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The Accumulation of Zn, Se, Cd, and Pb and Physiological Condition of *Anadara trapezia* Transplanted to a Contamination Gradient in Lake Macquarie, New South Wales, Australia.

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Abstract

The benthic bivalve, *Anadara trapezia*, was collected from a 'clean' reference site and transplanted along a suspected trace metal contamination gradient in Lake Macquarie, NSW. At monthly intervals, Zn, Se, Cd and Pb concentrations were measured in the surficial sediments and whole tissues of the cockle as well as their physiological condition (Scope for Growth).

Zinc, Cd and Pb concentrations in sediments decreased together, southward, with the highest concentrations in the Cockle Bay area, suggesting that this is the main source of contamination. Zinc, Cd and Pb concentrations were near or above ANZECC/ARMCANZ (2000) sediment quality guidelines at Cockle Creek, Warners Bay and Koorooa Bay. Significant differences in trace metal concentrations could not be attributed to grain size or Fe concentration differences. Se concentrations were highest in fine grain Fe rich sediments of Whiteheads Lagoon, and likely to be associated with power generation operations. Trace metal concentrations did not vary significantly over time.

Zinc, Cd and Pb concentrations in the tissues of *A. trapezia* followed a similar pattern to that of sediments. Zinc and Pb concentrations in cockles and sediments were highly correlated, indicating significant *exposure-dose* relationships. Selenium concentrations in the tissues of *A. trapezia* were higher after transplantation to the lake, however, Se concentrations were similar in all transplanted cockles, indicating that Se in contaminated sediments is not the major source of Se to organisms. There was a decline in the physiological condition of *A. trapezia* transplanted to Lake Macquarie after a 90-day period with marked differences in clearance rates and respiration rates at some locations and absorption efficiencies at all locations. The mean Scope for Growth value at the most contaminated location, Cockle Bay, was markedly lower than at other locations. A significant Zn *exposure-dose* response relationship indicates that Zn bioaccumulation is occurring in response to sediment contamination. A significant Cd *exposure-response* relationship indicates that Cd may be influencing the health of cockles. Significant Pb *exposure-dose*, *exposure-response* and *dose-response* relationships indicate that Pb probably is affecting the health of cockles in Lake Macquarie. Therefore, Zn, Cd and Pb concentrations in sediments are likely to be affecting the health of cockles in Lake Macquarie.

Introduction

Industrial development around Lake Macquarie, NSW, Australia (Figure 1) is extensive and consists of a Pb-Zn smelter, a fertiliser plant, steel foundry, collieries, sewage treatment works and two coal-fired power stations. The lake supports a large

recreational fishery and provides breeding and nursery grounds for many commercial fish species.

In comparison to other NSW estuaries, Lake Macquarie has significantly higher concentrations of trace metals¹ in its sediment, especially in the northern reaches (Batley, 1991; Kirby *et al.*, 200a, b; Peters *et al.*, 1999a, b; Roy and Crawford, 1984). A Pb-Zn smelter to the North of the lake has been in operation since 1897, and is a known source of Zn, Se, Cd, and Pb (SPCC, 1983, AWACS, 1995). Metal concentrations above background levels in the southern reaches and elevated metal concentrations near power stations indicate that the coal-fired power stations are also contributing trace metals to the lake (Kirby *et al.*, 2001a, b; Roach, 2005).

To evaluate if trace metals in Lake Macquarie sediments are bioavailable and likely to cause harm to benthic aquatic organisms we undertook a study to establish the *exposure-dose-response relationships* (Widdows and Donkin, 1992) of the sediment-dwelling cockle *Anadara trapezia* (which is indigenous to Lake Macquarie) transplanted along a suspected sediment trace metal contamination gradient in Lake Macquarie. Environmental trace metal concentrations were established in the sediments of Lake Macquarie and related to the uptake and accumulation of trace metals in *A. trapezia*. Finally, to indicate the biological response of the transplanted cockles the physiological measurements, clearance rate, absorption efficiency, oxygen consumption were made and the stress index Scope for Growth calculated (Bayne *et al.*, 1985; Widdows and Salkeld 1992).

Experimental

Study area and locations

Lake Macquarie is a large estuarine barrier lake about 100km north of Sydney on the southern outskirts of Newcastle, NSW (Figure 1). The lake extends 22km in a north-south direction, has a maximum width of about 10 km and a surface area of approximately 109 km². It is the largest coastal lake in eastern Australia and has a catchment area of approximately 622 km² (AWACS, 1995). The average depth of the lake is 6.7 m with a maximum depth of 11 m near Pulbah Island (Batley, 1987). A

¹ Trace metal refers to trace metals and metalloids

narrow connection to the ocean at Swansea reduces the tidal range to between 0.1 - 0.2 m, resulting in poor tidal flushing of the lake (AWACS, 1995). Also there are only small salinity fluctuations due to its relatively small catchment and associated freshwater inflows.

Five locations (Figure 1) were selected to examine how trace metal concentrations diminish with distance southward from the mouth of Cockle Creek. Locations were selected to follow the dispersion of sediments from Cockle Creek, the major freshwater input into Lake Macquarie and were of similar depths (~ 1.5 m). In addition a location with no industrial or sewage inputs was chosen at Bagnalls Beach, Port Stephens as a relatively pristine external control from which the cockle *A. trapezia* would be collected for transplant studies.

Study design and Statistical Analyses and sampling

Sediments

The sediment study was designed for a three-factor nested analysis of variance (Time, Location and Site). There were four sampling times and six locations (including control), with two sites nested in each location. Tukey's HSD test was performed on random times and raw data to determine where the significant differences occurred. Samples were collected by pushing an acid-washed 50-mL urine vial vertically down into the top 5 cm of the sediment layer. Vials were then withdrawn and frozen for transportation back to the laboratory.

Transplantation experiments

This study was designed for a four-factor nested analysis of variance (time, location, site and cage). Locations were the general area of sampling (Figure 1) while sites were specific areas at least 100 m apart, randomly chosen with locations for sampling. There were three sampling times (30, 60 and 90 days) and six locations (including control), with two sites nested in each location and two cages nested in each site. Tukey's HSD test was performed on cockles sampled after 60 days (time for cockles to reach a steady state with their environment) to determine where the significant differences occurred.

Transplantation experiments

***A. trapezia* Collection**

The Sydney cockle, *A. trapezia*, was chosen to assess bioaccumulation and effects as it is native to Lake Macquarie. It is a sediment-dwelling organism and been shown to accumulate trace metals in areas where sediments are contaminated with trace metals (Bately, 1987; Furner, 1979; Kirby *et al.*, 2001; Peters *et al.*, 1999a, b, Rai, 1998). Insufficient cockles were present at locations to use native cockles. Collection occurred over a three-day period in April 2000, shortly after the active spawning season, that occurs during the summer months (Rai, 1998). The collection of 450 cockles was from a native population at Bagnalls Beach, Port Stephens, located North of the study area. The area was chosen for its abundance of *A. trapezia*, close proximity to the study area and the relatively uncontaminated state of its environment (NSW Fisheries Research Institute Staff, Port Stephens *pers comm.*). Collection from a single population was favoured to decrease the genetic variability of the sample population, and to ensure that organisms had a similar exposure history to contaminants. To confirm that the collection site and cockles were relatively free from contaminants, a sub-sample of 22 cockles was used to determine baseline trace metal concentrations.

Transplantation of *A. trapezia*

In total, sixty plastic cages 35cm length x 30cm width x 10cm height were deployed on 10th April 2000, to randomly selected sites at the study locations. Divers, using SCUBA, embedded the cages into the benthic sediment of the lake. Cages were placed subtidally in 1-1.5 m of water. Cockles were unable to be caged at the 'source' location, as Bagnalls Beach proved to be a busy public beach, with a large tidal fluctuation, making the placement of control cages at this site prone to theft and impractical. Previous studies (Cain and Luoma, 1985; Couillard *et al.*, 1994a, b; DeWitt *et al.*, 1999; Martincic *et al.*, 1992) have concluded that cages per se have no treatment effects so this became an assumption for this study. Three days later on the 13th of April 2000, after sediment was allowed to equilibrate in the cages, SCUBA divers, deployed seven cockles into each cage. Cages were then secured to avoid predation and escape of the cockles. Three retrieval trips occurred, once a month, until July 2000. On each occasion two randomly chosen cages were retrieved from each site, nested within the locations, and five cockles randomly selected from each cage for metal analyses. For the same purpose ten cockles were collected from Bagnalls Beach each month. Cockles were depurated for 72 hours in fresh aerated seawater

from the location they were collected, then frozen at -20°C until dissection.

Examinations of cockle digestive systems showed no evidence of the presence of sediment.

Water samples were also collected at each location on each sampling occasion using a 1L acid-washed low density polyethylene container. A 50-ml subsample was filtered through a $0.45\ \mu\text{m}$ filter (Millipore HA), frozen on ice in the field and on return to the laboratory frozen at -20°C until analysed.

Trace metal analysis

Sediments

Sediments were oven dried at 60°C for 72 hours and hand sieved through a nylon mesh ($63\mu\text{m}$) and a 0.2 gram sub-sample weighed into a 50-ml polypropylene centrifuge tube. Five mL of nitric acid (Aristar Grade) was added, the tube was then capped and a fiberoptic probe placed in one of the sample tubes, and used as a temperature control for all vessels. Samples were digested in a CEM 2000 microwave oven, for 60 minutes at 60% power, which produced a stable temperature of 115°C . Samples were allowed to cool, diluted to a final volume of 50mL with deionised water, centrifuged and the supernatant removed for analysis.

Cockles

Thawed cockles were thoroughly washed of any detrital and epiphytic matter to reduce the chance of contamination. Tissues were frozen at -20°C prior to freeze-drying at -83°C (LABCONCO, Freezone Plus 6) for 24 hours and homogenised samples digested using a low-volume microwave digestion technique (Baldwin *et al.*, 1994). Digested samples were allowed to cool down to room temperature, transferred to 10-mL polyethylene centrifuge tubes and diluted to 10 mL with deionised water.

Trace Metal Analysis

Extracts were analysed for Zn, Se, Cd and Pb using a Perkin-Elmer Elan 6000 Inductively Coupled Plasma - Mass Spectrometer (ICP - MS) with a Scott double pass spray chamber and cross-flow nebuliser (Maher *et al* 2001). Certified reference materials (National Research Council Canada Marine Sediment BCSS-1; National

Bureau of Standards Estuarine Sediment 1646, National Institute Standards and Technology Oyster tissue 1566a; National Research Council Canada Lobster Hepatopancreas Tort-1, National Institute Environmental Studies Sargasso No. 9 and National Institute Environmental Studies Chlorella No. 3), were routinely digested and analysed with samples to determine the recovery rate of trace metals (Table 1) Water samples were analysed after on-line preconcentration with iminodiacetate resin and removal of salts (Warken *et al.*, 2000; Willie *et al.*, 1998). The accuracy of water analyses was confirmed by the analysis of two water CRMs, NASS-5 and SLEW-3 (Table 1)

Scope for growth measurements

Physiological response measurements (clearance rate, food absorption efficiency, respiration rate and ammonia excretion) were measured for a sub-sample of pre-deployment cockles (n = 40), Bagnall Beach cockles and all cockles collected 90 days after transplantation using published procedures (Bayne *et al.*, 1976, Bayne and Widdows, 1978). Trace metal concentrations in the algae feed to cockles, *Isochrysis galbana* (Tahitian strain), *Chaetoceros gracilis/muellrri* and *Tetraselmis species* (Reed Mariculture Inc., USA. were measured to detect any additional trace metal exposure that may have occurred through food intake during scope for growth trials. Trace metal concentrations in food were low (Zn: 12-72 $\mu\text{g g}^{-1}$; Se: < 0.02 $\mu\text{g g}^{-1}$; Cd: < 0.02 $\mu\text{g g}^{-1}$; Pb: < 0.02-2.3 $\mu\text{g g}^{-1}$ dry mass), and can be disregarded as a possible influence on cockles..

Other analyses

For particle size analysis a portion of the dried sediment was wet sieved through 63 μm mesh, oven dried and re-weighed to obtain the proportions of the silt/clay and sand fractions in the sediment samples

Results

Spatial and Temporal Variability in Sediment Trace Metal Concentrations

Initially a range of trace metals were measured in sediments (Ag, As, Cd, Co, Cu, Fe, Ni, Mn, Mo, Pb, Se, Sn and Zn), but only Zn, Se, Cd and Pb had elevated concentrations with respect to background concentrations. Bagnalls Beach had low Zn, Se, Cd and Pb concentrations relative to the locations in Lake Macquarie (Figure 2). This confirms that Bagnalls Beach, Port Stephens, was an appropriate choice to use as a less contaminated control and as a collection site for *A. trapezia*.

Zn, Cd and Pb

Zinc, Cd and Pb concentrations in surficial sediments differed significantly between the five locations (Table 2). The concentrations decreased in the order of locations, Cockle Bay > Warners Bay $\geq$ Koorooa Bay > Eraring Bay > Whiteheads Lagoon (Figure 2). The order of concentrations from highest to lowest at locations did not change with time other than for Zn and Pb at Koorooa Bay where considerable variation between sites occurred in July. Whole sediment Zn concentrations were near ANZECC/ARMCANZ (2000) sediment quality guidelines levels of concern (low: 200 $\mu\text{g g}^{-1}$, high: 410 $\mu\text{g g}^{-1}$) at Cockle Creek (183 $\mu\text{g g}^{-1}$). Whole sediment Cd concentrations were above or near ANZECC/ARMCANZ (2000) sediment quality guidelines levels of concern (low: 1.5 $\mu\text{g g}^{-1}$, high: 10 $\mu\text{g g}^{-1}$) at Cockle Bay (9.6 $\mu\text{g g}^{-1}$), Warners Bay (2.8 $\mu\text{g g}^{-1}$) and Koorooa Bay (1.54 $\mu\text{g g}^{-1}$). Whole sediment Pb concentrations were also above or near ANZECC/ARMCANZ (2000) sediment quality guidelines levels of concern (low: 50 $\mu\text{g g}^{-1}$, high: 220 $\mu\text{g g}^{-1}$) at Cockle Bay (95 $\mu\text{g g}^{-1}$) and Warners Bay (49 $\mu\text{g g}^{-1}$).

Zinc, Cd and Pb concentrations in sediments were significantly correlated and showed a strong relationship (Table 3) indicating they share a common source of contamination. Zinc, Cd and Pb concentrations in sediments were significantly negatively correlated with Fe concentrations (Table 3). When Whiteheads Lagoon data are removed from the correlations there are no longer any significant correlations of any kind. The similarity of grain size at Cockle Bay (< 63 μm , 28 $\pm$ 5 %), Warners Bay (< 63 μm , 27 $\pm$ 7%) and Koorooa Bay (< 63 μm , 29 $\pm$ 5 %) indicates that the significant differences in Zn, Cd and Pb concentrations at these locations cannot be

attributed to location grain-size effects. Whiteheads Lagoon had significantly different grain size ($< 63 \mu\text{m}$, $59 \pm 13 \%$)

Se

Se concentrations in sediments differed significantly between the five locations in Lake Macquarie (Table 2). Se concentrations were similar for Cockle Bay, Warners Bay, Koorooa Bay, and Eraring Bay, but significantly higher at Whiteheads Lagoon (Figure 2b). The order of concentrations from highest to lowest at sites and locations did not change with time. At Koorooa Bay considerable variation of Se concentrations between sites occurred in July. At Whiteheads Lagoon, Se concentrations varied each month. There are no ANZECC/ARMCANZ (2000) sediment quality guidelines levels of concern for Se.

Zn–Se, Cd–Se and Pb–Se correlations were negative and statistically significant (Table 3). A significant positive relationship was found between Se and Fe concentrations and Se concentration and grain size (Table 3), suggesting that higher Se concentrations at Whiteheads Lagoon may partially be explained by enriched Fe concentrations and grain size.

Spatial and temporal variability of trace metal concentrations in *A. trapezia* transplanted to Lake Macquarie

Zn, Cd and Pb

Zinc, Cd and Pb concentrations in whole tissues were significantly different among locations after 60 days (Table 4). Mean concentrations decreased in the order Cockle Bay > Warners Bay > Koorooa Bay > Eraring Bay > Whiteheads Lagoon and were significantly higher than Bagnalls Beach (Figure 3a). Concentrations reached a maximum after 60-days, with the exception of Zn and Cd in cockles from Warners Bay which had higher concentrations after 90-days (Figure 3a and 3c). Zn–Cd ($r^2 = 0.537$, $p = 0.0001$), Zn–Pb ($r^2 = 0.780$, $p = 0.0001$) and Cd–Pb ($r^2 = 0.612$, $p = 0.0001$) concentrations were strongly correlated suggested that co-accumulation of trace metals is occurring.

Se

Se concentrations in whole tissues were significantly different between locations after 60 days (Table 4). Mean concentrations decreased in the order Cockle Bay = Koorooa Bay = Whiteheads Lagoon > Warners Bay = Eraring Bay and were significantly higher than Bagnalls Beach (Figure 3). Se concentrations increased significantly with time (Table 4), reached a maximum after 60-days and slightly decreased after this time (Figures 3b).

Spatial variation of Physiological Responses in Lake Macquarie

Physiological data for cockles is given in Table 5. Clearance rates ($F = 7.21$; d.f. = 4,80; $p < 0.0001$), absorption efficiency ($F = 17.10$; d.f. = 4,80; $p < 0.0001$), respiration rates ($F = 13.41$; d.f. = 4,80; $p < 0.0001$) and Scope for Growth ($F = 5.65$; d.f. = 4,80; $p < 0.0001$) were significantly different among locations after 90 days. Clearance rate was reduced to $< 0.4 \text{ L g}^{-1} \text{ h}^{-1}$ at Cockle Bay, then increased southward along the contamination gradient. Food absorption efficiencies were similar throughout Lake Macquarie. Of the transplanted cockles, Cockle Bay cockles had the highest respiration rates ($> 23 \mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$). For Scope for Growth (Table 5) there was a general trend of low values in the north ($< 8 \text{ J g}^{-1} \text{ h}^{-1}$) with values increasing southward ($> 70 \text{ J g}^{-1} \text{ h}^{-1}$ at Eraring Bay). Partially lower values were evident at Whiteheads Lagoon ($52 \text{ J g}^{-1} \text{ h}^{-1}$). Mean values of Scope for Growth of cockles from Bagnall Beach varied from 36 - 58 $\text{J g}^{-1} \text{ h}^{-1}$ over the study period (Table 5) and considered indicative of “healthy” cockles.

Exposure-dose-response relationships

Exposure-dose

Trace metal concentrations measured 60 days after transplantation were used for these analyses, as this was the approximate timing that *A. trapezia* trace metal concentrations equilibrated with environmental contamination (Figure 2). Zinc and Pb concentrations in cockle tissues were strongly positively correlated with concentrations of these trace metals in sediments (Figures 4a and 7a). Strong correlations between Zn concentrations in *A. trapezia* and Cd and Pb concentrations in sediments ($r^2 = 0.873$, $p = 0.0532$; $r^2 = 0.953$, $p = 0.0122$ respectively) and whole Pb in *A. trapezia* and Cd in surficial sediments ($r^2 = 0.984$, $p = 0.0024$) were also found. These relationships probably are probably coincidental as Zn, Cd and Pb concentrations increase together in the sediments (Figure 2).

Dose-Response Relationships

Neither significant relationships nor trends were found between Se and Cd concentrations in the tissues of *A. trapezia*, their physiological responses and Scope for Growth (Table 6, Figures 5c and 6c).

There was a significant relationship between tissue Zn concentration and absorption efficiency (Table 5).

Clearance rate and Scope for Growth were significantly inversely correlated with Pb concentrations in *A. trapezia* (Table 6, Figure 7c) Absorption efficiencies were significantly positively correlated with Pb concentrations (Table 6).

Exposure-Response Relationships

No significant relationships were evident between sediment Zn and Se concentrations and the physiological responses of *A. trapezia* (Table 6). Significant inverse relationships were found between Cd and Pb sediment concentrations and clearance rates and Scope for Growth (Table 6; Figures 6b and 7b).

Exposure-Dose –Response Relationships

Significant Zn *exposure-dose* relationship (Fig 4a) indicates that Zn bioaccumulation is occurring in response to sediment contamination but no significant impairment of physiological responses was detected. The significant Cd *exposure-response* relationship (Figure 6b) indicates that Cd may be indirectly influencing the health of cockles. The significant Pb *exposure-dose*, *exposure-response* and *dose-response* relationships (Figures 7a, b, c) indicate that Pb is probably affecting the health of cockles in Lake Macquarie.

Discussion

Spatial and Temporal Variability in Sediment Trace Metal Concentrations

Zinc, Cd and Pb concentrations decreased in a southward direction in the order Cockle Bay > Warners Bay > Koorooa Bay > Eraring Bay > Whiteheads Lagoon (Figure 2), indicating Cockle Creek as the source of contamination. Correlation analysis (Table 3) showed a common source of Zn, Cd and Pb further supporting Cockle Creek as the contamination source. This was expected as the smelter and other industries located on Cockle Creek release Zn, Cd and Pb into Lake Macquarie (AWACS, 1995). Smelter waste that has accumulated in Cockle Creek in the past is most likely the main source of trace metals, especially during flood periods when highly contaminated sediments may be scoured and transported into the lake. The absence of efficient tidal flushing in Lake Macquarie ensures that bottom sediments receive and retain virtually all of the trace metals introduced to the lake.

Sediments of Lake Macquarie are enriched in trace metals. Trace metal concentration ratios relative to background concentrations are Zn: 1.5-10, Se: 2-27, Cd: 40-700, Pb: 3.5-58 with Zn, Cd and Pb concentrations at Cockle Creek and Warners Bay (Figure 2) near or above those considered to be detrimental to sediment dwelling organisms.

Selenium concentrations in sediments were low throughout the northern section of the lake ($1-2 \mu\text{g g}^{-1}$), while elevated concentrations ($4-9 \mu\text{g g}^{-1}$) were found in Whiteheads Lagoon (Figure 2b). Atmospheric deposits from power station stacks and the smelter at Cockle Creek are the likely sources of Se to northern sediments. Selenium most likely enters Whiteheads lagoon from the overflow channel from the power station's ash-fly dam. High concentrations of Se in the sediments of Lake Macquarie have been previously associated with power generation stations (Kirby *et al.*, 2001; Peters *et al.*, 1999a, b) and smelting operations (AWACS, 1995). One such study (Peters *et al.*, 1999) found a range of $5-12 \mu\text{g g}^{-1}$ (mean $8.3 \mu\text{g g}^{-1}$) of Se in surficial sediments located in Mannering Bay, west of Vales Point power station. This study found no evidence of elevated Se concentrations at the northern study locations situated near the lead-zinc smelter.

The elevated Se concentrations at Whiteheads Lagoon can partially be accounted for due to a high percentage of fine grain particles (59 % relative to 28 % at most of the

other sites) and the enrichment of Fe concentrations in surficial sediments ($30,000 \pm 9000 \mu\text{g/g}$ relative to $13,000 \pm 5000 \mu\text{g/g}$ at other sites). The amount of fine sediments in the lagoon is evidence of an extremely low energy depositional environment, where minimum transportation velocities are virtually non-existent, resulting in the precipitation and accumulation of fine particles. Trace metals, are known to be associated with fine grain particles (Luoma *et al.*, 1990). A strong Fe-Se relationship (Table 3) indicates that large amounts of Se may be adsorbed to the Fe particulates. Therefore, the flocculation of fine Fe-enriched particles in Whiteheads Lagoon probably accounts for the elevated concentrations of Se in these sediments.

Generally, sediment trace metal concentrations did not vary over time during the study (Table 2; Figure 2). Small variations at Cockle Bay, Warners Bay and Eraring Bay measured over the temporal scale of this study are probably due to heterogeneity of sediments rather than temporal variations. Temporal variability in concentrations of trace metals results from the deposition of new sediments to the lake that have lower or higher concentrations of trace metals. This temporal variation would occur over larger time scales, than used in this study, as new sediments are deposited at $<1 \text{ mm}$ per year to Lake Macquarie (Peters *et al.*, 1999a).

Zinc and Pb concentrations at Koorooa Bay varied significantly at one site in July compared to other months (Figures 2a and 2d) and Se concentrations at Whiteheads Lagoon varied each month (Figure 2b). There was evidence of land-derived sediments entering Koorooa Bay near the anomalous site during the study i.e. sediment plumes and local observations indicate that mine spoil was being used for foreshore reclamation work. These factors may explain, the variability found in sediment trace metal concentrations of the anomalous site in Koorooa Bay, especially during July 2000. Temporal variation of Se concentrations at Whiteheads Lagoon, is likely to be related to the variability of discharges from the Eraring power station fly-ash dam overflow channel.

Spatial and temporal variations in transplanted *Anadara trapezia* trace metal concentrations

Baseline concentrations of trace metals in *A. trapezia* collected from Bagnalls Beach were lower than trace metal concentrations measured in *A. trapezia* after

transplantation to Lake Macquarie (Figure 3). This justifies the initial decision of collecting *A. trapezia* from Bagnalls Beach to represent a lesser-contaminated location and establish baseline concentrations of trace metals. Small fluctuations in trace metal concentrations of Bagnall Beach cockles occurred over the 4-month study (data not presented) but were all below concentrations accumulated by transplanted cockles.

Zinc, Cd and Pb concentrations in transplanted *A. trapezia*, also decreased in a southward direction, in the order Cockle Bay > Warners Bay > Koorooa Bay > Eraring Bay > Whiteheads Lagoon (Figure 3). Zinc and Pb concentrations in *A. trapezia* were significantly correlated with concentrations of these trace metals in the surficial sediments of the lake (Figures 4a and 7a) indicating significant *exposure-dose* relationships. These relationships were strong, and 98% of Zn and 96% of Pb concentration variation in *A. trapezia* transplanted to Lake Macquarie was explained by the concentration of these trace metals in the surficial sediments that form their habitat. Overlying dissolved water concentrations of trace metals were <2-5 µg/l, <0.05 µg/l and <0.03 µg/l for Zn, Cd and Pb respectively over the period of this study. These findings are supported by previous findings of Bately, (1987) who found that Zn and Pb are in highly bioavailable forms in the sediments of Lake Macquarie and support the conclusions of Bately (1987) and Furner (1979), that bivalves in Lake Macquarie primarily accumulate Zn and Pb from contaminated surficial sediments. However, it is not known whether cockles accumulate trace metals from resuspended sediment particles or contaminated food derived from the sediment.

Selenium concentrations in *A. trapezia* were similar across all locations within Lake Macquarie, but concentrations were significantly greater than Se concentrations in *A. trapezia* from Bagnalls Beach (Figure 3). Selenium enrichment in the surficial sediments at Whiteheads Lagoon was not reflected in the tissues of *A. trapezia* (Figure 5a) and not in agreement with the previous measurements made on native *A. trapezia* and other biota from the southern section of Lake Macquarie (Jolley *et al.* 2004; Kirby *et al.*, 2001); Peters, *et al.*, 1999a). These studies concluded that Se concentrations in biota in close proximity to power stations were elevated. This study indicates that Se in Whiteheads Lagoon sediments is not in a bioavailable form to *A. trapezia*. Se in Whiteheads Lagoon is either associated with the high amounts of Fe found in the sediments or present as reduced species such as Se⁰ and selenium

sulphides due to the reducing conditions of the anoxic sediments. These forms of Se may be less available for biological uptake, furthermore, the lack of bioturbative activity in Whiteheads Lagoon, due to a paucity of organisms previously found at this location, may break an important link between abiotic (i.e. surficial sediments) and biotic (*A. trapezia*) trace metal uptake at this site. The resuspension of sediments by biota (i.e. bioturbation) is a potentially important mechanism by which sediment particulate material and their adsorbed contaminants are reintroduced to the water column, making contaminants available for consumption by higher organisms (Wainwright, 1990). Experimental evidence (Peters *et al.* 1999a) has indicated that the burrowing and feeding activities of benthic organisms in Lake Macquarie can increase Se flux to interstitial waters, resulting in higher concentrations of Se becoming available for other organisms in the ecosystem, including the benthic bivalve *A. trapezia*.

The uptake and retention of Zn, Cd and Pb in *A. trapezia* reached a maximum approximately 60 days after transplantation to Lake Macquarie (Figure 3). Zinc, Cd and Pb concentrations varied little over the next month, with the exception of cockles at Cockle Bay, where Cd and Pb concentrations slightly decreased and at Warners Bay in July where Cd and Pb concentrations increased. The increase at Warners Bay was for only one site that was visibly affected by land derived sediment inputs (Figures 2a and 2d). The increased variability in trace metal concentrations after 60 days also suggests that other biological and/or environmental processes could be affecting the accumulation of these trace metals.

Other studies (Andres *et al.*, 1999; Elliott *et al.*, 1985; Hickey *et al.*, 1995; Klumpp and Burdon-Jones, 1982; Martincic *et al.*, 1992; Romeril, 1971; Simpson, 1979; Wallner-Kersanach *et al.*, 2000), have found that equilibration of trace metals in bivalves with their environment can range between 30 days to 77 days (11 weeks) and longer for some species of clams. Such a wide range in equilibration times among species can be associated with previous exposure history, life stage of bivalve, associated metabolic activities associated with adjustment to a new environment, temperature changes, and food availability. Our results showed that *A. trapezia* reached “steady state” with its environment and doesn’t continue to accumulate trace metals as other bivalves such as *Mytilus edulis* do (Langston *et al.*, 1998). Thus,

A. trapezia is a good biomonitor as it reflects bioavailable trace metals in its environment.

Strong relationships between tissue Zn – Cd, Zn – Pb, Cd - Pb concentrations were also found, suggesting that the accumulation of these metals may be dependent on one another or have a common source. Sediment data in this study indicates a common source of Zn, Cd and Pb. Molluscs readily take up Cd, most likely via the same pathways as Zn and Cu (Simkiss and Taylor, 1995). Elliott *et al.* (1985) concluded that for the bivalve *Mytilus edulis*, the accumulation of Cd was greatly reduced in the presence of Zn, Pb and Cu; and, that in the presence of Cd and Pb, Zn accumulation was not directly proportional to exposure time. Laboratory uptake studies of trace metals will need to be conducted to determine the interaction of trace metals during uptake.

Spatial variation of Physiological Responses in Lake Macquarie

There was a decline in the physiological condition of *A. trapezia* transplanted to Lake Macquarie after a 90-day period (Table 5). By July, there was a marked difference in clearance rates at Cockle Bay and Warners Bay, absorption efficiencies at all locations, and respiration rates at Warners Bay, Koorooa Bay, Eraring Bay and Whiteheads Lagoon (Table 5). Mean Scope for Growth values at Cockle Bay were markedly lower than other locations in Lake Macquarie (Table 5).

The ability of *A. trapezia* to filter particulates (i.e. clearance rates) improved southward in the order Cockle Bay < Warners Bay ≤ Koorooa Bay ≤ Whiteheads Lagoon < Eraring Bay (Table 5). The decline in the condition of *A. trapezia* at Cockle Bay can be associated with the low food clearance rates (mean $0.31 \text{ L g}^{-1} \text{ h}^{-1}$) and the increase in oxygen consumption (mean $23.13 \mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$) associated with an animal under stress. As a result of enhanced oxygen consumption a marked reduction in the Scope for Growth or growth potential was recorded in cockles from the Cockle Bay mean compared to other locations (Table 5). Scopes for Growth values at all locations, except Cockle Creek, were similar to those measured in cockles from Bagnalls Beach ($36\text{-}58 \text{ J g}^{-1} \text{ h}^{-1}$) and consistent with values reported for 'healthy' cockles (Din and Ahamad, 1995). This indicates that organisms in the southern section of Lake Macquarie, other than at Cockle creek, are not under physiological

stress. However, direct comparisons should be made with caution as the water temperature was markedly higher at Bagnalls Beach (Table 5).

Absorption efficiencies were statistically similar throughout the lake; however, there was trends of decreasing efficiencies in a southward direction as feeding rates improve (Table 5). Decreases in the food absorption efficiencies of bivalves are known to be a function of high feeding rates. Similar results were found in a study on *Mytilus edulis* transplanted along a pollution gradient in Narragansett Bay, USA (Widdows *et al.*, 1980-81). Several bivalve species have also demonstrated that food absorption is independent of seston concentration, however, it is dependent on the seston quality, or percentage of organics (MacDonald *et al.*, 1998) i.e. absorption efficiencies increase with the quality of the food.

The observed decline in Scope for Growth of *A. trapezia* transplanted to Lake Macquarie was significantly correlated with the increasing concentrations of Cd in the surficial sediments (Figure 7b) and increasing concentrations of Pb in the surficial sediments (Figure 4d) and tissues of *A. trapezia* (Figure 7c). The significant Cd *exposure-response* relationship implies that Cd may be influencing the health of *A. trapezia*, probably through indirect effects on the cockle's health such as a decrease in the quality of food, rather than because of the bioaccumulation of Cd. The significant Pb *exposure-dose*, *dose-response* *exposure-response* relationships indicate that Pb concentrations are elevated sufficiently in Lake Macquarie surficial sediments to cause adverse effects on biota. Pb and Cd concentrations exceeded the ANZECC/ARMCANZ (2000) sediment quality guidelines levels of concern at several sites.

Trends were also evident between Scope for Growth and concentrations of Zn in *A. trapezia* (Figure 4c), however, caution must be exercised with interpreting this correlation as *A. trapezia* are known regulators of Zn (Rai, 1998) and it is unlikely that elevated Zn concentrations are causing the decline in the physiological condition of this organism. No significant *exposure-dose-response* relationships are evident for Se (Figure 2), indicating that the accumulation of Se in the tissues are not high enough to induce stress in the organism and limit its ability for growth and reproduction.

Natural stressors, such as temperature and salinity fluctuations, have been suggested as possible influences on the Scope for Growth. Bayne (1975, op. cit. Martin *et al.*, 1984) and Bayne *et al.* (1976) found Scope for Growth in mussels remained relatively independent of temperature from 10 – 20°C, and that the mussels suffer transient stress from reduced salinities, but recover within 2 –3 days. In the present study the natural stressors that are most likely to affect physiological responses, remained relatively constant along the length of Lake Macquarie (Table 5), furthermore, due to the nature of Lake Macquarie, in general, these environmental factors are known to vary little throughout the year (AWACS, 1995). Thus the reduction in the condition of *A. trapezia* transplanted to the northern section on lake can be assumed to be independent of temperature and salinity, and caused by anthropogenic stressors rather than natural environmental variables. Other contaminants such as organochlorine pesticides and ammonia in pore waters are low (NSW DEC, unpublished data), therefore are not stressors in this environment.

Risk assessment of contaminated sediments

Many papers have been published that relate *exposure-dose-response* for transplanted bivalves in contaminated waters (e.g. Gagne *et al.*, 2006; Zorita *et al.*, 2006) but none to our knowledge have applied this framework to bivalves transplanted to contaminated sediments. Zinc, Cd and Pb concentrations at Cockle Creek, Warners Bay and Koorooa Bay are above or near those considered to be detrimental to sediment dwelling organisms, based on ANZECC/ARMCANZ (2000) sediment quality guidelines. Clearly based on the criteria that *exposure-dose* and *dose-response* should be significant, Pb concentrations are likely causing adverse effects to the transplanted cockles and by inference on other sediment dwelling biota. The effects of Zn and Cd accumulation in sediments and biota are unclear. For Zn, *exposure-dose* is significant but *dose response* is not. For Cd, although the sediment quality guidelines are exceeded, *exposure-dose* and *dose-response* relationships are not significant. Zinc and Cd in sediment appear not to be directly affecting the health of transplanted cockles. These relationships, although only correlative, form the basis of a weight of evidence approach. Thus, on balance all three metals are probably contributing to the impaired physiological condition of the transplanted cockles. We are at present developing other measures of health based on the production of oxygen radicals in

cells and cell damage (Ercal *et al.* 2001) as additional lines of evidence to increase the confidence in making predictions about the effect of contaminated sediments on benthic organisms.

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Table 1 Recoveries of Zn, Se, Cd, and Pb from certified standard reference material. Values are means $\pm$ SE

CRM	Supplier	Zinc		Selenium $\mu\text{g g}^{-1}$ dry mass or $\mu\text{g l}^{-1}$		Cadmium		Lead	
		Certified	Found	Certified	Found	Certified	Found	Certified	Found
Oyster Tissue SRM 1566a	NIST	830 $\pm$ 57	769 $\pm$ 25 (93)	2.21 $\pm$ 0.24	2.1 $\pm$ 0.2 (97)	4.15 $\pm$ 0.38	4.7 $\pm$ 0.7 (113)	0.37 $\pm$ 0.01	0.4 $\pm$ 0.3 (102)
Lobster Hepatopancreas TORT – 1	NRCC	177 $\pm$ 10	194 $\pm$ 98 (110)	6.88 $\pm$ 0.47	5.6 $\pm$ 0.2 (81)	26.3 $\pm$ 2.1	23.9 $\pm$ 0.1 (91)	10.4 $\pm$ 2	11 $\pm$ 1 (104)
Sargasso CRM No. 9	NIES	15.6 $\pm$ 1.2	13.7 $\pm$ 0.4 (88)	0.05 ¹	B.D.L.	0.15 $\pm$ 0.02	0.17 $\pm$ 0.09 (113)	1.35 $\pm$ 0.05	1.12 $\pm$ 0.05 (83)
Chlorella CRM No. 3	NIES	20.5 $\pm$ 1	20 $\pm$ 1 (95)	N.A.	0.06 $\pm$ 0.01	0.026 ^b	B. D.L.	0.6 ^a	0.4 0 $\pm$ 0.02 (66)
Estuarine Sediment SRM 1646	NIST	138 $\pm$ 6	100 $\pm$ 3 (73)	0.6	0.5 $\pm$ 0.11 (84)	0.39 $\pm$ 0.7	0.35 $\pm$ 0.8 (90)	28.2 $\pm$ 1.8	20.7 $\pm$ 1.1 (73)
Marine Sediment BCSS-1	NRCC	119 $\pm$ 12	92 $\pm$ 4 (77)	0.43 $\pm$ 0.06	0.35 $\pm$ 0.13 (81)	0.25 $\pm$ 0.94	0.23 $\pm$ 0.1 (92)	22.7 $\pm$ 3.4	19.8 $\pm$ 0.6 (87)
Open Ocean Seawater NASS-5	NRCC	0.102 $\pm$ 0.039	0.11 $\pm$ 0.04 (108)	0.018 ¹	B.D.L.	0.023 $\pm$ 0.003	0.022 $\pm$ 0.008 (96)	0.008 $\pm$ 0.005	0.090 $\pm$ 0.007 (113)
Estuarine water SLEW-3	NRCC	0.201 $\pm$ 0.037	0.18 $\pm$ 0.03 (90)	N.A.		0.048 $\pm$ 0.004	0.043 $\pm$ 0.008 (90)	0.0090 $\pm$ 0.0014	0.008 $\pm$ 0.001 (89)

¹Reference values not certified

B.D.L. = Below Detection Limit $< 0.02 \mu\text{g g}^{-1}$ dry mass

N. A. = Not available

() = % Recovery

Table 2 Nested ANOVA results for spatial and temporal differences of Zn, Se, Cd and Pb concentrations in Lake Macquarie and Bagnalls Beach surficial sediments (n = 48). Tukey's results are presented in decreasing order (L –R) and locations underlined are not significantly different (p>0.05).

Metal	Source	Df	F value	p > F
Zinc	Location	5	25.79	0.0001
	Site(Location)	6	2.83	0.0417
	Time	3	1.00	NS
	Time*Location	15	1.83	NS
	Time*Site(Location)	18	19.40	0.0001
Selenium	Location	5	24.64	0.0001
	Site(Location)	6	2.19	NS
	Time	3	2.17	NS
	Time*Location	15	9.91	0.0001
	Time*Site(Location)	18	1.57	NS
Cadmium	Location	5	302.48	0.0001
	Site(Location)	6	6.20	NS
	Time	3	0.578	NS
	Time*Location	15	7.45	0.0001
	Time*Site(Location)	18	1.17	NS
Lead	Location	5	95.51	0.0001
	Site(Location)	6	2.50	NS
	Time	3	1.18	NS
	Time*Location	15	2.53	0.0317
	Time*Site(Location)	18	2.97	0.0006

Zn

Cockle Bay Koorooa Bay Warners Bay Eraring Bay Whiteheads Lagoon Bagnalls Beach

Se

Whiteheads Lagoon Koorooa Bay Cockle Bay Eraring Bay Warners Bay Bagnalls Beach

Cd

Cockle Bay Warners Bay Koorooa Bay Eraring Bay Whiteheads Lagoon Bagnalls Beach

Pb

Cockle Bay Warners Bay Koorooa Bay Eraring Bay Whiteheads Lagoon Bagnalls Beach

Table 3 Pearson's Correlation Coefficients (P values in italics) for trace metal, iron and silt concentrations in Lake Macquarie surficial sediments. Significant relationships are in bold where $p < 0.05$.

n		Zn	Se	Cd	Pb	Fe	% <63µm
120	Zn	1					
	Se	-0.3711 <i>0.0001</i>	1				
	Cd	0.5443 <i>0.0001</i>	-0.3752 <i>0.0001</i>	1			
	Pb	0.7747 <i>0.0001</i>	-0.4924 <i>0.0001</i>	0.8762 <i>0.0001</i>	1		
	Fe	-0.3943 <i>0.0001</i>	0.8269 <i>0.0001</i>	-0.4859 <i>0.0001</i>	-0.5577 <i>0.0001</i>	1	
10	% <63µm inc. W.L	-0.2344 0.5145	0.8032 <i>0.0051</i>	-0.1005 0.7824	-0.2091 0.5620	0.7837 <i>0.0073</i>	1
8	% <63µm excl. W.L	0.8348 <i>0.0099</i>	0.2859 0.4923	0.5936 0.1208	0.7907 <i>0.0195</i>	0.0482 0.9098	1

W.L = Whiteheads Lagoon.

Table 4 Nested ANOVA results for spatial and temporal differences of Zn, Se, Cd and Pb concentrations in transplanted *Anadara trapezia* tissues (n = 244) after 60-days. Tukey's results are presented in decreasing order (L –R) and locations underlined are not significantly different ($p>0.05$).

Source	df	Zn		Se		Cd		Pb	
		F value	p>F	F value	p>F	F value	p>F	F value	p>F
Location	5	9.28	0.0015	56.85	0.0001	5.64	0.0190	64.59	0.0001
Site(Location)	5	0.66	NS	0.14	NS	0.77	NS	1.34	NS
Cage (Site (Location))	10	0.39	NS	0.37	NS	0.40	NS	0.52	NS
Time	2	2.44	NS	9.99	0.0046	0.80	NS	5.82	0.0205
Time*Location	10	8.69	0.0031	0.71	NS	3.91	0.0148	2.86	0.0424
Time*Site(Location)	9	0.34	NS	2.31	NS	0.51	NS	0.42	NS
Time*Cage(Site(Location))	14	1.26	NS	1.46	NS	2.27	0.0085	2.34	0.0064

NS = not significant $p>0.05$

Zn: Cockle Bay Warners Bay Koorooa Bay Eraring Bay Whiteheads Lagoon

Se: Cockle Bay Whiteheads Lagoon Koorooa Bay Eraring Bay Warners Bay

Cd: Cockle Bay Warners Bay Eraring Bay Whiteheads Lagoon Koorooa Bay

Pb: Cockle Bay Warners Bay Koorooa Bay Eraring Bay Whiteheads Lagoon

Table 5 Summary of physiological responses of *A. trapezia* from Bagnalls Beach and cockles transplanted to Lake Macquarie after 90 days July 2000 (mean $\pm$ SE)

	Bagnalls Beach	Transplant location				
	n = 22	Cockle Bay n = 20	Warners Bay	Koorooa Bay n = 20	Eraring Bay n = 7	Whiteheads Lagoon n = 16
Clearance rate ($\text{Lg}^{-1}\text{h}^{-1}$)	1.65 $\pm$ 0.17	0.31 $\pm$ 0.35	0.84 $\pm$ 0.37	1.03 $\pm$ 0.35	1.78 $\pm$ 0.59	1.18 $\pm$ 0.51
Absorption efficiency (%)	39	85	86	78	70	69
Oxygen consumption ($\mu\text{mol O}_2 \text{g}^{-1}\text{h}^{-1}$)	24.48 $\pm$ 1.21	23.13 $\pm$ 2.10	14.37 $\pm$ 2.21	18.91 $\pm$ 2.10	15.17 $\pm$ 3.55	13.10 $\pm$ 3.00
C = Joules consumed	119.45	23.68	51.40	65.97	133.31	110.34
A = Joules absorbed	50.84	20.32	44.87	52.64	96.13	72.88
R = Joules respired	11.16	10.75	6.56	8.62	6.91	6.23
U = Joules excreted	0.46	1.43	0.50	1.02	16.18	1.67
Scope for growth ($\text{Jg}^{-1}\text{h}^{-1}$)	35.95 $\pm$ 4.79	7.96 $\pm$ 19.11	37.09 $\pm$ 20.27	41.63 $\pm$ 19.11	70.35 $\pm$ 32.30	52.51 $\pm$ 27.36
Environmental Factors						
Water Temperature ($^{\circ}\text{C}$)	22.3	13.8	14.4	14.7	15.3	15.2
Salinity (‰)	33	34	35	35	35	35

Clearance rate

Cockle Bay Warners Bay Koorooa Bay Whiteheads Lagoon Eraring Bay

Absorption efficiency

Warners Bay Cockle Bay Koorooa Bay Eraring Bay Whiteheads Lagoon

Respiration rates

Cockle Bay Koorooa Bay Eraring Bay Warners Bay Whiteheads Lagoon

Scope for Growth

Cockle Bay Warners Bay Koorooa Bay Whiteheads Lagoon Eraring Bay

Table 6 Pearson's Correlation Coefficients (P values in italics) for *A. trapezia* trace metal concentrations and physiological responses and sediment trace metal concentrations. Location means were used for analysis. Significant relationships are bold where $p < 0.05$. (n=5)

		Clearance rate	Absorption efficiency	Respiration rates	Scope for Growth
<i>A. trapezia</i>	Zn	-0.823 <i>0.0873</i>	0.992 <i>0.0009</i>	0.405 <i>0.4985</i>	-0.806 <i>0.0999</i>
	Se	-0.706 <i>0.1827</i>	0.337 <i>0.5789</i>	0.600 <i>0.2843</i>	-0.687 <i>0.2004</i>
	Cd	-0.179 <i>0.7742</i>	0.467 <i>0.4279</i>	-0.473 <i>0.4209</i>	-0.089 <i>0.8857</i>
	Pb	-0.915 <i>0.0293</i>	0.939 <i>0.0177</i>	0.582 <i>0.3029</i>	-0.915 <i>0.0295</i>
Sediment	Zn	-0.504 <i>0.3867</i>	0.475 <i>0.4183</i>	0.637 <i>0.2480</i>	-0.532 <i>0.3557</i>
	Se	0.365 <i>0.5462</i>	-0.668 <i>0.2177</i>	-0.408 <i>0.4954</i>	0.452 <i>0.4446</i>
	Cd	-0.881 <i>0.0483</i>	0.747 <i>0.1469</i>	0.835 <i>0.0783</i>	-0.927 <i>0.0233</i>
	Pb	-0.895 <i>0.0403</i>	0.858 <i>0.0626</i>	0.854 <i>0.0657</i>	-0.932 <i>0.0209</i>

Figure 1. Sampling locations in Lake Macquarie, NSW, Australia

Figure 2 Spatial and temporal variability in mean trace metal concentrations of Lake Macquarie surficial sediments during the four-month study period. BB = Bagnalls Beach, CB = Cockle Bay, WB = Warners Bay, KB = Koorooa Bay, EB = Eraring Bay, WL = Whiteheads Lagoon. Mean data for individual sites is presented and means joined for ease of visual examination. Crossing lines indicate an interaction between site and time.

----- ANZECC/ARMCANZ (2000) low sediment quality guidelines expressed on a < 63 μm basis

- - - - - ANZECC/ARMCANZ (2000) high sediment quality guidelines expressed on a < 63 μm basis

Figure 3 Spatial and temporal variability in mean trace metal concentrations of *Anadara trapezia* at Bagnalls Beach and transplanted to Lake Macquarie. BB = Bagnalls Beach, CB = Cockle Bay, WB = Warners Bay, KB = Koorooa Bay, EB = Eraring Bay, WL = Whiteheads Lagoon. Where significant interactions occurred between time and cages nested within sites (locations), mean data for individual sites is presented and means joined for ease of visual examination.

Figure 4 Exposure-dose-response for Zn. *Indicates significance ($p < 0.05$)

Figure 5 Exposure-dose-response for Se. *Indicates significance ($p < 0.05$)

Figure 6 Exposure-dose-response for Cd. *Indicates significance ($p < 0.05$)

Figure 7 Exposure-dose-response for Pb. *Indicates significance ($p < 0.05$)

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

◆ Cockle Bay □ Warners Bay ▲ Koorooa Bay ✕ Eraring Bay ✖ Whiteheads Lagoon

◇ Cockle Bay □ Warners Bay △ Koorooa Bay × Eraring Bay * Whiteheads Lagoon

Fig 6

Fig 7