginning and end of the experimental period for analysis of metal concentrations in the sediments and in the pore waters. Wet sediments were filtered through 0.45 μm cellulose-acetate membrane filters to collect the pore water before being oven dried at 60°C for 48 hours to a constant weight. For each treatment two replicate 1 gram of dried sediment were digested using 20 ml 1M HCl for 4 hours following the methods of Snape et al. (2004) before being filtered through 0.45 μm cellulose-nitrate membrane filters (Snape et al. 2004). Pore water samples were prepared by dilution of 5 ml pore water extract into 50 ml total using Mili-Q de-ionised water acidified with 1ml high purity HNO_3. Metal analysis was performed by ELEMENT High Resolution ICP-MS (Finnigan-MAT, Bremen, Germany) at the University of Tasmania's Central Science Laboratory.

5.2.3 Animal Exposures

Mature *Echinocardium cordatum* were hand-collected by divers from 3 to 4 m depth in the middle reaches of the Derwent Estuary in Tasmania in June 2003. The Derwent Estuary has records of elevated levels of iron and lead in some sediments, particularly in the upper reaches and the possible implications of this for test results is addressed in the discussion. The urchins were buried in the sand to depths of between 10 and 20 cm at densities of 1 - 5 urchins m^{-2}. The urchins were acclimated in 70-l aquaria with 6 cm depth of sand for one week to ensure any animals that had been damaged during collection were identified.

Urchins were exposed to four concentrations of copper-spiked sediment and two concentrations of lead-spiked sediments plus clean control sediment for 60 days. Tables 5.2 and 5.3 give details of the metal concentrations in sediments and pore-waters at the beginning and end of the exposure period respectively. The concentrations of spiked sediments were aimed to achieve a sub-lethal exposure level. Tolerances of *Echinocardium cordatum* had previously been assessed in a

pilot study in which urchins were exposed for 10 days to sediment spiked with soil that was contaminated with a suite of metals and hydrocarbons. In this pilot experiment it was found that mortality of adult *E. cordatum* was not significantly increased at concentrations ≤ 350 mg Cu kg^{-1} and ≤ 1,250 mg Pb kg^{-1}.

For each treatment concentration three replicates were used, with three urchins in each replicate. This gave a density of urchins for a given surface area was not greater than is found in animals in the areas where the specimens were collected. Experimental containers consisted of 4-l plastic tubs containing 1.5 litres of sediment, which provided an adequate depth of sediment for the urchins to completely bury. Three litres of clean, filtered (23 μm) seawater was added without disturbing the sediment and aeration provided throughout the experiment. Every three days, one litre of the overlying seawater was replaced and the salinity in the tubs adjusted to 33.5 $^{0}/_{00}$ by the addition of Milli-Q de-ionised water.

5.2.4 Growth Measurements of Urchins

In order to assess the approximate growth of animals over the 60-d period, three urchins were injected through the peristome with 100 mg tetracycline kg urchin wet $weight^{-1}$ and maintained under identical conditions to the control urchins for the period of the experiment. At the end of the experiment these animals were sacrificed and the tests cleared of tissue by soaking for 4 hours in 1:1 sodium hypochlorite and then rinsed with de-ionized water. The position of the fluorescent tetracycline marker in the test plates was viewed with UV light under a Leica dissecting microscope. Any area of the carbonate test plates outside the fluorescent marker represents new growth subsequent to the injection date (Kobayashi and Taki 1969).

5.2.5 Metal Analysis of Urchin Shells

At the end of the 60-d exposure period all of the urchins were sacrificed. Gut and gonad tissue and coelemic fluid were dissected out and frozen for later analysis. The carbonate test material from each animal was frozen separately. Metal concentrations in the tests of four urchins from each treatment (two urchins from each of two replicates) were analysed by ICP-MS at the University of Tasmania's Central Science Laboratory.

For each urchin the sternal plates was separated out and cleared of tissue by soaking for 48 hours in 30% analytical grade hydrogen peroxide. Test plates were then rinsed four times in Milli-Q de-ionised water before being dried for 24 hours at 40°C. The dry test plates were broken in half and each part was completely digested in 1 ml 20% high purity HNO_3 and diluted to 20 ml total volume with Milli-Q de-ionised water. Aliquots of the digest were analysed by ELEMENT High Resolution ICP-MS (Finnigan-MAT, Bremen, Germany). Indium was added to urchin samples at a concentration of 100 $\mu g\ l^{-1}$ as an internal standard. External calibration used multi-element mixed standards from QCD Analysts, USA, and calibration accuracy was checked by running a NIST 1640 Standard Reference Water for each calibration made. The final results were normalised to a constant percentage of calcium (33.7%), based on the average calcium for all samples, to reduce the effects of any weighing errors resulting from the extremely low density of the shell material or other analytical errors.

To assess how metals were incorporated into the test material over time, three test plates from each of 6 urchins (one control, one from the second highest copper concentration and two from the each of the highest copper and lead concentrations) were further analysed by Proton Induced X-ray Emission (PIXE) imaging. High-energy heavy-ion microprobe facilities at the Australian Nuclear Science and Technology Organisation were used to map 2 mm^2 and 0.65 mm^2 areas of interambulacral and ambulacral plates of exposed and control urchins using a 3meV beam. Calibration of the ion-probe was undertaken daily using NIST NBS-278 or SRM-278 Standard Reference Obsidian. Elemental mapping was undertaken from the data using the CSIRO Dynamic Analysis Method (Ryan 2003) for the outer and inner surfaces of the test plates and of cross sections through the plates. The detection limits for PIXE are considered to be in the 50 - 100 ppm range for most elements, although the calcium carbonate matrix of the urchin tests interferes to some extent with detection of some ions, including copper. By extending the period of analysis for the areas of interest the detection limits of the instrument can be realistically reduced to approximately 10 ppm. Samples were analysed by the ion probe for between 20 minutes and 1 hour, which was considered adequate for mapping of significant differences in most elements.

5.3 Results

5.3.1 Analytical Precision and Limitations

In the ICP-MS analysis the average concentrations of metals in the acid blanks were < 0.3 μg l^{-1} for all elements of interest except iron, which was 1.37 μg l^{-1}. There was < 10% variation between reference and measured concentrations of the standards for the ICP-MS results, which is considered to be an adequate level of accuracy for the analysis of these samples.

For the PIXE analysis there was good agreement between reference and measured concentrations for the elements of interest. However, the background signal of the calcium carbonate combined with the degree of error meant that some metals could not be confidently analysed at the concentrations present. Copper, zinc and bromine were all present in concentrations too low to be mapped using the PIXE method, although lead was present in concentrations high enough to be clearly measured above the background signal. Iron was also above the detection limits in some samples.

5.3.2 Sediment and Pore water Metal Concentrations

The grain size distributions for the sediments used in the experiment are provided below in Table 5.1.

Table 5.1 Sediment Grain Size Distributions

Treatment	Grain Size as Percentage (%) of Total Weight			
	< 68 μm	68 – 124 μm	125 – 250 μm	> 250 μm
Control	3	5	62	31
Copper 1	4	5	60	31
Copper 2	5	5	59	31
Copper 3	5	6	58	31
Copper 4	7	6	56	31
Lead 1	7	6	56	31
Lead 2	9	7	53	31

The average concentrations of lead and copper in the spiked sediments and pore waters are provided in Table 5.2. The concentrations of copper in the sediment were between 50% and 62% of the nominal spiking concentration with concentrations of < 1 mg kg^{-1} in the sediments that had not been spiked with copper. Lead concentrations were also generally < 1 mg kg^{-1} in sediments that had not been spiked with lead, and in the spiked sediments were approximately 25% of the nominal concentrations.

Initial lead concentrations in the pore water of two of the control replicates were surprisingly high. Sediment and final pore water lead concentrations in the same replicates were not obviously elevated, suggesting that this result is due to sample contamination of the pore water during sampling or analysis.

Table 5.2 Metal concentrations in sediments and filtered pore water (FPW) at the commencement of the exposure period

Treatment	Copper			Lead		
	Nominal mg kg^{-1}	Sediment mg kg^{-1}	FPW ug l^{-1}	Nominal mg kg^{-1}	Sediment mg kg^{-1}	FPW µg l^{-1}
Control	0.00	0.57	22.95	0.00	0.56	516.79*
Copper 1	3.80	2.22	7.56	0.00	1.76	106.70
Copper 2	7.60	4.94	8.10	0.00	0.67	53.24
Copper 3	15.20	7.83	10.57	0.00	0.59	22.35
Copper 4	30.40	14.89	20.41	0.00	0.65	31.61
Lead 1	0.00	0.66	6.11	136.14	32.00	166.59
Lead 2	0.00	0.92	17.59	204.41	57.73	396.38

* High average may be due to contamination after sampling

Pore water lead concentrations in a third of the replicates decreased over the 60 d exposure period. The decrease in individual replicates was between 13.5 and 1,049.3 µg l^{-1} and was greatest in two of the controls and in the lead treatments. The average decrease in pore water lead in both concentrations of lead-spiked sediments was approximately 75% over the 60 days. Pore water copper concentrations also decreased by up to 32 µg l^{-1} over the experimental period. The decrease in pore water copper in the copper-spiked sediments ranged from 3

and 33 μg l^{-1}. The change in pore water concentrations of lead and copper over the 60 days are shown in Table 5.3.

In contrast to the decreasing concentrations of copper and lead there was a significant increase in the pore water iron concentrations in all treatments over time (Table 4). This increase ranged from 63.3 μg Fe l^{-1} to 965.9 μg Fe l^{-1}. In all but one control replicate pore water iron had increased by >250 μg l^{-1}, with an average increase across all treatments of 469.1 μg Fe l^{-1}. The final pore water iron concentrations for each treatment were on average 13 times higher and up to 22 times higher than the initial concentrations.

Table 5.3 Average change in pore water copper and lead concentrations over the experimental period

Treatment	Sediment (mg kg^{-1})		Filtered Pore Water (ug l^{-1})			
	Day 1		Day 1	Day 60	Day 1	Day 60
	Cu	Pb	Cu		Pb	
Control	0.57	0.56	22.95	3.46	516.79	34.56
Copper 1	2.22	1.76	7.56	3.49	106.70	28.27
Copper 2	4.94	0.67	8.10	6.39	53.24	21.64
Copper 3	7.83	0.59	10.57	3.89	22.35	22.17
Copper 4	14.89	0.65	20.41	5.57	31.61	19.80
Lead 1	0.66	32.00	6.11	1.29	166.59	41.19
Lead 2	0.92	57.73	17.59	1.31	396.38	104.28

Table 5.4 Average change in pore water iron concentrations over the experimental period

Treatment	Sediment mg kg^{-1}	Filtered Pore Water μg l^{-1}	
	Day 1	Day 1	Day 60
Control	221.6	34.4	97.7
Copper 1	187.1	50.9	421.0
Copper 2	281.1	23.2	552.1
Copper 3	212.7	35.3	451.3
Copper 4	256.6	59.2	767.5
Lead 1	240.2	68.5	668.8
Lead 2	223.9	42.2	546.3

5.3.3 Urchin Growth

All of the three urchins injected with tetracycline at the beginning of the experiment showed some evidence of growth when the tests were viewed under ultraviolet light. Growth was variable between and within urchins, with the tetracycline mark at the outer margins of some plates and up to 40 μm inside the margin of the plate in other parts of the urchin. Growth was also not uniform around the edges of each plate, with the greatest growth to be seen at the boundary between the interambulacral and the ambulacral series. There was also evidence of approximately 10 – 20 μm of new growth around the margins of the foot plates of two of the urchins.

5.3.4 Metal Concentrations in Urchin Tests Measured by ICP-MS

Table 5.5 summarises the metal concentrations in *Echinocardium cordatum* following exposure to the metal-spiked sediments.

There was a high degree of heterogeneity in the measured copper concentrations in the tests of individual urchins and between urchins from the same treatment. Although there was evidence of metal uptake in copper-exposed urchins, copper was not significantly increased in urchins from any of the treatments relative to the controls ($P = 0.05$). In all replicates the urchin copper concentration was > 5 times higher than pore water copper, and was 500 times higher in one animal. If this one outlying animal is excluded from the analysis, urchin copper concentrations were on average 39 times greater than that of the initial pore water concentrations. Urchin copper concentrations were between 2% and 86% of the sediment copper concentrations (average 16%).

In the control urchins copper concentrations were 0 – 0.516 mg Cu kg^{-1} (average 0.221 mg Cu kg^{-1}). Where urchins had been exposed to copper spiked sediments the concentrations in the test material ranged from 0.029 mg Cu kg^{-1} up to 6.945 mg Cu kg^{-1} (average 1.170 mg Cu kg^{-1}). Average copper concentrations in urchins from the copper-spiked sediments were higher than in those from unspiked sediment. However, urchin copper concentrations did not increase in order of treatment. The highest copper levels were measured in urchins from the

second highest copper treatment with very high copper (6.945 mg Cu g^{-1}) in one animal from this treatment. With this outlying result excluded, there was a positive correlations between urchin copper concentrations and concentrations in both the sediment and the pore water ($R^2 = 0.41$ for both).

Consistently high lead concentrations were found in all urchins relative to levels reported in other studies (Auernheimer and Chinchón 1997, Duquesne and Riddle 2002) with no significant or apparent trend of different lead concentrations in animals from spiked and unspiked sediments. Urchins from sediments not spiked with lead had test concentrations from 40.6 mg Pb kg^{-1} to 60.0 mg Pb kg^{-1} (average 49.5 mg Pb kg^{-1}). Urchins from lead-spiked sediments had average lead concentrations of 48.1 mg Pb kg^{-1}. Urchin lead concentrations were from 45 times up to > 6,000 times higher than the pore water concentrations and between 0.8 and 130 times higher than the sediment lead concentrations.

Iron concentrations in urchin tests varied from 16.3 to 65.9 mg Fe kg^{-1} (average 24.5 mg Fe kg^{-1}). By far the highest iron concentration of 65.9 mg kg^{-1} was measured in a control urchin, compared to a maximum concentration of 27.9 mg Fe kg^{-1} in urchins from sediments spiked with either copper or lead. Urchin iron concentrations were between 240 and ~ 2,000 times the pore water iron concentrations, and from 5% to 30% of the sediment iron concentrations. The pore water and urchin iron concentrations showed a strong negative correlation with an R^2 value of 0.346.

5.3.5 Elemental Mapping

From the results of the PIXE analysis, it was apparent that metal concentrations were not homogenous, with lead and iron concentrations varying by up to an order of magnitude across the surface and throughout transverse sections of the test plates. However, the differences in metal concentration did not appear to relate to the patterns of new growth on the margins of the test plates in any of the samples, and there was no correlation between the concentrations of the two metals in different regions of each sample. The elemental mapping using the Dynamic Analysis software also did not show up patterns of metal distribution that could be related to the formation of growth bands within the urchin tests.

Table 5.5 Metal concentrations in *Echinocardium cordatum* in metal-spiked sediments as measured by ICP-MS

Replicate Average (mg kg^{-1})				Treatment Average (mg kg^{-1})			
	Cu	Pb	Fe		Cu	Pb	Fe
Control	0.221	49.608	65.955	Control	0.221	49.608	65.955
Cu 1 a	1.004	46.455	16.303	Cu 1	0.456	53.243	17.348
Cu 1 b	0.044	60.030	18.393	Cu 2	0.337	45.917	24.994
Cu 2 a	0.232	40.887	52.425	Cu 3	2.748	50.033	23.038
Cu 2 b	0.442	50.947	24.564	Cu 4	1.072	50.092	20.515
Cu 3 a	0.178	40.660	18.127	Pb 1	0.057	48.948	19.955
Cu 3 b	5.319	59.406	27.950	Pb 2	0.510	47.169	11.824
Cu 4 a	1.283	57.318	21.754				
Cu 4 b	0.861	42.866	19.278				
Pb 1 a	0.057	48.948	19.955				
Pb 2 a	0.510	47.169	11.824				

5.4 Discussion

Echinocardium cordatum appeared to increase concentrations of copper in the carbonate test as a result of exposure to copper spiked sediments. The copper concentrations in *E. cordatum* in this study were similar to the concentrations measured in the temperate urchins *Paracentrotus lividus* and *Arbacia lixula* (Portocali et al. 1997) but were higher than those found in the Antarctica species *Abatus nimrodi* from chronically contaminated sediments (Chapter 2). The uptake of copper by *E. cordatum* was highly variable and therefore non-significant compared to controls, however there was a positive correlation between sediment, pore water and urchin copper concentrations. There were decreases in pore water copper concentrations during the experiment, and these may be related to the uptake of copper by the urchins, but may also have been due to loss from the pore water during the regular exchange of the overlying seawater in the containers.

The high degree of variability of copper uptake by the urchins may be due to a number of factors. Variable growth between and within urchins, as observed in tetracycline labelled urchins over the experimental period, could result in a difference in the amount of substitution of metal ions for calcium as new

carbonate is precipitated. Differences in metal concentrations in the soft-tissues of sea urchins have previously been correlated with non-environmental factors including age, sex and reproductive state of the animal (Portocali et al. 1997). These factors were not assessed in the present study, but may also affect metal uptake into the carbonate test. It is also possible that a longer period is required for equilibration between the animal and environmental metal concentrations.

Copper uptake in relation to actual exposure concentrations has not previously been reported for any urchin species other than the Antarctic urchins *Abatus ingens* and *A. nimrodi.* In one study of adult *Abatus* spp. from contaminated sites in Antarctica there was no evidence of significantly increased copper in urchins (Duquesne and Riddle 2002). However, elevated copper concentrations were found in analysis of the test of *Abatus nimrodi* collected from a site with sediment concentrations up to approximately 11 mg kg^{-1}, with a gradient of metal concentration proportional to the urchins' proximity to contamination sources (Chapter 2). The contamination at this site dates back to the 1950s, and given the slow growth rates and limited motility of *Abatus*, these animals may have been exposed to contaminated sediments for many years.

The high lead concentrations in all urchins including those from control sediments suggest that lead was accumulated by the urchins prior to the experimental exposure. Decreases in the pore water concentrations of lead during the experiment may relate to some uptake by the urchins, although the changes in pore water concentration may also have resulted from loss during periodic water exchanges. Pre-experimental uptake is also supported by the fact that urchin lead concentrations were generally many times higher than concentrations in the spiked sediments, as well as by the lack of correlation between urchin concentrations and those in the experimental sediments or pore waters. Sediment lead concentrations in the Derwent Estuary have been reported up to 8,100 mg Pb kg^{-1} and commonly exceed 1,000 mg kg^{-1}, with concentrations of approximately 10 mg kg^{-1} near to the collection site of the urchins (TAFI 2002). The movement of both urchins and sediment throughout the estuary mean that exposure of the test animals to high lead levels in the past is quite possible. The uptake of lead from the environment is supported by findings of elevated lead levels in both regular temperate urchins (Auernheimer and Chinchón 1997) and in

Abatus spp. (Duquesne and Riddle 2002) from contaminated sites. Lead concentrations in the *E. cordatum* tests were similar to levels in regular urchins exposed to sediment lead concentrations of 1,800 mg kg^{-1}(Auernheimer and Chinchón 1997), but were up to 50 times higher than was found in *Abatus nimrodi* exposed to sediment concentrations of 43.5 mg Pb kg^{-1} (Chapter 2). As with copper, an equilibration period greater than the 60 days of the present experiment may be needed for urchins to either uptake or depurate lead to reach equilibrium with the environmental concentrations.

The iron concentrations in the urchins are also likely to have been the result of previous environmental exposure. Iron levels resulting from historical contamination of the Derwent Estuary have been reported in excess of 30,000 mg Fe kg^{-1} (TAFI 2002), providing a very large potential source of this metal for the urchins throughout their life. Sediment iron concentrations near to the area where the urchins were collected have been reported up to approximately 4,700 mg kg^{-1} (TAFI 2002). *Abatus nimrodi* from an Antarctic site with sediment iron concentrations of 2,600 mg kg^{-1} had iron levels up to 10 times higher than were found in the present study (Chapter 2). Iron concentrations in temperate regular urchins in areas with local beach sediment iron concentrations of 132,375 mg kg^{-1} (Auernheimer and Chinchón 1997) were up to 5 times higher than for *E. cordatum* in the present study.

The substantial increase of iron in the pore water throughout the experiment in all but one control replicate was an interesting and unexpected result. The replicate without increased pore water iron was the same one in which the iron concentration in the urchin test material was the greatest. It is possible that the increase in pore-water iron is the result of desorption of iron from the sediments during the exposure period. Iron concentrations in the urchins were up to 2,000 times the pore water concentrations, and it is also possible that the increase in pore water iron during the experiment was the result of urchins depurating iron over the 60-d period. Depuration or iron by the urchins would be consistent with the conclusion of Stronkhorst et al. (1999) that *Echinocardium* have low lipid content and high metabolic rates, with accordingly high elimination rates of tin. However, it does not explain the lack of depuration by the control urchin, which may have been an isolated metabolic effect. It may also be that iron already

present within the skeletal material was exchanged for the spiked-metals, although there is no correlation between metal concentrations in the skeleton or changed metal concentrations in the pore water. Although the cause of this effect is uncertain, these results suggest that iron may be relatively readily exchanged between the urchins and the environment. The affinity for iron uptake by urchins from the environment is also supported by the reported high concentrations of iron in urchin shells and tissues from contaminated sites (Bohn 1979, Auernheimer 1997, Guo 2000).

Abnormal morphology and structure of the tests and reduced growth rates have been reported in regular (Dafni 1980) and irregular (Chapter 2) urchins collected from polluted marine sites, however a causative link with the contamination has not been established. In the case of *Abatus nimrodi* from contaminated sites in Antarctica the size, shape and growth of the urchins correlated with the proximity of the urchins to the contamination source and to the concentration of metals in the test. Growth of sand-dollars has also been shown to be reduced in sediments contaminated with metals (Casillas et al. 1992, Rice et al. 1995). The results of the present study further suggest that there is a relationship between the presence of certain metals in the environment and the concentrations in the urchin tests. If substitution of other ions for calcium in the carbonate test of the urchins affects the strength or development of the test this could explain the altered morphology and growth of urchins from contaminated sites.

The apparent uptake of metals throughout the test material rather than in distinct growth bands means that these urchins are not useful for reconstructing historic levels of environmental metal contamination in the same way that bivalves are used. This result also supports the hypothesis that the carbonate of urchin tests is not a stable material, and may be subject to a greater extent of ongoing resorption and modification than has been suggested by other authors (Raup 1966, Swan 1966). However the close association of burrowing heart urchins with the sediment and pore water, and its ability to incorporate trace metals into the test mean that these urchins may be useful for identifying the presence of bioavailable metals in these phases.

5.5 Conclusions

In this pilot study uptake of copper in the tests of *E. cordatum* correlated with the concentrations of this metal in sediments and pore waters, with the variable degree of copper uptake possibly related to individual animal differences or insufficient equilibration time. Lead and iron were apparently present in the urchins in high concentrations from previous environmental exposure, and the final concentrations of these metals in the urchins did not reflect experimental sediment and pore water concentrations.

It appears that *Echinocardium cordatum* does incorporate trace metals from the environment into the carbonate test. However, further study is needed to assess the time necessary for the urchins to equilibrate with environmental concentrations for different metals, and the relationship of metal uptake to other factors. Additionally, adequate replication is required to overcome the variability between and within individual animals. Further study is also required to calibrate the uptake of metal observed in the laboratory with uptake of metals by animals under field conditions. The uptake of metals throughout the test plates rather than only in new growth means that urchin plates are not suitable as a temporal record of contamination. However, the continued reworking of the carbonate and the ability of urchin species to depurate metals provides opportunity for use of these animals in monitoring of bioavailable environmental contamination. The intimate association of this abundant species with sediment and pore water also provides opportunities for monitoring of sediment contamination in places where other suitable biomonitoring species are unavailable.

6 Effects of Diesel Contaminated Sediment on Burial and Survival of the Temperate Heart Urchin *Echinocardium cordatum* (Pennant) in Laboratory Exposures

Abstract

Diesel fuel is a common contaminant in marine environments, and is a source of hydrocarbons in marine sediments particularly in shallow, nearshore environments. This research assessed the toxic effects of diesel-spiked sediments over a 10 day period to the common heart urchin species *Echinocardium cordatum*. Urchins were exposed to a range of diesel concentrations in a static renewal test with daily observations of burrowing behaviour and mortality. This species was highly sensitive to the diesel, responding by beginning to emerge from the sediment almost immediately in the hydrocarbon treatments. Emerged urchins demonstrated signs of paralysis and impaired righting ability. All emerged urchins subsequently died, with mortality increasing both with time and hydrocarbon concentration. Mortality over 10 days was significantly increased at hydrocarbon concentrations $\geq$ 51.5 mg kg dry sediment^{-1} compared to 100% survival of the control urchins. Urchins surviving after 10 days at the lowest tested hydrocarbon concentration of 42 mg kg^{-1} all emerged from the contaminated sediment, and were unable to rebury in clean sediment at the end of the experiment. Toxic effects, including mortality of urchins, occurred at hydrocarbon concentrations less than have been reported in sediments affected by oil spills or terrestrial runoff. The rapid effects of diesel on *E. cordatum*, and the observed responses of emergence and paralysis even at sub-lethal exposure levels, suggest that local populations of this species would be severely affected by a diesel spill in shallow waters.

6.1 Introduction

Hydrocarbon contamination of marine environments has occurred in many intertidal and shallow subtidal zones close to urban and industrial areas. Aside from land-sourced pollution, large volumes of fuel and oil products are transported throughout the world's oceans in addition to the bunker fuels used by shipping. Spillage from these sources occurs as a result of incidents during

loading and refuelling, shipping accidents, and routine discharges (Siripong 1988, Raaymakers 1994). It is estimated that the average annual input of petroleum to marine waters is around 1,300 thousand tonnes, and may be as high as 8,300 thousand tonnes (The National Academy of Sciences 2002). Even in Antarctica, although remote from large-scale human development, oil pollution is seen as the most likely significant environmental threat (CoMNAP 2000).

In Australian waters alone there were a total of 48 reported oil spills of more than 10 tonnes between 1970 and 1999, with 18 of these being greater than 1,000 tonnes (AMSA 2003). Many more unreported and smaller spills are also believed to occur and may commonly involve diesel fuels, which are extensively used by ships and especially in smaller recreational and fishing vessels (Raaymakers 1994). Diesel products are considered particularly toxic due to the high proportion of aromatic hydrocarbons that are more soluble and potentially more bioavailable than higher molecular weight hydrocarbons (Anderson et al. 1974, Lysyj and Russell 1974). Sub-lethal toxic responses and mortality have been reported for a number of marine species exposed to diesel fuel. Diesel was found to be the most toxic of a number of hydrocarbon products tested on gastropods and among the most toxic to cladocerans (Das and Konar 1988). Recruitment of a range of Antarctic benthic species was reduced in diesel-contaminated sediment compared to clean sediments and sediments contaminated with a range of heavy-metals (Stark et al. 2003). Although the high toxicity of diesel is to some extent mitigated by the rapid volatilisation of the aromatic fractions (Neff et al. 2000) this is less true at low temperatures where diesel may persist in soils and sediments for a considerable period (Howarth 1989, Ferguson et al. 2003).

Hydrocarbons may enter marine sediments directly from the water column, particularly in shallow waters affected by wave action, and also from contaminated soils and particulates entering the marine environment. Lighter oils in particular may quickly adsorb to suspended sediment particles (Johannes and Betzer 1975). Following the *Exxon Valdez* spill hydrocarbon concentrations in shallow subtidal sediments of up to 22.7 mg kg^{-1} were reported (Wolfe et al. 1996). Sediments in Puget Sound affected by urban runoff have been found to have polycyclic aromatic hydrocarbon concentrations up to 15,000 mg kg^{-1} (Casillas et al. 1992). Although hydrocarbons in the water column may degrade

quite rapidly, long-term persistence of organic pollutants in sediments has been reported in many locations (Cabioch et al. 1978, Burns et al. 1994, Dodge et al. 1995) including Antarctica (Lenihan et al. 1990, Kennicutt et al. 1992, Green and Nichols 1995). More than 27 years after the *Arrow* oil spill in 1970 subtidal sediments in the area still contained hydrocarbon concentrations up to 850 mg kg^{-1} (Lee et al. 1999). The low temperatures in cool temperate and polar regions further retards the degradation rates of hydrocarbons (Howarth 1989, Ferguson et al. 2003).

The burrowing heart urchin *Echinocardium cordatum* is considered to be the most widely distributed spatangoid in the world, with records of the species from New Zealand to Norway (Hyman 1955), although recent genetic research is investigating whether it is a single species (J-P Féral pers. comm.). These urchins are relatively long-lived, with an estimated life span of 15 years (Buchanan 1966). *Echinocardium* spp. are gonochoric, with seasonal spawning and the development of planktotrophic larvae that take up to 40 days to metamorphose and settle (Gordon 1926, Moore 1936, Kashenko 1994). Despite annual spawning, periods of up to 5 years have been observed between successful recruitment of juveniles (Buchanan 1967). *E. cordatum* has been collected from a range of depths from intertidal zones to depths of 100 m (Moore 1936, Buchanan 1966, Rosenberg et al. 2002), and is relatively tolerant of different sediment types and grain sizes from coarse sands to muddy sediments (Higgins 1975). Densities of adult *E. cordatum* may commonly reach 10 urchins m^{-2} (Buchanan 1966, personal observation) and have been reported up to 20 m^{-2} (Bird et al. 1999). There urchins play a major role in the bioturbation of sediments, thereby potentially influencing the flux of contaminants between the sediment and the surrounding seawater (Reynoldson 1987, Crane et al. 1996, Bird et al. 1999).

Spatangoid urchins, including *E. cordatum*, feed directly on the sediments in which they burrow. The burrowing depth of *E. cordatum* varies depending on the type of sediment, but may be up to 20 cm in coarse sands. The urchins maintain a funnel to the sediment surface through which water is circulated around the body. Urchins may collect food particles from the sediment surface as well as ingesting both surface and subsurface sediment and detritus (Moore 1966, Duineveld and Jenness 1984, Hollertz 2002). Organic matter is stripped from the sediment and is

concentrated in the second gut loop for absorption (de Ridder et al. 1984). The direct ingestion of sediment and contact with the overlying water mean that this species is potentially exposed to contamination in the sediment, pore waters and in seawater.

Despite the abundance and broad geographic distribution of the species, there has been little reported toxicity research using *Echinocardium cordatum* or any other irregular urchin species. The studies that do exist are often limited to use of these urchins as an indicator species in studies of pollution effects on benthic communities (eg. Cabioch et al. 1978, Lenihan et al. 1995, Lenihan et al. 2003). Cabioch et al (1978) reported on the mass mortality of *E. cordatum* following the *Amoco Cadiz* oil spill in Northern Brittany. Lenihan et al. (1995) found reduced burrowing by the Antarctic spatangoid urchin *Abatus shakletoni* in sediments heavily contaminated with both hydrocarbons and metals. Densities of *A. shakletoni* in manipulative field studies were also reduced in response to copper contamination in sediments (Lenihan et al. 2003). Published studies reporting toxicity testing using *Echinocardum cordatum* include testing of oil-spiked sediments (Brils et al. 2002) and the bioaccumulation and toxicity of tributyltin (Stronkhorst et al. 1999). Gasoil was found to cause significant toxicity at concentrations ≥ 255 mg kg^{-1} with a 14-day LC_{50} of 190 mg kg^{-1}. Hydraulic oil was less toxic with a 14-day LC_{50} of 1,064 mg kg^{-1} (Brils et al. 2002). Other testing of irregular echinoids in laboratory concentration-response experiments include fertilisation, juvenile growth and survival of sand-dollars in toxicity assessments of contaminated seawater and sediments (Casillas et al. 1992, Rice et al. 1995, Nipper et al. 1997). A number of studies of adult regular urchins (Axiak and Saliba 1981) and seastars (O'Clair and Rice 1985, Hooten and Highsmith 1996, Ryder et al. 2001) have shown sensitivity to oil pollution in seawater and sediments. Abnormal or retarded development of regular urchin larvae has also been found in tests of hydrocarbon contaminated water and sediments (Quiniou et al. 1999, Nascimento et al. 2000).

A particularly useful behavioural feature of *Echinocardium cordatum* is their emergence from the sediment as a response to sub-lethal stress or before dying (Stronkhorst et al. 1999, personal observation). Mass emergence of urchins has been observed in response to hypoxia (Nilsson and Rosenberg 1994). In

experiments and where air hoses were accidentally dislodged, emergence of urchins was observed as dissolved oxygen in the water dropped below ~ 4 mg l^{-1}. Once aeration was reintroduced the urchins reburied very rapidly. In other instances where animals have been damaged during collection they almost invariably emerge from the sediment before dying (personal observation).

The aim of this study were to develop toxicity testing protocols for a temperate heart urchin species that could also be applied to Antarctic species, and to undertake an initial assessment of the sensitivity of a temperate heart urchin species to hydrocarbon-contaminated sediments. This chapter describes the methods used to test the toxicity of diesel-spiked sediments and the effects of these sediments on the burrowing behaviour and survival of adult *Echinocardium cordatum* in a 10 day laboratory exposure.

6.2 Materials and Methods

6.2.1 Sediment Spiking

Clean sand for use in the experiments was collected from the d'Entrecastreaux Channel, South East Tasmania (147°250'E 43°083'S) and was sieved through 1 mm mesh to remove large native infauna, small rocks and shell fragments. The sediment was spiked with a low-paraffin diesel (Special Antarctic Blend), which is used in large quantities at Australian research stations in Antarctica and so was of particular interest for comparison of polar and temperate species' sensitivities. Special Antarctic Blend (SAB) is characterised by a higher proportion of n-alkanes and lower molecular weight components (C9 –C12) than most Australian diesel fuels (P. Harvey pers. comm.).

Seven concentrations of SAB-spiked sediment plus a clean control were used in the test. For each concentration, 10.5 kg of wet sediment was hand-mixed with 5 litres of clean, filtered (23 μm) seawater for 5 minutes in 20-l buckets to form a homogenous slurry. Diesel was first added to 100 ml of seawater and then mixed vigorously by hand into the sediment slurry for 5 minutes. The buckets were covered and left for 48 hours to settle before the overlying water was poured off and replaced with clean seawater. The sediment was again stirred to a slurry for 5 minutes and allowed to stand for a further 24 hours before having as much

overlying water as possible drained off. This spiking method is similar to the method reported by Brils et al. (2002), which was shown to have high reproducibility and achieve very comparable oil concentrations to field-contaminated sediments. At the beginning of the experiment duplicate samples were collected from each batch of spiked-sediment and frozen for analysis of total recoverable hydrocarbons. Hydrocarbon analysis was done at the Analytical Services Tasmania, Sandy Bay Laboratory using by GC-FID (Varian Instruments) following dichloromethane extraction.

6.2.2 Urchin Exposures

In August 2001 adult *Echinocardium cordatum* with an average length of approximately 45 mm were hand-collected by divers from the d'Entrecastreaux Channel from depths of 2 to 8 m. Urchins were found buried up to 20 cm deep in clean sand. The densities at the collection sites were generally between 3 – 8 urchins m^{-2}. After collection the urchins were transferred to aquarium facilities at Australian Antarctic Division where they were maintained in 70-l aquaria with a 6 cm deep layer of sand. Aeration and biofiltration were used to maintain water quality, with up to 12 animals in each aquarium. Urchins are easily maintained under these conditions, with 100% survival for periods in excess of 3 months. Urchins were acclimated in aquaria, for 5 days before use in the experiment at a density of between 10 and 15 urchins in approximately 10 litres of sediment. Any urchins that emerged from the sediment during this period were discarded.

Three replicate 4-l plastic containers were used for each treatment. Sediment depth in the containers was 5 cm with 10 cm of overlying seawater. Water temperature was maintained at 15°C ± 2°C, being the approximate average seawater temperature in southern Tasmania. A 12-h light cycle was used and containers were covered and aerated. Every second day 1 litre of the overlying seawater was replaced to ensure low ammonia levels and to minimise the effects of any diesel that may have leached from the sediment into the water. At the two highest spiked SAB concentrations a slight sheen was observed on the water surface at the time of the first water change, but was not apparent at any later time.

Urchins were added randomly to the experimental containers with three urchins placed in each replicate. Daily observations were made of the degree of

emergence of the urchins from the sediment over the next 10 days. An urchin was classified as emerged if more than half of the test was above the sediment surface. Mortality of the urchins was defined by a complete lack of spine or tube-foot movement even in response to prodding or inversion, and any dead urchins were removed from the containers following each observation. Daily measurements were made of pH, dissolved oxygen, salinity and temperature. The salinity was adjusted every second day to ~34 $^{0}/_{00}$. At the completion of the experiment the ability of surviving urchins to rebury in clean sediment was tested.

6.2.3 Statistical Analysis

Dunnett's one-tailed hypothesis testing was used to determine the highest concentration not causing significant effects (NOEC) and the lowest concentration causing significant effects (LOEC). All values were calculated on a 0.05 level of significance. Logistic regression was used to calculate LC_{50} values, with 95% fiducial limits presented in brackets in the results. All analyses were based on untransformed data, and results calculated on the average measured total recoverable hydrocarbon concentrations for each treatment.

6.3 Results

6.3.1 Effectiveness of Sediment Spiking Method

The final concentrations of hydrocarbons in the spiked sediment varied substantially between replicates and there was not a high degree of correlation with the amount of diesel added. Most of the measured hydrocarbons in each treatment were in the C10-C14 fractions. The average measured hydrocarbons and fractions for each treatment are presented in Table 6.1.

Dissolved oxygen levels in all replicates were > 6.6 mg l^{-1} throughout the experiment, except where all urchins in a replicate had died between observations in which case the dissolved oxygen reduced to as low as 4 mg l^{-1}, possibly as a response to increased bacterial activity. Seawater pH ranged between pH 6.6 and pH 8.0, but dropped as low as pH 5.6 following the death of urchins in a replicate. Following the removal of dead urchins and partial replacement of the seawater pH and dissolved oxygen levels returned to the normal range. Salinity ranged from 34.0 $^{0}/_{00}$ to 34.9 $^{0}/_{00}$ throughout the experiment

Table 6.1 Nominal and Measured Hydrocarbons in Spiked Sediment

Nominal Concentration	Measured Concentration (mg kg dry sediment^{-1})				
mg SAB kg wet sediment^{-1}	TRH	C6 –C9	C10-C14	C15-C28	C29+
0	< 5	< 5	< 5	< 5	< 5
80	42	< 5	37	< 5	< 5
176	51.5	< 5	48	< 5	< 5
367	61	< 5	53.5	5	< 5
798	94	< 5	82	7	< 5
1756	51.5	< 5	43.5	6	< 5
3672	89	< 5	78	6.5	< 5
7983	86	< 5	74	7	< 5

6.3.2 Emergence and reburial

All of the urchins burrowed completely into the sediment within 5 minutes of being placed into the treatment containers, although at measured concentrations ≥ 89 mg kg^{-1} some urchins began to emerge from the sediment within 30 minutes. The urchins in the control treatment all remained buried throughout the 10 day exposure, except for a single animal that was partially emerged at the time of the 96-h observation. At the end of the exposure period all of the control urchins reburied within 5 minutes when transferred to aquaria with clean sediment.

A feature of the emergence endpoint was the repeated attempts by living urchins to rebury for the first two days of the experiment. This resulted in slight increases in the number of animals buried in some replicates between 24-h and 48-h observations. During the initial 48 hours urchins on the surface produced large amounts of mucus. Emerged animals were also often observed to have greatly extended tube feet that were apparently paralysed. After 48 hours there was no reburial of animals in the spiked sediments, with a steady increase in emergence over the following days. Patterns of the relationship between emergence over time and sediment hydrocarbon concentration are shown in 6.1. The emergence and mortality of the urchins over time are summarised in Tables 6.2 and 6.3.

After 6 hours 30% of the urchins in the two highest dosed treatments had emerged from the sediment, although this result was not significantly different from the

other treatments due to the high degree of variation between replicates. After 24 hours there was emergence of some urchins in all diesel treatments, with emergence of 30% of the animals at TRH concentrations of 42 mg kg^{-1}, 45% at 51.5 mg kg^{-1}, 66% at 86 mg kg^{-1} and 100% emergence at 89 mg kg^{-1}. The EC_{50} at 24 hours was 56.9 mg kg^{-1}.

After 48 hours emergence was 89% at 86 mg kg^{-1} and ranged from 11% to 78% in the other concentrations. Emergence was significantly increased relative to controls in all concentrations ≥ 42 mg kg^{-1} and the 48–h EC_{50} was 86.9 mg kg^{-1} (22.3 – 101.8 mg kg^{-1}).

After 72 hours 100% of the urchins had emerged at 89 mg kg^{-1}, with average emergence rates of 33% to 89% at concentrations ≥ 42 mg kg^{-1}. Emergence was significantly increased at concentrations ≥ 61 mg kg^{-1} and the 72-h EC_{50} was 72 mg kg^{-1} (19 – 98.5 mg kg^{-1}).

By 96 hours the average emergence was 89% at 42 mg kg^{-1} and 100% at all higher concentrations. The 96-h EC_{50} was 24 mg kg^{-1} (17.7 – 44.1 mg kg^{-1}). The single urchin still buried in the 42 mg kg^{-1} treatment had emerged at the 168-h observation. In all other concentrations of diesel there was no change in emergence after 96 hours, at which time animals on the surface began to die.

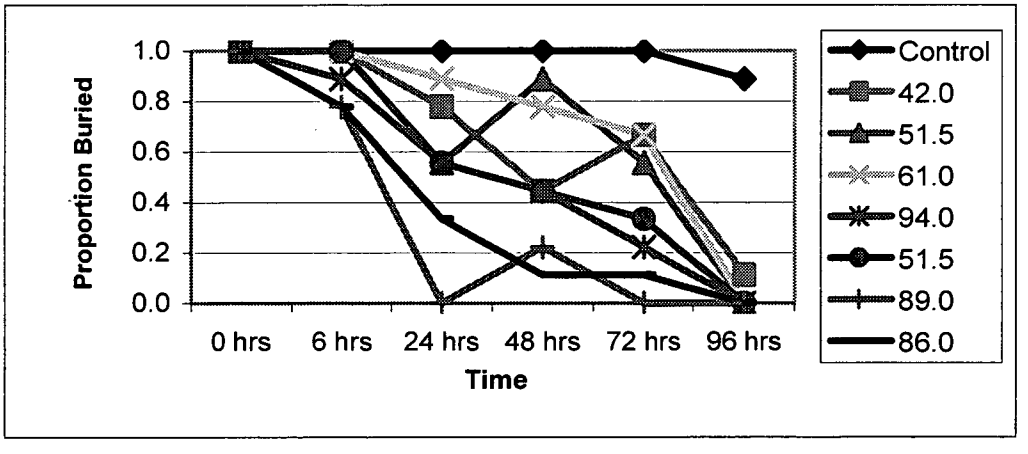

Figure 6.1 Proportion of *Echinocardium cordatum* urchins buried in diesel spiked sediment (concentrations expressed in mg TRH kg dry $sediment^{-1}$)

Table 6.2 Burial of *Echinocardium cordatum* in diesel-spiked sediments

TRH mg kg^{-1}	Proportion of Urchins Buried				
	6 hrs	24 hrs	48 hrs	72 hrs	96 hrs
NOEC	86.0	86.0	<42	61.0	<42
LOEC	>86	>86	42.0	94.0	42.0
F Value	0.398	0.243	0.004	0.024	0.000
EC50	>86	56.9	86.9	72.0	24.0
95% low			22.3	19.0	17.7
95% high			101.8	98.5	44.1

6.3.3 Mortality

Survival of urchins in the control sediment over the experimental period was 100%. Mortality of urchins in the diesel-spiked sediment was first observed at 120 hours. At this time 100% of the urchins were still alive in concentrations ≤ 61 mg kg^{-1} but mortality was significantly increased at 89 mg kg^{-1} with an average mortality of 45% in this treatment. The calculated 120-h LC_{50} was 107 mg kg^{-1}.

By 144 hours an average of 11% of the urchins had died in the 61 mg kg^{-1} treatment, with significantly increased mortality at all higher concentrations. Average mortality was 64% in both the 86 and the 94 mg kg^{-1} treatments, increasing to 89% at 89 mg kg^{-1}. The 144-h LC_{50} was 84.4 mg kg^{-1} (77.3 – 90.2 mg kg^{-1}).

At 168 hours some mortality had occurred at all concentrations ≥ 51.5 mg kg^{-1}, with mortality significantly increased at ≥ 86 mg kg^{-1}. At 51.5 mg kg^{-1} an average of 33% of the urchins had died, increasing to 56% at 61 mg kg^{-1}. At 89 mg kg^{-1} mortality was 100%. The 168 hour LC_{50} was 62.2 mg kg^{-1} (58.2 – 67.3 mg kg^{-1}). Some mortality had occurred in all but the control treatments by 192 hours. At the lowest tested concentration of 42 mg kg^{-1} an average of 22% of the urchins died, increasing to 67% at 51.5 and 61 mg kg^{-1}, with 100% mortality at 86 mg kg^{-1}.

Mortality was significantly increased at all concentrations ≥ 51.5 mg kg^{-1} for this and all subsequent time periods. The 192 hour LC_{50} was 48.4 mg kg^{-1} (42.9 – 52.3 mg kg^{-1}).

At 216 hours urchin mortality had increased to 33% at 42 mg kg^{-1} and to 78% at 51.5 mg kg^{-1}. By 240 hours 100% mortality had occurred at 61 mg kg^{-1}. Except for the control treatment all urchins still alive at this time were on the surface of the sediment, moving only very slightly and showing obvious signs of decay, such as loss of spines. The 216-h LC_{50} was 44.3 mg kg^{-1} (35.9 – 48.5 mg kg^{-1}) and the 240-h LC_{50} was 45.1 mg kg^{-1} (42.8 – 47.0 mg kg^{-1}).

Any urchins still alive at the end of the 10 day exposure were placed onto clean sediment. None of the animals from any of the spiked sediment concentrations were able to rebury and all subsequently died within 3 days. Figure 6.2 illustrates the pattern of survival of the urchins over the 10-d exposure.

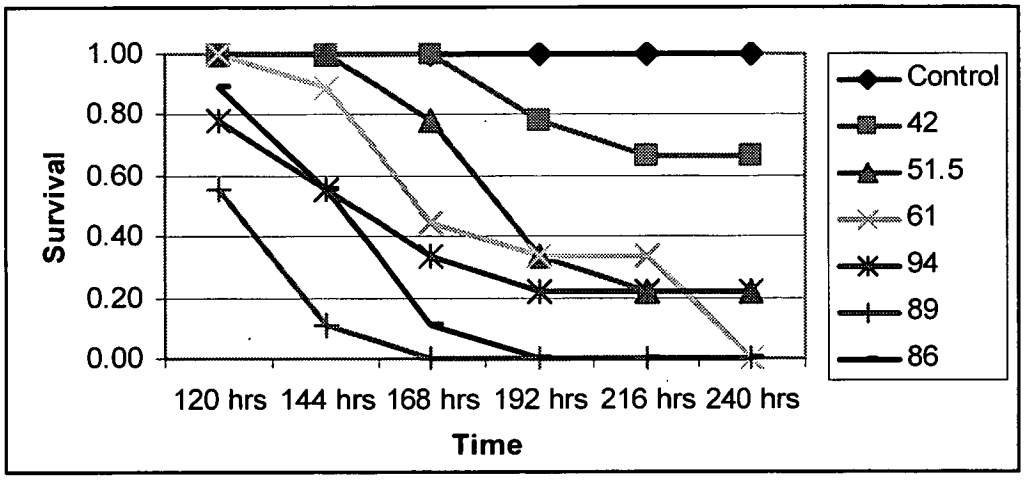

Figure 6.2 Survival of *Echinocardium cordatum* in diesel spiked sediments (concentrations expressed in mg TRH kg dry $sediment^{-1}$)

Table 6.3 Survival of *Echinocardium cordatum* in diesel-spiked sediments

TRH	Proportion of Urchins Alive					
mg kg^{-1}	120 hrs	144 hrs	168 hrs	192 hrs	216 hrs	240 hrs
Control	1.00	1.00	1.00	1.00	1.00	1.00
42.0	1.00	1.00	1.00	0.78	0.67	0.67
51.5	1.00	1.00	0.67	0.33	0.22	0.22
61.0	1.00	0.89	0.64	0.33	0.33	0.00
94.0	0.78	0.46	0.33	0.22	0.22	0.22
51.5	1.00	1.00	0.78	0.33	0.22	0.22
89.0	0.56	0.11	0.00	0.00	0.00	0.00
86.0	0.89	0.46	0.11	0.00	0.00	0.00
NOEC	51.5	51.5	61.0	42.0	42.0	42.0
LOEC	89.0	89.0	94.0	51.5	51.5	51.5
F Value	0.002	0.007	0.013	0.018	0.040	0.029
EC50	107.0	84.4	62.2	48.4	44.3	45.1
95% low		77.3	58.2	42.9	35.9	42.8
95% high		90.2	67.3	52.3	48.5	47.0

6.4 Discussion

The low levels of hydrocarbons measured in the spiked sediment compared to the amount initially added and the degree of heterogeneity of hydrocarbon concentrations between replicates suggests that the spiking technique used in this experiment could be improved. The apparent low adsorption of diesel to the sediment may be a result of the relatively coarse grain sizes of the sediment. Poor adsorption of organic compounds to coarse sediments has previously been reported (Northcott and Jones 2000b), and far higher concentrations of hydrocarbons were measured in finer sediments that had been spiked using almost identical methods to the present study (Chapter 7). For large volumes of material, such as used in this study, longer mixing and equilibration times may improve adsorption and homogeneity. Despite the variation of TRH concentrations, in general toxicity was observed to increase uniformly between replicates in order of

increasing measured concentration. Despite the inability to establish a logarithmic series of hydrocarbon concentrations in the sediments, analysis of the actual levels of TRH in the sediments indicated that the *Echinocardium* were exposed to a range of hydrocarbon concentrations. Additionally, the concentrations used elicited a range of responses by the urchins. If the analytical method did not achieve 100% recovery of bioavailable hydrocarbons in the spiked sediment, as has been reported by other authors (Northcott and Jones 2000a), this may explain both the consistent urchin response in replicates and the greater toxicity of sediments that were more heavily spiked but had lower measured TRH.

The effect of the diesel on the burrowing behaviour of *E. cordatum* was very rapid, with urchins starting to emerge from spiked sediments within 30 minutes at high hydrocarbon concentrations and significant increases in emergence within 24 hours. By 48 hours at least some of the urchins had emerged from the spiked sediment at all concentrations and by 96 hours only one urchin remained buried at 42 mg kg^{-1} with all of the urchins at higher concentrations fully emerged. None of the emerged urchins survived and many showed signs of decay even while still moving. Urchins at the surface would be extremely unlikely to survive for long under natural conditions. Aside from the increased risk of predation of emerged urchins, the test of this species is very fragile and if emerged from the sediment they are likely to be damaged by normal water and sediment movement.

The inability of treated animals to right themselves once inverted also represents a toxic effect, with these urchins normally able to right within a few minutes (personal observation). Impaired righting ability and paralysis have been observed in other echinoderms in response to hydrocarbon contamination. O'Clair and Rice (1985) noted apparent narcotic effects including paralysis and inversion in the seastar *Evasterias troschelli* in response to the water accommodated fraction of crude oil. Similar effects, including slow righting and low levels of tube-foot activity, were noted in the regular urchin *Paracentrotus lividus* with hydrocarbon concentrations $\geq$ 22 mg l^{-1} (Axiak and Saliba 1981). Inversion and temporary paralysis was also observed in the Antarctic ophiuroid *Ophiura crassa* exposed to diesel contaminated sediments (Chapter 7). Where normal movement has been impaired, urchins will be unable to move away from contaminated sediments even where only a small area has been affected.

In addition to the effects on burrowing behaviour and movement, diesel-contaminated sediments caused high levels of mortality in *E. cordatum.* The first definite mortalities occurred at 120 hours, although prior to this affected urchins were obviously not healthy, with floppy spines and tube-feet and showing marked discolouration. It is considered extremely unlikely that any animals affected in this way would have recovered even if removed from the contaminated sediment earlier in the exposure. Mortality increased steadily over time and with increasing hydrocarbon concentration. By 168 hours all of the urchins in the highest concentration of 89 mg kg^{-1} had died and 24 hours later there had been mortalities in all treatments except the control. By 240 hours there had been greater than 75% mortality in all treatments above 42 mg kg^{-1} and all urchins exposed to diesel at any concentration subsequently died. Significantly increased mortality of *E. cordatum* in these experiments occurred at hydrocarbon concentrations less than those reported in sediments that have been impacted by terrestrial pollution or marine spills (Lenihan et al. 1990, Wolfe et al. 1996, Lee et al. 1999, Snape et al. 2001). Compared to the toxicity values reported for this species by Brils et al. (2002), diesel-contaminated sediment appears to be ~ 4 times more toxic to *E. cordatum* than gasoil and 20 times more toxic than hydraulic oil. *E. cordatum* was far more sensitive to SAB in sediment than *Ophiura crassa*, in which animals survived in concentrations up to 2,800 mg kg^{-1} (Chapter 7).

The extent of exposure to sediment contamination by this species may depend on depth to which urchins are buried and the sediment type. Urchins are generally more deeply buried in coarse sands than fine sediments (Buchanan 1966), although the depth of penetration of contaminants into coarse sediment may also be greater. *E. cordatum* may also potentially be exposed to contaminants from the sediment surface and overlying water through the respiratory funnel (de Ridder et al. 1984). The circulation of water from above the sediment around the body of the urchin (Moore 1936, de Ridder et al. 1984) and reworking of substratum as the urchins burrow may also increase the depth to which hydrocarbon contamination penetrates the sediment.

6.5 Conclusions

Diesel fuel in sediments is highly toxic to the irregular urchin *Echinocardium cordatum*, with low concentrations eliciting a very rapid response causing urchins to emerge from the sediment and exhibit signs of paralysis within 24 to 48 hours. At high concentrations urchins emerge from the sediment within minutes of exposure. All urchins that were exposed to diesel-contaminated sediments in this experiment subsequently died. Although it took 120 hours for affected animals to begin to die, recovery of animals even after short-term contact with the contaminated sediment is considered highly unlikely. Due to the 100% survival of the control urchins, and the consistent response of urchins with increasing hydrocarbon concentrations and time of exposure, *Echinocardium cordatum* appears very suitable for assessing the toxicity of hydrocarbon contaminated sediments to adult echinoderms. Further advantages of using *E. cordatum* in toxicity testing are the cosmopolitan distribution and often high abundances of the species, and the ease with which urchins can be collected and maintained under laboratory conditions.

Considering the key role that burrowing urchins play in the reworking of marine sediments (Reynoldson 1987, Crane et al. 1996, Bird et al. 1999), the exclusion of these urchins from an area due to hydrocarbon contamination would likely have severe and long-lasting effects on the local benthic ecosystem. Additionally, through the reduced aeration and bioturbation of sediments that would result from reduced heart urchin populations microbial degradation of the hydrocarbon pollution may be retarded, further inhibiting recovery of the impacted area.

7 Toxicity of Dispersed and Undispersed Diesel in Sediments to the Antarctic Ophiuroid *Ophiura crassa*

Abstract

The toxicity of diesel contamination in sediments to Antarctic ophiuroids was examined in a 10-day test in which adults of the abundant Antarctic species *Ophiura crassa* were exposed to diesel-spiked sediments. Antarctic marine sediments were artificially contaminated with a range of concentrations of Special Antarctic Blend diesel (SAB) and SAB that had been pre-treated with the synthetic dispersant Dasic Slickgone™. Movement of the ophiuroids was affected by both undispersed and dispersed diesel in sediments at hydrocarbon concentrations less than have been recorded in some Antarctic contaminated marine sites. Undispersed diesel generally had an impact on ophiuroid movement within 24 hours, although there was some recovery of affected animals over a longer period. Dispersed SAB was very much more toxic to this species, with EC_{50} values for complete lack of movement as low as 209 mg SAB kg dry sediment^{-1}. Additionally, ophiuroids moved more slowly and were unable to right themselves even at concentrations not causing a total cessation of movement. Ophiuroids are one of the most abundant fauna of Antarctic marine benthic habitats and impacts on these organisms resulting from sediment contamination would almost certainly have wider ecosystem impacts.

7.1 Introduction

Contamination of marine sediments has occurred in the vicinity of many coastal research stations around the Antarctic continent and sub-Antarctic islands. Sources of contamination include the historic dumping of waste directly into marine environments, accidental spills, and terrestrial runoff. As an example, soils from the site of an abandoned fuel farm at Casey contain up to 47,600 mg kg^{-1} total petroleum hydrocarbons (Cole et al. 2000, Snape et al. 2001). In areas of contaminated soil or where waste has been disposed of in landfills, the seasonal melt waters that run through these sites may become contaminated with dissolved metals and hydrocarbons or may entrain contaminated soil particulates. These melt waters then discharge into the near shore marine environments, resulting in elevated levels of heavy metals and hydrocarbons in the receiving

marine sediments (Scouller et al. 2000, Snape et al. 2001). Sediments from Brown Bay, which lies adjacent to the abandoned Thala Valley waste disposal site near Casey Station, have concentrations of up to 200 mg kg^{-1} petroleum hydrocarbons sourced from fuel and mineral lubricating oils (Snape et al. 2001). Marine sediments in Winter Quarters Bay near McMurdo Station have hydrocarbon concentrations as high as 4,500 mg kg^{-1} (Lenihan et al. 1990).

Where there is a spill of fuel or oil in shallow marine environments there is a high probability of hydrocarbons entering marine sediments. Total recoverable hydrocarbon concentrations up to 22.7 mg kg^{-1} were reported in sediments affected by the *Exxon Valdez* oil spill (Wolfe et al. 1996). Polycyclic aromatic hydrocarbon concentrations of up to 15,000 mg kg^{-1} have been measured in Puget Sound sediments affected by urban runoff (Casillas et al. 1992). More than 27 years after the *Arrow* oil spill hydrocarbon concentrations up to 950 mg kg^{-1} were measured in Nova Scotia sediments (Lee et al. 1999).

The potential for direct fuel spills in the Antarctic marine environment is considerable. Large volumes of diesel and other fuels used for shipping, terrestrial and air transport, and electricity generation are transported to Antarctica by sea. Australia's four permanent Antarctic and sub-Antarctic stations were supplied with more than 2 million litres of diesel in the 2001/2002 season alone, in addition to other fuels and lubricating oils (http://www.aad.gov.au). The extent of risk to the Antarctic environment from fuel contamination has been recognised by the Council of Managers of National Antarctic Programs (CoMNAP) as being one the most significant threats to Antarctic marine environments (CoMNAP 2000). As a result a number of documents recommending strategies to prevent spills (CoMNAP 1992b, c) and the preparation of spill contingency plans (CoMNAP 1992a) have been produced.

While the risk of accidental spillage of fuel during ship-to-shore transfer is well recognised and reduced as far as is possible, resources on Antarctic stations for dealing with a large fuel or oil spills are limited and there is little chance of getting additional resources or expertise to the site. The lack of data on the toxicity of hydrocarbons or dispersants to Antarctic marine species and ecosystems means that there is limited information to allow informed decision-

making regarding the most appropriate response. Factors such as low air and water temperatures and slow microbial degradation mean fuel or oil spilled in Antarctic marine or terrestrial environments will degrade only very slowly as a result of natural processes (Ferguson et al. 2003). As a consequence spill response strategies that are accepted as best practice in temperate or tropical regions may not be appropriate for Antarctic conditions.

Changes to natural benthic invertebrate communities apparently related to contamination of marine sediments have been reported in Arthur Harbour near McMurdo Station (Lenihan 1992, Lenihan and Oliver 1995) and in Brown Bay near Casey Station (Stark 2000, Stark and Riddle 2000, Thompson et al. 2003). Recruitment of benthic infauna to sediments that had been artificially contaminated with diesel fuel was significantly reduced compared to recruitment to control sediments (Stark et al. 2003).

There have been very few studies assessing the toxicity of contaminated sediments to Antarctic marine species. Lenihan et al. (1995) conducted a number of whole sediment assays using contaminated marine sediments from the vicinity of McMurdo Station. These studies showed effects on the survival and behaviour of a variety of Antarctic crustacean species, as well as on the burial response of the echinoid *Abatus shackletoni* and the survival of benthic communities transplanted to contaminated sites. In these studies actual concentrations of heavy metals or hydrocarbons in the tested sediments were not reported. As a result it is not possible to determine the substance responsible for the observed effects, or to account for potential synergistic or antagonistic effects of the combined contaminants. Cleveland et al. (1997) also conducted toxicity testing of sediments from Winter Quarters Bay and McMurdo Sound using standardized USEPA protocols. In these tests the Winter Quarters Bay sediments elicited a toxic response in Microtox testing and avoidance behaviours and inhibited burrowing by amphipods (Cleveland et al. 1997). The use of temperate amphipod and bacterial species, while allowing for some characterisation of sediment toxicity, does not provide information on the toxicity of these sediments to Antarctic species.

Limited published data are available on the toxicity of hydrocarbon contamination to echinoderm species, and these are mostly restricted to temperate species. Impacts on natural populations of seastars were reported following the Exxon Valdez spill (Dean et al. 1996). Righting response and tube-foot activity were reduced, followed by high levels of mortality, in the regular urchin *Paracentrotus lividus* exposed to sunken crude oil (Axiak and Saliba 1981). O'Clair and Rice (1995) reported reduced righting responses and apparent narcotic effects in seastars at seawater hydrocarbon concentrations > 0.72 mg l^{-1}. Avoidance of contaminated sediments and reduced growth of seastars were also reported in response to oil-contaminated sediments (Ryder et al. 2001). The temperate irregular echinoid *Echinocardium cordatum* was found to be highly sensitive to diesel-contaminated sand, with a 96-h EC_{50} of 24 mg kg^{-1} for emergence from the sediment and subsequent death of the urchins (Chapter 6).

Previous studies of the toxicity of dispersants to marine invertebrates are limited to tropical and temperate species, and to crude or fuel oils rather than lighter fuels such as diesel. It is generally concluded that dispersant-oil combinations are more toxic to marine organisms than dispersant alone (Harrison et al. 1990, Burridge and Shir 1995, Singer et al. 1998, Lane and Harrison 2002), although it has not been determined whether this is a consequence of the increased concentration of bioavailable hydrocarbons in water and sediments from dispersal of oil, or from the inherent toxicity of the dispersant itself. Additionally, dispersed oil products have an increased potential to come into contact with benthic marine organisms due to their greatly increased surface area and depth distribution compared to undispersed oil (Trudel and Ross 1987, McCay and Payne 2001).

Ophiura crassa (Figure 7.1) is a common Antarctic ophiuroid that is very abundant in the Casey area. The species is found on both hard and soft substratum (personal observation) and stomach contents of dissected animals consist largely of fine detrital matter with some small mineral sediment particles. Antarctic ophiuroids commonly feed on a variety of detrital matter as well as preying on small invertebrates and other faunal species (Dearborn 1977). Sediment and detritus have been found to make up a large proportion of both the weight and the volume of stomach contents in ophiuroids (Dearborn 1977, Dearborn et al. 1996), making them potentially vulnerable to sediment-bound

contaminants. The objectives of this study were to use a series of tests to determine threshold concentrations and identify toxic effects of sediments contaminated with diesel and chemically-dispersed diesel on adults of the Antarctic ophiuroid *Ophiura crassa.*

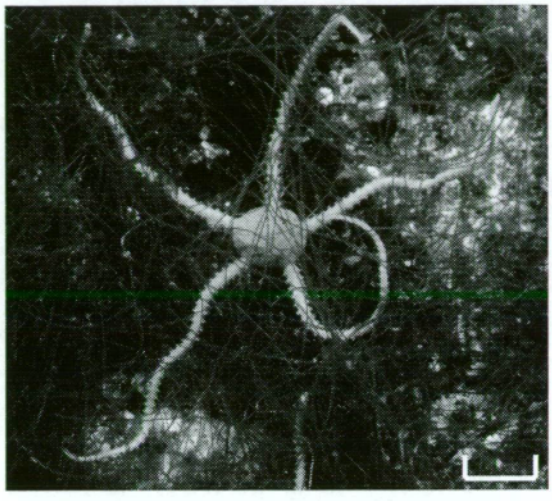

Figure 7.1 ***Ophiura crassa*** **2cm**

7.2 Materials and Methods

7.2.1 Ophiuroids Collection and Maintenance

Specimens of *Ophiura crassa* with a disc diameter of between 1.0 and 1.5 cm were collected by divers from uncontaminated sites in Newcombe Bay, Wilkes Land from depths of between 6 and 12 m and immediately transported in buckets of seawater to aquarium facilities at Casey Station. Water temperature was maintained at normal summer seawater temperatures of –1.0°C ± 0.5°C, with aeration and clean sediment to provide a food source and habitat structure. The ophiuroids were acclimated to the above laboratory conditions for a minimum of 3 days prior to use in experiments, with 100% survival over this time. Only animals that were actively moving were used in the experiments. Although some ophiuroids were missing arms or part thereof at the time of collection, probably as a result of partial predation, this species does not appear to readily shed arms in response to handling. Generally only intact ophiuroids were used in the experiments, although a few animals with regenerating arms were used due to insufficient numbers of intact animals. Ophiuroids were randomly assigned to experimental containers.

7.2.2 Transport of Ophiuroids

Due to time constraints, for the first of the experiments using dispersed diesel ophiuroids were transported by ship to aquarium facilities at the Australian Antarctic Division headquarters in Kingston, Tasmania. Ophiuroids were transported in 60-litre drums of aerated seawater with small amounts of macroalgae included to provide habitat structure and food in the form of epiphytes and attached microfauna. The drums were filled to capacity to reduce water movement due to the motion of the ship and were maintained at 1°C during transport, with exchange of 25% of the water volume every three days. The ophiuroids tended to stay within the macroalgae, which floated mid-water in the drums and further protected them from water movement. The success of the transport method was demonstrated by the 100% survival of animals during a 4-week ocean voyage.

7.2.3 Sediment Spiking Methods

The experimental sediment was fine, silty sand (grain size as % of total weight: 19.7% <63 μm; 71.9% 63μm -2 mm; 0.45% >2mm) that is characteristic of the sediments in the majority of soft-bottom habitats around Casey Station, and typical of the low energy environments found under sea-ice. Sediments were collected by divers from an uncontaminated site in O'Brien Bay and transported back to Casey Station with a layer of overlying water to prevent drying. Sediments were stored wet at approximately -1 °C. Prior to spiking, sediments were sieved through 500 μm plankton mesh to remove larger infauna, rocks and shell fragments. The sieved sediments were then mixed to a slurry with 500 ml of filtered (23 μm) seawater for each kilogram of wet sediment. Special Antarctic Blend (SAB) low-paraffin diesel fuel was added to 100 ml of seawater and vigorously mixed into the slurry by hand for one minute. The spiked sediments were then covered and allowed to settle for 48 hours at - 1°C. At the end of the settling period the overlying water was discarded, an additional 500 ml clean filtered seawater added, and the sediment again mixed to a slurry for one minute and allowed to stand for a further 24 hours. At the end of the second settling period the overlying water was again discarded. A very similar spiking method has proven to achieve highly reproducible results and very comparable oil concentrations to field-contaminated sediments (Brils et al. 2002). The process of

washing the sediment was intended to remove surplus diesel that was not adsorbed to sediment particles and to reduce the hydrocarbon contamination of overlying water in the experimental containers. Seven concentrations of SAB and a control sediment were spiked separately and used in each experiment. At the beginning of the experiment, triplicate samples of the sediment from each concentration were collected and frozen. On returning to Australia the sediments were analysed by GC-FID following dichloromethane extraction for actual concentrations of total recoverable hydrocarbons. All toxicity values were calculated on actual measured hydrocarbon concentrations.

The spiking process for dispersed SAB was similar to that used for undispersed SAB. In the first of the dispersed SAB experiments sediments were stored for a longer period (6 weeks) prior to spiking as they had to be shipped back to Australia. The sieved sediment was covered with a shallow layer of seawater and maintained at close to 1°C during transport and subsequent storage in Kingston. The second experiment using dispersed SAB was conducted at Casey using freshly collected and sieved sediments. In the spiking process, SAB was added to 100 ml of clean seawater, immediately followed by the addition of Dasic Slickgone ™ at the recommended rate of one part dispersant to 20 parts SAB by volume. The spiked seawater was then stirred into the sediment slurry. Pre-dispersion of SAB did not greatly affect final sediment total recoverable hydrocarbon (TRH) concentrations, except at the higher concentrations where it appeared to slightly increase the TRH in the sediment. However the ratio of the different hydrocarbon fractions was clearly affected by the addition of dispersant, with a slight increase in the C6 – C9 fraction and a substantial decrease in the C15 – C28 fraction. Table 7.1 details the TRH levels compared to the initial concentration of dispersed and undispersed diesel added.

Table 7.1 Hydrocarbon concentrations in sediments spiked with dispersed and undispersed diesel

Nominal Concentration mg kg dry sediment^{-1}	Average Measured TRH mg kg dry sediment^{-1}			
	1 Undispersed SAB	2 Undispersed SAB	3 Dispersed SAB	4 Dispersed SAB
0	0.0	0.0	0.0	0.0
18	< 5	Not used	Not used	Not used
37	36.5	31.0	37.0	60.0
80	83.5	98.5	109.0	125.7
176	156.0	341.5	224.0	264.3
367	546.5	558.0	532.0	561.7
798	670.5	924.5	853.0	1213.3
1756	1445.0	1825.0	2310.0	2366.7
3672	Not used	4400.0	5200.0	4076.7

7.2.4 Exposure of Ophiuroids

In each of the four experiments, 300 g of the spiked sediments were added to 4-litre plastic tubs to provide a sediment depth of approximately 2 cm. Two litres of clean, filtered (23 μm) seawater was added to each tub to provide an overlying water depth of 10 cm. Tubs were arranged randomly and maintained at $-1.0°C \pm 0.5°C$ in water baths. Continuous low lighting levels were used and the tubs were shaded to prevent stress to the animals, which would normally be sheltered from high light levels by sea-ice cover or by taking refuge under algae or rocks during summer (personal observation). Three replicates were used for each spiked sediment concentration and the control sediments. In the first SAB experiment 5 ophiuroids were used in each replicate, with 4 used in subsequent experiments. The tubs were covered, with continuous aeration and replacement of half the volume of overlying water every third day to maintain high water quality during the experiments.

Ophiuroids were checked daily for 10 days to determine the number of animals that did not move within a two-minute period, even in response to gentle prodding. In some instances this lack of movement was temporary and the animals were active again at a later observation period, suggesting a temporary narcotic effect. Additionally, it was noted that in some of the contaminant treatments ophiuroids were often found inverted and apparently unable to right themselves although still moving. The righting time of animals was examined as a potential experimental endpoint, but due to the high variability to right, even for an individual animal, and the influence of factors such as light exposure on righting time this was rejected as not being useful. It was also found that animals in higher concentrations of diesel moved far more slowly than those in controls or in very low concentrations of diesel or dispersed diesel. While this response was not quantified for the purposes of statistical interpretation, these differences in the activity levels of the ophiuroids are discussed.

7.2.5 Statistics

The statistical analysis used was Dunnett's one-tailed hypothesis testing to determine the highest concentration not causing significant effects (NOECs) and the minimum concentration causing significant effects (LOECs), with all values calculated on a 0.05 level of significance. Logistic regression analysis was used to calculate EC_{50} values. All statistical analysis was based on untransformed data.

7.3 Results

All results are based on the mean concentration of total recoverable hydrocarbons (TRH) in each treatment concentration reported as mg TRH kg dry sediment^{-1} at the commencement of the experiment. All reported LOEC values are calculated on a 95% confidence level. Calculated EC_{50} values along with the 95% confidence intervals for each experiment are provided in Table 7.2.

7.3.1 Undispersed SAB – Experiment 1

Over the 10 days of the experiment all of the ophiuroids in the control sediment continued to move actively. Ophiuroids at SAB concentrations < 670.5 mg kg^{-1} also continued to move throughout the experiment, with the exception of one

animal in the 36.5 mg kg^{-1} treatment, which was only observed to be moving intermittently throughout the period and one animal at 546.5 mg kg^{-1} that was not moving at the 120-h observation. The number of animals moving was significantly reduced relative to controls at concentrations ≥ 670.5 mg kg^{-1} at 24, 192 and 216 hours, and at concentrations ≥ 1,445 mg kg^{-1} for all time periods except the final observation at 240 hours. At the time of the final observation any ophiuroids not moving in each container were inverted and observed for 5 minutes. Ophiuroids that previously had shown no sign of movement all moved at least slightly during this time.

Over the course of the experiment EC_{50} values for ophiuroid movement varied, with the lowest values being recorded in the first 48 hours of exposure. The 24-h EC_{50} value was 1,066 mg kg^{-1}, decreasing to 1,033.5 mg kg^{-1} by 48 hours. Over the following observation periods up to 216 hours EC_{50} values ranged from 1,656 mg kg^{-1} up to 4,490 mg kg^{-1}, with most values between 2,600 and 2,800 mg kg^{-1}.

7.3.2 Undispersed SAB – Experiment 2

All ophiuroids in the control treatments continued to move actively throughout the 10-day experimental period. After 24 hours the number of animals moving at concentrations ≥ 924.5 mg kg^{-1} was significantly reduced relative to controls. At all later observations movement was significantly reduced at concentrations ≥ 1,825.0 mg kg^{-1}, except for the 120-h and 240-h observations where the movement was not significantly reduced at concentrations ≤ 4,400.0 mg kg^{-1}.

The calculated 24-h EC_{50} values for ophiuroid movement was 1,507 mg kg^{-1}. Throughout the remainder of the experiment the EC_{50} values varied from 1,311 mg kg^{-1} at 144 hours up to 3,264 mg kg^{-1} at 96 hours, with no apparent relationship to the time of exposure. The only exception to this was for the 240-h observation at which time a greater number of animals moved in response to being inverted, resulting in a very high EC_{50} value of > 16,000 mg kg^{-1}.

7.3.3 SAB Dispersed with Dasic Slickgone – Experiment 3 (Kingston)

In the control treatments and at hydrocarbon concentrations ≤ 109 mg kg^{-1} 100% of the test animals continued to move throughout the experiment. At 24 hours ophiuroid movement was significantly reduced relative to controls at concentrations ≥ 224 mg kg^{-1}, and from 48 to 96 hours movement was significantly reduced at concentrations ≥ 532 mg kg^{-1}. From 144 hours until the end of the experiment movement was significantly reduced at concentrations ≥ 853 mg kg^{-1}.

After 24 hours the EC_{50} value for ophiuroid movement was 342 mg kg^{-1}, increasing to 835 mg kg^{-1} by 120 hours. From 144 hours ti 192 hours the calculated EC_{50} value decreased from 607 mg kg^{-1} to 282 mg kg^{-1} and at 240 hours had increased to 674 mg kg^{-1}.

7.3.4 SAB Dispersed with Dasic Slickgone – Experiment 4 (Casey)

To examine possible increased sensitivity of the ophiuroids due to stress caused by transport from Antarctica to Australia a second experiment was conducted at Casey in December 2002. This experiment used freshly collected sediments and ophiuroids. Ophiuroid movement in the controls and in the lower concentrations in this experiment was found to be more variable than in the first dispersed-SAB experiment. In the control treatments at least one out of the total of 12 ophiuroids was not moving during all but one of the observation periods, although the same animals had generally resumed moving by the following day. In the lowest tested concentration of 60 mg kg^{-1} all ophiuroids continued to move throughout the exposure period except for one animal that was immobile during the first observation period only. The lowest concentration to cause significantly reduced movement compared to controls was 264.7 mg kg^{-1} at both the 24-h and 168-h observations. Movement was significantly reduced at concentrations ≥ 561.7 mg kg^{-1} at 48 hours and 72 hours. At all other observation periods movement was significantly reduced at concentrations ≥ 1,213.3 mg kg^{-1}.

The lowest EC_{50} value for ophiuroid movement was 197 mg kg^{-1} at 24 hours. The EC_{50} increased to 704 mg kg^{-1} at 48 hours, and reduced againg to 487 mg kg^{-1} at 72 hours. From 96 hours onwards the EC_{50} value varied between 567 mg kg^{-1} and

1,238 mg kg^{-1} independent of increasing exposure time. At the 240 hour observation period the EC_{50} was 1,220 mg kg^{-1}.

Table 7.2 Calculated EC_{50} Values for Ophiuroid Movement in Sediments Contaminated with Dispersed and Undispersed SAB (95% fiducial limits shown in brackets)

EC_{50} Values for Ophiuroid Movement (mg TPH kg dry sediment^{-1})				
Time	**Experiment 1 SAB**	**Experiment 2 SAB**	**Experiment 3 Dispersed SAB**	**Experiment 4 Dispersed SAB**
24 hours	1,066.4 (796.4 – 1,660.7)	1,507.3 (1,194.3 – 1,987.4)	342.1 (272.1 – 425.7)	197.1 (23.01 – 300.59)
48 hours	1,033.5 (862.2 – 1,260.1)	2,608.6 (1,819.8 – 4,638.9)	540.2 (396.0 – 737.2)	704.0 (441.1 – 1,125.7)
72 hours	4,489.5 (2,102.7 – 822,296.7)	1,438.1 (1,244.4 – 1,679.2)	570.1 (442.3 – 730.8)	487.0 (383.5 – 615.1)
96 hours	2,850.7 (1,748.7 – 54,723.8)	2,277.5 (1,563.7 – 4,096.8)	756.4 (551.3 – 1,063.4)	828.1 (594.5 – 1,144.3)
120 hours	2,640.4 (1,776.8 – 30,648.7)	3,264.3 (2,499.4 – 4,812.6)	835.4 (675.9 – 1,072.7)	1,238.5 (1082.7 – 1,405.6)
144 hours	2,622.7 (1,860.5 – 60,746.8)	1,311.4 (1,087.7 – 1,624.5)	607.1 (467.9 – 789.7)	878.6 (698.8 – 1,094.7)
168 hours	1,656.2	2,255.2 (1,560.2 – 3,972.2)	574.5 (432.5 – 763.9)	566.9 (421.2 – 758.6)
192 hours	2,835.5 (1,640.20 – 53,088.7)	1,459.6 (1,316.1 – 1,585.5)	281.7 (226.9 – 348.2)	742.1 (507.3 – 1,079.5)
216 hours	2,835.5 (1,640.20 – 53,088.7)	1,645.7 (1,354.4 – 2,064.0)	694.7 (551.5 – 889.4)	928.4 (721.5 – 1,182.1)
240 hours		16,374.2	673.7 (614.5 – 738.7)	1,220.0

7.4 Discussion

In general *Ophiura crassa* was tolerant to undispersed SAB in sediment at the concentrations tested, with all ophiuroids surviving throughout the 10-day exposure period. However, movement of the animals was significantly affected by the treatment at concentrations within levels that may be found in contaminated marine sediments in Antarctica (Lenihan et al. 1990, Snape et al. 2001). The hydrocarbon concentrations causing significantly reduced movement were also within the range that has been measured in subtidal sediments following oil spills in the marine environment and chronic pollution (Meador et al. 1990, Lee et al. 1999). The calculated EC_{50} values for both experiments using

undispersed-SAB were greater than 1,000 mg kg^{-1} for all observation periods, but were generally less than 3,000 mg kg^{-1}. The concentrations causing toxic effects for these ophiuroids is far higher than has been reported for temperate seastar or urchin species exposed to the water accommodated fraction of oil (Axiak and Saliba 1981, O'Clair and Rice 1985, Ryder et al. 2001). *Ophiura crassa* were also far less sensitive to diesel-contaminated sediment than the temperate heart urchin *Echinocardium cordatum* (Chapter 6). One reason for the limited toxicity of contaminated sediments to the ophiuroid may be that sediment or sediment pore water are not major exposure routes for this species.

Interestingly, the effects of the contaminated sediments on movement of the ophiuroids did not follow a pattern of increased toxicity with time. In Experiment 1 (undispersed SAB) the greatest effect was observed in the first 48 hours of exposure, and fluctuated throughout the remainder of the experiment independently of exposure time. In the second undispersed-SAB experiment high toxicity was also apparent in the first 24 hours, and then continued to vary independently of time. The reduced toxicity at later times may be a consequence of degradation of the hydrocarbons over the experimental period, particularly of the more toxic lighter fractions. Similar recovery over time of urchins and seastars exposed to the water accommodated fraction of crude oil has been reported by other authors (Axiak and Saliba 1981, O'Clair and Rice 1985). Temporary narcotic effects were also noted in seastars exposed to oil-contaminated sediments (Ryder et al. 2001). The periodic partial exchange of the overlying water may also have resulted in dilution of any diesel that could have entered the water column from the sediment and affected the animals. Analysis of hydrocarbon concentrations in both the sediment and the water throughout the exposure period, and toxicity testing of this species with diesel in seawater, would be useful in determining the effects of changing concentrations and contaminant partitioning.

As has been reported for a wide range of marine species (Trudel and Ross 1987, Harrison et al. 1990, Curran et al. 1997, Singer et al. 1998, Lane and Harrison 2002, Edwards et al. 2003) the addition of a synthetic dispersant to the diesel resulted in considerably increased toxicity to ophiuroids in both experiments. Movement of the ophiuroids was significantly affected at concentrations as low as

224.0 mg kg^{-1} in the first dispersed-SAB experiment and 264.6 mg kg^{-1} in the second. The EC_{50} of dispersed-diesel was generally less than half, and in most cases was three to five times lower than for undispersed-diesel. Additionally, the dispersed-diesel was more consistently toxic over time, with a clear pattern of increased response with increasing concentrations, and less variation in either LOEC or EC_{50} values at different observation times. Whether the increased response is due to toxicity of the dispersant itself or of the dispersant-diesel mixture is unclear. Toxicity of oil dispersants to a range of marine invertebrates and flora has been reported (Burridge and Shir 1995, Singer et al. 1995, Curran et al. 1997, Lane and Harrison 2002). Studies of the effects of oil, dispersed-oil and dispersant alone on reproduction of corals demonstrated that the increased toxicity of dispersed-oil could be clearly attributed to that of the dispersant itself (Lane and Harrison 2002).

In the first dispersed-diesel experiment using ophiuroids that had been transported to Tasmania there was greater toxicity observed for most time periods than in the second of these experiments conducted at Casey. This may be the result of the ophiuroids being stressed during transport and an extended time in captivity, although all animals used appeared healthy at the commencement of both experiments. Further replication of this experiment, and exposures to a reference toxicant, using animals acclimated for a consistent period would clarify the possible effects of stress on chemical sensitivity of this species.

Ophiuroids in all but the lowest hydrocarbon concentrations tended to move far more slowly than those in the control treatments. This species of ophiuroid is generally highly active and movement of healthy animals could be easily observed within a few seconds, particularly in response to light used during the observations. Although for the purposes of the experiment movement was defined as any active movement of the animals' arms, such movement was often very slow and slight in extent in exposed animals. Interestingly, it was generally the inverted animals that continued moving in the higher hydrocarbon concentrations after ophiuroids that were normally oriented, with their oral surface against the sediment, had ceased to move. The righting response is very strong in ophiuroids, with healthy animals of this species generally righting within one minute (personal observation). It may be that this righting response caused

the animals to continue moving despite the narcotic effects of hydrocarbon. It is also possible that reduced exposure due to the inability of inverted animals to ingest contaminated sediment was the cause of this reduced toxicity.

Although exposure to diesel and dispersed-diesel in sediments did not always result in mortality, the consequences of even temporarily reduced movement of ophiuroids may be severe. Not only are animals unable to feed effectively, they will be at increased risk of predation by other species. Following the breaking up of sea-ice in summer this species is frequently found sheltering under rocks and macro-algae, although during periods of sea-ice cover they are more commonly found on exposed rocks or sediments. Presumably this movement to a different habitat is a light-avoidance response, although it would also reduce vulnerability of the animal to predation. Ophiuroids with reduced motility will be more vulnerable to other hazards including burial by sediments or exposure to anchor-ice that this normally highly motile species may be able to avoid. In particular, an animal with limited motility would be unable to move away from a contamination source to cleaner sediment, increasing the risk of prolonged effects or mortality.

Although only movement was examined in this experiment, it is possible that other physiological and metabolic effects would occur in response to exposure to sediment contamination. Sub-lethal effects including reduced reproductive success, permanently damaged locomotive or sensory facilities, or toxicity to juveniles, would also seriously compromise the ability of this species to persist in contaminated sites. That this ophiuroid is abundant, and is found at contaminated sites with reduced faunal assemblages, suggests that this particular species may be unusually tolerant of sediment contamination. For the purposes of assessment of contaminated sites in Antarctica and in the establishment of meaningful water and sediment quality guidelines there is an urgent need for further ecotoxicological research of a range of Antarctic marine organisms.

7.5 Conclusions

This study demonstrates that diesel contamination in sediments has the potential to impact on an important Antarctic benthic species at concentrations that have been reported in some Antarctic marine sediments. In particular the addition of a

chemical dispersant may result in increased toxicity to ophiuroids as well as having the potential to increase both water-column and sediment concentrations of hydrocarbons. The exclusion of ophiuroids from an Antarctic benthic habitat represents a significant change in the species composition and potentially to the ecology of these environments.

This work represents the only known published study to quantify hydrocarbon concentrations resulting in toxic effects to an Antarctic species. For a framework for dealing with existing contaminated sites or future oil or fuel spill events to be developed, more information is urgently needed on the sensitivity of a range of species to hydrocarbon contamination both in water and sediments.

8 General Discussion

8.1 Review of Objectives and Summary of Findings

Anthropogenic contamination in Antarctica is an issue of concern at an international level. To the general public environmental harm resulting from human activities in Antarctica is unthinkable, and the Antarctic Treaty nations have undertaken to minimise environmental damage caused by their operations. This attitude has led to the enactment of the Madrid Protocol, in which it is clearly stated that future pollution of the Antarctic should be rigorously avoided, and that where possible historic contamination should be removed and affected sites cleaned up. The caveat the Madrid Protocol places on the remediation of contaminated sites is that the remediation activities must not result in greater environmental harm than would result from leaving the sites undisturbed.

Unfortunately, much of the information needed to make informed decisions about the degree of environmental harm caused either by existing contamination or the potential impacts of remediation is not available. Research in Antarctica is logistically difficult and expensive, and there are substantial knowledge gaps in many fundamental aspects of Antarctic ecology and biology, particularly regarding marine benthic species. There is also only extremely limited information on the toxicity of contaminants such as hydrocarbons and heavy metals to Antarctic species, and presently there are no established protocols for toxicity testing using Antarctic organisms.

Near-shore marine environments are likely to be receiving environments for contamination from Antarctic research stations through sewage and wastewater discharge and from potential fuel or oil spills. Additionally, the sites of historic waste disposal are often in coastal areas where contaminants may be transferred by melt-streams carrying dissolved metals and organics or contaminated soil into adjacent marine habitats. The transfer of contamination through soil movement and the presence of metals and organic pollutants in marine sediments have been identified as major pollution issues in Antarctic marine environments in the vicinity of research stations (Cole et al. 2000, Scouller et al. 2000, Snape et al.

2001). It is important to determine the sensitivities of Antarctic marine benthic species to these contaminants in order to determine priorities and targets for site remediation, and to anticipate the likely consequences of leaving sites undisturbed or making an effort to clean them up. An understanding of the comparative sensitivities of Antarctic and temperate marine species is also needed to facilitate the application of toxicity data from other, more accessible, regions to the development of Antarctic water and sediment quality guidelines.

This project has aimed to develop ecotoxicological testing methods that are relevant and applicable to:

- Antarctic benthic species that may be exposed to anthropogenic contaminants;
- the ecological role of these species in the community;
- the likely route of exposure of these species to contamination; and
- different contaminant types and related species, including those from other geographic regions.

The project also aimed to identify some of the effects of contamination and threshold concentrations of some substances on a range of Antarctic echinoderm species characteristic of soft-sediment, nearshore environments.

Abatus spp. heart urchins are widely distributed and abundant in Antarctic benthic marine habitats. Due to their role in the bioturbation of marine sediments in these otherwise low energy environments, *Abatus* urchins are potentially keystone species in soft-sediment habitats. Through the oxygenation and movement of sediments they may influence the cycling of nutrients and of anthropogenic contaminants between the sediments, the pore waters and overlying waters, and the subsequent bioavailability of these contaminants (Reynoldson 1987, Bird et al. 1999, Sandnes et al. 2000). Through burrowing and ingestion of sediment *Abatus* urchins are directly exposed to heavy metals, hydrocarbons and other anthropogenic pollutants in the sediments and pore waters. Unlike the majority of temperate or tropical urchin species, all 11 described species of *Abatus* are brooders (David et al. 2001), making them unsuitable for the commonly used fertilisation and larval development toxicity testing applied to regular urchins in other parts of the world.

This study identified clear evidence of deformity and altered patterns of growth in *Abatus nimrodi* from Brown Bay near Casey Station. Urchins from the bay, which is polluted with a range of heavy metals and hydrocarbons, were on average wider and flatter relative to their length than urchins from uncontaminated sites. Deformities in the form of sunken interambulacral plate series, depressions of the aboral or oral part of the test, and skewing or asymmetry of the test were found in more than 50% of the urchins collected from the most contaminated sites. Such deformities were completely absent in urchins from uncontaminated locations. The urchins from the contaminated bay were also smaller, and with narrower growth lines in the test plates, suggesting that these urchins may be growing more slowly than urchins from other locations. The correlation of the test abnormalities and smaller size with the proximity of the urchins to the contamination source and to heavy metal concentrations in sediments from the areas where the urchins were collected strongly suggests that these effects are directly related to the presence of contamination.

There have been previous reports of the incidence of deformities in regular urchins from other regions, some of which were collected from areas subject to anthropogenic pollution. These studies suggest that the observed deformities relate to weaknesses in the test or abnormalities in the structure of the individual test plates (Moore 1973, Dafni 1980), although how these abnormalities relate to exposure of the urchins to contamination has not been demonstrated. In this study it was found that the incidence of abnormal growth patterns of *Abatus* collected from Brown Bay correlated with the concentrations of copper, iron and lead measured in the carbonate tests of urchins from these locations. Uptake of metals from the environment has been demonstrated in other urchin species, although the relationship between environmental exposure and metal accumulation by the urchins is poorly understood, with differences in whole-animal concentrations related to size, age, gender and reproductive status (Portocali et al. 1997). In the present study, the temperate heart urchin *Echinocardium cordatum* exposed for 60 days to copper-spiked sediments showed evidence of accumulation of this metal in the carbonate test. The variability of growth and metal accumulation between urchins suggests that a longer period is required for these urchins to equilibrate metal concentrations with the environment. This is further supported by the high concentrations of lead and iron measured in the urchins, including

control animals, which is likely to have been the result of long-term environmental exposure prior to the experiment. During the 60-day exposure there was also some sign of depuration of iron from the urchin tests, with increasing concentrations of iron in the sediment pore water between the beginning and end of the experiment. Adult *Abatus nimrodi* collected from contaminated sites in Antarctica showed clearer patterns of metal accumulation consistent with the metal levels in sediments from the same area. The extended period of exposure of these Antarctic urchins to the long-term contamination at these sites may have resulted in better equilibration with environmental concentrations.

Bivalves are routinely used for biological monitoring of metals and other contaminants in the environment. The annual growth rings in bivalve shells provide a temporal record of contamination levels at the time the shell material was produced (eg. Mauri et al. 1990, Berkman and Nigro 1992, Vander Putten et al. 2000). Because of their abundance in soft sediment environments and likely exposure to contamination in the sediment or pore water, it was considered that urchin tests may prove similarly useful in reconstructing historic levels of metal contamination in these environments. However, fine-scale elemental mapping of the tests of urchins exposed to heavy metals in the field and laboratory showed no distinct patterns of deposition. Concentration of metals appeared to be much the same throughout the carbonate test, both across the surface and in transverse sections of test plates. This demonstrates that the urchin test is far more active in terms of dissolution and deposition during the life of the animal than for bivalves. The very porous test material is filled with tissue, and it is likely that this contact with living tissue results in ongoing metabolism of the test material (Raup 1966, Swan 1966). The uniform distribution of metals throughout the tests means that spatangoid urchins are unsuited for reconstruction of past environmental metal concentrations. However, the relatively sedentary habit and ability of the urchins to uptake and depurate metals from the test may make them suitable for monitoring bioavailable metals in sediments and pore waters.

Understanding the effects of a substance on a species requires study of more than one life stage of the organism. Reproductive processes and juvenile life stages have generally been shown to be far more sensitive to chemical or other stress

than mature animals (eg. Heslinga 1976, Truhaut 1977, Howarth 1989, McGee et al. 1993, Traunspurger and Drews 1996), and impacts at this level may have equivalent or greater effects on populations than toxicity to adults. In the case of Antarctic urchin species, with greatly extended periods of growth and development, understanding the effects on immature life stages is highly relevant. The juvenile development over several months for these Antarctic urchins represents a much longer period in which sensitive life stages may be exposed to contamination compared with temperate species. In contrast to the ease of observing the development of non-brooding species that metamorphose and settle within a few weeks, the development of brooded juveniles from spawning to maturity is very difficult to study. Juvenile development has only been partially described for three species of *Abatus*, and this information is based on the range of developmental stages of preserved specimens removed from the pouch of female urchins (Schatt 1988, Pearse and McClintock 1990, Anderson 1998). However, the ability to collect live juveniles from the brood pouches of female urchins provides opportunities for research of the biology and growth of the immature life stage of these urchins over time.

In developing methods for toxicity testing using juvenile *Abatus* it was found that the juvenile urchins could be easily removed live from the brood pouches of adult females, transported to Australia, and successfully maintained in aquaria for in excess of one year. The principal requirements for keeping the juveniles alive are the maintenance of high water quality and minimising handling and physical disturbance. The ability to rear these urchins from lecithotrophic juveniles to the independent, feeding stage provides a valuable opportunity for a range of research from basic biology through to long-term ecotoxicological studies. If combined with field studies verifying the parallel development of laboratory-reared juveniles with those living under natural conditions, studies of growth and development of juveniles in the laboratory will lead to a far greater understanding of the biology of these animals.

In the present work it was found that the juvenile *Abatus* grew very slowly, with average increases in length of only 1.2 mm over a one year period. If this growth rate is the same for animals in the brood pouch this supports the hypothesised longevity of these species, for which ageing techniques have not been developed.

This slow juvenile growth also suggests that the growth-ring series measured in mature *Abatus nimrodi* from around Casey may represent periods of annual growth, making some of these urchins at least 15 years old. Increased knowledge of the biology and ecology of these species also has direct application in anticipating the potential effects of contamination on individual urchins and populations, and of diminished populations on the ecosystem. Similar culturing techniques could feasibly be applied to a range of Antarctic brooding echinoderms. Transport and laboratory maintenance of Antarctic species also allow for research to be conducted in other places, thereby overcoming the disadvantages of expense, limited resources and restricted field-study seasons associated with research in Antarctica.

Toxicity testing over 4 and 10 days using pre-feeding juvenile *Abatus nimrodi* and *Abatus ingens* demonstrated the suitability of this life stage for experiments on the effects of metal contaminants in seawater. These relatively advanced spined-juvenile urchins were highly sensitive to copper and zinc, with high rates of mortality resulting from concentrations that have been found to effect fertilisation and early larval development in regular urchins and sand dollars (Heslinga 1976, Nacci et al. 1986, King and Riddle 2001). Juvenile *Abatus* urchins were also far more sensitive to copper than has been reported from tests using temperate polychaete worms (Reish et al. 1976) or amphipods (Ahsanullah et al. 1988). The protocols developed for testing juvenile *Abatus* have potential for application to a wide range of related species and for the testing of other contaminants in seawater. By raising the juveniles to later developmental stages where they begin to burrow and ingest sediment, these animals may also prove suitable for testing of contaminants in sediment and pore water. Extended laboratory exposures of developing *Abatus* will also be valuable in studies of long-term toxicity and metal accumulation by the urchins, and may help to explain the mechanisms of altered growth and development observed in urchins from contaminated sites.

In developing methods for exposing adult heart urchins to contamination, the temperate heart urchin *Echinocardium cordatum* was used for toxicity testing against hydrocarbons in sediment. In a 10 day static-renewal test, *E. cordatum* were found to be highly sensitive to diesel fuel in sediment, with animals responding by emerging rapidly from the spiked sediments before dying. The

response of the urchins increased both with hydrocarbon concentration and as a function of exposure time, with all of the exposed urchins dying within 12 days of the commencement of exposure. Prior to death, the motility and righting response of the urchins was affected, the exposure reducing the ability of the animals to move away from even small-scale or localised contamination and increasing their vulnerability to other threats such as predation. The sensitivity of *E. cordatum* to diesel in sediments was between 4 and 20 times higher than has been reported for this species exposed to other hydrocarbon products (Brils et al. 2002). *Echinocardium* was also far more sensitive to diesel-spiked sediments than an Antarctic ophiuroid species tested in the present study using the same methodology. If the sensitivity of *Abatus* spp. urchins to hydrocarbons is found to be similar to that of *Echinocardium*, the 100% mortality of exposed urchins suggests that any spill of diesel in a shallow Antarctic marine environment is likely to have a large impact on resident populations of heart urchins. Additionally, it is unlikely that populations would recover while hydrocarbon contamination of the sediments persists. Particularly in the Antarctic context, hydrocarbons may remain for a considerable time in soil and sediments due to low rates of volatilisation and biodegradation. The long-term exclusion of burrowing urchins from an area would likely result in changes to the redox status and physical structure of the sediments. The subsequent lessened oxygenation may further retard the degradation of the hydrocarbons, prolonging the environmental impact of the contamination.

The protocol used for testing the toxicity of contaminated sediments on *Echinocardium* was very effective, with 100% survival of urchins in controls and highly consistent results between replicates. Attention needs to be paid to water quality throughout the experiment, as both pH and dissolved oxygen concentrations in the water decreased rapidly in response to animal mortality. The spiking method for adding hydrocarbon contaminants to sediment needs to ensure homogenous concentrations and good adsorption, particularly where coarse sands or large sediment volumes are to be used. *Echinocardium cordatum* proved to be well suited to this type of toxicity testing, particularly due to its habit of emerging from the sediment and the presence of obvious signs of poor-health of urchins prior to mortality, allowing for the observation of sub-lethal effects. This testing method should be readily applicable to other contaminant types and to

other echinoderm species, although the emergence endpoint would be unsuitable for some *Abatus* species that do not burrow so completely into the sediment.

Ophiuroids are another group of echinoderms that are abundant throughout the Antarctic and sub-Antarctic as well as in many temperate marine environments. *Ophiura crassa* is a relatively small species compared with many Antarctic ophiuroid species, and is found in abundance in soft-sediment and mixed-substratum habitats around Casey Station. As well as being common in uncontaminated locations, these ophiuroids have been collected from Brown Bay, suggesting that they are reasonably tolerant to sediment contamination. This species proved to be easy to keep in aquaria and to transport, allowing for ongoing experimental work in locations other than Antarctica.

In a series of 10 day experiments *Ophiura crassa* adults were found to be far more tolerant to diesel-contaminated sediments than *Echinocardium cordatum*. The concentrations of hydrocarbon in sediment that caused toxic responses in *O. crassa* were also higher than has been reported for other temperate seastar or urchin species (Axiak and Saliba 1981, O'Clair and Rice 1985, Ryder et al. 2001). Ophiuroids exposed to the hydrocarbon-spiked sediments suffered temporary narcotic effects including reduced righting ability, obviously slowed movement and, at higher concentrations, complete paralysis. In nearly every instance the ophiuroids recovered over the exposure period. Similar symptoms of narcosis have also been observed in other echinoderms and invertebrate species exposed to hydrocarbons (Axiak and Saliba 1981, O'Clair and Rice 1985, Das and Konar 1988, Ryder et al. 2001). The recovery of the ophiuroids over time may be related to gradually decreasing hydrocarbon concentrations due to water exchanges or degradation of the hydrocarbons throughout the experimental period. Although the limited toxicity observed in these experiments may indicate that *O. crassa* is relatively insensitive to hydrocarbons, it may also suggest that sediment is not a major exposure route for this species.

Pre-treatment of the diesel with a synthetic dispersant prior to sediment-spiking resulted in far greater toxicity to *Ophiura crassa* than undispersed-diesel. Ophiuroids ceased to move at hydrocarbon concentrations five times lower than had caused temporary narcosis where dispersant was not used. Ophiuroids were

affected within 24 hours of exposure, and there was very little recovery of affected animals. This increased response may have been due to the toxicity of the dispersant itself, or of the dispersant-diesel conjugate. It may also be that dispersal of the diesel resulted in ongoing elevated concentrations of hydrocarbons in the water column, which may represent a more available source of exposure for this species. The dispersal of fuel spills may be of value in preventing stranding of slicks on shorelines or in protecting foraging seabirds and seals, but may result in substantial impacts on benthic species and associated ecological effects.

8.2 Comparison of the sensitivities of Antarctic and temperate species and suggested future directions for research

There are only a very limited number of published studies that allow direct comparison of results between Antarctic and temperate species, and research is needed using organisms from both regions to address this deficiency. However, differences in the biology and ecology of related species from different regions need to be taken into account in developing test methods that will allow comparative research to be undertaken. For example, there is potential to adapt the exposure methods used for *Echinocardium cordatum* in the present study to *Abatus* spp., although larger experimental aquaria and careful attention to water quality would be required.

Care should also be exercised in interpreting the results of testing from different regions. For example, the Antarctic species, *Ophiura crassa*, was apparently far less sensitive to hydrocarbons than some temperate echinoderms, but this may actually be due to different exposure route of the contaminant. Testing of *O. crassa* against the water-accommodated fraction of diesel would help to clarify the exposure route and sensitivity of this species. The testing protocol used for exposing *O. crassa* to hydrocarbon-spiked sediments in the present study could also readily be applied to many species of temperate or tropical ophiuroids, allowing for a more complete comparison of the relative sensitivity of the different species.

In exposures of *Abatus* spp. to metals in seawater, concentrations of copper and zinc causing mortality of juveniles were low compared to the reported toxicities for many temperate invertebrates. The slow development of these urchins, with the potential for extended exposure of this life-stage, combined with high sensitivity may mean that these urchins are more vulnerable to contamination than related temperate species. Also, the low reproductive output and limited dispersal of brooding species compared to those with planktonic larval development (Pawson 1994, Poulin and Féral 1994) may increase the potential impacts on populations resulting from metal pollution. Pulses of heavy metals entering the marine environment as a result of soil disturbance or hydrological changes during remediation of contaminated-sites are likely to result in reduced recruitment of *Abatus* and mortality of recently recruited juveniles in the affected area. Where concentrations of metals are below the levels causing mortality there is still a threat of sub-lethal effects, such as the observed deformity and reduced growth of adult *Abatus* from Brown Bay. These morphological effects may impact on the functional role of these urchins in these environments and also may reduce reproductive success. The ecological impacts of reduced heart urchin numbers may be significant in Antarctic environments, where lower densities of benthic species and little wave or current action mean there is less sediment movement and chemical cycling than in many shallow temperate or tropical marine environments.

Direct toxicity of a contaminant to a species is only one way that pollution may affect the environment. Impacts on food supply due to toxicity to microbial communities with subsequent alterations in the food-chain and changes to habitats, such as may result from reduced populations or functioning of a key species, could also severely impact on benthic ecosystems over time. To fully understand the implications of contamination in Antarctic marine environments and to set meaningful water and sediment quality guidelines far more research is required, including:

- the toxicity of different substances on a wide variety of organisms at different functional levels;
- synergistic or antagonistic effects of multiple contaminants;
- effects of substances on multi-species systems;
- field-based testing, including recruitment studies; and

- combined biological and chemical studies that will assist in understanding the role of Antarctic species in cycling of contaminants in marine systems.

8.3 Methods Development for Antarctic Ecotoxicology

Because of the adaptations of many Antarctic species and the unique ecology of the systems in which they live, it is neither possible nor appropriate to simply transfer testing protocols used for temperate species to the Antarctic context. Many Antarctic groups differ markedly from their temperate relatives in terms of size, longevity, feeding, reproductive strategies and behaviour. This was demonstrated clearly during the present study in a number of ways. For example, the testing protocol developed for exposing the temperate urchin *Echinocardium cordatum* to contaminated sediment used emergence from the sediment as one of the experimental endpoints. It was also possible to easily determine the health of *E. cordatum* from the extent of spine movement and colouration of the urchins. In contrast, the Antarctic urchins *Abatus nimrodi* and *Abatus ingens* only partially bury in sediments. Additionally, the Antarctic urchins moved very slowly even when healthy, and it was not possible to readily assess the health of the animal by visual observation.

The different reproductive strategies of many Antarctic echinoids mean that the most common toxicity testing methods used for temperate urchins are not applicable to these species. The processes of spawning and gamete fertilisation are still not described for *Abatus* spp. or other brooding species. The use of the juveniles of brooded species is an alternative that allows for testing of younger life stages that may be more sensitive to contamination than adults. Testing of juveniles of temperate echinoderms is not commonplace, but is possible and would be valuable in the comparison of sensitivities of temperate and Antarctic species.

Improved understanding of the most likely means of exposure of different Antarctic species to contaminants is also needed. For example the studies of *Ophiura crassa* in which little response to diesel-spiked sediment was observed may easily be interpreted as meaning that this species is particularly tolerant to hydrocarbons. However, it may be that *O. crassa* would have responded very

differently to the water-accommodated fractions of diesel in seawater. This hypothesis was partly supported by the recovery of the ophiuroids over time in the undispersed-diesel experiment and the increased toxic effects in the dispersed-diesel experiment. Researchers need to consider the results of their studies critically in view of the limited knowledge of Antarctic species, and be open to adapting testing protocols and ideas as the research progresses.

Often the major considerations in toxicological testing are cost-efficiency and the applicability of the protocol to large-scale commercial testing of substances. In practice, there is no circumstance in which these concerns should overshadow the importance of collecting high quality data and the correct interpretation of results. These concerns are even less appropriate in the Antarctic context, where the priority is for identification of the tolerances of a few key species to a limited number of substances to which they are likely to be exposed. The majority of the expense associated with polar research is due to travel and the logistic difficulties of living and working in a remote and harsh environment. The cost of the testing is therefore of limited importance compared to the need for developing methods that allow for the efficient collection of useful data with the limited time and resources available.

Field-collection of animals and sediments is often problematic in Antarctica, and alternatives to collection by divers would be advantageous in terms of both safety considerations and the practical limitations on diving in Antarctic environments. Further work on developing methods for maintaining test organisms for extended periods in the laboratory will help by reducing the number of collections required. Methods for transport of live organisms to other countries will reduce the time researchers need to be working with the limited resources generally available at Antarctic research stations and will also allow for longer-term testing to be conducted. Extended laboratory and field studies are essential to learn more about the biology of the test species and the likely exposure pathways of these species to contamination. Long-term studies are also important to better understand the potential effects of toxicity on populations and the communities in which these species exist. The potential for successful transport and aquarium maintenance of juvenile urchins and adult ophiuroids was demonstrated in the present study, and is likely to be applicable to a range of Antarctic species.

A consideration that is important in all toxicity testing work but rarely acknowledged in the literature is the need to minimise any impacts on the environment resulting from research practices. Effects on populations due to large-scale collection of organisms must be avoided, and test species and collection sites carefully chosen with this in mind. Additionally, the deployment of *in-situ* experiments must consider the possible impacts at the experimental site. All experiments should be carefully planned and executed to maximise the chance that the research goals are achieved and that test organisms are not wasted. Most importantly, researchers must consider the relative value of the data against the damage caused in obtaining it.

8.4 Conclusions

The specific findings of the individual toxicity tests conducted during this project have contributed to the very limited existing knowledge on the effects of contamination on Antarctic species. However, the real value of this research is the way in which it highlights the differences between Antarctic species and their relatives from other areas and to emphasise the futility of simply applying toxicity testing methods from other areas to Antarctica. This study also serves to identify techniques for testing of metals and hydrocarbons in sediments and seawater that are applicable to a range of Antarctic and related temperate echinoderm species.

One of the first priorities for studying impacts of pollution in Antarctica is to learn more about the biology of key species and the ecological systems in which they live. This information is essential to allow targeting of limited research resources and to understand the wider consequences of observed toxic effects on the environment. Toxicity testing protocols must integrate this ecological information with emerging information on the behaviour of chemicals in cold regions to ensure testing is applicable to the likely modes of exposure to contamination. Techniques for maintenance and transport of suitable test species are particularly valuable in view of the limited resources available and difficulties of working in Antarctica. Testing of a range of related Antarctic and temperate species is needed to better understand the relative sensitivities of Antarctic organisms, with test results considered carefully in terms of the adaptations and unique ecology of species from the different regions.

While the work presented in this thesis is only a beginning, it has identified some specific threats to Antarctic echinoderms from contamination and also some of the key issues in the development of methods in Antarctic ecotoxicology. It is hoped that this work has laid a useful foundation for the development of further meaningful and relevant ecotoxicological research, and that this research will contribute to the management of human impacts in Antarctica and the protection of its unique marine environments.

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