

# **Transplanted Oysters & Resident Mud Crabs as Biomonitors in Spillway Creek**

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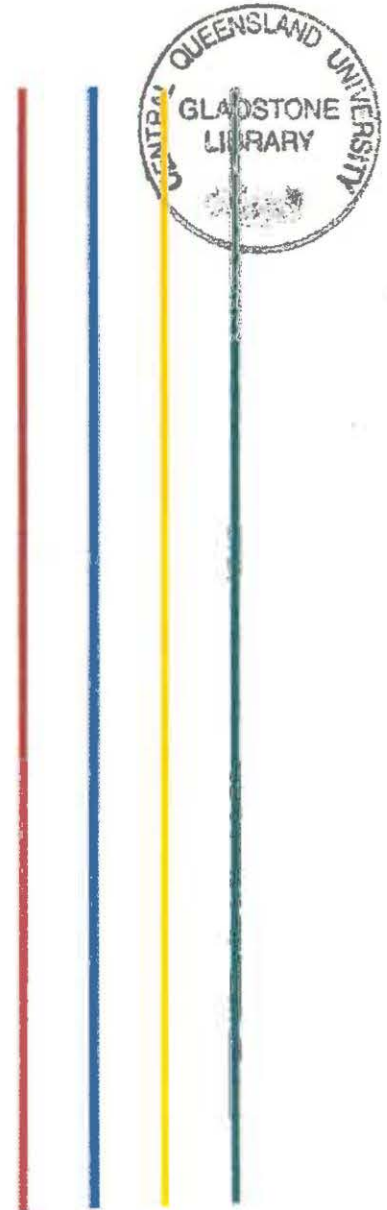
**Centre for Environmental Management**

**Central Queensland University**

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**Central Queensland  
UNIVERSITY**

# **Transplanted Oysters and Resident Mud Crabs as Biomonitors in Spillway Creek**



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**Authors: Leonie Andersen, Andrew Storey, Amy Sinkinson and  
Nick Dytlewski**

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### **ABSTRACT**

Transplanted oysters and resident mud crabs were used as biomonitoring tools to assess spatial differences in fluoride and metal (aluminium (Al), arsenic (As), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), selenium (Se) and zinc (Zn)) concentrations in Spillway Creek. Oysters obtained from an oyster lease from an uncontaminated area were deployed for a three month period at four sites within Spillway Creek and two external control sites. After retrieval, oysters (soft tissue) were analysed for concentrations of fluoride and nine metals. Between site comparisons included oysters from the oyster lease.

Mud crabs were collected from the same sites over a two week period immediately prior to oyster retrieval and assessed for the presence of rust spot shell disease. Hepatopancreas (liver) and muscle tissues of mud crabs were also analysed for fluoride and metal concentrations and between site comparisons made. Concentrations of fluoride and metals in oysters and mud crabs were also compared to food guidelines. The mud crab results were also compared to the findings of a previous study 'Fluoride and metals in Spillway Creek Crustacea' (Andersen et al., 2001). Analyses of water metal and fluoride concentrations in Spillway Creek were also undertaken by BSL on one occasion.

Despite elevated concentrations of fluoride in water samples closer to the discharge channel there were no between site differences in fluoride accumulations in oysters. Concentrations were, however, elevated in mud crab muscle from mud crabs closer to the discharge channel compared to sites near the mouth of Spillway Creek and the external reference sites, although the site separation was not statistically significant. A similar trend of fluoride accumulation in mud crab muscle was evident in the previous study. Although mean concentrations in mud crab hepatopancreas tended to be more elevated in the Spillway Creek sites these were not significantly different to reference sites. Concentrations in mud crab muscle were not at such a level as to pose a human health risk from the consumption of mud crab meat.

Nickel was elevated in Spillway oysters closer to the discharge suggesting exposure to bioavailable nickel, however a reverse trend of accumulation was evident for copper, zinc and to a lesser extent iron. Lilly Island oysters often had some of the highest metal concentrations, with oysters from the lease area often the lowest. Wild Cattle appeared to be a suitable control site. There also appeared to be an accumulation of selenium in mud crabs closer to the discharge channel. Apart from selenium there appeared to be no site trend for metal accumulations in mud crab tissue, which was similar to the findings of the previous study. Metal concentrations in mud crabs or oysters were not outside the boundaries of current food guidelines.

There also appeared to be no relationship between water metal concentrations sampled on one occasion and biota metal concentrations. Water concentrations in Spillway Creek are known to be variable and the findings highlight the benefits of biologically monitoring, representing average ambient bioavailable contaminant concentrations over a time period.

The differences in fluoride and metal concentrations in oysters and mud crabs in this study are likely to be due to a combination of their accumulation strategies and the nature of the exposure being pulse rather than continuous.

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### 1.1. GENERAL INTRODUCTION

#### 1.1.1. Background

Boyne Smelters Limited (BSL) located on Boyne Island, Queensland is Australia's largest aluminium smelter producing over 510 000 tonnes of aluminium per year. As part of the production process, suspended solids from process water from the smelter settle in a pond on the site and then pass through a reed bed. The water exiting the reed bed is then discharged via a licensed release point into a man-made channel (Spillway Channel), which then flows into the receiving marine environment of Gladstone Harbour/Port Curtis via Spillway Creek.

A research-monitoring project "Fluoride and Metals in Spillway Creek Crustacea" conducted by the Centre for Environmental Management (CEM) in collaboration with BSL was completed in 2001. The pilot study was instigated to assess the extent of effects of local anthropogenic inputs into Spillway Creek including those from BSL, on mud crabs (*Scylla serrata*), fiddler crabs (*Uca coarctata*) and banana prawns (*Penaeus merguensis*). The project was one of the first to introduce to Gladstone Industry the use of marine organisms as bioindicators in monitoring programs. Although there appeared to be trends of metal and fluoride accumulations in crustaceans at sites closest to the discharge channel, these were not always consistent or significant (Andersen et al., 2001).

With the release of the new ANZECC/ARMCANZ (2000) guidelines there has been a move to include more biological test methods in water quality assessment and monitoring of receiving waters, particularly in the compilation of an Environmental Impact Assessment (EIS). Biological indicators are regarded as essential tools in the assessment of the impacts of effluent discharges on aquatic ecosystems. Ideally bioindicators should be relatively sedentary or resident to the area of interest, abundant, long lived, be available for sampling all year and have a wide distribution (MacFarlane et al., 2000). They should also be relatively tolerant of environmental stressors, be net accumulators of the pollutants in question and provide sufficient tissue for individual analysis (Rainbow, 1995). The oyster *Saccostrea glomerata*, which is common to Queensland coastal areas, meets these criteria.

Suspension feeders like mussels and oysters respond to metal sources in dissolved and suspended phases making them suitable heavy metal biomonitors (Rainbow, 1995). Marine bivalves also readily accumulate fluoride in their shells and soft tissues

(Hemmens & Warwick, 1972, Wright & Davison, 1975). Mussels (and oysters) have become widely used for monitoring contamination in coastal and estuarine ecosystems. The technique has been widely referred to as “Mussel Watch” and the International Mussel Watch Program, was recently expanded into the Asia/Pacific region by the Mussel Watch Committee (Jeng et al., 2000).

Difficulties arose in the previous study mainly due to the lack of abundance of collectable biota in Spillway Creek, therefore limiting number of replicate samples for analysis. The problem of lack of abundance can be overcome by transplanting biomonitors into the area to be studied and has several advantages (Odzak et al., 2001). Oysters can be brought into an area selected for monitoring where they may not have been previously abundant. This serves to increase the number of sites, which can be selected for monitoring allowing increased site specificity. The number of samples available for analysis can also be increased thereby reducing variation and placing no limitations on the proposed scope of analyses.

Oysters obtained from oyster leases from unimpacted areas are of uniform size and have similar environmental histories, which also reduces some of the variability encountered when sampling resident biota. The use of transplanted oysters in this study would identify if spatial differences exist in contaminant loads in Spillway Creek, as oysters are sessile compared with mud crabs. Resident oysters are recreationally, an important fished species in Port Curtis.

Mud crabs are also an important recreational and commercially fished species in Port Curtis. Concerns had been raised previously about the higher prevalence of shell disease and metal concentrations in Port Curtis mud crabs compared with mud crabs from other areas in Queensland (Andersen & Norton, 2001). Metals and fluoride concentrations and the prevalence of shell disease in mud crabs were investigated in the previous study into Spillway Creek crustacea. Although none of the 92 crabs that were critically examined in the previous study had shell lesions, further sampling would provide a more comprehensive understanding of contaminant loads and health of mud crabs in Spillway Creek.

### **1.1.2. Aims**

The project aimed to:

1. Assess the concentration and spatial distribution of fluoride (F) and nine metals; (aluminium (Al), arsenic (As), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), selenium (Se) and zinc (Zn)) in muscle and hepatopancreas of resident mud crabs and whole tissues of transplanted oysters within Spillway Creek and two external reference sites, prior to the extension of reduction lines at BSL.
2. Compare concentrations of fluoride and metals in mud crabs in Spillway Creek with concentrations found in mud crabs in the previous study 'Fluoride and Metals in Spillway Creek Crustacea' (Andersen et al., 2001) and with reference data from elsewhere in the region including the relevant metals in the Australia New Zealand Food Standards Codes (ANZFA, 1999a, 2002).

3. **Assess the external appearance of mud crabs in Spillway Creek and the external reference sites for occurrence of shell lesions and compare results to other mud crab sites within Port Curtis determined in previous studies (Andersen & Norton, 2001).**

## **1.2. METHOD**

### **1.2.1. Location of study area**

Six sites were selected for the collection of mud crabs and deployment of oysters with the locations being recorded using a Global Positioning System (GPS–WGS84)(Table 1). Four sites (A –D) were located along Spillway Creek at approximately 1 km intervals from the upper reaches of the estuary to the mouth, allowing for the detection of a gradient of fluoride and metals should it exist (Figure 1).

An external reference site (LI) was located in Lilly Creek behind Lilly Island opposite the mouth of Spillway Creek (Figure 1). This site is potentially influenced by anthropogenic inputs into the Boyne River including those from QAL. A new control site was located in Wild Cattle Creek (WC). The new site was established closer to Colosseum Inlet as it appeared from the results of the previous study that the original control site (near the boat ramp) may have been influenced by anthropogenic inputs and therefore was not a suitable control (Andersen et al., 2001) (Figure 1).

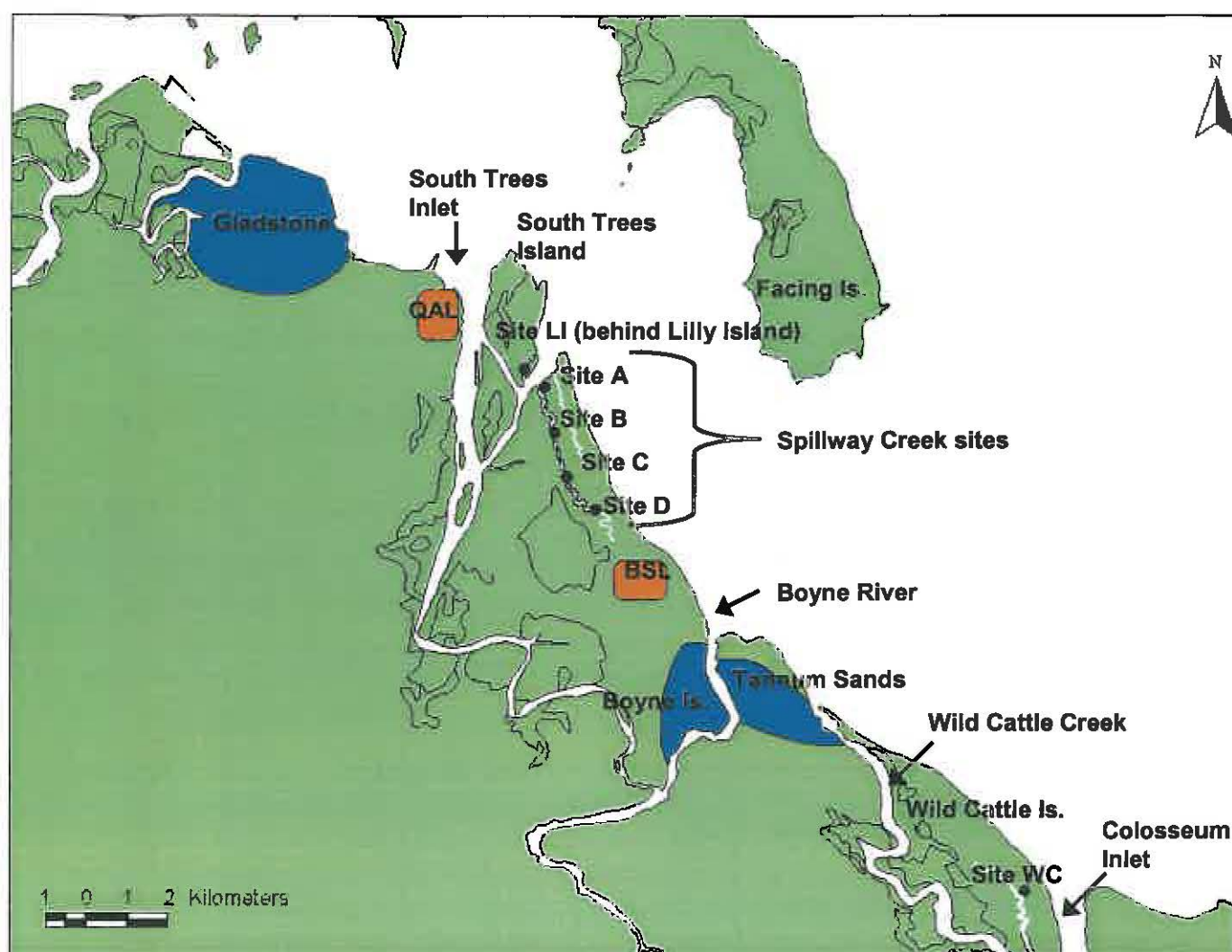
Site A (at the mouth of Spillway Creek) and Site B were at the same locations as those selected in the previous study (Andersen et al., 2001) (Figure 2). Sites C and D (closet to the source) were relocated for this study to expand the monitoring area and to ensure sites were spatially equidistant. Although Site C was moved closer to the mouth and Site D closer to the discharge point for the placement of oysters, the sites remained within the vicinity of where mud crabs had been caught previously (Figure 2) and did not constitute a significant change in position from the earlier study.

### **1.2.2. Water quality**

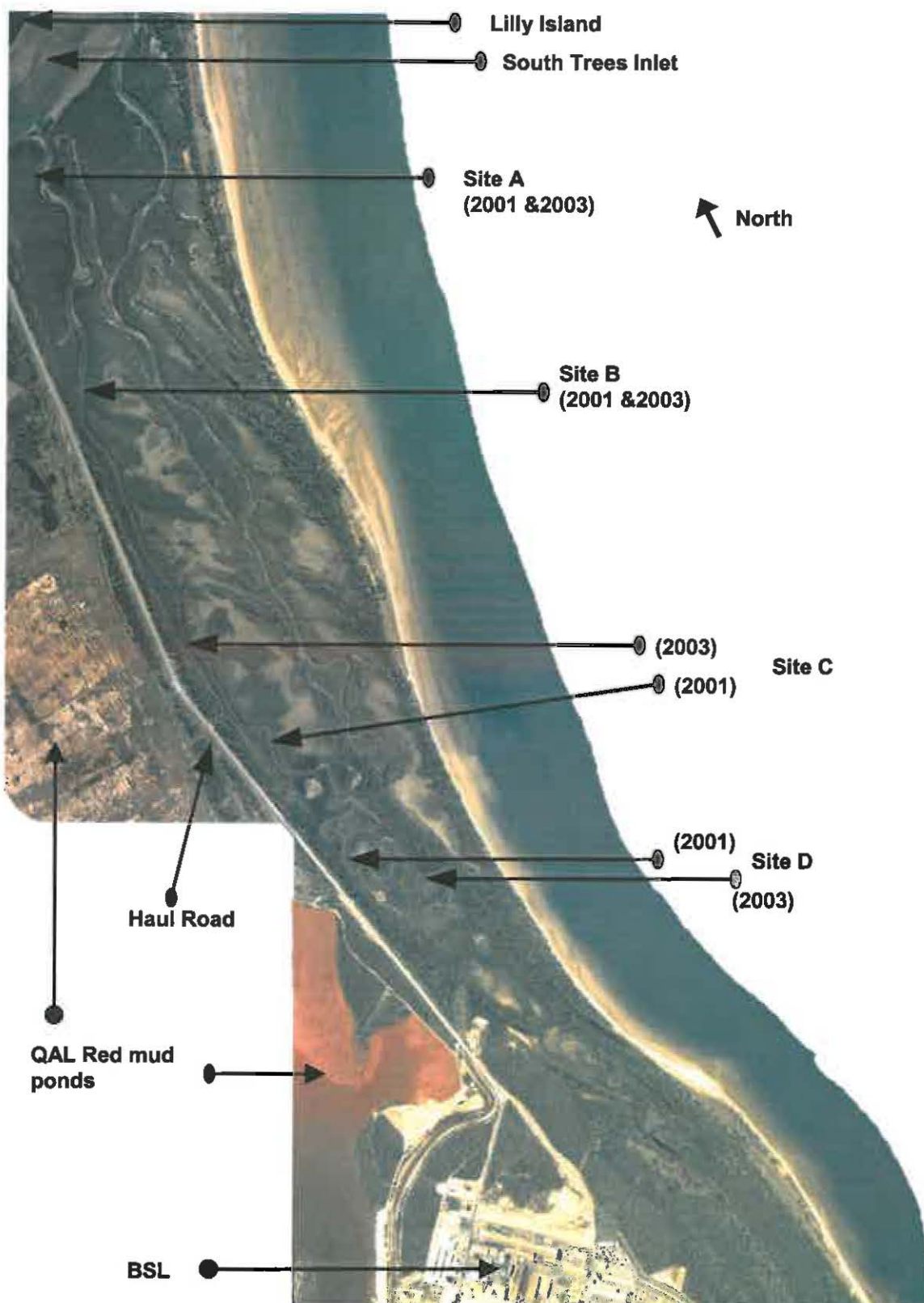
Water physicochemical properties were measured in surface waters at each site on one occasion using a TPS 90 FLMV monitoring unit. Boyne Smelters also undertook total metal and fluoride analyses of water samples in the vicinity of the four sites within Spillway Creek on 25<sup>th</sup> February 2003. No water samples were collected at Lilly Island or Wild Cattle Creek. Samples were sent to a NATA certified laboratory (Australian Government Analytical Laboratories (AGAL)) for total metal analysis. (BSL Report No. RN345729 - 11/03/03) and fluoride was analysed on site at BSL.

### **1.2.3. Oysters**

Oysters of approximately the same age and size (approximately 60mm shell length) (Figure 3) were obtained from an oyster lease from a known uncontaminated site (as determined by water quality monitoring conducted by QDPI – Laurie McGrath pers. comm.) in Eastern Moreton Bay.



**Figure 1.** Location of sites A to D in Spillway Creek, Site LI at Lilly Island and the control site (WC) in Wild Cattle Creek.



**Figure 2.** Aerial photograph depicting the location of Spillway Creek sites in the previous study (2001) in comparison to the relocated sites in this study (2003) in relation to BSL.

The following sampling and deployment procedure were employed:

**1. Baseline**

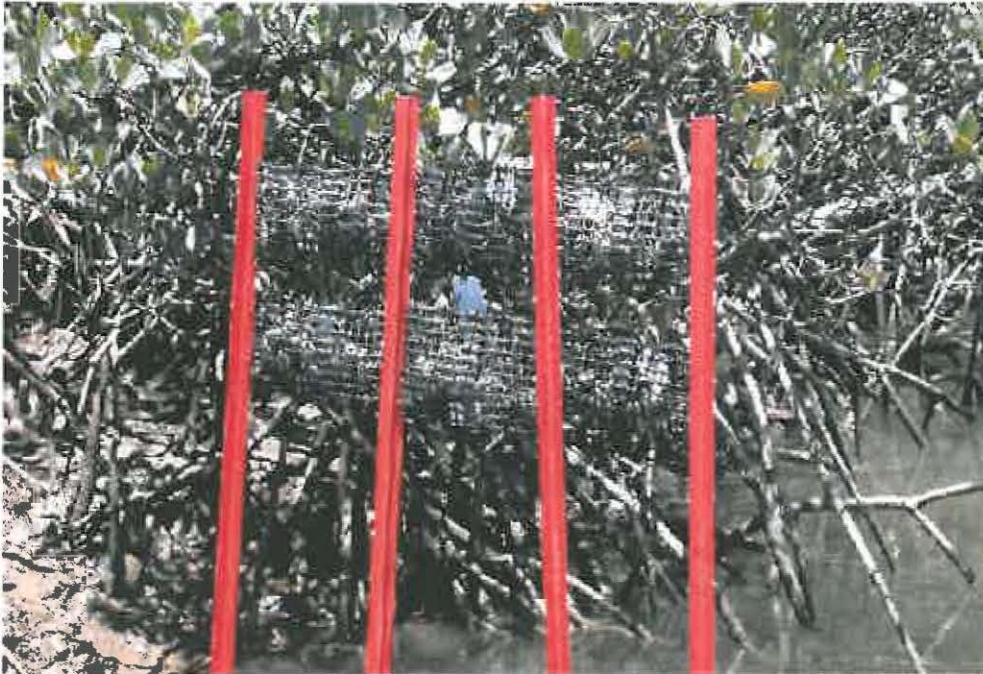
A random sub sample of specimens taken from the oyster lease was selected to determine the baseline concentrations of fluoride and metals prior to deployment. The tissues of eight individual oysters were pooled to form one homogenate/composite replicate and five replicates were analysed.



**Figure 3.** Oyster (*Saccostrea glomerata*)

**2. Deployment**

Twelve oysters were placed into mesh (20 x 20 mm) trays that were secured with electrical zip ties. Eight oysters would be processed from each tray, with the extra oysters available as spares in case of mortalities. Six trays were attached in two rows to three plastic star pickets (Figure 4). On the 20<sup>th</sup> November 2002, two sets of star pickets with oyster trays attached were deployed in the intertidal zone on either side of the Creek at each site (Figure 5). The second set of oysters acted as a spare in case of loss or theft. Deployed oysters were marked with signs indicating a research project was in progress (Figure 6); no theft or losses occurred through interference.



**Figure 4.** Oysters inside oyster mesh trays attached to star pickets.



**Figure 5.** Securing oyster stakes at Site C.



**Figure 6.** Location of oysters at Site D. The top of the star pickets can be seen (arrowed) under the water surface.

### 3. Retrieval

Three months after deployment (21<sup>st</sup> February 2003) oysters trays were retrieved from each site. Individual oysters were scrubbed and refrigerated prior to processing. Whole oyster soft tissue was removed from the shell (taking care to avoid contamination) rinsed in demineralized water (Milli Ro Plus®) and blotted dry. For each site five replicate samples were prepared, each replicate being a composite of eight individual oysters. Samples were prepared as per the baseline replicates. Composites were stored frozen in plastic specimen vials prior to drying.

#### 1.2.4. *Mud crabs*

Sites were sampled within 100m either side of the GPS reference point repeatedly between the 12<sup>th</sup> and 21<sup>st</sup> of February 2003 using standard collapsible mesh posts baited with mullet or kangaroo until the required number of crabs had been obtained (Figure 7). As the previous study had determined that no gender differences existed in metal and fluoride concentrations of mud crabs from Spillway Creek (Andersen et al., 2001), samples consisted of mud crabs of mixed gender. Specimens of 160 –190 mm carapace width were targeted to reduce the variability in bioaccumulation caused by possible age differences. The carapace widths of crabs were measured, specimens

examined for the presence of shell disease, and selected crabs tied for transport to the laboratory. Any unwanted specimens were immediately released after examination.

Crabs were processed within 24 hrs of capture. Specimens were anaesthetized by chilling at  $-5^{\circ}\text{C}$  for at least one hour prior to dissection. Samples of hepatopancreas (liver) and muscle tissue were placed separately in plastic specimen vials and stored frozen prior to analyses.



**Figure 7.** Catching mud crabs in collapsible mesh pots.

#### **1.2.5. Laboratory analyses**

All samples were oven dried for 48 hours at  $65^{\circ}\text{C}$ . For the oyster and muscle tissues this was followed by grinding for 5 minutes in a 'shatter-box' grinding mill equipped with a fused stabilised zirconia grinding head that grinds material to  $<50\ \mu\text{M}$ . The hepatopancreas, however, failed to dry sufficiently by oven drying, remaining too oily to be ground. Therefore the hepatopancreas samples were additionally freeze dried in a Virtis Sentry™ freeze drier with an Alcatel vacuum pump. Samples were then of a sufficient homogenous toffee like consistency and did not require grinding.

Samples for accelerator-analysis at the ANSTO facility were prepared by creating a solid, robust disk of compacted material. This was achieved by placing a small amount of the loose, dried material ( $\sim 0.3\ \text{g}$ ) was placed in a metallic cup, which in turn was inserted into a pressing die and subjected to a high compression. For the hepatopancreas samples the material remained stable within the metallic cup and

therefore compression was not required. The disks were placed in a vacuum chamber and irradiated with high-energy protons in the Australian Nuclear Science and Technology Organisation's (ANSTO) Van de Graaff particle accelerator.

The protons interact with fluorine, aluminium and other metals in the material creating characteristic X-rays and also, high energy nuclear radiations called gamma rays, both of which have precisely defined energies. These unique energies enable the unambiguous signatures of fluorine and metals to be observed and measured. The intensity of these X-rays and gamma rays is a measure of the amount of these elements in the material. The intensity (and hence concentration) was measured relative to the standard reference materials NBS1632a, which has a certified concentration of fluorine and aluminium and NBS278, which has a suite of other heavier elements. All results are reported on a dry weight basis.

#### **1.2.6. Statistical analyses**

Within each tissue type (hepatopancreas and muscle from resident mud crabs and whole body tissue from transplanted oysters), one-way analysis of variance (ANOVA) was used to test for differences in mean concentration of each metal between sites (viz. the four locations in Spillway Creek (Sites A, B, C & D) and the two control locations (Lilly Island, (LI) and Wild Cattle Creek, (WC)). For transplanted oysters, a baseline sample (BL), comprising of randomly selected oysters from the Oyster Lease retained prior to deployment, was also included. Baseline samples were included to indicate metal levels in the uncontaminated oyster lease area relative to ambient metal concentrations the Gladstone Harbour system.

Prior to analyses homogeneity of sample variances were tested with Levenes and Brown/Forsyth tests. Where necessary within each tissue type, data for selected metals were  $\log_{10}(x+1)$  transformed to achieve equality of sample variances and therefore satisfy the assumptions of the ANOVA test. For metals that were below detection limit, half of the mean detection limit for the metal was used to allow analysis. *A posteriori* Tukeys HSD multiple comparison test was then used to locate differences for significant main effects. Results for all tests were tabulated. Between-site differences in fluoride concentrations and levels of all metals which showed significant site differences, were plotted for each tissue type.

### 1.3. RESULTS

#### 1.3.1. Water quality

The GPS location of sites and associated physicochemical water parameters of each site are reported in Table 1.

**Table 1.** Physicochemical surface water parameters and GPS locations from sites in Spillway Creek (A-D) Lilly Island (LI) and Wild Cattle Creek (WC).

Site		Location	Temp ° C	Conductivity mS/cm	pH	Dissolved O <sub>2</sub> ppm
A	Lat.	23° 53.060	28.8	43.8	8.5	6.82
	Long.	151° 19.114				
B	Lat.	23° 53.653	28.7	44.9	8.5	5.31
	Long.	151° 19.238				
C	Lat.	23° 54.220	28.7	45.5	8.5	5.14
	Long.	151° 19.539				
D	Lat.	23° 54.661	29.5	35.7	8.1	4.46
	Long.	151° 19.988				
LI	Lat.	23° 52.820	27.4	45.6	8.5	5.11
	Long.	151° 18.898				
WC	Lat.	23° 59.663	28.6	46.7	8.3	5.89
	Long.	151° 25.414				

At all locations the water parameters measured were within ANZECC/ARMCANZ (2000) guidelines for fresh and marine water quality. Sites A to D also conformed to the quality characteristics of release waters required of BSL under its environmental authority.

**Table 2.** Total metal and fluoride concentrations (mg/L) in water samples from BSL Sites SK1 – SK5, which are in the vicinity of project Sites A to D in Spillway Creek.

BSL Site	Location vicinity	Total elements mg/L							
		Al	As	Cr	Cu	F	Ni	Pb	Zn
<b>SK1</b>	<b>Site A</b>	0.75	<0.001	<0.001	0.001	1.06	<0.005	<0.001	0.011
<b>SK2</b>	<b>Site B</b>	0.76	<0.001	<0.001	0.002	1.11	<0.005	<0.001	0.011
<b>SK3</b>	<b>Site C</b>	0.68	<0.001	<0.001	0.001	1.08	<0.005	<0.001	0.013
<b>SK5</b>	<b>Site D</b>	0.52	<0.001	<0.001	<0.001	3.33	<0.005	<0.001	0.011

Analyses of water samples (total metals) collected by BSL in the vicinity of the Spillway Creek sites determined that only three (Al, Cu and Zn) of the seven metals analysed were above limits of detection. Concentrations for the three metals appeared similar for all sites. The concentration of fluoride at Site D was higher than the other three sites.

### 1.3.2. Oysters

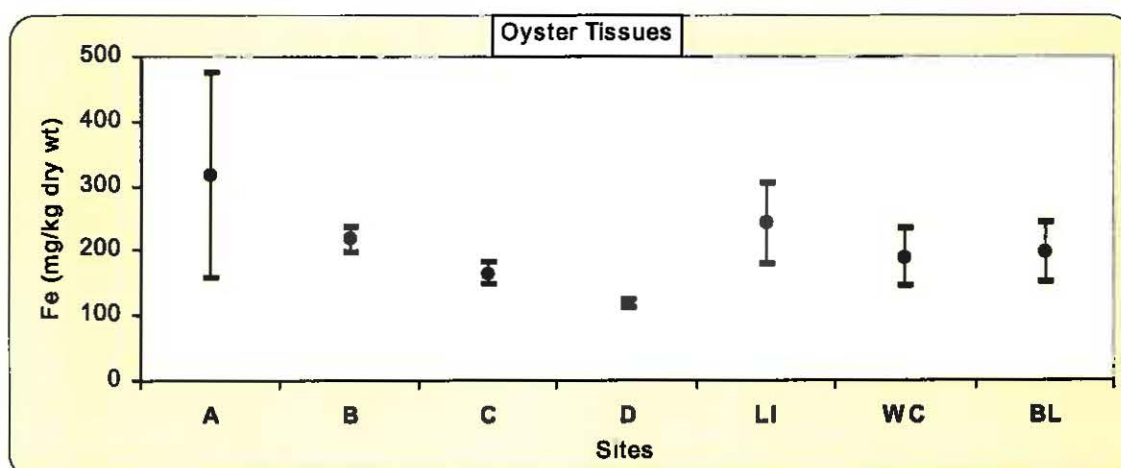
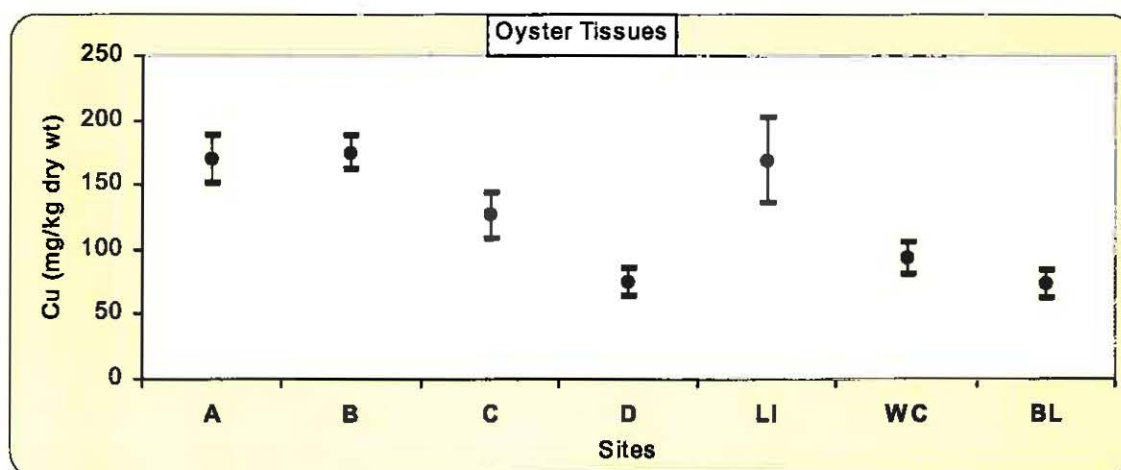
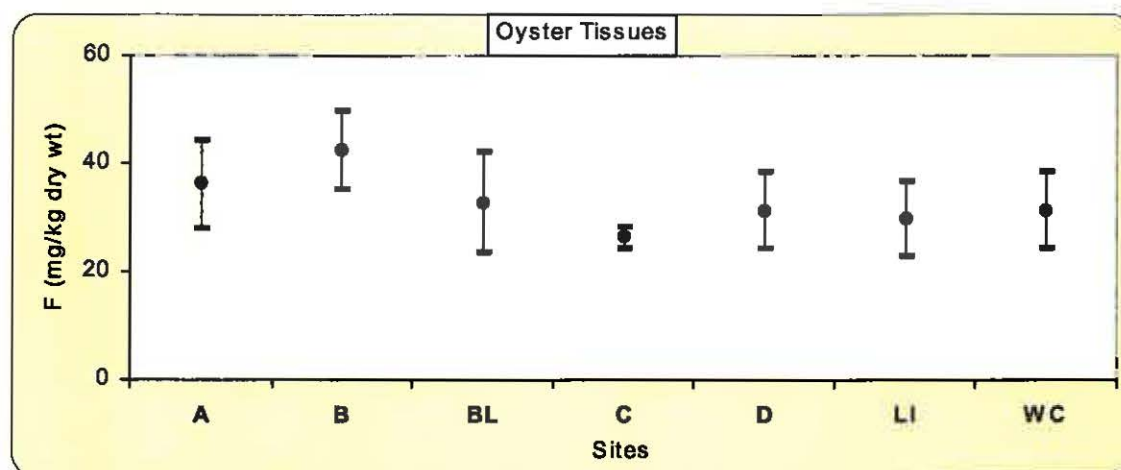
#### 1.3.2.1. Fluoride and metal analyses

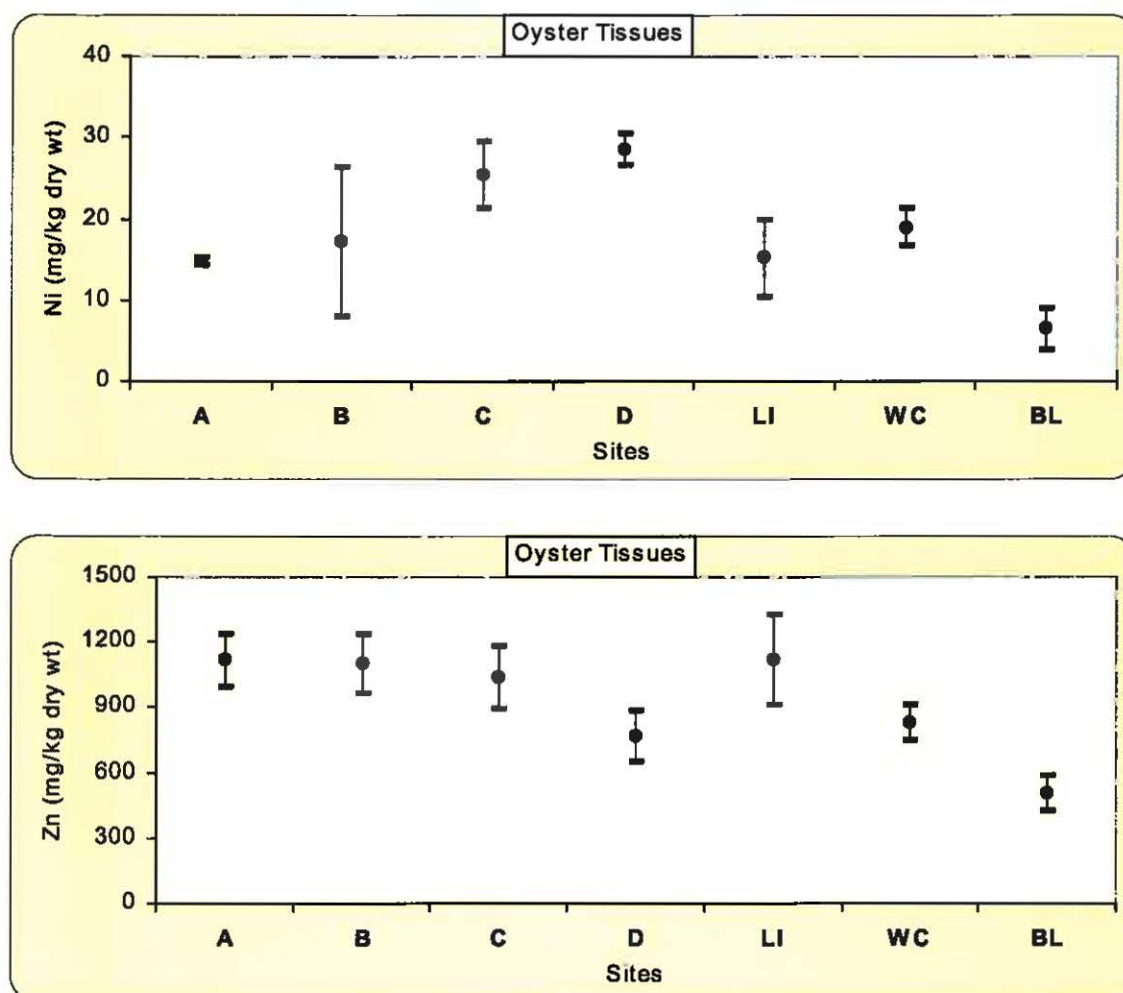
Of the ten metals tested in oyster tissues, Fe, Ni, Cu and Zn showed significant site differences (Table 3). Within Fe, metal concentrations in specimens deployed to site A were greater than at site D, with no other significant between-site differences (Table 3, Figure 8). Within Ni, there was a range of between-site differences in metal concentration, with the highest levels recorded in specimens deployed at sites D, C and WC, and the lowest concentrations at sites LI, A and BL. For Cu in oysters, levels in specimens from sites B, A & LI were significantly greater than all other sites. Site C was higher than WC, D and BL, within which there were no differences (Table 3, Figure 8).

**Table 3.** Summary of one-way ANOVAs on metal concentrations in oyster tissues from Spillway Creek sites (A, B, C & D), control sites at Lilly Island (LI) and Wild Cattle Creek (WC) and the oyster lease prior to deployment (Baseline (BL)). Where indicated,  $\log_{10}(x + 1)$  transformations were applied to achieve equality of variances. Tukey's HSD multiple comparison test was used to locate between-level differences for significant main effects. A common line joins levels not significantly different at  $p < 0.05$  and effects are in descending order. Arithmetic mean values are presented for metal concentrations in each sample, with geometric means presented for  $\log_{10}$  transformed data. Between site differences for fluoride and all significant metals are plotted in Figure 8. Mean (+ 1 SE) concentrations of each metal and limits of detection are presented in Appendix 1 (\* = all values were below detection limit).

Metal	df	F	P	Tukeys HSD Range Test						
Al	6,28	0.89	ns	A (196.4)	LI (152.2)	BL (123.8)	C (123.8)	D (123.8)	B (123.8)	WC (123.8)
As	6,28	1.33	ns	B (24.6)	C (24.2)	WC (23.3)	LI (23.3)	A (23.3)	BL (21.4)	D (19.4)
Cr*	6,28	-	-							
Cu	6,28	24.14	<0.0001	B (175.4)	A (170.2)	LI (169.4)	C (126.6)	WC (94.0)	D (75.4)	BL (74.0)
F	6,28	1.98	ns	B (42.4)	A (36.2)	BL (32.8)	D (31.4)	WC (31.4)	LI (29.8)	C (26.4)
Fe	6,28	3.05	0.0200	A (316.2)	LI (242.4)	B (218.2)	BL (198.0)	WC (190.4)	C (166.0)	D (119.4)
Ni	6,28	10.21	<0.0001	D (28.6)	C (25.4)	WC (19.0)	B (17.2)	LI (15.2)	A (14.8)	BL (6.4)
Pb*	6,28	-	-							
Se	6,28	0.99	ns	LI (14.0)	WC (13.6)	C (13.4)	D (13.0)	B (12.0)	A (11.8)	BL (10.4)
Zn	6,28	11.89	<0.0001	LI (1119)	A (1117)	B (1102)	C (1039)	WC (830)	D (768)	BL (505)

This indicates elevated levels at most locations in comparison with baseline conditions, suggesting ambient levels in the Gladstone Harbour area in general are elevated relative to the oyster lease. Within Zn, levels at site LI, A & B were greater than at site D & baseline (BL), with intermediate levels at sites C & WC (Table 3, Figure 8). As for Cu and Ni, levels appeared generally elevated compared with baseline concentrations. There was no significant between-site difference for fluoride in oyster tissues, as can be seen in Figure 8.





**Figure 8.** Comparison of mean metal concentrations (mg/kg dry weight; means  $\pm$  95 % C.I.) in oyster tissues from the four sites in Spillway Creek (A, B, C & D), the two control sites (Lilly Island (LI); Wild Cattle Creek (WC) and the oyster lease (Baseline (BL)).

### 1.3.3. Mud crabs

#### 1.3.3.1. Prevalence of shell disease

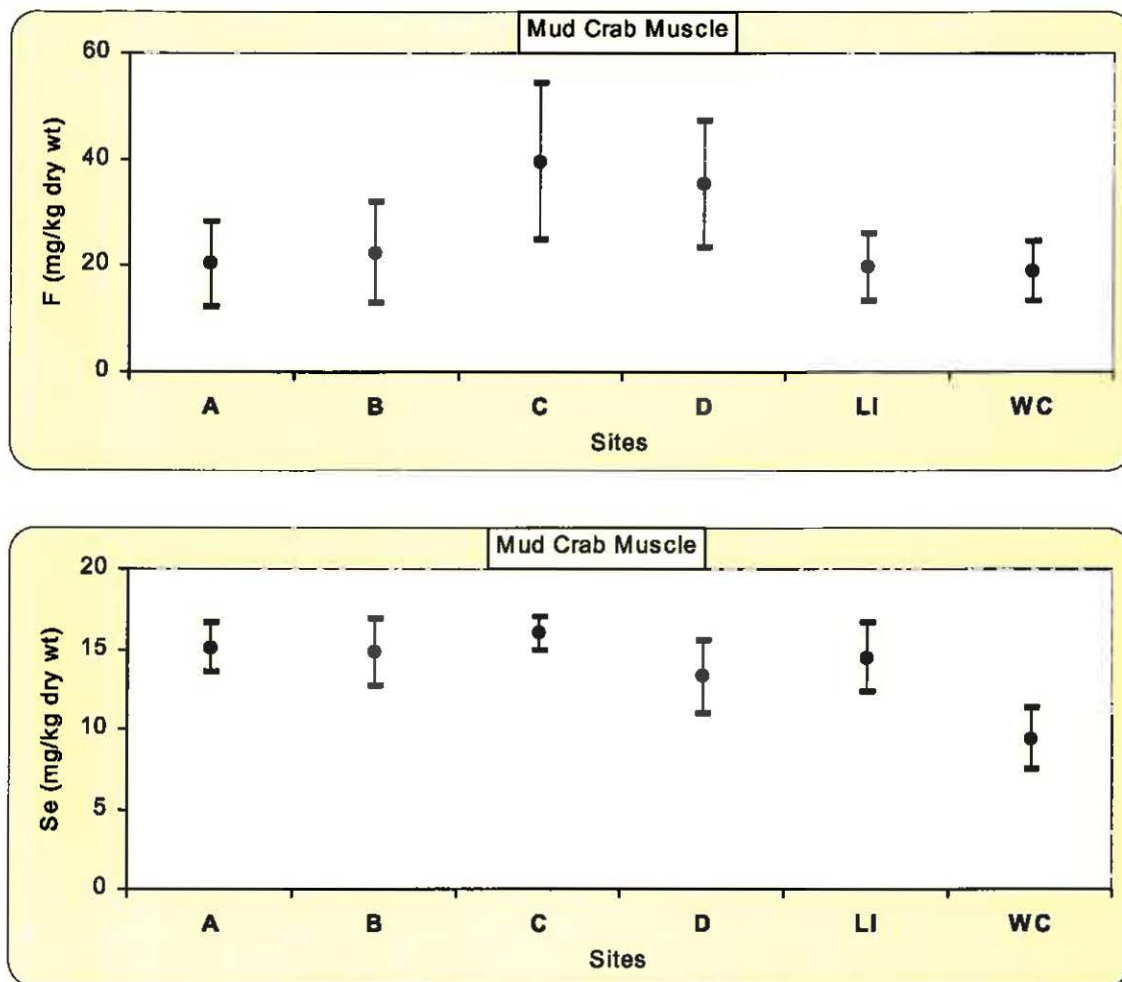
103 mud crabs ranging from 110 mm to 200mm carapace width were captured and critically examined for the presence of shell lesions. Four crabs (3.8%) were determined to have rust spot shell lesions as defined by a grading system developed in a previous study on mud crab shell disease (Andersen & Norton, 2001) and all were captured from the Spillway Creek sites. The lesions were considered minor with only one lesion being slightly perforated.

#### 1.3.3.2. Muscle fluoride and metal analyses

Of the ten metals tested in mud crab muscle tissue, only F and Se showed a significant site difference (Table 4). Within Se, metal concentrations were significantly greater at sites C, A, B & LI than at site WC, with no significant difference between Site D and all other sites (Table 4, Figure 9). Although ANOVA detected a significant site difference for Fluoride, the multiple comparison test was not able to locate the site difference, reflecting the relatively high within-site variance and that the Tukey's test is not as statistically powerful as the ANOVA. Examination of the plot (Figure 9), suggests a trend for high F concentrations in mud crab muscle collected from Sites C & D relative to the other sites.

**Table 4.** Summary of one-way ANOVAs on metal concentrations in mud crab muscle tissue from Spillway Creek sites (A, B, C & D) and control sites at Lilly Island (LI) and Wild Cattle Creek (WC). Where indicated,  $\log_{10}(x + 1)$  transformations were applied to achieve equality of variances. Tukey's HSD multiple comparison test was used to locate between-level differences for significant main effects. A common line joins levels not significantly different at  $p < 0.05$  and effects are in descending order. Arithmetic mean values are presented for metal concentrations in each sample, with geometric means presented for  $\log_{10}$  transformed data. Between site differences for fluoride and all significant metals are plotted in Figure 9. Mean (+ 1 SE) concentrations of each metal along with limits of detection are presented in Appendix 1 (\* = all values were below detection limit).

Metal	df	F	P	Tukeys HSD Range Test					
Al	5,54	0.63	ns	WC (153.1)	C (144.3)	LI (140.6)	D (123.8)	A (123.8)	B (123.8)
As	5,54	1.07	ns	B (28.5)	D (26.6)	C (24.7)	WC (23.7)	LI (23.3)	A (22.9)
Cr*	5,54	-	-						
Cu (log)	5,54	1.43	ns	B (98.0)	WC (90.4)	LI (87.1)	C (84.6)	A (67.7)	D (55.6)
F	5,54	3.21	0.0130	C (39.6)	D (35.5)	B (22.5)	A (20.3)	LI (19.6)	WC (19.2)
Fe	5,54	0.67	ns	WC (114.7)	D (107.3)	A (84.1)	B (77.8)	LI (72.4)	C (55.5)
Ni	5,54	2.03	ns	A (23.7)	D (22.5)	B (18.0)	WC (17.7)	C (16.1)	LI (9.7)
Pb	5,54	0.63	ns	B (5.3)	D (5.2)	A (3.8)	WC (3.7)	C (3.3)	LI (3.3)
Se	5,54	5.85	0.0002	C (16.0)	A (15.1)	B (14.8)	LI (14.5)	D (13.3)	WC (9.4)
Zn	5,54	1.48	ns	B (623.1)	WC (562.6)	A (551.1)	C (548.7)	LI (547.2)	D (519.1)



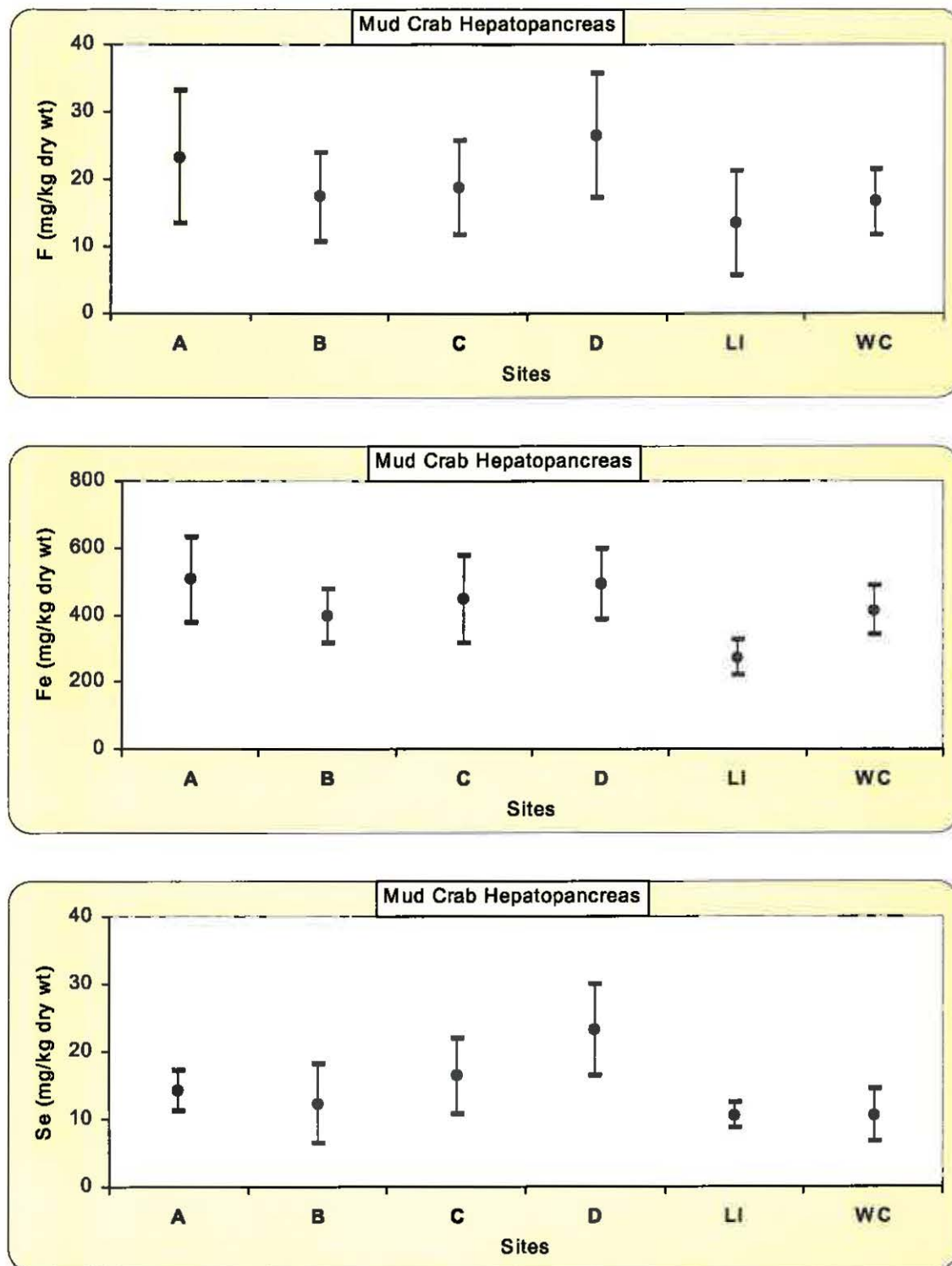
**Figure 9.** Comparison of mean metal concentrations (mg/kg dry weight; means  $\pm$  95 % C.I.) in mud crab muscle tissues from the four sites in Spillway Creek (A, B, C & D), and the two control sites (Lilly Island (LI); Wild Cattle Creek (WC)).

#### 1.3.3.3. *Hepatopancreas fluoride and metal analyses*

Of the ten metals tested in mud crab hepatopancreas tissues, only Fe and Se showed significant site differences (Table 5). Within Fe, metal concentrations at sites A & D were greater than at site LI, with no other significant between-site differences. Within Se, metal concentrations at site D was significantly greater than at sites B, LI and WC, with no other significant between-site differences (Table 5, Figure 10). There was no significant between-site difference for fluoride in mud crab hepatopancreas, as can be seen in Figure 10.

**Table 5.** Summary of one-way ANOVAs on metal concentrations in mud crab hepatopancreas tissue from Spillway Creek sites (A, B, C & D) and control sites at Lilly Island (LI) and Wild Cattle Creek (WC). Where indicated,  $\log_{10}(x + 1)$  transformations were applied to achieve equality of variances. Tukey's HSD multiple comparison test was used to locate between-level differences for significant main effects. A common line joins levels not significantly different at  $p < 0.05$  and effects are in descending order. Arithmetic mean values are presented for metal concentrations in each sample, with geometric means presented for  $\log_{10}$  transformed data. Between site differences for Fluoride and all other significant metals are plotted in Figure 10. Mean ( $\pm 1$  SE) concentrations of each metal and limits of detection are presented in Appendix 1.

Metal	df	F	P	Tukeys HSD Range Test					
Al	5,54	1.00	ns	WC (148.8)	A (123.8)	C (123.8)	D (123.8)	LI (123.8)	B (123.8)
As (log)	5,54	0.65	ns	D (19.5)	A (17.8)	LI (16.2)	C (15.2)	B (14.8)	WC (14.3)
Cr	5,54	1.10	ns	WC (7.8)	B (7.7)	A (6.3)	C (6.3)	LI (6.3)	D (6.3)
Cu (log)	5,54	2.24	ns	D (901.4)	LI (713.8)	A (641.1)	C (416.1)	B (295.1)	WC (230.4)
F	5,54	1.45	ns	D (26.4)	A (23.3)	C (18.7)	B (17.4)	WC (16.6)	LI (13.4)
Fe	5,54	2.75	0.0277	A (506.0)	D (493.3)	C (447.1)	WC (414.5)	B (397.0)	LI (273.0)
Ni	5,54	1.67	ns	LI (14.7)	WC (13.7)	A (12.9)	D (10.0)	B (8.5)	C (7.9)
Pb	5,54	1.00	ns	WC (3.7)	A (3.3)	C (3.3)	D (3.3)	LI (3.3)	B (3.3)
Se	5,54	3.84	0.0048	D (23.3)	C (16.4)	A (14.3)	B (12.3)	LI (10.6)	WC (10.6)
Zn	5,54	1.65	ns	A (432.1)	D (432.0)	B (357.6)	WC (355.9)	C (296.8)	LI (257.2)



**Figure 10.** Comparison of mean metal concentrations (mg/kg dry weight; means  $\pm$  95 % C.I.) in mud crab hepatopancreas tissues from the four sites in Spillway Creek (A, B, C & D), and the two control sites (Lilly Island (LI); Wild Cattle Creek (WC)).

## 1.4. DISCUSSION

### 1.4.1. Water quality

One week prior to the commencement of crabbing and 16 days prior to the removal of oysters a large rain event deposited approximately 570 mm on the Gladstone area over four days. The salinity measured at all sites was therefore less than could be expected for estuarine conditions due to the recent inundation of fresh water to the system. Rainfall of this extent had not been seen in the Gladstone area for some time. The amount of rain could have served to flush the system of contaminants thereby diluting bioavailable metal and fluoride concentrations or conversely may have caused additional contaminants from stormwater sources to be flushed into the system. Flows into Spillway Channel are dominated by releases from the Smelter operations, stormwater runoff from the Smelter and infrequent stormwater overflow from the QAL Red Mud Dam No 1 (SKM, 2002).

Oysters are known to close their shells for extended periods during heavy rainfall until suitable conditions (salinity and suspended sediment levels) resume (Laurie McGrath, oyster lease, pers. comm.). The oysters and mud crabs were a measure of time-averaged concentrations of contaminants over the whole three-month period. Therefore it is considered that the short period of exposure time to dilute or conversely contaminated water after the rain event would have had little effect on oyster concentrations overall. Depuration rates for some metals in this species are slow with half-lives in the order of 53 to 231 days (Ke & Wang, 2001). Mud crabs are osmoregulators and were caught within 14 days of the rain event. Moore, (1971) determined that for blue crabs (*Callinectes sappidus*) returned to natural seawater after a 30-day exposure to 128 ppm of fluoride, there was a depuration lag phase of at least ten days before a reduction in muscle or hepatopancreas fluoride concentrations was detected. Therefore it is unlikely that metal or fluoride concentrations in oysters or mud crabs were significantly affected overall, by the rain event.

The concentration of total metals in water samples taken by BSL at all Spillway sites was comparable, being either negligible or low and this was generally reflected in the non-significant overall site differences in accumulation of metals by biota. Despite a decreasing gradient of accumulation of nickel in oysters from Site D to Site A, the concentration of nickel in water sampled on this occasion was below detection limits. Concentration of fluoride in water, however, was greatest at Site D compared to all other sites and this correlates with the trend for accumulation of fluoride in mud crab muscle tissue from Site D and to a lesser extent Site C, although significant site differences in accumulations of fluoride were not detected in oysters or mud crab hepatopancreas.

### 1.4.2. Oysters

#### Fluoride

Despite the reported ability of oysters to readily accumulate fluoride in the soft tissue (Hemens & Warwick, 1972, Wright & Davison, 1975), there was no between site difference in oyster fluoride concentrations. Concentrations in oysters from Spillway sites were not significantly different to those in oysters from the two control sites or the Baseline (lease) oysters. Nell and Livanos, (1988) demonstrated a linear uptake of fluoride by oyster (*Saccostrea glomerata*) spat. Concentrations in whole spat (shell

and soft tissue) ranged from 45 to 204 ppm dry weight in oysters exposed to fluoride additions from 0 to 30 ppm. Mean concentration in transplanted oysters (soft tissue only) across all sites including Baseline in this study ranged between 26.4 and 42.4 ppm, which was similar to the concentration in control oysters (no addition of fluoride) from the study by Nell and Livanos, (1988).

### *Metals*

There was a gradient of increasing nickel concentrations in oyster tissue from Sites A to D despite negligible concentrations of nickel in water samples analysed on one occasion. This suggests oysters closer to the discharge channel are being exposed to greater bioavailable forms of nickel and highlights the benefits of biological monitoring averaging out ambient pollutant bioavailability over time (Phillips & Segar, 1986). This is an acknowledged advantage over water sampling, which provides only a snapshot in time. However, concentrations in oysters at all Spillway sites were not significantly different to those from Wild Cattle Creek or Lilly Island, although Sites C and D were elevated in comparison to Site A and Baseline oysters.

A reverse gradient of accumulation was observed for copper and zinc with concentrations in oysters decreasing from Sites A to D. Iron to a lesser extent followed a similar trend. The uptake patterns for copper and zinc were almost identical at individual sites over the sampling period. Oysters from Lilly Island and Sites A and B tended to have significantly higher concentrations of these two metals than Site D and Baseline suggesting alternate anthropogenic exposures to these two metals outside of Spillway Creek. Copper and zinc have been previously demonstrated to be elevated in oysters from inner harbour sites compared to outer harbour sites in Gladstone and reference sites (Andersen unpub data, CRC unpub data). There appeared to be no relationship between the concentrations of copper and zinc in water samples from Spillway creek and those in oysters.

### *Food guidelines*

There are no current ANZFA, (2002) recommended food guidelines for the metals for which significant site differences were detected. The previous ANZFA, (1999a) guideline had set maximum limits for copper and zinc at 70 mg/kg and 1000 mg/kg respectively for oysters. After conversion to wet weight assuming an average moisture content of 75% for oyster meat (Andersen, unpub), the mean concentrations at all sites for copper (18.5 – 43.5 mg/kg) and zinc (126.25 – 279.75 mg/kg) were determined to be below the previous recommended levels.

### **1.4.3. Mud crabs**

#### **1.4.3.1. Shell disease**

Although the prevalence of rust spot shell disease (3.8%) in mud crabs was higher than that recorded in the 2001 study (0%), it was still below the 5% prevalence, which is considered to represent natural background levels of shell disease in inshore populations of crustaceans (Sindermann, 1989). The prevalence was also less than the 14.9% average combined prevalence of rust spot shell disease recorded in mud crabs from Port Curtis harbour examined in the 1999/2000 mud crab season (Andersen & Norton, 2001).

#### **1.4.3.2. Mud crab muscle**

### *Fluoride*

Although ANOVA detected a significant between site difference in fluoride concentrations in mud crab muscle, the post hoc Tukey's test could not identify which sites were significantly different from each other. The means plot (Figure 9), however, indicates that accumulations in mud crab muscle from Sites D and C were greater than those from Sites B and A, which were only slightly more elevated than those from Lilly Island and Wild Cattle. There was no significant difference in fluoride concentrations between sites for mud crab muscle in the 2001 study although there was a trend for higher concentrations in Spillway Creek sites, in particular Sites D and B compared to Wild Cattle.

### *Food guidelines*

Results suggest that there is some fluoride accumulation occurring in mud crab muscle in sites closer to the discharge channel. Although the ANZFA, (2002) food code does not set a limit for fluoride in food, in New Zealand fluoride is permitted at a concentration of 15 ppm wet weight in shellfish (ANZFA, 1999b). Converting the dry weight results to wet weight, assuming the average moisture content of mud crab meat is 75% (Andersen and Norton, unpub.) gives a mean concentration range of 4.8 – 9.9 ppm at all sites, which is below this recommended limit.

### *Metals*

Apart from Selenium where only one site (Wild Cattle) differed significantly from the majority of other sites except Site D, there were no significant between site differences or trends in metal concentrations in mud crab muscle. A number of metals showed significant between site differences in the previous study, however, additional site comparisons were made between Ayr, Gladstone and Baffle Creek. Similarly no obvious trend of metal accumulation at any one particular site or sites was detected in the 2001 study. Selenium was not measured in the previous study.

### *Food guidelines*

There is no recommended maximum permitted level for selenium in the current ANZFA (2002) guidelines due to this metal and a number of other metals being deleted from the guidelines in a risk assessment review of contaminants in food in 1999 (ANZFA, 1999a). The previous recommended level for selenium was 1 mg/kg. Concentrations of selenium in mud crab muscle (after conversion to wet weight using the conversion rate above) at all sites including Wild Cattle ranged from 2.35 – 4mg/kg, which is above the previous recommendation. The review recommended deleting selenium from the current ANZFA, (2002) food code due to their being 'no cause for concern about the dietary exposure to selenium for high consumers of specific food commodity groups'. As mud crab meat is deemed an 'occasionally' consumed food i.e. consumed less than once a week by 75% of the population (ANZFA, 1999a), there is likely to be minimal risk of selenium toxicity from the consumption of mud crab meat from Spillway Creek or the control sites.

#### *1.4.3.3. Mud crab hepatopancreas*

### *Fluoride*

Although mud crab hepatopancreas from Spillway Creek sites, in particular Site D tended to have greater accumulations of fluoride, these were not significant.

### *Metals*

Apart from iron for which no trend could be identified, the only metal for which significant site differences were established was selenium. There were also significant site differences identified for selenium in mud crab muscle but no trend among sites could be established. However, for mud crab hepatopancreas there appeared to be a trend for accumulations of selenium in Spillway Creek compared to Lilly Island and Wild Cattle with Site D in particular being significantly elevated compared to the two controls. Selenium was not measured in water samples from Spillway Creek in this study nor was it measured in mud crab hepatopancreas in the 2001 study.

## 1.5. GENERAL DISCUSSION

One of the aims of this study was to compare concentrations of fluoride and metals in mud crabs to those found in the previous 2001 study (Andersen et al., 2001). Between-laboratory variations in results are known to occur despite the same techniques being used for extraction and analyses. When different methods of analyses are used, results are even less comparable. In the previous study fluoride and metals were analysed separately at two or more different laboratories. For metals, samples were microwave acid digested prior to analyses by ICP-MS with results reported on a wet weight basis. For fluoride, samples were ashed prior to acid digestion with a fluoride electrode used to measure the fluoride. In this study the fluoride and metals were analysed simultaneously using ANSTO's Van de Graff Particle Accelerator, where samples are placed in a vacuum and the fluoride and metal ions generated from the bombardment of protons, are measured. The techniques differ so widely that it was decided that temporal statistical comparisons could not be meaningfully made using the two data sets. A parallel could be drawn, however, from trends in accumulations of metals or fluoride from either study.

Although known quantities of fluoride are released from BSL into Spillway Channel there was no site difference detected in fluoride accumulation in oysters in Spillway Creek. Estuarine and marine invertebrates and fish appear to be more tolerant to fluoride toxicity than their freshwater counterparts, probably as a consequence of the elevated content of calcium and chloride in seawater (Comargo, 2003). It is likely that insoluble fluorides, including complexes with aluminium, are formed as fluoride discharged from the smelter mixes with seawater. Therefore the bioavailability of fluoride ions (and, consequently, their toxic action) is reduced with increasing water hardness (Comargo, 2003). Although the majority of uptake of fluoride is thought to be directly from the water (Hemens & Warwick, 1972), digestion of fluoride particles may offer an alternate route of fluoride uptake in oysters to the dissolved phase. However, there is also evidence that oysters preferentially select and sort particulates, retaining the organic and rejecting the inorganic fraction (Barille et al., 1997, Hawkins et al., 1998) and this ability may have reduced the uptake of fluoride in this species.

Although a linear uptake of fluoride has been demonstrated for this species of oyster in chronic exposure experiments up to 30ppm (Nell & Livanos, 1988), it is possible that at the low exposure concentrations experienced by oysters in Spillway Creek, no accumulation differences could be demonstrated between Spillway oysters and control oysters. Mean water fluoride concentrations around Site D ranged from 1.3 mg/L to 6.2 mg/L in a two-year period from 1994-1996, but concentrations recorded downstream (in the vicinity of Sites A-C) were similar to background levels (~ 1.0 mg/L) (Yezdani, 1996).

In exposure experiments Moore, (1969)(cited in (Hemens & Warwick, 1972)) demonstrated that oysters (species not specified) accumulated fluoride when subjected to continual fluoride exposure. Similar linear uptakes have been demonstrated for other species such as prawns (McClurg, 1984, Andersen et al., 2002) and crabs (Wright & Davison, 1975). Hemens and Warwick (1972) suggested that from calculations based on Moore's published data, the majority of the fluoride was accumulated in the body fluids of oysters and that true tissue accumulation of this element may not have occurred. Additionally Moore, (1969) determined that fluoride

was lost as fast as it had been gained on return to fluoride free water, also suggesting that the fluoride was not chemically bound within the oyster. As the exposure to fluoride in Spillway Creek is likely to be of a pulse type nature with concentrations fluctuating widely due to tidal inundation and variable discharge concentrations, it is possible that the influx/efflux rate of fluoride in oysters remained constant and offers another explanation as to why no site difference in fluoride accumulation could be detected in oysters.

Fluoride is known to accumulate in crustaceans including mud crabs, particularly in the carapace but also in the muscle and hepatopancreas (Moore, 1971, Wright & Davison, 1975, Andersen et al., 2001), however a longer retention time of fluoride in the tissues is likely compared to oysters. Trends for accumulations of fluoride in muscle and hepatopancreas tissue from upper Spillway sites compared to lower Spillway and control sites are apparent from this and the previous study. Concentrations, however, do not appear to be of such a significant level as to cause harm to mud crabs or pose a human health risk from eating mud crab meat. The mean concentrations in mud crab meat in this study are less than those determined for whole prawns in the control group in a fluoride exposure experiment (Andersen et al., 2002), using the same ANSTO method of fluoride analyses.

The conflicting results for mud crabs and oysters could also be related to their feeding and uptake mechanisms. Fluoride is known to accumulate and biomagnify during transfer through food chains with some serious adverse effects for those animals at the top of the chain (Groth, 1975). Oysters are filter feeders and therefore are only one step up in the food chain from plants. Mud crabs, however, are known to feed on slow moving benthic organisms including molluscs, worms and smaller crustaceans (Thimdee et al., 2001) and are relatively high in the estuarine food web (Andersen & Norton, 2001). The opportunity to accumulate biomagnified fluoride through food sources is therefore more likely for this species.

Metal concentrations in baseline oysters from the oyster lease were generally lower than concentrations in oysters from the majority of sites in Gladstone including Wild Cattle, and most likely reflect the differences in ambient water column metal concentrations of the two locations. Lilly Island tended to have some of the most elevated oyster metal concentrations, perhaps indicating this location receives additional anthropogenic sources of inputs of metals. Wild Cattle appeared overall to be a suitable control site.

The gradient for accumulation of nickel in oysters from Site A to D and the reverse trend for accumulation of copper, zinc and iron are interesting. A similar trend of accumulation was not seen in mud crabs. Antagonistic interactions for uptake between metals have been demonstrated (Phillips, 1990). The uptake of arsenic has been negatively correlated with the uptake of both copper and zinc in *S. glomerata* (Mackay et al. cited in Cunningham, 1979) although an interaction between copper, zinc and nickel has not been recorded.

Selenium featured as having significant site differences in concentrations in both mud crab muscle and hepatopancreas, although for mud crab muscle no pattern of accumulation could be established. There does appear to be some accumulation of selenium in hepatopancreas at Site D although the significance of this is not known.

Selenium was not significant in oysters and is not currently measured in water samples collected by BSL. Aluminium did not feature as significant in either species.

Differences in metal accumulations between oysters and mud crabs are likely to be due in part to their differing accumulation strategies. Oysters are net accumulators and therefore generally reflect ambient bioavailable water metal concentrations (Rainbow, 1990). In contrast mud crabs are metal regulators. Regulators have the ability to maintain constant body metal concentrations over a wide range of ambient metal bioavailabilities (Rainbow, 1990), although some accumulation does occur.

In conclusion, the finding of some elevations of fluoride in mud crabs from sites closer to the discharge channel support the findings of the previous 2001 study. Concentrations of fluoride were not elevated in oysters from Spillway Creek compared to controls and oysters from the lease. Selenium appeared to more elevated in upper Spillway mud crabs and Nickel more elevated in upper Spillway oysters compared to reference sites. A reverse pattern of accumulation was observed for copper and zinc in Spillway oysters. However, concentrations of fluoride and metals in tissues were not consistently more elevated than those at control sites. The consumption of mud crab meat from any of the study sites does not pose a human health risk.

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## 1.8. APPENDICES

**Appendix 1.** Mean (+ 1 SE) concentration (mg/kg dry wt) of each metal in each tissue type at each site in Spillway Creek (A, B, C & D), the two control sites (Lilly Island, LI; Wild Cattle Creek, WC), and the oyster lease (Baseline, BL) (oysters only). Mean limits of detection (LOD) are shown in bold.

Tissue	Site	Al	As	Cr	Cu	F	Fe	Ni	Pb	Se	Zn
Mud crab Hepatopancreas	<b>LOD</b>	<b>247.5</b>	<b>3.5</b>	<b>12.6</b>	<b>3.8</b>	<b>18.9</b>	<b>8.8</b>	<b>4.3</b>	<b>6.7</b>	<b>3.9</b>	<b>4.2</b>
	<b>A</b>	123.8 (0)	18.3 (1.5)	6.3 (0)	687.1 (85.7)	23.3 (5.0)	506.0 (65.8)	12.9 (2.0)	3.3 (0)	14.3 (1.6)	432.1 (62.5)
	<b>B</b>	123.8 (0)	17.1 (3.3)	7.7 (1.0)	526.3 (175.5)	17.4 (3.4)	397.0 (41.0)	8.5 (1.9)	3.3 (0)	12.3 (3.0)	357.6 (80.9)
	<b>C</b>	123.8 (0)	16.5 (2.1)	6.3 (0)	671.1 (169.4)	18.7 (3.6)	447.1 (66.0)	7.9 (2.3)	3.3 (0)	16.4 (2.9)	296.8 (34.3)
	<b>D</b>	123.8 (0)	22.8 (4.2)	6.3 (0)	1240.0 (311.5)	26.4 (4.7)	493.3 (55.0)	10.0 (2.1)	3.3 (0)	23.3 (3.5)	432.0 (58.7)
	<b>LI</b>	123.8 (0)	16.5 (1.1)	6.3 (0)	827.0 (128.9)	13.4 (4.0)	273.0 (27.4)	14.7 (2.0)	3.3 (0)	10.6 (0.9)	257.2 (30.1)
	<b>WC</b>	148.8 (25.0)	16.2 (2.9)	7.8 (1.5)	518.5 (166.9)	16.6 (2.5)	414.5 (37.6)	13.7 (2.9)	3.7 (0.4)	10.6 (1.9)	355.9 (45.0)
Mud crab Muscle	<b>A</b>	123.8 (0)	22.9 (1.5)	6.3 (0)	70.2 (6.4)	20.3 (4.1)	84.1 (17.0)	23.7 (3.2)	3.8 (0.5)	15.1 (0.8)	551.1 (26.6)
	<b>B</b>	123.8 (0)	28.5 (2.4)	6.3 (0)	106.1 (12.9)	22.5 (4.8)	77.8 (9.3)	18.0 (1.4)	5.3 (2.0)	14.8 (1.1)	623.1 (27.1)
	<b>C</b>	144.3 (20.5)	24.7 (2.2)	6.3 (0)	102.8 (20.0)	39.6 (7.5)	55.5 (10.0)	16.1 (1.4)	3.3 (0)	16.0 (0.6)	548.7 (16.4)
	<b>D</b>	123.8 (0)	26.6 (3.1)	6.3 (0)	63.5 (10.9)	35.5 (6.0)	107.3 (34.8)	22.5 (6.8)	5.2 (1.9)	13.3 (1.2)	519.1 (25.5)
	<b>LI</b>	140.6 (16.8)	23.3 (1.2)	6.3 (0)	108.1 (21.3)	19.6 (3.2)	72.4 (11.4)	9.7 (2.3)	3.3 (0)	14.5 (1.1)	547.2 (24.6)
	<b>WC</b>	153.1 (29.3)	23.7 (1.8)	6.3 (0)	101.0 (15.5)	19.2 (2.9)	114.7 (45.7)	17.7 (2.8)	3.7 (0.4)	9.4 (1.0)	562.6 (43.6)
Oysters	<b>A</b>	196.4 (72.6)	23.0 (1.9)	6.3 (0)	170.2 (9.5)	36.2 (4.1)	316.2 (80.8)	14.8 (0.2)	3.3 (0)	11.8 (0.7)	1116.8 (62.0)
	<b>B</b>	123.8 (0)	24.6 (1.5)	6.3 (0)	175.4 (6.9)	42.4 (3.7)	218.2 (10.6)	17.2 (4.7)	3.3 (0)	12.0 (0.9)	1102.2 (67.4)
	<b>C</b>	123.8 (0)	24.2 (1.4)	6.3 (0)	126.6 (9.4)	26.4 (1.0)	166.0 (8.2)	25.4 (2.1)	3.3 (0)	13.4 (1.5)	1039.4 (73.9)
	<b>D</b>	123.8 (0)	19.4 (0.7)	6.3 (0)	75.4 (5.6)	31.4 (3.6)	119.4 (3.3)	28.6 (1.0)	3.3 (0)	13.0 (0.5)	768.4 (60.9)
	<b>BL</b>	123.8 (0)	21.4 (0.5)	6.3 (0)	74.0 (5.4)	32.8 (4.7)	198.0 (23.5)	6.4 (1.3)	3.3 (0)	10.4 (0.9)	504.8 (42.2)
	<b>LI</b>	152.2 (28.4)	23.2 (2.3)	6.3 (0)	169.4 (16.8)	29.8 (3.6)	242.4 (32.3)	15.2 (2.4)	3.3 (0)	14.0 (2.1)	1118.6 (105.6)
	<b>WC</b>	123.8 (0)	23.2 (1.6)	6.3 (0)	94.0 (6.3)	31.4 (3.7)	190.4 (21.9)	19.0 (1.1)	3.3 (0)	13.6 (1.5)	830.2 (41.9)