Cardno
Ecology Lab
Shaping the Future

Marine and Freshwater Studies

# Ex HMAS Adelaide Artificial Reef

## Bioaccumulation Monitoring
## Survey 1

**Job Number: EL1112024**

**Prepared for Department of Primary Industries – Catchments and Lands**

**March 2012**

**Cardno (NSW/ACT) Pty Ltd**
**Trading as Cardno Ecology Lab**

ABN 95 001 145 035
Level 9
203 Pacific Highway
St Leonards
New South Wales 2065
Australia
**Telephone: 02 9496 7700**
International: +61 2 9496 7700
ecologylab@cardno.com.au
www.cardno.com.au

Cover Image: Preparing oyster trays for deployment, photographer Brendan Alderson, Cardno Ecology Lab.

## Document Control

| Report Number | Status | Date | Author | | Reviewer | |
|---|---|---|---|---|---|---|
| 1112024 C | Final | 27 March 2012 | Kate Reeds | KR | Marcus Lincoln Smith | MLS |

# Executive Summary

The Ex-HMAS Adelaide “the vessel’ was gifted from the Australian to the NSW Government for the specific purpose of scuttling the ship as an artificial dive reef off the Central Coast of NSW. In accordance with the Artificial Reef (Sea dumping) permit, the Department of Primary Industries - Catchments and Lands, must implement a Long Term Monitoring and Management Plan (LTMMP) which was prepared in March 2011.

The LTMMP covers environmental and structural monitoring for the first five years post-scuttling and forms the basis for ongoing monitoring and maintenance over the operational life of the vessel as a dive site, which is estimated to be 40 years. A requirement of the LTMMP is that bioaccumulation monitoring be undertaken to determine whether resident biota (i.e. biota in direct contact with the superstructure) are likely to be affected by zinc chromate paint, which may have been used originally on the aluminium alloy of the vessel.

Sydney rock oysters (*Saccostrea glomerata*) were selected as sentinel organisms and sourced from oyster growers in the Tuncurry/Forster area. They were attached to the vessel (monitoring sites) in PVC trays and to a special marker buoy approximately 35 m from the ship (used as a control site). At the time of supply, additional oysters were sourced from the supplier to provide information on baseline levels of zinc and chromium. Oysters were deployed for 10 weeks from November 2011 to January 2012. The oysters attached to the special marker buoys, were lost due to adverse weather, hence environmental control samples were not retrieved.

Samples attached to the mid ship and stern of the vessel were successfully retrieved. All samples, including baseline controls were sent to an NATA accredited laboratory for analysis of zinc and chromium concentrations in oyster soft tissue.

The mean concentration of chromium and zinc was marginally higher in oysters at the monitoring sites than the baseline, although this was not statistically significant.

# Table of Contents

**Executive Summary ..... i**

**Glossary ..... iii**

**1 Introduction ..... 4**

1.1 Background and Aims ..... 4

1.2 Study Area ..... 5

**2 Existing Information ..... 6**

2.1 Bioaccumulation Studies ..... 6

2.2 Toxicity of Chromium in the Marine Environment ..... 6

2.3 Toxicity of Zinc in the Marine Environment ..... 7

2.4 Previous Studies ..... 7

**3 Study Methods ..... 9**

3.1 Sampling Design ..... 9

3.2 Field Methods ..... 11

3.3 Laboratory Methods ..... 11

3.4 Data Analyses ..... 12

**4 Results ..... 13**

4.1 General Findings ..... 13

4.2 Comparison between Monitoring Sites and Baseline Controls ..... 13

**5 Discussion ..... 15**

**6 Acknowledgements ..... 16**

**7 References ..... 17**

**8 Appendices ..... 19**

## List of Tables

Table 1: Concentrations of Chromium and Zinc in Oysters Deployed at Monitoring Locations on the Ex-HMAS Adelaide and from Baseline Controls Transplanted from Forster/Tuncurry in November 2011. ..... 14

## List of Figures

Figure 1: Location of Ex-HMAS Adelaide Artificial Reef and Dive Site. The approximate location and orientation of the ship is indicated by the yellow line. ..... 8

Figure 2: Positions of Control and Vessel Monitoring Sites Selected for the Deployment of Oysters. ..... 10

Figure 3: Preparing oyster trays for deployment. ..... 11

## List of Appendices

Appendix A: Laboratory Results

Appendix B: Results of Univariate Statistical Analysis

# Glossary

| | |
|---|---|
| Artificial Reef | A structure or formation placed on the seabed for the purpose of increasing or concentrating populations of marine plants and animals or for the purpose of being used in human recreational activities. |
| ANZECC/ARMCANZ | Australian and New Zealand Environment Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand. |
| Baseline Control | Levels of contaminants present in oysters from their original source (i.e. the aquaculture facility from which they were obtained). |
| Background Control Sites | Special marker buoys near to the vessel used to provide an indication of background levels of contaminants. |
| Bioaccumulation | The accumulation of substances, such as pesticides or heavy metals in an organism. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is eliminated. |
| DSEWPaC | Department of Sustainability, Environment, Water, Population and Communities. |
| d.w. | Dry Weight. |
| $EC_{50}$ | Half maximal effective concentration.  Refers to the concentration of a toxicant which induces a response halfway between the baseline and maximum after some specified exposure time. |
| EP&A Act | Environmental Planning & Assessment Act 1979. |
| LAT | Lowest Astronomical Tide. |
| $LC_{50}$ | Lethal Concentration that kills 50 % of test animals in a given time. |
| LOEC | Lowest-observed-effect-concentration.  Means the lowest treatment (i.e., test concentration) of a test substance that is statistically different in adverse effect on a specific population of test organisms from that observed in controls. |
| LTMMP | Long Term Monitoring and Management Plan. |
| Monitoring (“Vessel”) Sites | Sites located on the vessel. |

# 1 Introduction

## 1.1 Background and Aims

Cardno (NSW/ACT) trading as Cardno Ecology Lab Pty Ltd was commissioned by the NSW Department of Primary Industries – Catchments and Lands (DPI Catchments and Lands), to undertake the post-scuttling environmental monitoring for the Ex-HMAS Adelaide artificial reef and dive site.

The Ex-HMAS Adelaide "the vessel" was gifted from the Australian to the NSW Government for the specific purpose of scuttling the ship as an artificial reef off the Central Coast of NSW. A comprehensive environmental assessment was undertaken for the project in accordance with state and federal environmental legislation. This included obtaining approval under the NSW Environmental Planning and Assessment Act 1979 (EP&A Act) and obtaining an Artificial Reef (or Sea Dumping) Permit issued under the Environment Protection (Sea Dumping) Act 1981 from the federal Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC).

Sea Dumping Permits ensure that appropriate sites are selected, materials are suitable and appropriately prepared, that there are no significant adverse impacts on the marine environment and that the reef does not pose a danger to marine users. A condition of the Permit is that DPI Catchments and Lands must implement a Long Term Monitoring and Management Plan (LTMMP) which was prepared in March 2011.

The LTMMP covers environmental and structural monitoring for the first five years post-scuttling and forms the basis for ongoing monitoring and maintenance over the 40 year operational life of the vessel as a dive site. The frequency of monitoring and the methodologies used will be reviewed periodically during the life of the Plan. The scope of work to be carried out by Cardno Ecology Lab is for a two year period post-scuttling, which follows on from baseline investigations in April/May 2011 and includes:

- Reef communities;
- Sediment quality; and
- Bioaccumulation studies.

This Progress Report outlines the methodology and findings for the first (of two) bioaccumulation surveys. These surveys were to be carried out six months post scuttling and again at 12 months post-scuttling. This report presents the results of the six month post-scuttling survey which was scheduled for October 2011. Due to adverse weather conditions, this survey was carried out approximately 7 months post-scuttling (November 2011). The second is scheduled for April 2012.

The main aim of the bioaccumulation study as outlined in the LTMMP is to determine whether resident biota (i.e. biota in direct contact with the superstructure), are likely to be affected by zinc chromate paint, which may have been used originally on the aluminium alloy. Zinc chromate was routinely used as an anticorrosive application on the topside of naval vessels, although it is understood that the more recent coating formulations did not contain chromium salts. Following scuttling, the zinc chromate paint (if present) is expected be subjected to corrosion and microbial attack and will likely deteriorate over time. Most available toxicological information is based on OH&S type exposure, where zinc chromate is a suspected carcinogen due to the presence of hexavalent chromium; the primary route of exposure being through inhalation of dust. The environmental fate of zinc chromate in the marine environment is less well known. It is usually described as insoluble, or very slightly soluble in Material Safety Data Sheets (Worley Parsons 2011a).

While the environmental fate of zinc chromate in the marine environment is not well understood, it is assumed that zinc and chromium will be liberated into the marine environment through processes involving dissolution and flaking. The zinc and chromium may potentially affect marine organisms that live in direct association with the vessel via accumulation within their tissues ("bioaccumulation"). Biomonitoring of marine fouling organisms was proposed as part of the LTMMP. The LTMMP recommends that until a substantial amount of marine growth has developed on the vessel, active biomonitoring involving the deployment and collection of sentinel organisms from a non-impacted / comparatively clean location (e.g. an aquaculture facility) would be deployed to the Ex-HMAS Adelaide for a period of six to eight weeks and then analysed to determine concentrations of zinc and chromium. Bivalves such as mussels or oysters are commonly used as sentinel species for this purpose.

This progress report outlines the following:

- Description of sampling dates, times, weather conditions and tidal height;
- Description of the methods used to deploy and retrieve sentinel organisms;
- Results and interpretation of laboratory analyses;
- Discussion of findings; and
- Reports of any condition or occurrence that may influence results of the study.

## 1.2 Study Area

The Ex-HMAS Adelaide artificial reef and dive site is located within Bulbararing Bay, approximately 1.87 km offshore from Avoca Beach. The ship lies at a depth of approximately 32 m to 34 m of water at Lowest Astronomical Tide (LAT) and is embedded approximately 1 m into the flat, sandy, seabed. There is a minimum of 6 m of sand overlying bedrock. The vessel is orientated with the bow facing into the prevailing ESE swell direction (**Figure 1**).

The ship is 138.1 m in length, with a beam of 14.3 m and an original displacement of 4,200 tonnes. The hull is made of steel and the superstructure of aluminium alloy. Heights are approximately 12 m to the main deck, 18 m to the bridge, 24 m to the top of the foremast (the mast closest to the bow), and 39 m to the top of the mainmast (NSW Government 2011). Preparation for scuttling involved the removal of the main mast structures for safety and navigation reasons and stripping of machinery, hatches and any items that could pose a risk to divers or the environment. Potential contaminants such as fuels, oils, heavy metals, batteries and electrical items containing polychlorinated biphenols (PCBs) were removed. Diver access holes were cut into the sides of the hull, floors and ceilings to allow extra vertical access between decks and also to allow light to penetrate. Further holes were also made to allow air to escape during the scuttling process (NSW Government 2011).

The Ex-HMAS Adelaide was prepared to meet DSEWPaC standards which were specified during the months of preparation prior to scuttling. DSEWPaC had conducted a series of inspections to confirm that its detailed requirements were achieved. The original clean-up process included removing loose or flaking paint in accordance with DSEWPaC's requirements.

# 2 Existing Information

## 2.1 Bioaccumulation Studies

Bioaccumulation is a dynamic indicator of water quality and ecosystem integrity and has gained universal acceptance as a measure of the bioavailable fraction of contaminants in the aquatic environment (Phillips 1980).

While direct measurements of metals in the sediments within and adjacent to the Ex HMAS Adelaide (and at reference locations) have been carried out as part of the LTMMP, the observed concentrations of metals vary according to different chemical, hydrographical and geological processes. Direct measurements of metal concentrations in the sediment and surrounding waters do not represent the metal loads actually available to biota (Bryan and Langston 1992, Hatje *et al*. 2003). Deploying filter feeding sentinel organisms such as bivalve molluscs to assess accumulation of metals is a relatively simple way of inferring metal bioavailability and assessing metal concentrations over both long and short periods of time (Rainbow 2006). Bivalve molluscs are filter feeding organisms which actively filter dissolved and suspended matter from the water by pumping water through specialised filtration structures. They are therefore suitable organisms to test for water contamination and the accumulation of contaminants or toxins (Huber 2010). Mussels and oysters, tolerate a wide range of temperatures, salinity, concentrations of suspended sediments and dissolved oxygen (Anderson 2001). These animals are able to accumulate certain contaminants in tissue to high concentrations without lethal effects. As these organisms are sedentary and easy to sample they provide an attractive biomonitoring tool (Phillips 1980). The Sydney Rock Oyster (*Saccostrea glomerata*), formerly known as *Saccostrea commercialis*, is commonly used in New South Wales (NSW) as a biomonitoring species because it is ubiquitous on the east coast, it survives transplantation and exposure to contaminants, accumulates contaminants to concentrations proportional to ambient waters and is readily available from commercial growers (Brown and McPherson 1992; Scanes 1996; Scanes and Roach 1999; Spooner *et al*. 2003, Hedge *et al*. 2009). The accumulation of metals by *S. glomerata* can occur via the gills in dissolution (Förstner *et al*. 1989; Simpson *et al*. 1998) or as particulates via digestion (Wang and Fisher 1999). There are several studies which document the use of Sydney rock oysters as sentinel organisms in toxicity studies on the east coast of Australia (e.g. Hedge *et al*. 2009, Scanes 1996, Scanes and Roach 1999). These studies may provide an indication of the concentration of metals which may be expected to occur in moderately urbanised coastal areas. Less information is available regarding the levels of metal toxicants in blue mussels. As such, the Sydney rock oyster was selected as the preferred test organism for this study. The species also accumulates contaminants to concentrations proportional to ambient waters (Brown and Macpherson 1992) and is readily available from commercial growers, as opposed to blue mussels, which can only be sourced from the far south coast of NSW.

## 2.2 Toxicity of Chromium in the Marine Environment

Chromium occurs naturally in the trivalent chromium (III) and hexavalent, chromium (VI) forms (Hart 1982). The form of chromium affects toxicity to aquatic organisms and the behaviour of chromium in the aquatic environment. Precipitation of chromium hydroxide is thought to be the dominant removal mechanism for chromium (III) in natural water (ANZECC/ARMCANZ 2000). Chromium (VI) may bioaccumulate to some degree and chromium (III) may be bioavailable from suspended material (ANZECC/ARMCANZ 2000). Pawlisz *et al*. (1997) reported marine toxicity data for chromium. Cr (III) was shown to affect the filtering rate of the mussel *Perna perna* at $EC_{50}$ of 2 µg/L. The lowest acute $EC_{50}$ reported for Cr (III) was 1600 µg/L for nauplii of *Tisbe battagliai* over 96 h. The 7-d LOEC (lowest observed effect concentration) for reproduction of this species was 320 µg/L. For Cr (VI), Pawlisz *et al.* (1997) reported marine acute toxicities to Australian crab *Portunus pelagicus* of 1300 µg/L and to the Australian amphipod *Allorchestes compressa* of 5560 µg/L. Several other species had similar toxicities. The most sensitive fish was flatfish *Citharichthys stigmaeus* with a 21-d $LC_{50}$ of 5000 µg/L. Short-term (2−4 d) acute toxicities to marine fish were all above 16 000 µg/L. Cr (VI) is considered more toxic to marine organisms than Cr (III). For example, the diatom *Nitzschia closterium*, isolated from estuarine waters near Sydney at 33 ‰ salinity, had a 72-h $EC_{50}$ of 2.4 mg/L for Cr (VI), compared to a 72-h EC50 of >5.0 mg/L for Cr (III) (Florence & Stauber1991). Fertilisation of the macroalga (*Hormosira banksia)*, isolated from Port Phillip Bay, was insensitive to Cr (VI), with an $EC_{50}$ of 360 mg/L. In studies with the Australian sand crab *Portunus pelagicus*, deleterious sub-lethal effects were found at Cr (VI) concentrations of 300 µg/L (Mortimer & Miller 1994) while the 96-h $LC_{50}$ for the Tasmanian blenny, a tidepool fish, was reported as 2.6 mg/L (Stauber *et al*. 1994a).

The ANZECC/ARMCANZ water quality guideline for chromium (at the 95 % protection level) is 4.4 µg $L^{-1}$. In marine and estuarine conditions, high sulfate concentrations make chromium toxicity unlikely, except at very polluted sites (ANZECC/ARMCANZ 2000). A recommendation of 5 µg $L^{-1}$ (dissolved annual average) is broadly accepted for the protection of saltwater life, although where there is concern that the health of communities in sites of nature conservation importance may be compromised as a result of the presence of particularly sensitive species, a lower value may be used as a guideline.

## 2.3 Toxicity of Zinc in the Marine Environment

Zinc is an essential trace element required by most organisms for their growth and development. It is found in most natural waters at low concentrations (ANZECC/ARMCANZ 2000).

Mance and Yates (1984) reviewed data on the toxicity of zinc to marine organisms. Similar to chromium, invertebrates were generally more sensitive than the fish species investigated while effects on marine macro and microalgae were noted at concentrations slightly lower than reported for invertebrates. The apparent development of increased tolerance was noted as a complicating factor. They reported the toxicity and bioaccumulation of zinc to be greater at lower salinities. Hunt and Hedgecott (1992) proposed a guideline value of 10 µg $L^{-1}$ as appropriate for the protection of saltwater life. This value (also expressed as a dissolved annual average) was based on the lowest, most reliable NOECs (No Observed Effects Concentrations) reported for a range of organisms. In Australia, ANZECC/ARMCANZ guidelines (at the 95 %) protection level is 15 µg $L^{-1}$.

Neither chromium nor zinc are listed as toxicants for which possible bioaccumulation and secondary poisoning effects require special consideration in terms of the ANZECC/ARMCANZ water quality guidelines. For some chemicals (e.g. mercury and PCBs), this is the main issue of concern, rather than direct effects of toxicants. Metals such as chromium, zinc and copper, can accumulate in shellfish without causing harm to the animals.

Acute toxicity testing of chromium and zinc has been carried out for several different groups of marine species and are published in the ANZECC Guidelines. These guidelines are not directly relevant to the current study as water quality testing was not carried out in conjunction with the bioaccumulation study.

## 2.4 Previous Studies

As per the requirements of the LTMMP, the first bioaccumulation study (implemented by Worley Parsons) took place one week after scuttling of the Ex-HMAS Adelaide (April 2011). Blue mussels, sourced from Eden Sea Farms aquaculture facility (southern NSW) were used as the test organism. Mussels were deployed at three monitoring sites attached to the vessel ("vessel sites") and two control sites on mooring lines approximately 35 m from the vessel to provide an indication of background concentrations of metals. Mussel samples collected directly from the aquaculture facility were also tested to determine baseline levels of contaminants. Mussels were retrieved from the monitoring sites after a six week deployment period. Mussel bags from the control sites were lost as they were attached to moorings which became displaced.

The mean concentration of chromium in mussel tissues from baseline controls was 0.67 mg/$kg^{-1}$ d.w (S.D= 0.1 mg/$kg^{-1}$ d.w). Mean zinc concentration in the in the baseline controls was 152 mg/kg with a standard deviation of 29.5 mg/$kg^{-1}$ d.w.

A comparison of mean concentrations of metals in tissue among the three impact sites found that concentrations from the bow, stern and mid-section were generally similar. When data were combined, the vessel samples had a mean chromium concentration of 1.4 mg/$kg^{-1}$ d.w (S.D. = 0.47 mg/$kg^{-1}$ d.w). Zinc had a mean value of 178 mg/$kg^{-1}$ d.w (S.D. = 44.4 mg/$kg^{-1}$ d.w). Overall, there were no statistically significant differences in metal concentrations in tissues among the three vessel sites after a six week deployment period.

Significant differences in metal concentrations in mussel tissue between the baseline controls and vessel sites were evident. *Post-hoc* testing identified significant differences in the concentrations of chromium, but no differences in zinc. Although an increase in metal concentrations was observed between the baseline control mussels and the vessel samples, the significant increase noted for chromium could not be directly attributed to the presence of the vessel without consideration of environmental control concentrations as the references would have provided a measure of background concentrations. Given the limited amount of data available regarding metal concentrations in blue mussels in the study region, broader comparisons of the data with expected ambient levels were not made.


| Boundary of Dive Site | Easting (MGA 94) | Northing (MGA 94) |
|---|---|---|
| A | 356428.713 | 6296117.693 |
| B | 356538.438 | 6296341.142 |
| C | 356850.615 | 6296188.618 |
| D | 356742.410 | 6295963.310 |

**Figure 1: Location of Ex-HMAS Adelaide Artificial Reef and Dive Site.** The approximate location and orientation of the ship is indicated by the yellow line.

# 3 Study Methods

## 3.1 Sampling Design

The sampling design outlined in the LTMMP included multiple monitoring and control sites. This included three vessel monitoring sites at the bow, mid-ship and stern of the vessel and two control sites attached to mooring buoys approximately 20 m to the port and starbord of the of the vessel. At the time of deployment it was advised by DPI (catchments and Lands) that only one special marker buoy (located approximately 35 m to the bow of the vessel) was available as a control site. Both lots of control samples were therefore attached to the special marker buoy as indicated in **Figure 2**.

As per the recommendations of DPI (Catchments and Lands), attaching the control oyster trays to the special marker buoy mooring lines was considered to be preferable over attachment to the dive mooring lines (recommended in the LTMMP) as they were stronger and less likely to be damaged or tampered with by divers ascending or descending the lines (Worley Parsons 2011b). For consistency between control and monitoring locations, depth of attachment at the control sites was the same as for the vessel monitoring sites (i.e. ~ 25 m).

Three oyster trays (each containing approximately 30 oysters) were attached at each site which is similar to Worley Parsons (2011b). The sampling design includes the 'After, Control, Impact components of the widely accepted BACI' (Before, After, Control, Impact) sampling approach which makes use of multiple controls in space and time (Underwood 1991, 1992, Long *et al*. 1996). As no sampling was undertaken prior to the scuttling of the Ex-HMAS Adelaide, no 'before' data are available to compare with the 'after' data and therefore relies strongly on environmental controls. This will allow changes at a potential impact location to be evaluated against variation measured at multiple control locations. The design requires that sampling be done in at least two external control locations in addition to the potential impact locations. In summary, the sampling design would yield a total of 45 samples including:

- x 3 vessel monitoring sites (bow, stern and mid ship) attached to ship;
- x 2 control background control locations (bow special marker buoy) approx. 35 m from ship;
- x 3 trays at each location (90 mussels per location);
- x 30 oysters within each tray.
- **= Total of 450 oysters (15 samples)**

In addition, three samples (each of 30 oysters) were used as 'baseline controls' as a measure of concentrations of zinc and chromium in oysters prior to deployment.

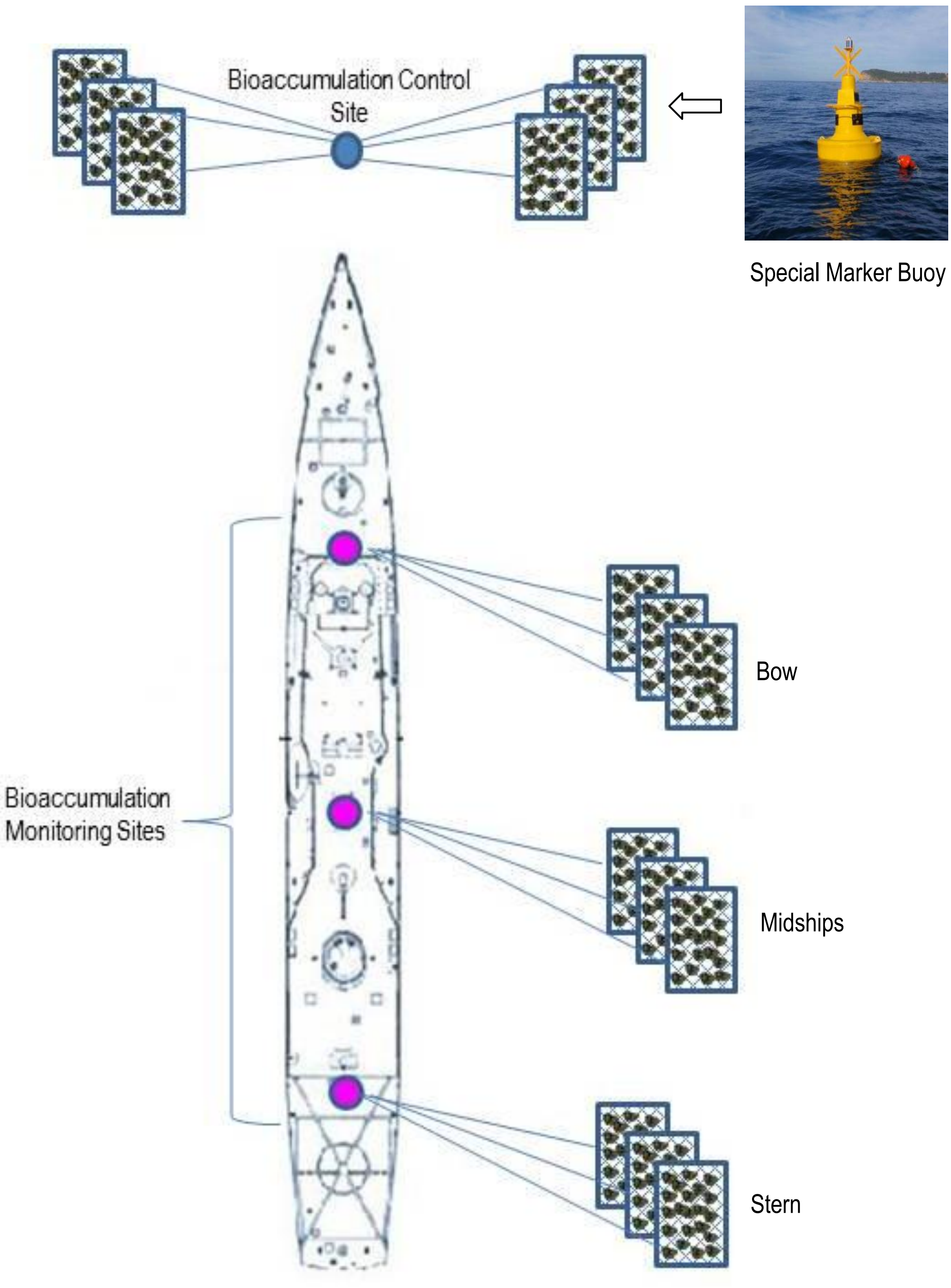


**Figure 2: Positions of Control and Vessel Monitoring Sites Selected for the Deployment of Oysters.**

## 3.2 Field Methods

Sydney rock oysters (*Saccostrea glomerata*) were sourced from a supplier in Tuncurry/Forster (Trevor Dent Oyster Suppliers) and delivered to Cardno's Sydney laboratory 24 hours prior to deployment, where they were maintained at a temperature of approximately 20°C. As oysters can survive for several weeks at this temperature, the minimal time spent out of water was not considered to have any impact on the survivorship of the test organisms once deployed. It is possible that size and growth rate may affect the rate of bioaccumulation in oysters (Pearson 1993 Phillips 1980, Richards and Chaloupka (2008). Therefore only organisms of a similar age and size (bottle grade) were used to minimise the effects of growth dilution. Individual oysters were attached to the inside of purpose built oyster trays approximately 100 cm length x 50 cm width x 10 cm deep (**Figure 3**). The trays were constructed of robust UV stable oyster mesh (2 cm x 2 cm) and were fitted with a float at one end and a weight at the bottom to ensure that they sit upright and stable in the water column. The use of oyster trays does not affect the accumulation of metals by organisms (Cain and Luoma1985). The oyster trays were attached to the vessel (monitoring sites) and mooring lines (control sites) by divers using a combination of marine grade rope, cable ties and small stainless steel shackles. Once secured, the trays were left in place for a period of approximately 10 weeks to allow sufficient time for any bioavailable chromium or zinc to be assimilated into the oyster tissue. Once collected, oysters were removed from the trays and placed in polyurethane bags labelled internally and externally with the site, date, time and replicate number. Bags containing oysters were chilled and transported back to the Cardno laboratory in eskies to be prepared for tissue analysis.

**Figure 3: Preparing oyster trays for deployment.**

## 3.3 Laboratory Methods

Oysters were opened carefully with stainless steel knives and the soft tissue was dissected from their shells using a wooden spatula to avoid the risk of metal contamination. The soft tissue for each oyster was placed into a chilled polyurethane bag and the shell was discarded. Samples were then frozen and dispatched to an NATA accredited laboratory for analysis of metal concentrations. As there is no standard methodology for analysis of hexavalent chromium, samples were analysed for total chromium and zinc.

Under the LTMMP, sampling and analysis of oyster tissue is required to be reported in dry weight (wet weights are needed if comparison with food standards are required, however, this was not the case). Tissue from each of the 30 oysters making up each sample were freeze-dried and homogenised into one composite sample to reduce the effect of intraspecific variability between individuals. These samples were analysed for chromium and zinc using digestion by concentrated nitric acid (or a mixture of nitric and hydrochloric acids) by heating on top of a boiling water bath. Elements were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and/or Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Laboratory QA / QC was undertaken. Laboratory Sample Receipts and original Reports of Analysis from the baseline control and monitoring site oysters are provided in **Appendix 1**. The level of reporting (LOR) was 0.01 mg/kg.

## 3.4 Data Analyses

Mean and standard errors were calculated for concentrations of chromium and zinc at all sites and for the baseline controls. Permutational Analysis of Variance was used to determine any spatial differences between the concentrations of chromium and zinc in mussel tissues between the three impact sites baseline controls and where possible at background control sites. The PERMANOVA+ routine in PRIMER v6 was used to undertake all statistical analysis.

# 4 Results

## 4.1 General Findings

Oysters were deployed on the Ex-HMAS Adelaide on 24 November 2011 and retrieved on 20 January 2012 (a period of approximately 10 weeks). Conditions at the time of deployment were calm with approximately a 1 m swell and a light wind from the SSE. Visibility was approximately 5 m. Conditions at the time of retrieval were similar with a light breeze from the SE/E and visibility of 4 - 5 m, however, due to unknown circumstances (likely to be extreme weather), several of the oyster trays were lost and could not be retrieved. These included all of the environmental controls attached to the special marker buoys. Since deployment of further oyster trays at a different time period would not have produced comparable results, analysis was only done to compare metal concentrations at the monitoring sites with those of the baseline control oysters. Monitoring samples attached to the vessel were retrieved from the mid ship and stern only.

Results of the tissues analysis are presented in **Table 1**. Receipts of laboratory testing are provided in **Appendix A.**

The mean concentration of chromium in the baseline control samples was 0.21 mg/kg $^{-1}$ d.w (S.E= <0.1). The mean concentration of chromium in monitoring samples collected from the mid ship was 0.53 mg/kg $^{-1}$d.w (S.E = 0.29). The concentration of chromium from the stern was 0.23 mg/kg$^{-1}$ d.w. Means and standard errors were not calculated due to lack of replicate samples.

The mean concentration of zinc in the baseline control samples was 866.67 mg/kg $^{-1}$ d.w. (S.E. 44.7). The mean concentration of zinc in monitoring samples collected from the mid ship was 1033.33 mg/kg $^{-1}$d.w (S.E = 73.11). The concentration of zinc from the stern was 800 mg/kg$^{-1}$ d.w. Moisture content measured across all samples was similar.

## 4.2 Comparison between Monitoring Sites and Baseline Controls

The mean concentration of both chromium and zinc was marginally higher at the monitoring sites than for the baseline control samples (**Table 1**). Univariate statistical analysis did not, however, indicate that these differences were statistically significant ($P$= 0.543, Chromium), ($P$=0.373, Zinc).

**Table 1: Concentrations of Chromium and Zinc (dry weight) in Oysters Deployed at Monitoring Locations on the Ex-HMAS Adelaide and from Baseline Controls Transplanted from Forster/Tuncurry in November 2011.** (n=7)

| | Sample ID | Trace Metals | | Moisture Content (%) |
|---|---|---|---|---|
| | | **Chromium (mg/kg)** | **Zinc (mg/kg)** | |
| | LOR | 0.01 | 0.01 | n/a |
| Baseline Controls | BASELINE CONTROL R1 | 0.22 | 890.00 | 79.70 |
| | BASELINE CONTROL R2 | 0.24 | 930.00 | 79.60 |
| | BASELINE CONTROL R3 | 0.17 | 780.00 | 80.00 |
| | **Mean** | **0.21** | **866.67** | **79.77** |
| | **S.E** | **0.02** | **44.85** | **0.12** |
| Monitoring Sites | MID SHIP R1 | 0.26 | 1010.00 | 80.00 |
| | MID SHIP R2 | 1.10 | 1170.00 | 77.70 |
| | MID SHIP R3 | 0.23 | 920.00 | 79.60 |
| | **Mean** | **0.53** | **1033.33** | **79.10** |
| | **S.E** | **0.29** | **73.11** | **0.71** |
| | STERN R1 | 0.23 | 800.00 | 78.10 |
| | **Mean** | n/a | n/a | n/a |
| | **S.E.** | n/a | n/a | n/a |

# 5 Discussion

The mean concentrations of both chromium and zinc tended to be larger at the vessel monitoring sites than for the baseline control samples, although this was not statistically significant. A similar trend was observed in the previous study (Worley Parsons 2011b), but direct comparisons between the two studies cannot be made, as different species and animals of different sizes and ages (likely to bioaccumulate at different rates), were used. Thus, in the absence of appropriate environmental controls, it cannot be determined whether zinc and chromium potentially leached from the Ex-HMAS Adelaide has resulted in elevated levels of these metals in filter feeders living in association with the vessel. The statistical power of the analyses performed in the current study was also limited by the small number of samples retrieved after the 10 week period of deployment.

Zinc and chromium are essential elements for many marine organisms and as such, readily bioaccumulate. Zinc levels observed in the tissues of oysters in the present study are similar to background levels recorded at their source and would not be of toxicological significance. The levels of zinc recorded in oyster tissues are also similar or below that recorded in tissues of the same species in a recent bioaccumulation study of Port Hacking and Botany Bay (Hedge *et al*. 2009). The next bioaccumulation survey is scheduled to take place in April 2012.

# 6 Acknowledgements

This report was written by Kate Reeds and reviewed by Dr Marcus Lincoln Smith. Field Work was done by David Cummings, Brendan Alderson, Craig Blount and Marcus Lincoln Smith. Laboratory work was done by NMI Sydney.

# 7 References

Anderson, D.T., (2001). Invertebrate Zoology. Oxford University Press, South Melbourne.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for Fresh and Marine Water Quality, National Water Quality Management Strategy No. 4, Australian and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand, October 2000.

Brown, K.R., McPherson, R.G., (1992). Concentrations of copper, zinc and lead in the Sydney rock oyster, *Saccostrea commercialis* (Iredale and Roughley) from the Georges River, New South Wales. Science of the Total Environment 126, 27-33.

Bryan, G.W., Langston, W.J., (1992). Bioavailability, accumulation and effects of heavy-metals in sediments with special reference to United Kingdom estuaries – a review. Environmental Pollution 76, 89–131.

BOM (2011). Bureau of Meterology. Climate Statistics. Viewed November 2011. http://www.bom.gov.au/nsw/

Cain, D.J., Luoma, S.N., (1985). Copper and silver accumulation in transplanted and resident clams (Macoma–Balthica) in South San Francisco Bay. Marine Environmental Research 15, 115–135.

Florence TM & Stauber JL 1991. The toxicity of heavy metals to aquatic organisms. In Proceedings of IIR Conference on environmental monitoring, Sydney.

Förstner, U., Ahlf, W., Calmano, W., (1989). Studies on the transfer of heavy metals between sedimentary phases with a multi-chamber device. Combined effects of salinity and redox variation Marine Chemistry 28, 145–158.

Hatje, V., Apte, S.C., Hales, L.T., Birch, G.F., (2003). Dissolved trace metal distributions in Port Jackson estuary (Sydney Harbour), Australia. Marine Pollution Bulletin 46, 719–730.

Hedge L.H., Knott, N.A., Johnston E.L. (2009). Dredging related metal bioaccumulation in oysters. Marine Pollution Bulletin 58 (2009) 832–840.

Huber M. (2010). Compendium of Bivalves. A Full-color Guide to 3'300 of the World's Marine Bivalves. A Status on Bivalvia after 250 Years of Research. Hackenheim: Conch Books. pp. 901 pp.

Hunt, S. M. and Hedgecott, S. (1992) Revised environmental quality standards for zinc in water. Water Research Centre Report No. DoE 2686/1, 107 pp.

Long, B. G., Dennis, D. M., Skewes, T. D., Poiner, I. R. (1996). Detecting an environmental impact of dredging on seagrass beds with a BACI sampling design. Aquatic Botany 53: 235-243.

Mance, G. and Yates, J. (1984) Proposed environmental quality standards for List II substances in water. Zinc. Water Research Centre, Technical Report TR209.

Mortimer MR & Miller GJ 1994. Susceptibility of larval and juvenile instars of the sand crab, *Portunus pelagicus* (L.), to sea water contaminated by chromium, nickel or copper. Australian Journal of Marine and Freshwater Research 45, 1107–1121.

NSW Government (2011). Life Before Scuttling – History of the HMAS Adelaide. NSW Government, Queens Square, Sydney.

Pawlisz AV, Kent RA, Schneider UA & Jefferson C 1997. Canadian water quality guidelines for chromium. Environmental Toxicology and Water Quality 12, 123−183.

Pearson, B. (1993). Bioaccumulation of Heavy Metals in Sydney Rock Oyster (*Saccostrea commercialis*) (Iredale and Roughley). Masters Thesis, University of Technology, Sydney.

Phillips, D.J.H., 1977. Use of Biological indicator organisms to monitor trace-metal pollution in marine an estuarine environments – review. Environmental Pollution 13, 281–317.

Rainbow, P.S., (2006). Biomonitoring of trace metals in estuarine and marine environments. Australasian Journal of Ecotoxicology 12, 107–122.

Richards, R.G., Chaloupka, M (2008). Does oyster size matter for modelling trace metal bioaccumulation?. Science of the Total Environment (389) 539 – 544.

Scanes, P.R., (1996). Oyster Watch: Contaminants in Deployed Oysters in Sydney. NSW Environment Protection Authority, Sydney.

Scanes, P.R., Roach, A., (1999). Determining natural 'background' concentrations of trace metals in oysters from New South Wales, Australia. Environmental Pollution 105, 437-444.

Simpson, S.L., Apte, S.C., Batley, G.E., (1998). Effect of short term resuspension events on trace metal speciation in polluted anoxic sediments. Environmental Science and Technology 32, 620–625.

Spooner, D.R., Maher, W., Otway, N., (2003). Trace metal concentrations in sediments and oysters of Botany Bay, NSW, Australia. Archives of Environmental Contamination and Toxicology 45, 92-101.

Stauber J.L, Gunthorpe L, Deavin J, Munday B & Ahsanullah M 1994a. Validation of new marine bioassays using synthetic treated and untreated bleached eucalypt kraft mill effluents. National Pulp Mills Research Program Technical Report 8, CSIRO, Canberra.

Wang, W.-X., Fisher, N.S., (1999). Delineating metal accumulation pathways for marine invertebrates. The Science of the Total Environment 237–238, 459–472.

Worley Parsons (2011a). Ex-HMAS Adelaide Artificial Dive Reef - Long Term Monitoring and Management Plan. Report for the Land and Property Management Authority (LPMA), WorleyParsons, March 2011.

Worley Parsons (2011b). Ex-HMAS Adelaide Artificial Dive Reef -. Bioaccumulation study (Draft). Report for the Land and Property Management Authority (LPMA), WorleyParsons, June 2011.

WorleyParsons (2011c). Ex-HMAS Artificial Reef Project – Marine Sediment Quality Survey. Report for the Land and Property Management Authority (LPMA), WorleyParsons, July 2011.

# 8 Appendices

**Appendix A: Laboratory Results**
**Appendix B: Results of Univariate Statistical Analysis**

## Appendix A: Laboratory Results

Australian Government
National Measurement Institute

# REPORT OF ANALYSIS



Report No. RN899277

| | | | |
|---|---|---|---|
| Client | : CARDNO ECOLOGY LAB<br>LEVEL 9, THE FORUM<br>203 PACIFIC HIGHWAY<br>ST LEONARDS NSW 2065 | Job No.<br>Quote No.<br>Order No.<br>Date Sampled<br>Date Received | : CARD20/120125<br>: QT-01735<br>:<br>: 20-JAN-2012<br>: 25-JAN-2012 |
| Attention | : KATE REEDS | Sampled By | : |
| Project Name : | | | |
| Your Client Services Manager | : BRIAN WOODWARD | Phone | : (02) 94490151 |

| Lab Reg No. | Sample Ref | Sample Description |
|---|---|---|
| N12/002019 | . | OYSTER TISSUE ZERO CONTROL R1 JOB NO EL1112024 |
| N12/002020 | . | OYSTER TISSUE ZERO CONTROL R2 JOB NO EL1112024 |
| N12/002021 | . | OYSTER TISSUE ZERO CONTROL R3 JOB NO EL1112024 |
| N12/002022 | . | OYSTER MID SHIP R1 JOB NO EL1112024 |

| Lab Reg No. | | N12/002019 | N12/002020 | N12/002021 | N12/002022 | |
|---|---|---|---|---|---|---|
| Sample Reference | Units | . | . | . | . | Method |
| Trace Elements | | | | | | |
| Chromium | mg/kg | 0.22 | 0.24 | 0.17 | 0.26 | NT2_46 |
| Zinc | mg/kg | 890 | 930 | 780 | 1010 | NT2_46 |
| Moisture Content | | | | | | |
| Moisture | % | 79.7 | 79.6 | 80.0 | 80.0 | NT2_49 |

N12/002019
-N12/002025.
Results are expressed on a dry weight basis.

Lisa Liu, Analyst
Inorganics - NSW
Accreditation No. 198

9-FEB-2012

# REPORT OF ANALYSIS


Report No. RN899277

| | | | |
|---|---|---|---|
| Client | : CARDNO ECOLOGY LAB<br>LEVEL 9, THE FORUM<br>203 PACIFIC HIGHWAY<br>ST LEONARDS NSW 2065 | Job No.<br>Quote No.<br>Order No.<br>Date Sampled<br>Date Received | : CARD20/120125<br>: QT-01735<br>:<br>: 20-JAN-2012<br>: 25-JAN-2012 |
| Attention | : KATE REEDS | Sampled By | : |
| Project Name | : | | |
| Your Client Services Manager | : BRIAN WOODWARD | Phone | : (02) 94490151 |

| Lab Reg No. | Sample Ref | Sample Description |
|---|---|---|
| N12/002023 | . | OYSTER MID SHIP R2 JOB NO EL1112024 |
| N12/002024 | . | OYSTER MID SHIP R3 JOB NO EL1112024 |
| N12/002025 | . | OYSTER STERN R1 JOB NO EL1112024 |

| Lab Reg No. | | N12/002023 | N12/002024 | N12/002025 | | |
|---|---|---|---|---|---|---|
| Sample Reference | Units | . | . | . | | Method |
| Trace Elements | | | | | | |
| Chromium | mg/kg | 1.1 | 0.23 | 0.23 | | NT2_46 |
| Zinc | mg/kg | 1170 | 920 | 800 | | NT2_46 |
| Moisture Content | | | | | | |
| Moisture | % | 77.7 | 79.6 | 78.1 | | NT2_49 |

Lisa Liu, Analyst
Inorganics - NSW
Accreditation No. 198

9-FEB-2012

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

Accreditated for compliance with ISO/IEC 17025.
This report shall not be reproduced except in full.
Results relate only to the sample(s) tested.

This Report supersedes reports: RN899223 RN899270

**Appendix B:** Permutational Analysis of Variance (PERMANOVA) comparing concentrations of zinc and chromium at control and monitoring locations in relation to the Ex-HMAS Adelaide. Significant factors are highlighted in bold. Data was not transformed as the data was normally distributed.

**A. Chromium**

| Source of Variation | df | SS | MS | Pseudo-F | P(perm) | Unique perms |
|---|---|---|---|---|---|---|
| Treatment (Monitoring vs Baseline) | 1 | 0.1029 | 0.1029 | 0.92221 | 0.543 | 22 |
| Residual | 5 | 0.5579 | 0.11158 | | | |
| Total | 6 | 0.6608 | | | | |

Estimates of components of variation

| Source | Estimate | Sq.root |
|---|---|---|
| S(Tr) | 0.002532 | 0.050316 |
| V(Res) | 0.11158 | 0.33404 |
| Total | 0.384356 | |

**B. Zinc**

| Source | df | SS | MS | Pseudo-F | P(perm) | Unique perms |
|---|---|---|---|---|---|---|
| Tr | 1 | 20119 | 20119 | 1.1839 | 0.3726 | 31 |
| Res | 5 | 84967 | 16993 | | | |
| Total | 6 | 105090 | | | | |

Estimates of components of variation

| Source | Estimate | Sq.root |
|---|---|---|
| S(Tr) | 911.67 | 30.194 |
| V(Res) | 16993 | 130.36 |
| Total | 160.554 | |