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# **Metal bioaccumulation through food web pathways in Port Curtis**

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THE UNIVERSITY OF  
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CRC for Coastal Zone  
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# ***Metal Bioaccumulation through Food web Pathways in Port Curtis***

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**2005**

# ***Metal Bioaccumulation through Food web Pathways in Port Curtis***

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## ***Executive summary***

Port Curtis is an international trades port supporting major local industries as well as a large commercial and recreational fishing industry. Previous studies in the harbour (CRC Coastal Zone, 2005), have determined that despite relatively low metal concentrations in the water column there appears to be some accumulations of some metals in the marine organisms that inhabit the harbour. Aquatic animals can absorb metals through exposure in sediments and the water column but also via their diet, through metals accumulated in their food source. This route of exposure was investigated in this study.

There are several techniques available for understanding the diet of organisms including gut content analysis and direct observation of feeding behaviour. However, consumption of food does not necessarily mean the food is assimilated or taken up by the organisms. It may be rejected or excreted. Stable isotopes of carbon and nitrogen sampled in the tissues of plants and animals can give an indication of the energy source, and the position in the food chain of those organisms, respectively. Using the mud crab as an example of an animal at the top of the food chain, stable isotope analyses was undertaken of mud crabs and a number of organisms thought to be part of the mud crab diet, in order to establish a food web of Gladstone mud crabs. Animals and plants within the mud crab food web from a number of sites within (Boat Creek, Graham Creek and Black Swan) and outside of Port Curtis (Yellow Patch) were also examined for metal accumulations in order to identify site differences and pathways of metal accumulations in Port Curtis.

A food web including mud crabs, other crustaceans, fish, molluscs and a variety of plants was identified in Port Curtis. In general the food web was not unlike those established for other estuarine embayments. There were no ecologically significant site or gender differences among male and female mud crabs in terms of their carbon and nitrogen signatures indicating crabs from all four sites have similar diets and trophic positions. Carbon isotopes suggested that prawns were feeding either directly or indirectly on blue green algae (*Lyngbya majuscula*) and this was supported by observations of pigment from the algae being visually evident in the prawns. The finding may have consequences for consumers should the toxin produced by the algae follow similar uptake pathways to the pigment and accumulate in the prawn muscle tissue. Mud crabs were identified as one of the dominant predators in the food chain. Mangrove carbon contributed to the diet of very few organisms. It appears that very few species rely on mangroves as a predominant food source but are more likely to be dependent on benthic organic matter and algae. This finding supports those of other authors.

Yellow Patch (reference site) organisms tended to have the lowest aluminium accumulations compared to all the other sites but in contrast the highest arsenic concentrations. This was highlighted in oysters, which reflected the metal concentrations of their food source (seston) at these sites. This does not necessarily imply contamination of arsenic at the reference site but may simply be lack of antagonism for uptake from other metals such as copper and zinc found elevated at inner harbour sites. For mud crabs some metal accumulations appeared to be related to the carbon sources of mud crabs at each site. It is possible that mud crabs at each site are reflecting the metal concentrations of the predominant organism consumed at that particular site.

An attempt was made to identify biomagnification of metals by organisms in the food web. Biomagnification of metals occurs when increasing accumulation of metals occurs with increases in trophic levels and has been identified in some trophic food webs and predator-prey relationships. Although metal consumer-prey relationships and perhaps bioamplification were identified, biomagnification per se was not demonstrated in this study.

Although there were very few significant site differences in metal accumulations of organisms from inner harbour sites there was a trend for these organisms to be more enriched in metals than those from the unimpacted reference site outside the harbour. Sites were chosen along a transect in the direction of The Narrows to represent increasing distances from anthropogenic inputs. It was previously assumed that The Narrows was likely to be a lesser impacted reference area due to receiving flows from the Fitzroy area. Recent findings by phase 2 of the Contaminants Pathways Project suggests The Narrows could be a sink (or source) for dissolved metals in Port Curtis and that metal concentrations may not decrease appreciably until outside of Port Curtis (Brad Angel pers. com.). In addition the recently published CRC Port Curtis hydrodynamic model suggests a reduced flushing of the harbour and a greater retention time of the water body than reported in previous models. A longer retention time of the water body containing slightly elevated dissolved metals could explain the anomalous bioaccumulation of some metals into aquatic organisms, through extended exposure and increased bioavailability due to entry into the food chain. The finding has ramifications for management and regulatory authorities when considering the total load of metal being discharged into Port Curtis. Continual monitoring of biota metal loads is warranted.

In summary the study highlights the complexity of metal pathways that are likely to occur in dietary interactions in estuarine food webs. Although uptake of metals from dissolved routes is still an equally important consideration, dietary exposure also plays a significant role. The adage 'you are what you eat' may hold true for carbon sources, but not necessarily for metals accumulated by consumers in sophisticated mangrove ecosystems.

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## INTRODUCTION

Gladstone situated 550 km north of Brisbane is an industrial city adjacent to a deep estuarine embayment (Port Curtis). It is an international trades port, which also supports a large commercial and recreational fishing industry (QDEH, 1994). Despite being Queensland's largest multi-cargo port supporting major industries in the area (GPA, 2002) and there being several potential sources of metal contamination, there have only been a small number of metal bioaccumulation studies undertaken. The Coastal CRC Contaminant Risk Assessment Project determined that despite concentrations of dissolved metals in water being below levels of regulatory concern, concentrations of some metals in biota were enriched relative to those at control sites (CRC Coastal Zone, 2005). Studies prior to this had flagged concentrations of some metals; in particular copper and zinc in mud crabs (Andersen & Norton, 2001) and copper in seagrass (Prange, 1999) and fiddler crabs (Andersen et al., 2002) as potentially anomalous in Port Curtis relative to background levels. An investigation into not only the sources of contaminants to biota (i.e. the differentiation of natural versus anthropogenic) but also the mechanism of uptake was warranted.

Marine organisms can accumulate trace metals from the dissolved phase and from ingested food (Fisher & Reinfelder, 1995). Metals are often significantly accumulated by marine and estuarine species thereby allowing them to be utilised as bioindicators of bioavailable metals in the aquatic medium (Phillips, 1990). Aquatic organisms from many trophic levels take up metals in dissolved forms through permeable surfaces, such as gills and other membranes through both active and passive processes (Wright, 1995, Rainbow, 1997, Ralph & Burchett, 1998). Diet is another important source of metal uptake for some heterotrophs (Chou et al., 2000, Ke & Wang, 2001) through the consumption of previously accumulated metals in their food source. It was hypothesised that the dietary route of metal accumulation could account for the observed elevated levels in biota, and therefore this pathway/mechanism was investigated in this study.

The use of stable isotopes as an alternative to gut content analyses have been used successfully to define aquatic food web interactions (France, 1998, Fantle et al., 1999, Kang et al., 1999, Melville & Connolly, 2003, Carmichael et al., 2004). The examination of gut contents does not give a true indication of the relative importance of food items, because consumption does not necessarily mean assimilation of these items (Kang et al., 1999). All animals ultimately rely on autotrophic sources (i.e. algae, plankton, sea grass, mangroves, terrestrial riparian plants etc), but for a carnivore at the top of the food chain there may be a number of intermediate prey separating the carnivore from the autotroph(s) at the base of its trophic pathway.

Carbon and nitrogen have more than one isotope and the isotopic composition of natural materials such as animal and plant tissue change in predictable ways as these elements cycle upward through the food web and these changes can be measured (Peterson & Fry, 1987). Isotopic compositions or ratios of heavy and light isotopes  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  are expressed as Delta ( $\delta$ ) values, which are the relative per ml difference (‰)(parts per thousand) between the sample and conventional standard reference materials (Peterson, 1999). Increases in the  $\delta$  value denote an increase in the amount of heavy isotope component (the sample is therefore enriched in  $^{13}\text{C}$  or  $^{15}\text{N}$ )

and therefore will have a heavier  $\delta$  value. Conversely a sample depleted in  $^{13}\text{C}$  or  $^{15}\text{N}$  will have a more negative or lighter  $\delta$  value.

Carbon isotopes allow organic sources to be traced through benthic consumers (Rodelli et al., 1984), whereas nitrogen isotopes provide information on trophic level of an organism (Peterson, 1999). Chemical reactions cause fractionation of stable isotopes therefore changing the  $\delta$  value. For example in the metabolism of nitrogen, the light isotope is concentrated in nitrogenous excretion products while the heavy isotope is retained in the body tissues. Therefore the  $\delta^{15}\text{N}$  value in the consumer becomes enriched compared to the food consumed (Peterson, 1999).

Conversely very little fractionation of carbon occurs during trophic transfer (the majority occurs primarily at the primary production phase (photosynthesis)). Therefore carbon isotopes can be used to differentiate between sources of organic matter from atmospheric to riverine or oceanic sources (Peterson, 1999). For example in marine systems there has been a clear separation demonstrated between C-4 marsh grasses, phytoplankton and upland C-3 plants with respect to their  $\delta^{13}\text{C}$ . The animals that consume each of these plants will be similar in their carbon isotopic compositions ( $\delta^{13}\text{C}$ ) to those of the plants they consumed (Peterson & Fry, 1987).

Although current studies in Port Curtis are investigating the accumulation rates of metals in transplanted oysters (Andersen et al., unpub.), very little work to date has been done to ascertain the pathways of metal accumulation to the top order consumers in Port Curtis. A previous study using stable isotopes determined there was a significant difference in carbon signatures of mud crabs from Port Curtis compared to those from the reference site in Ayr (North Queensland). The difference could have been due to a natural spatial variability in the same carbon source such as algae or alternatively that the mud crabs from the two locations were consuming different diets (different carbon sources). The study also determined that there was a trend for a correlation between hepatopancreas copper concentrations and muscle  $\delta^{13}\text{C}$  suggesting the diets of mud crabs may be a major source of copper in Gladstone mud crabs.

Therefore if mud crab diets from the two locations were different, the results, although speculative, inferred that the Gladstone mud crabs might be consuming something in their diet, which had accumulated copper, but was not available in the diet of Ayr mud crabs (Andersen, 2003). Biomagnification of mercury into food webs has been demonstrated despite low ambient concentrations of the metal in the environment (Bowles et al., 2001) and therefore it is possible that a similar mechanism occurs in Port Curtis whereby organisms such as plankton or algae are biomagnifying metals into the Port Curtis food web.

Using the mud crab as an example of a higher trophic consumer, organisms that were thought to be part of the mud crab food web were selected for isotopic analyses from one site in Port Curtis. The same organisms from three sites within and one reference site out side of Port Curtis were analysed for metal accumulation.

The aims of the project were to:

1. Establish an estuarine food web in Port Curtis using stable isotopes of C and N

2. Determine if there are site or gender differences in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  in mud crabs from Port Curtis
3. Determine the metal concentrations of organisms within the food web
4. Establish if site differences exist in metal concentrations in those organisms
5. Ascertain pathways of metal uptake within the food web

## METHODS

### 2.1. Study area

Three sites: Boat Creek (Site 1), Graham Creek (Site 2) and Black Swan (Site 3) were selected within Port Curtis along a NW transect towards The Narrows representing increasing distances from likely sources of anthropogenic inputs (Figure 1). Yellow Patch (Site 4) (Figure 1), an unimpacted oceanic reference site on the eastern side of Curtis Island was selected for comparison. The location of each site was recorded using the Global Positioning System (GPS) (WGS 84) and the sites are listed in Table 1.

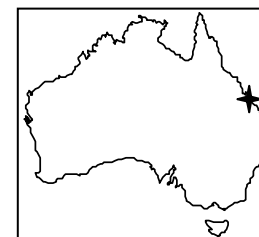
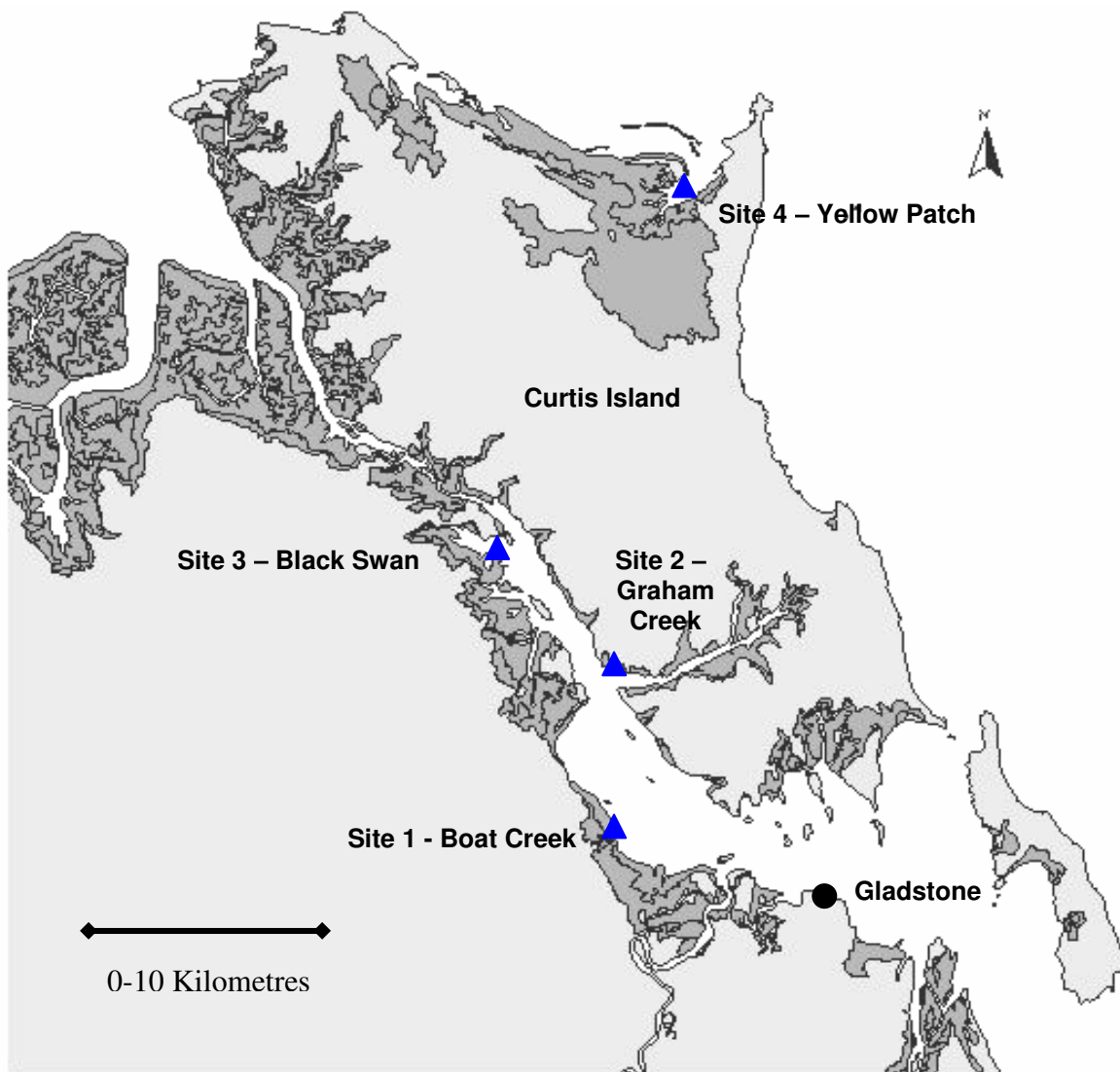
**Table 1.** GPS location of each site for the collection of specimens

Site	Description	Latitude/Longitude
1	Boat Creek	23° 48.793 / 151° 09.824
2	Graham Ck.	23° 43.954 / 151° 11.720
3	Black Swan	23° 41.200 / 151° 07.417
4	Yellow Patch	23° 50.459 / 151° 12.117

### 2.2. Specimens

Initially samples of mud crabs were collected from all four sites to determine if there were site differences in mud crab carbon signatures. The majority of samples of other specimens for isotopes were all collected from Site 2 except the June 2004 samples, which were collected from Site 3. The majority of samples were collected from April to June 2001 except for seston and mangrove snails at Site 4 and mullet at Site 2, which were collected in October 2002. Samples of epiphytic and filamentous algae and prawns and repeated samples of macroalgae and POM were collected in June 2004. The results would provide a generalised overview of a mud crab food web, however, as there are known to be some temporal effects on isotopic signatures (Loneragan et al., 1997), sources and consumer signature matches should be treated with some caution. June 2004 samples were also analysed for isotopes at a separate laboratory (The University of Western Australia) from previous samples (CSIRO Marine Research, Hobart).

Specimens for metal analyses were collected from all four sites. Although all sample specimens were sent for isotope analyses for inclusion in the food web, samples of macroalgae, filamentous algae, seagrass and prawns were not located at the Site 4 reference site and therefore samples from all other sites were excluded from metal analyses. Apart from the fiddler crabs all samples were analysed for metals at the same laboratory (NMI, Pymble) in December 2002 and June 2004.



**Figure 1.** Location of sampling sites in Port Curtis; Site 1 – Boat Creek, Site 2 – Graham Creek and Site 3 – Black Swan, and reference site outside of Port Curtis; Site 4 – Yellow Patch. Shaded areas indicate mangrove zones.

### **2.2.1. Mud crabs (*Scylla serrata*)**

Mud crabs (155 – 180 mm carapace width) (Figure 2) were caught using standard baited mesh pots, tied and processed within 24 hours of capture. Specimens were anaesthetized by chilling at -5°C for at least one hour prior to dissection. Samples of hepatopancreas for metals (n = 5 males) and muscle for isotopes (n = 5 males) were placed separately in plastic specimen vials and stored frozen prior to analyses.

In order to determine if there were site or gender differences in isotopes of mud crabs, five male mud crabs from sites 1, 3 and 4 in addition to five males and five females from Site 2, were also collected for isotopic analyses in June 2001.

### **2.2.2. Fiddler crabs (*Uca coarctata*)**

Male fiddler crabs (Figure 3) were collected by hand from the intertidal zone from each site. Crabs were rinsed onsite in seawater to remove sediments, placed in plastic zip lock bags and put on ice for transport to the laboratory. After identification, entire replicate crabs of similar size were further rinsed in deionized water (Milli-RO®) and frozen whole prior to metal (n=7) or isotope (n=5) analyses.

### **2.2.3. Metopograpsus (*Metopograpsus frontalis*)**

Grapsid crabs (Figure 4) of mixed gender (mostly males) were collected by hand from the prop roots and trunks of mangroves (*Rhizophora stylosa*) in the intertidal zone. Samples were treated similarly to the fiddler crabs and frozen for metal (n=5) and isotope (n=5) analyses.

### **2.2.4. Banana Prawns (*Penaeus merguensis*)**

Prawns (Figure 5) were caught using a standard cast net, placed in plastic zip lock bags and put on ice for transport to the laboratory. After identification individual prawns were measured from rostrum to tail tip, shelled and deveined. Muscle tissue was rinsed in deionized water (Milli-RO®), blotted and frozen for isotope (n=3) analyses. As this species was not found at Site 4 metal analyses was not completed.

### **2.2.5. Mullet (*Mugil cephalus*)**

Five mullet (Figure 6) from each site were caught using a standard cast net, placed in plastic zip lock bags and put on ice for transport to the laboratory. Fish were rinsed, measured from nose to tail tip and weighed. A fillet of muscle from each specimen was frozen for metal (n=5) and isotope (n=3) analyses.

### **2.2.6. Mud whelks (*Telescopium telescopium*)**

Whelks (Figure 7) were collected from each site by hand from the mud flats in the intertidal zone at low tide, depurated in site water for at least 48 hours and stored frozen. Shell length was measured prior to cracking the shell open in a vice and removing the soft tissue excluding the hind gut and operculum. The tissue was rinsed in deionized water (Milli-RO®), blotted, weighed and frozen for metal (n=5) and isotope (n=5) analyses.

### 2.2.7. Mangrove snails (*Nerita balteata*)

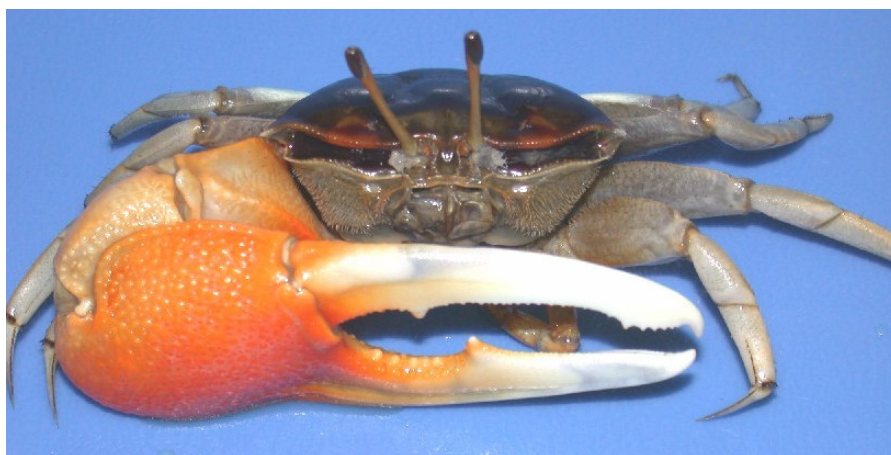
Snails (Figure 8) were collected by hand from the trunks and prop roots of mangroves (*Rhizophora stylosa*), depurated in site water for at least 48 hours and stored frozen. After the shell aperture was measured the snail was cracked open in a vice to remove the soft tissue, discarding the operculum. The tissues were weighed, rinsed in deionized water (Milli-RO®), blotted and frozen for metal analyses (n=5 pooled composites of 3 snails) and isotope analyses (n=5 individual samples).

### 2.2.8. Oysters (*Saccostrea glomerata*)

Whole oysters (Figure 9) were collected from rocks in the intertidal zone and depurated in site water for at least 48 hours. Whole soft tissues from each oyster were removed, rinsed in deionized water (Milli-RO®), blotted and frozen for metal (n=5 pooled composites of 9 oysters (3 only at Site 4) and isotope (n=5 pooled composites of 2 oysters).



**Figure 2.** Mud crab (*Scylla serrata*)



**Figure 3.** Fiddler crab (*Uca coarctata*)



**Figure 4.** Grapsid crab (*Metopograpsus frontalis*)



**Figure 5.** Banana prawn (*Penaeus merguensis*)



**Figure 6.** Mullet (*Mugil cephalus*)



**Figure 7.** Mud whelks (*Telescopium telescopium*)



**Figure 8.** Mangrove snails (*Nerita balteata*)



**Figure 9.** Oyster (*Saccostrea glomerata*)

### **2.2.9. Mangrove leaves (*Rhizophora stylosa*)**

Leaves of fresh new growth were collected by hand, placed in plastic zip lock bags and put on ice for transport to the laboratory. Leaves were rinsed in deionized water (Milli-RO®), blotted and bagged frozen for metal (n=5 composite samples) and isotope (n=3 composite samples) analyses.

### **2.2.10. Seagrass (*Zostera capricorni*)**

Seagrass was collected by hand while diving and rinsed on site before being placed in plastic zip lock bags and put on ice for transport to the laboratory. After identification the samples were sorted and only the cut leaf including epiphytes was retained for analyses. Samples were rinsed in deionized water (Milli-RO®), blotted and bagged frozen for isotope (n=3 composite samples) analyses. As this species was not found at Site 4 metal analyses were not completed.

### **2.2.11. Macroalgae (*Catenella nipae*)**

Strands of red algae were collected by hand off the prop roots of mangroves (*Rhizophora stylosa*) and rinsed on site before being placed in plastic zip lock bags and put on ice for transport to the laboratory. Algae was identified, rinsed in deionized water (Milli-RO®), blotted and bagged frozen for isotope (n=3 composite samples) analyses. As this species was not found at Site 4 metal analyses were not completed.

### **2.2.12. Filamentous algae (*predominantly Lyngbya majuscula*)**

Benthic mats of algae were collected by hand off the mud flats in the intertidal zone at low tide rinsed on site before being placed in plastic zip lock bags and put on ice for transport to the laboratory. Algae was identified, rinsed in deionized water (Milli-RO®), blotted and bagged frozen for isotope (n=3 composite samples) analyses. As this species was not found at Site 4 metal analyses were not completed.

### **2.2.13. Seston (*zoophytoplankton*)**

Samples were collected by trawling a 20 µm plankton net just below the surface of the water for approximately 100m. The collected residue was then scraped into a plastic specimen jar, iced for transport to the laboratory and frozen for metal analyses (n=5). For isotopes the samples were diluted with deionized water (Milli-RO®), filtered through 0.45 µm filter paper through a Nalgene® pump and the paper retained frozen for isotope (n=3) analyses.

### **2.2.14. Epiphytic algae**

The surfaces of rocks were rubbed into a bucket of seawater in order to resuspend the attached algae. Sample water was filtered through 0.45 µm filter paper through a Nalgene® pump and the paper retained frozen for metal and isotope (n=3 composites of 3 papers) analyses.

### **2.2.15. Particulate organic matter (POM)**

The top 1-2cm of fine surface sediment was scraped from the intertidal zone at low tide. Samples were sieved on site with the 20-250 µm fraction retained for analyses. The residue was then scraped into plastic specimen jars, iced for transport to the laboratory and frozen for metal (n=3) and isotope (n=3) analyses.

## 2.3. Laboratory analyses

### 2.3.1. Elemental analyses

Samples were analysed for 8 different metals: Aluminium (Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Selenium (Se) and Zinc (Zn) and reported on a wet weight basis.

Analyses were performed using USEPA 6010, 6020, AOAC, 16<sup>th</sup> Edition, Method 986.15, 974.14. The sample was homogenised using a blender equipped with a stainless steel or titanium blade. A sub sample (~1 g) was digested with re-distilled nitric acid. After making up to the appropriate volume the digest was analysed for trace elements using Inductively Coupled Plasma – Mass Spectrometer (ICPMS) and or Inductively Coupled Plasma – Emission Spectrometer (ICPAES). Each analysis batch included blanks, duplicates, matrix spikes and reference materials containing known amounts of trace metals and metalloids.

### 2.3.2. Isotope analyses

Specimens were defrosted, placed into glass Petri dishes and oven dried at 60°C for 48 hours. Dried samples were ground to a fine powder using a mortar and pestle and refrozen for storage prior to isotope analyses.

Powdered samples were weighed into tin cups (Elemental Microanalysis, UK) and analysed using a Carlo Erba NA 1500 CNS analyser interfaced via a Conflo II to a Finnigan Mat Delta S isotope ratio mass spectrometer operating in the continuous flow mode. Combustion and oxidation were achieved at 1090°C and reduction at 650°C. Where necessary the carbon signal was diluted using helium.  $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$  were expressed as the relative per mil (‰) difference between the sample and conventional standards (the primary standards are for carbon, Vienna Pee Dee Belemnite (VPDB), and for nitrogen,  $\text{N}_2$  in air. The formula used to express these is:

$$\delta = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000\text{‰}$$

### 2.3.3. Two end point mixing models

By using a two end-point mixing model, the contribution of the dominant carbon sources (mangrove/macroalgae – seston/POM/filamentous algae – seagrass/epiphytes) to the assimilated carbon in each higher consumer level can be estimated. Although it may appear from the  $\delta^{13}\text{C}/\delta^{15}\text{N}$  plot that a consumer is attaining 100% of its carbon from an autotroph directly beneath, in reality it may obtain an equal proportion of carbon from more enriched and more depleted autotrophs, which is reflected as an averaged middle range carbon signature in the consumer. Although a higher level consumer may rely on more than two main autotrophs the two end-point mixing model allows a reasonable estimation of the contribution of the dominant carbon source. Carbon isotope data were converted to percentage of assimilated carbon derived from algal sources using the following model:

$$P_A = (\delta^{13}\text{C}_{\text{consumer}} - f - \delta^{13}\text{C}_{\text{sourceB}}) / (\delta^{13}\text{C}_{\text{sourceA}} - \delta^{13}\text{C}_{\text{sourceB}})$$

Where  $P_A$  = proportion of carbon in consumer A; and  $f$  = isotopic fractionation (‰). In all cases, an isotopic fractionation ( $f$ ) of 0.2 was used (Peterson & Fry, 1987). Sources of carbon chosen for each consumer were two autotroph endpoints enclosing the carbon signature of the consumer eg. depleted mangroves as one end point source and the more enriched filamentous algae as the other. Therefore if the equation determined that 35% of carbon for a certain consumer was derived from mangroves, by default 65% of carbon would be derived from algae.

## 2.4. Statistical analyses

### *Mean comparisons - metals*

For any metal concentration reported as “below detection level”, half the detection level was used as the nominal concentration to allow comparison with other sites/samples. If data were heteroscedastic, either square root,  $\log_2$  or  $\log_{10(x+1)}$  transformation was applied to achieve homogeneity (= equality) of sample variances. As ANOVA is a fairly robust procedure, untransformed data is presented where equality of variances could not be achieved. Between-site ( $n=4$ ) differences in the concentration of each metal were tested using parametric one-way ANOVA, with *a posteriori* Tukeys HSD multiple range test applied to locate site differences where there was a significant main effect and results tabulated. Mean concentration ( $\pm 1$  SE of the mean) of each metal at each site were plotted to allow interpretation of between-site differences.

### *Mean comparisons – mud crab isotopes*

To determine if there was a gender difference in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of male and female mud crabs ( $n = 10$ ) from Site 2, a student's t-test was performed. If there was no significant gender difference data were combined for future comparisons. If there was a significant gender difference detected, female crabs from Site 2 were excluded from future male site comparisons. Site differences in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of male crabs were tested using parametric One-Way ANOVA, with *a posteriori* Tukeys HSD multiple range test applied to locate site differences where there was a significant main effect. Mean concentration ( $\pm 1$  SDEV of the mean) of signature at each site was tabulated to allow interpretation of between-site differences.

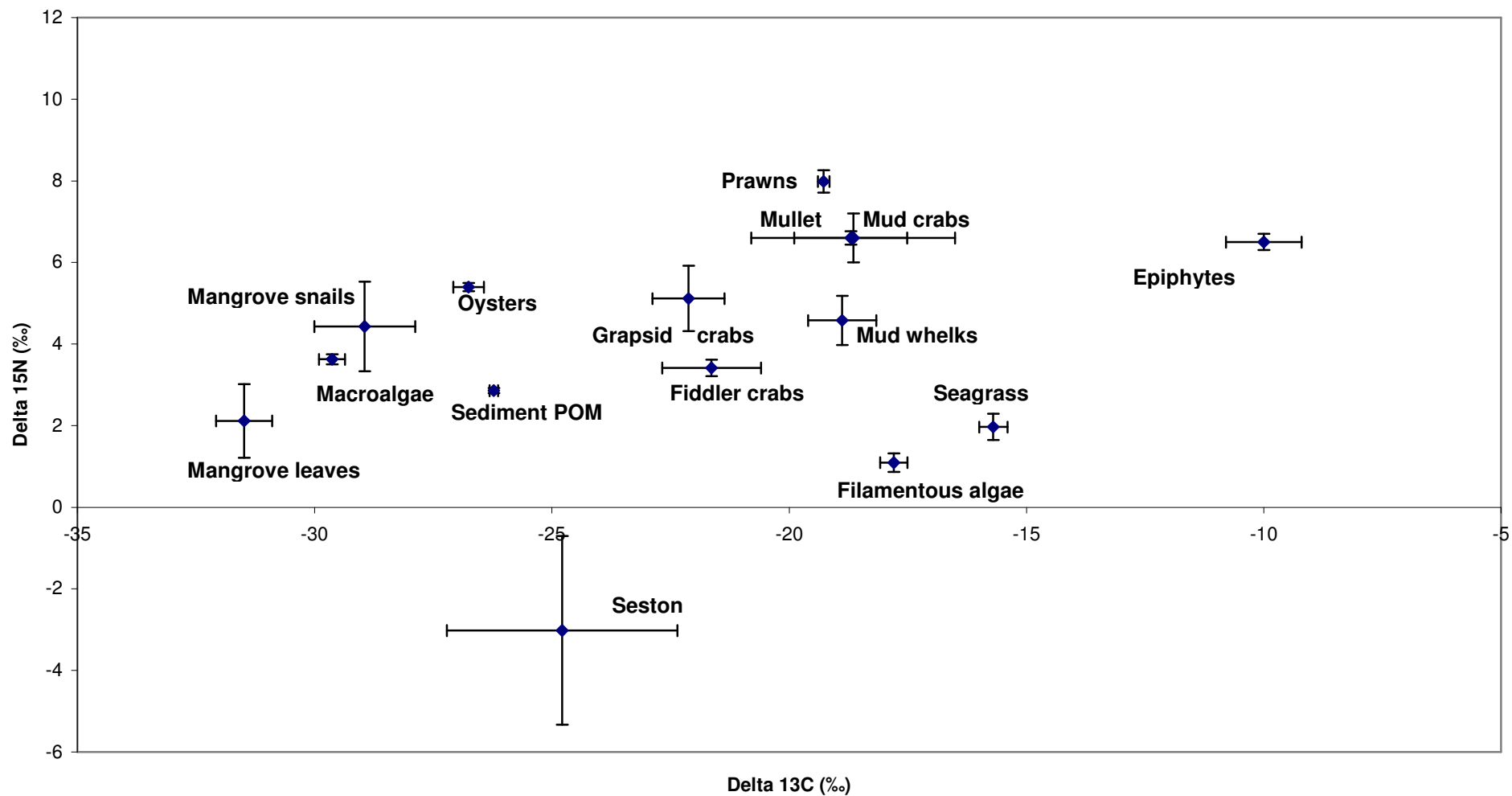
## RESULTS & DISCUSSION

### 3.1. ISOTOPES

The mean isotopic values ( $\pm 1$ SD) of the specimens within the Port Curtis food web are presented in Table 2 and plotted in Figure 10.

**Table 2.** The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $\pm 1$ SD) of selected primary producers and consumers in a Port Curtis estuarine food web.

Specimens	Mean $\delta^{13}\text{C}$ (‰) ( $\pm$ SD)	Mean $\delta^{15}\text{N}$ (‰) ( $\pm$ SD)
<i>Primary producers</i>		
Particulate organic matter	-26.2 (0.1)	2.9 (0.1)
Epiphytes	-10.0 (0.8)	6.5 (0.2)
Seston	-24.8 (2.4)	-3.0 (2.3)
Filamentous algae	-17.8 (0.3)	1.1 (0.2)
Macroalgae	-29.6 (0.3)	3.6 (0.1)
Seagrass	-15.7 (0.3)	2.0 (0.3)
Mangrove leaves	-31.5 (0.6)	2.1 (0.9)
<i>Molluscs</i>		
Oysters	-26.8 (0.3)	5.4 (0.1)
Gastropods		
Mangrove snails	-28.9 (1.1)	4.4 (1.1)
Mud whelks	-18.9 (0.7)	4.6 (0.6)
<i>Fish</i>		
Mullet	-18.6 (2.1)	6.6 (0.6)
<i>Crustaceans</i>		
Prawns	-19.3 (0.1)	8.0 (0.3)
Metopograsmus	-22.1 (0.8)	5.1 (0.5)
Uca	-21.6 (1.0)	3.4 (0.2)
Mud crabs	-18.7 (1.2)	6.6 (0.2)



**Figure 10.** Relationship of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  1SD) of selected primary producers and consumers in a Port Curtis food web.

### 3.1.1. Mud crabs

There was no significant gender difference in  $\delta^{13}\text{C}$  among male and female mud crabs from Site 2 ( $df = 1, 8, p > 0.05$ ), therefore data from this site were combined in future comparisons. It was conceivable that female mud crabs could have different carbon sources to male mud crabs. Due to the migratory spawning habits of female mud crabs they are likely to spend more time in off-shore habitats than males. Although little is known of their habits during this time, it is unlikely that they spend time feeding. Therefore time spent in this more enriched habitat should not necessarily influence their carbon signatures.

Ideally when comparing carbon signatures of specimens from different sites, the data should be standardised across sites by calculating the contribution of the dominant carbon sources to the assimilated carbon in each higher consumer level using a two end-point mixing model. This approach reduces the likelihood of spatial differences in signatures of primary sources (algae) giving the appearance of site differences in carbon signatures of the consumers (mud crabs). Standardisation would require knowledge of the carbon signatures of not only mud crabs at each site, but signatures of the main dominant, carbon sources (algae>seston>mangroves) to allow valid between site comparisons. Although carbon signatures were established for mud crabs at all sites, they were not obtained for other specimens at all sites. Therefore standardisation was not undertaken on mud crab carbon signatures prior to comparison. There was no significant site difference in  $\delta^{13}\text{C}$  among mud crabs from all four sites ( $df = 3,21, p > 0.05$ ), although male mud crabs from Site 1 tended to have the lowest carbon signatures (Table 3) compared to all crabs from other sites.

Mud crabs feed on a wide range of prey, however they tend to take advantage of the most dominant and abundant prey available at each location (Hill, 1976). Therefore mud crabs from different sites could potentially rely on different carbon sources. Although it has not been significantly demonstrated in this study, Gladstone mud crabs were previously shown to have more enriched  $\delta^{13}\text{C}$  ( $-16.37\text{‰} \pm 0.7 \text{ SE}$ ) compared to mud crabs from Ayr ( $-18.3\text{‰} \pm 0.6 \text{ SE}$ ) (Andersen & Norton, 2001). Although this may suggest different dietary sources at the two locations, it could also reflect spatial differences in the  $\delta^{13}\text{C}$  signature of the primary carbon sources (algae) supporting the respective food webs.

**Table 3.** The mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $\pm 1\text{SD}$ ) of male mud crabs from Sites 1 –4 and female mud crabs from Site 2.

Site	Gender	Mean $\delta^{13}\text{C}$ (‰) ( $\pm \text{SD}$ )	Mean $\delta^{15}\text{N}$ (‰) ( $\pm \text{SD}$ )
1	Male	-17.9 (1.0)	7.9 (0.4)
2	Male	-19.1 (1.0)	6.0 (0.4)
2	Female	-18.2 (1.3)	7.1 (0.3)
3	Male	-19.3 (1.2)	6.6 (0.5)
4	Male	-19.5 (0.6)	7.5 (0.5)

There were significant gender differences in  $\delta^{15}\text{N}$  among male and female mud crabs from Site 2 ( $df = 1, 8, p = 0.002$ ) (Table 3). Female crabs from Site 2 had more enriched nitrogen signatures than males from the same location. It is possible that crabs from different locations could occupy different trophic positions. Cabanna and Rasmussen (1994) reported a wide variation in mean  $\delta^{15}\text{N}$  (7.5 – 17.5 ‰) of adult lake trout from 24 Canadian lakes. The variation was attributed to between lake effects where the length of the food chain leading to the trout was different in the lakes i.e. in the shortest food chains the trout were feeding directly on zooplankton whereas in other lakes where intermediate consumers were available, trout were higher order consumers.

There was also a significant site difference in  $\delta^{15}\text{N}$  among male mud crabs from all sites (Table 4). However, despite the statistical difference in  $\delta^{15}\text{N}$  in crabs in this study there is unlikely to be a biologically significant difference. The small range in signatures across all sites (1.9‰) represents less than one trophic position, therefore it is unlikely that mud crabs share different trophic positions at each site in Port Curtis.

**Table 4.** One-way ANOVA comparing  $\delta^{15}\text{N}$  in male mud crabs from four sites. An *a posteriori* Tukeys range test was applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean nitrogen values (shown above).

Specimen	df	F	p	$\delta^{15}\text{N}\text{‰}$			
				Tukey's Multiple Range test			
Mud crabs	3,16	19.429	<0.0001	6.0	6.6	7.5	7.9
				2	3	4	1

### 3.1.2. Overview of the food web

In summary, mullet, mud crabs and prawns tended to share a similar trophic position and carbon signature in the food web and along with mud whelks, relied predominantly on filamentous algae and to a lesser extent epiphytes, seston and seagrass for their primary carbon sources. Due to its poor quality the seagrass was analysed with epiphytes being retained and so may have taken on some of this signature. Apart from mud whelks, mud crabs are also likely to feed on the grapsid and fiddler crabs, which appear to in turn to feed on seston, sediment organic matter (POM) and filamentous algae. Similarly oysters as filter feeders retained the same carbon signatures as the seston and suspended POM that they feed on.

The prawns in this study do not appear to be detritivores but rather consumers of meiofauna that in turn feed on benthic algae. The grapsid crabs and mangrove snails were both collected amongst the macroalgae but despite this, only the mangrove snails shared the same carbon signature as the macroalgae. Mangrove carbon and POM are likely to be the snail's predominant carbon sources. The fiddler and grapsid crabs were in close proximity in the food web, but are likely to have different carbon sources due to their contrasting feeding habits. Filamentous algae appeared to be a predominant carbon source for many consumers including prawns (observed though

pigment tracing). In contrast very few organisms (apart from the snails) relied on mangrove carbon as a predominant energy source.

Difficulties arise when attempting to compare the carbon and nitrogen signatures of some of the specimens collected in this study to those reported in the literature due to the different collection techniques, particularly for primary producers. In many studies the sampling technique is not completely described and the terminology used for the identification of specimens inconsistent among comparative studies. Care must also be taken when comparing carbon signatures among different studies due to the potential spatial and temporal differences of primary sources. However, generally the food web established in this study for Port Curtis was similar in structure to estuarine food webs of other authors.

### **3.1.3. Primary producers and sediments**

There was a large range in carbon signatures ( $-13.6‰$  to  $-31.5$ ) among the major primary producers (epiphytic algae, filamentous algae, seston, POM (sediment organic matter), seagrass, macroalgae and mangrove leaves). In some cases the signatures overlapped and this may be due in part to collection techniques where samples are fairly homogeneous eg. sediment organic matter (POM) and seston.

The POM was still enriched compared to the carbon signature of mangroves and in contrast had a similar signature to the seston ( $-24.8‰ \pm 2.4$ ), which includes the zoophytoplankton. The finding supports those of more recent studies (Bouillon et al., 2002a, Fry & Ewel, 2003) suggesting that mangrove derived organic matter is unlikely the principal source of organic matter in sediments, but rather imported phytodetritus and benthic microalgae. Ambler et al., (1994) noted that microscopically detritus is composed primarily of algal sources. However, Thimdee et al., (2004) observed that the organic matter in each area originated from the photosynthetic residents dominating that area i.e. the isotopic composition of detritus in mangroves, the inner bay and offshore were close to those of mangroves, seagrass and algae, and plankton, respectively.

The  $\delta^{13}\text{C}$  of the seston collected in this study most likely represented a composite signature of a number of autotrophic sources representing suspended organic matter containing zoophytoplankton, mangrove detritus and suspended sediments. The  $\delta^{15}\text{N}$  values ( $-.3.0 \pm 2.3‰$ ) reported here for seston are unusually depleted in comparison to other studies ( $5.02 - 6.7‰$ ) (Loneragan et al., 1997, Carmichael et al., 2004, Thimdee et al., 2004).

The mean  $\delta^{13}\text{C}$  ( $-13.6 \pm 6.3‰$ ) of the epiphytic algae scraped from rocks in the intertidal zone was highly variable. The large variation appeared to be due to one anomalous outlier sample ( $-20.8 ‰$ ). As the percentage carbon recovered from this particular sample was low in comparison to the other two samples, the results for this sample were disregarded on this basis. The  $\delta^{15}\text{N}$  of epiphytes was also unusually enriched for an autotroph.

The filamentous algae were predominantly a blue green algal species (*Lyngbya majuscula*) with a carbon signature of ( $-17.8 \pm 0.3‰$ ) similar to that found by Connolly and Guest (2004) in Port Curtis for this species.

The  $\delta^{13}\text{C}$  of the red macroalgae collected off prop roots in this study ( $-29.6 \pm 0.3\text{‰}$ ) was fairly depleted. France (1998) noted that many organisms (both auto and heterotrophs), which have a close association with mangroves (regardless of whether they are ingesting mangroves directly) are  $^{13}\text{C}$  depleted in mangrove swamps. Kang et al., (1999) also noted a relatively wide range of  $\delta^{13}\text{C}$  values ( $-11.1$  to  $-20.1\text{‰}$ ) for the different species of macroalgae they studied. This more depleted result can sometimes be attributed in part to the collection technique where pneumatophores from mangrove prop roots can become included in the sample allowing it to take on the more depleted signature of the mangroves (Andrew Storey, unpub). However, the algae collected on this occasion were abundant with elongated branches and rinsed well, therefore a mixed sample is unlikely. Due to the anomalous result, isotopic analyses for the macroalgae were performed on a second set of samples collected on another occasion and analysed at a different laboratory. A similar depleted result was obtained ( $\delta^{13}\text{C} = -32.5, \pm 0.6\text{‰}$ ). The mean  $\delta^{15}\text{N}$  values for both collections were 3.6 and 5.5‰ respectively.

Previous research in seagrass ecosystems have suggested that the seagrass algal epiphytes may be more important in terms of nutrition to animals than the seagrass itself (Moncreiff & Sullivan, 2001). Because of the short leaf length and poor quality, epiphytes were not removed from the leaves of seagrass in this study, but the  $\delta^{13}\text{C}$  ( $-15.7 \pm 0.3\text{‰}$ ) was similar to the carbon signatures of a combination of signatures from similar seagrass species and their epiphytes, determined by Melville and Connolly, (2003) for Moreton Bay and Loneragan et al., (1997) during a wet season sampling in Northern Australia.

The  $\delta^{13}\text{C}$  for mangrove leaves in this study ( $-31.5 \pm 0.6\text{‰}$ ) was slightly more depleted than for similar species in other studies (range  $-27$  to  $-29\text{‰}$ ) (Newell et al., 1995, Bouillon et al., 2002a, Connolly & Guest, 2004, Thimdee et al., 2004) as was the trend in this study for the food web as a whole. The  $\delta^{15}\text{N}$  value ( $2.1 \pm 0.9\text{‰}$ ) however, was in the range reported for the same authors ( $2 - 5.7\text{‰}$ ).

Bouillon et al (2002a) determined that there were three major types of primary carbon sources available for invertebrates on the sediment surface: mangrove litter, imported plant detritus and microphytobenthos. Autotrophs in this study could be placed into three main groups according to their  $\delta^{13}\text{C}$  values: the depleted mangroves and macroalgae, sources with middle values consisting of POM (sediment organic matter) seston and filamentous algae, and more enriched autotrophs such as seagrass and epiphytic algae. The three groups likely form the basis of energy sources for consumers in the mud crab food web in Port Curtis.

#### **3.1.4. Molluscs**

The mean  $\delta^{13}\text{C}$  of the filter feeding oysters in this study was  $-26.8 \pm 0.3\text{‰}$  and was likely a reflection of the carbon signature in the food source (seston, suspended POM). As macroalgal detritus contains relatively high amounts of nitrogen that can readily be assimilated (Kang et al., 1999), it is possible that suspended organic matter from this group of locally abundant autotrophs may have also contributed to the diet of the oysters, therefore contributing to the more depleted carbon signature. The two

end-point seston/mangrove mixing model for oysters determined that 70% of carbon was derived from seston.

Differences in  $\delta^{13}\text{C}$  of filter feeders in comparison to their food sources may well be due in part to selective/preferential ingestion and/or selective assimilation of certain components of suspended particulate matter (Ambler et al., 1994, Kasai et al., 2004). The unwanted matter is ejected as pseudofaeces.  $\delta^{15}\text{N}$  signatures may vary due to species specific, differences in fractionation that may occur in both bivalves and crustaceans (Carmichael et al., 2004). The nitrogen signatures of oysters in this study were within the range reported for similar species in other studies (Loneragan et al., 1997, Thimdee et al., 2004).

The herbivorous mangrove snails in this study collected off the prop roots and branches of mangroves had a depleted  $\delta^{13}\text{C}$  ( $-28.9 \pm 1.1\text{‰}$ ), suggesting they attain at least some of their carbon from mangrove sources. Rodelli et al., (1984) came to the same conclusion for a similar species (*N. articulata*) in this genus. The  $\delta^{15}\text{N}$  value in this study was about 3‰ higher than mangroves being consistent with this suggestion. Although the macroalgae had a similar carbon signature to the snails the  $\delta^{15}\text{N}$  value was also similar putting them in a similar trophic position, suggesting the snails may not be feeding directly on the macroalgae. Sediment organic matter (POM) is also likely to contribute to the snail's diet. The two end-point mangrove/POM mixing model for mangrove snails determined that 56% of carbon was derived from mangroves. Bouillon et al., (2002a) reported a similar finding for a few species mangrove-inhabiting gastropods and suggested that mangrove derived carbon and sediment organic matter could contribute in roughly equal portions to their diet, but that their trophic position is likely to be complex.

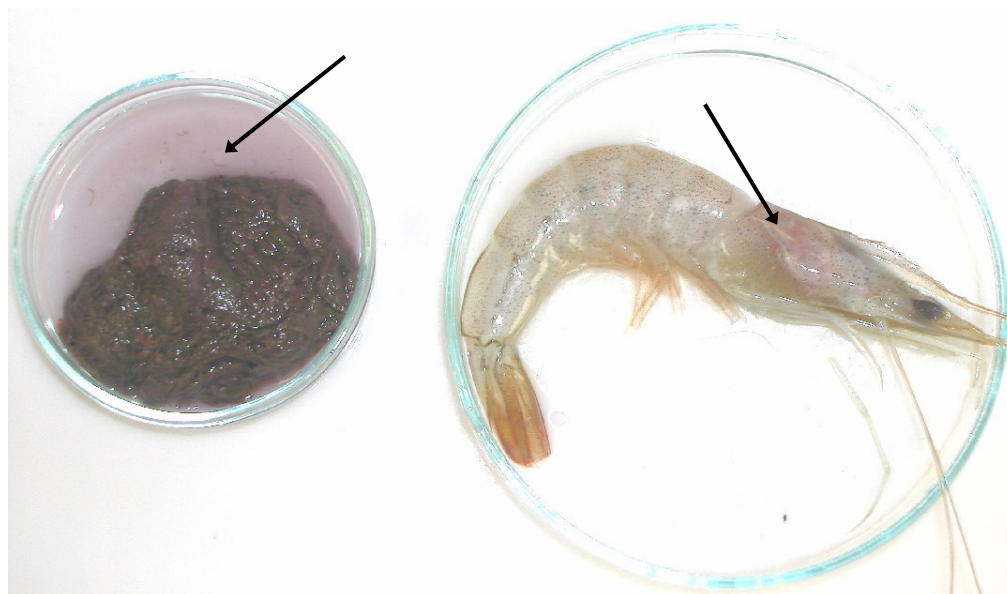
In contrast the other surface-grazing gastropod in this study (mud whelks) were more enriched in  $^{13}\text{C}$  ( $-18.9 \pm 0.7\text{‰}$ ) than their counterparts and similar to that determined by Newell et al., (1995), suggesting they do not rely on mangroves as a carbon source. However from the  $\delta^{15}\text{N}$  values it appears they share a similar trophic position to the mangrove snails. Mud whelks are likely to feed on the filamentous algae (and perhaps unicellular algae/diatoms) on the substrate on which they live, being approximately 3‰ higher in  $\delta^{15}\text{N}$  than the algae. Other authors indicate that these types of deposit feeders are also likely to utilize a combination of sediment POM and benthic microalgae (Bouillon et al., 2002a), which is probably the case in this study. The two end-point filamentous algae/POM mixing model for mud whelks determined that 85% of carbon was derived from the algae.

### **3.1.5. Crustaceans and fish**

Mullet shared almost the same carbon signature ( $-18.6 \pm 2.1\text{‰}$ ) and trophic position ( $6.6 \pm 0.6\text{‰}$ ) as mud crabs. A wide range of signatures are reported by other authors ( $-15.4$  to  $-26.3$  for  $\delta^{13}\text{C}$  and  $6.6$  to  $12.3$  for  $\delta^{15}\text{N}$ )(Thimdee et al., 2001, Melville & Connolly, 2003, Thimdee et al., 2004) indicating that different species of fish feed on different foods and occupy a variety of trophic positions. Although mangroves are unlikely to be a substantial contributor for some fish species, they may have some importance as a nutrition source for fish found over unvegetated habitats (Melville & Connolly, 2003). The two end-point filamentous algae/mangrove mixing model for fish determined that 92% of carbon was derived from the algae.

The  $\delta^{13}\text{C}$  of prawns in this study ( $-19.3 \pm 0.1\text{‰}$ ) was similar to that of the filamentous algae ( $-17.8 \pm 0.3\text{‰}$ ), which were identified predominantly as *Lyngbya majuscula* (a blue-green algae) with some green algal filaments interspersed within. Visual confirmation that the prawns were possibly feeding on the algae directly or on smaller invertebrates that had fed on the algae, were obtained when after 24 H, a break-down of the algae caused it to release purple pigments (most likely phycoerythrin and phycocyanin)(Larelle Fabbro, pers. comm.). The same colouring was noted in the digestive tracts of the prawns that had been captured in the vicinity of the algal samples (Figure 11).

The higher  $\delta^{15}\text{N}$  of prawns ( $8.0 \pm 0.3\text{‰}$ ) compared to the algae ( $1.1 \pm 0.2\text{‰}$ ) suggests a higher position in the food chain of approximately two trophic levels considering fractionation of nitrogen of  $\sim 3\text{‰}$  per trophic level. This suggests the prawns were feeding on primary consumers that may have been feeding directly on the algae. This may have consequences to higher order consumers feeding on the prawns, due to the toxin producing properties of the algae. If the prawns are accumulating algal toxins contained in the algae, then potentially these toxins may be transferred up the food chain. Accumulation of algae toxins, have been noted in freshwater mussels consuming blue green algae and their toxins (Andersen, Fabbro and Eaglesham, unpub). Should the toxin accumulate in the prawn muscle tissue this could also have human health ramifications for consumers of banana prawns in Port Curtis.



**Figure 11.** Blue green algae (*Lyngbya majuscula*) demonstrating released pigment (arrowed) and the same pigment observed in the hepatopancreas (liver) of a banana prawn from the same site (also arrowed).

The contribution of mangroves or mangrove detritus to the diet of prawns in this study appears to be limited and supports the findings of other researchers (Primavera, 1996, Thimdee et al., 2004) that prawns are not predominantly detritivores. Both authors suggested macro and micro algae, seagrass, epiphytes or seston, or combinations of these producers as alternate carbon sources for both juvenile and adult prawns. Rodelli et al., (1984) also observed mangrove detritus in the gut contents of penaeids

but their  $\delta^{13}\text{C}$  values did not reflect mangrove food sources, suggesting they weren't assimilating the ingested mangrove carbon. The two end-point filamentous algae/mangrove mixing model for prawns in this study determined that 88% of carbon was derived from the algae. Seagrass (or more likely seagrass epiphytes), epiphytes and seston are also likely to contribute to the prawn diet either directly or indirectly (through ingestion of small detritivorous invertebrates). Dittel et al., (1997) confirmed through laboratory experiments that post larvae are likely to feed on meiofauna that in turn feed on benthic algae in sediments.

In contrast Chong et al. (2001) disputed these findings and suggested that mangrove-derived carbon is a primary source for juvenile prawns inhabiting upper estuaries in Malaysia contributing up to 84% of the diet. A consensus of opinion from the combined research is that mangrove dependency becomes increasingly less in adult prawn populations as they move further offshore (Rodelli et al., 1984, Newell et al., 1995, Loneragan et al., 1997) and may also have a wet/dry seasonal influence (Loneragan et al., 1997).

The grapsid and fiddler crabs in this study appear to have some carbon sources in common to prawns (algae, POM, seston), however as their trophic position is lower it is likely they rely more directly on the autotrophs than the intermediate consumers. Although the  $\delta^{13}\text{C}$  of the grapsid crabs ( $-22.1 \pm 0.8\text{‰}$ ) was slightly more depleted than that of the *Uca* ( $-21.6 \pm 1.0\text{‰}$ ) suggesting perhaps a greater reliance on mangrove sources, there was some overlap between the two groups. The percentage carbon for the two species in the two end-point filamentous algae/mangrove, mixing model was similar at 67 and 70% respectively and for the seston/epiphyte mixing model, 83 and 80% respectively. Rodelli et al (1984) noted a wide intragroup variation in  $\delta^{13}\text{C}$  of intertidal crabs but was still able to separate the more enriched *Uca* from the depleted grapsids.

The burrowing fiddler crabs feed by scraping the organic matter from the surface, subsequently separating the food in the buccal cavity and discarding the sediment (Miller, 1961). They depend more on bacteria, diatoms and small meiofauna rather than large algal cells and have the ability to preferentially sort for these food items. They do not rely directly on mangrove detritus (Hogarth, 1999). In contrast some grapsid species are known to chop up and devour fallen leaves (McNae, 1968) as well as propagules and seedlings (Hogarth, 1999) taking them down into their burrows, although the grapsid in this study is non-burrowing (McNae, 1968). Food sources for intertidal crabs is likely to be species specific, however, in general it appears that although their diets are heterogenous including mangrove litter to some extent, they rely more on sediment organic matter (including benthic microalgae, and cyanobacteria) as their major food source (Rodelli et al., 1984, France, 1998, Bouillon et al., 2002a).

With a potential carapace width of over 20 cm, the mud crab is the largest invertebrate predator found in the mangroves (Hogarth, 1999) and is usually considered to be the top predator of the mangrove benthic community (Bouillon et al., 2002a). The  $\delta^{15}\text{N}$  of mud crabs ( $6.6 \pm 0.2\text{‰}$ ) in this study was slightly more depleted than the prawns ( $8.0 \pm 0.3\text{‰}$ ) caught at the same site but the range of  $\delta^{15}\text{N}$  ( $6.0 - 7.9\text{‰}$ ) of mud crabs from all sites suggests they occupy a reasonably dominant position in this estuarine food

web. Nitrogen signatures were within the range reported for other studies (6.54 – 12.0‰)(Bouillon et al., 2002a, Connolly & Guest, 2004, Thimdee et al., 2004).

The  $\delta^{15}\text{N}$  of young horseshoe crabs (*Limulus polyphemus*) increases as they age suggesting that they sought larger prey and moved up trophic steps as they grew (Carmichael et al., 2004), therefore an ontogenetic change in diet. Thimdee et al. (2001) also suggested that smaller juvenile mud crabs fed on plant material, possibly mangrove leaves and detritus with the inclusion of invertebrates (molluscs and crustaceans) in the diet as the crab increased in size. In contrast, adult mud crabs were likely to prefer invertebrates (crabs, shrimps, molluscs and worms) and some fish rather than plant material.

The  $\delta^{13}\text{C}$  of mud crabs in this study ( $-18.7 \pm 1.2\text{‰}$ ) suggests that mud whelks, fiddler and grapsid crabs could make up part of their diet. Crushed mud whelk shells are sometimes found scattered around mud crab burrows and it is unlikely that any other predator would have the ability to open these shells (Hogarth, 1999). It is probable the mud crab prefers slow moving benthic organisms, but differences in the proportion of predominant species which constitute the mud crab diet in any particular location are likely to result mainly from differences in the availability of prey (Hill, 1976). Although oysters could possibly be included in the diet of mud crabs determined by Thimdee (2004) for an estuary in Thailand, due to their depleted carbon signature they do not appear to feature in the diet of mud crabs from Port Curtis. In contrast, the abundant grapsid and fiddler crabs are likely to be a staple in the Port Curtis mud crab diet.

Although mud crabs often reside in amongst the mangroves, like prawns, it is unlikely that they feed directly on mangrove detritus. The two end-point filamentous algae/mangrove mixing model for mud crabs determined that 92% of carbon was derived from the algae. Thimdee et al (2004) also concluded that mangrove detritus was not a major food source of the mud crab. It appears that very few species rely on mangroves as the predominant food source and that most species are likely to be more dependent on benthic organic matter and algae. This supports the finding of other authors that algal carbon rather than vascular plant carbon (mangroves) contributes the most to consumers (Newell et al., 1995, Loneragan et al., 1997, Bouillon et al., 2002b, Jennerjahn & Ittekkot, 2002). However, it is possible that decomposing mangroves supply N and P nutrients to edible benthic bacteria and algae in the substrate, with this mangrove nutrient subsidy helping to support estuarine food webs (Fry & Ewel, 2003). The extent of the contribution of bacterial biomass is mostly unresolved and also requires further attention (Bouillon et al., 2003).

## **3.2. METALS**

### **3.2.1. General metals**

For Cd, concentrations in most organisms concentrations were below LOD (<0.01 mg/kg) and are therefore not reported here. Generally for most metals (except Al and As) one particular site did not stand out consistently as having more or less metal accumulations in organisms than any other site. There was a definite trend for organisms from Site 4 to have the lowest Al concentrations compared to all other sites but in contrast Site 4 organisms tended to have the highest As concentrations. The inner harbour sites (IHS) Boat creek (Site 1), Grahams Creek (Site 2) and Black Swan

(Site 3) did not show significant site differences or trends in accumulations for most metals, however on the whole organisms from these sites tended to have more elevated metal concentrations than those from Yellow patch (Site 4).

The finding of little site differences in metal accumulations in organisms from IHS in Port Curtis may be related to the finding of more elevated dissolved metal concentrations in The Narrows. Originally Port Curtis sites were selected in a transect to reflect increasing distances from anthropogenic influences. It was previously surmised that The Narrows received flows from the Fitzroy area and were assumed to represent a less impacted reference area. Therefore sites were chosen in this direction. More recent work by the CRC Contaminant Pathways Project has determined that dissolved metal concentrations in the water column may actually increase through the Narrows (Brad Angel, pers. com). Whether this is due to the Fitzroy area contributing to metal loads in the Narrows or different physicochemical conditions and reduced flushing through the Narrows, is yet to be determined.

Results from the CRC Port Curtis hydrodynamic model (Herzfeld et al., 2004) were also not available at the time of site selection. The model suggests a greater retention time of the water body in Port Curtis (approximately 19 days) compared what was previously assumed to be a rapid turnover. The reduced flushing and a longer retention time of water in the harbour could contribute to the finding of more elevated dissolved metals in the water column. With more metals available there is a greater likelihood of contaminants being taken up into phytoplankton and therefore the food web. Therefore in light of these recent findings the lack of significant site differences within Port Curtis is not surprising. Site differences in metal accumulations within Port Curtis may be hard to establish, because some dissolved metal concentrations do not decrease appreciably until outside of Port Curtis in the direction of off shore areas. If substantiated, then this finding may have some bearing on the selection of reference and control sites for monitoring projects in Port Curtis in the future.

Oysters reflected the metal concentrations of seston at each site for Al and As, and to a lesser extent Cu and Zn in this study suggesting a link with the suspended particulate matter for uptake of these metals. Ke and Wang (2001) determined that this species of oyster obtains greater than 50% of Se and Zn uptake from food sources rather than dissolved metals in the water column. The majority of Se is also accumulated from food sources in clams (>95%)(Luoma et al., 1992), and copepods (>98%)(Wang & Fisher, 1998). Additionally >50% of Zn was obtained from ingested food in copepods (Wang & Fisher, 1998). In contrast to estuarine oysters (*Crassostrea rivularis*), the species used in this study has a very low efflux (elimination) rate for Zn (Ke & Wang, 2001), which may partly account for elevated Zn concentrations in resident oysters in Port Curtis.

#### **3.2.1.1. Aluminium (Al)**

Although in the main there were few significant sites differences in Al accumulations in organisms, there was a definite trend for Site 4 to have the lowest accumulations compared to all other sites (Table 5, Figure 12). Mud whelks were the only organisms for which Al concentrations were lower at some IHS compared to Site 4. In many cases the differences in mean concentrations were quite large; for seston and POM, concentrations were almost two to three times higher at IHS, for mullet two to ten times higher and for oysters at least six times more elevated than at Site 4. Al is a

particle reactive metal and likely to have little penetration into algal cytoplasm (Fisher & Reinfelder, 1995), therefore the low values in seston and oysters at Site 4 could be due to a site-specific geochemical behaviour.

#### **3.2.1.2. Arsenic (As)**

Arsenic tended to show an opposite trend in accumulations to aluminium (Table 6, Figure 13). Higher accumulations of As were found in all organisms from Site 4 except for POM, epiphytes, mangrove leaves, mullet and fiddler crabs. Seston had the most dramatic differences in As accumulation with Site 4 seston having at least 21 times more As than its IHS counterparts. This was reflected in the oysters from Site 4, which had accumulated two and a half times more As than IHS oysters.

Arsenic was found to be elevated in the hepatopancreas of Ayr mud crabs (reference site) compared to Gladstone mud crabs in two consecutive years sampling (Andersen & Norton, 2001). Oysters transplanted to outer harbour sites were also found to accumulate greater concentrations of As than oysters from inner harbour sites (Andersen, unpub.). It is possible that more elevated concentrations of As in the more oceanic organisms is not due to increased concentrations of As but perhaps a lack of antagonism for uptake from other available metals at those sites. Both synergistic and antagonistic uptake interactions between metals have been demonstrated previously in bivalves and algae (Frayse et al., 2002, Wang et al., 2004). Mackay et al. (1975) noted the uptake of As was negatively correlated with the uptake of both Cu and Zn in oysters. Therefore considering the more elevated concentrations of Cu and Zn at inner harbour sites in seston and some other organisms it is feasible that a greater exposure to Cu and Zn at these sites may have antagonised the uptake of As in comparison to Site 4.

#### **3.2.1.3. Chromium (Cr)**

For most organisms there were very few significant site differences or trends in accumulations of Cr (Table 7, Figure 14). Site 4 POM and seston tended to have accumulated much less Cr than at other sites, however for grapsid and fiddler crabs the opposite trend occurred.

#### **3.2.1.4. Copper (Cu)**

There were significantly lower copper accumulations in fiddler crabs from Site 4 compared to all other sites (Table 8, Figure 15). Similarly a trend occurred for lower copper concentrations in POM, seston, mangrove snails and grapsid crabs from Site 4 compared to IHS. Cu concentrations in seston were at least two to three times lower at Site 4 than for other sites. However, the opposite trend occurred for epiphytes and mud whelks. Low concentrations of copper were found in Port Curtis sediments (Apte et al., 2005) and this may have influenced the copper loads in organisms associated with sediments such as mud whelks.

#### **3.2.1.5. Nickel (Ni)**

Like Cr there were few significant sites differences in Ni accumulations between organisms (Table 9, Figure 16). Seston from Site 4 had significantly more elevated concentrations of Ni than all other sites.

#### **3.2.1.6. Selenium (Se)**

There were significantly lower concentrations of Se in POM at Site 4 compared to all other sites and a similar trend occurred in epiphytes with concentrations in both autotrophs from Site 4 being below LOD (Table 10, Figure 17). Alternatively Se was significantly more elevated in Site 1 crustaceans including fiddler and grapsid crabs compared to all other sites. There was also a tendency for Se to be more elevated in mud crabs from this site. Luoma et al. (1992) determined that >95% of Se in the clams they studied was obtained from food rather than from Se in the water column. Unfortunately metal concentrations were not measured in the filamentous algae, a potential food source for the intertidal crabs, which may have also identified elevated Se in this Site 1 food source (or the intermediate consumers harbouring within the autotroph). Se was more elevated in Site 3 POM, another potential food source, than all other sites.

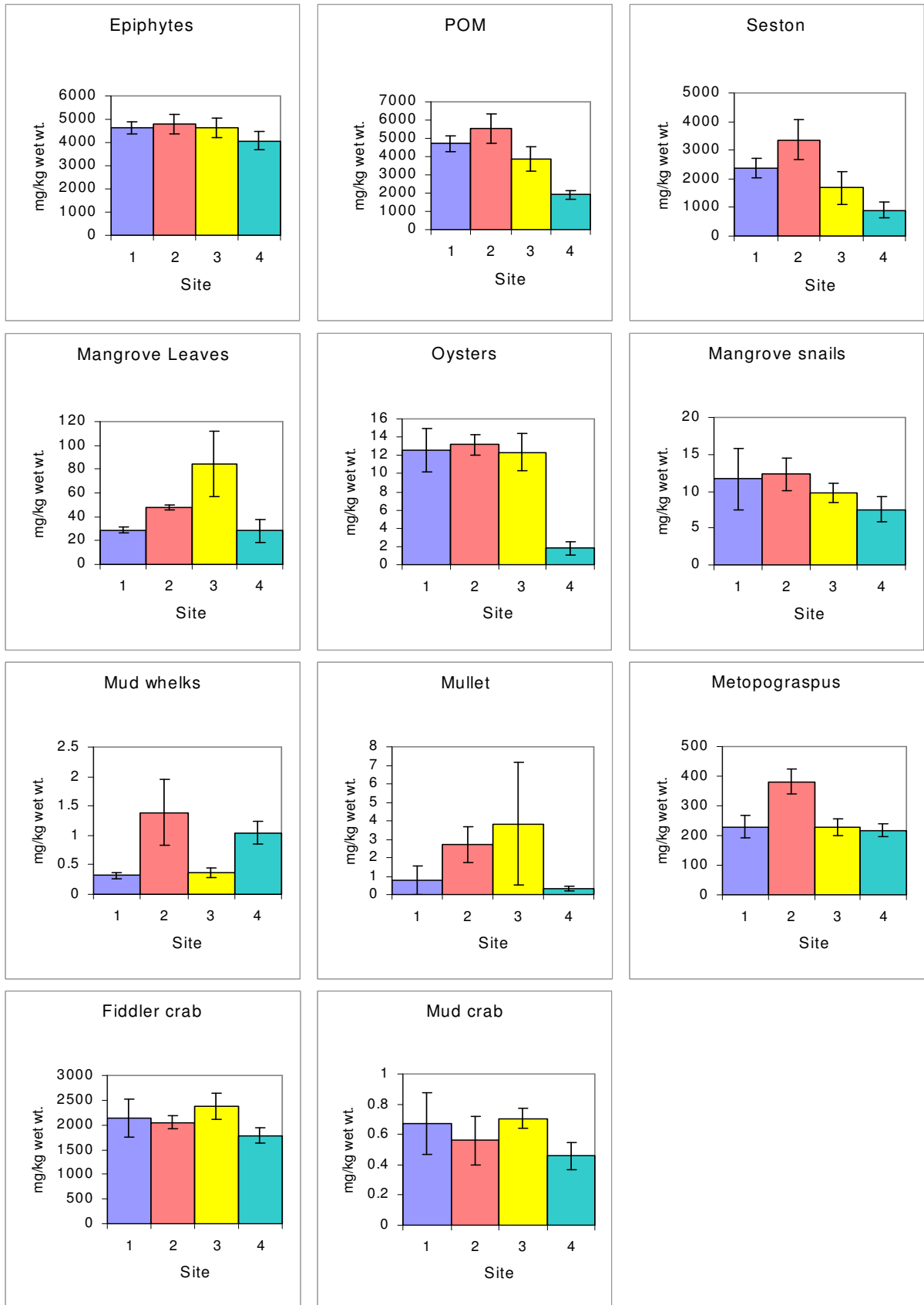
#### **3.2.1.7. Zinc (Zn)**

Fiddler crabs from Site 2 and epiphytes from Site 4 had significantly higher accumulations of Zn than organisms from other sites (Table 11, Figure 18). There was a trend for Zn in POM, seston and oysters to be lower at Site 4 compared to other sites. In a similar trend to Cu, concentrations of Zn in seston at Site 4 were two to four times lower than at other sites. Again this was reflected in the oysters to a lesser extent, with a tendency for the lowest concentrations of Zn to occur in oysters from Site 4.

**Table 5.** One-way ANOVA comparing aluminium concentrations in biota from three inner harbour sites in Port Curtis (1-3) and an outer reference site (4). Data were transformed where indicated and *a posteriori* Tukeys range test applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean concentration (shown above). \*Where equality of variances could not be achieved through sqrt, log<sub>2</sub> or log<sub>10</sub>(x+1) transformation, then untransformed data are presented.

Specimen	df	F	p	Aluminium			
				Tukey's Multiple Range test			
POM	3,8	7.397	0.011	1910 4	3870 3	4710 1	5543 2
Epiphytes	3,8	0.688	ns	4070 4	4623 3	4640 1	4803 2
Seston	3,16	4.394	0.019	910 4	1680 3	2374 1	3360 2
Mangrove leaves	3,16	3.190	*0.052	28.28 4	28.80 1	48.00 2	84.00 3
Oysters	3,16	10.369	<0.0001	1.79 4	12.36 3	12.52 1	13.2 2
Mangrove snails	3,16	0.683	ns	7.52 4	9.82 3	11.64 1	12.30 2
Mud whelks	3,16	2.779	*0.075	0.32 1	0.37 3	1.04 4	1.39 2
Mullet	3,16	0.862	ns	0.33 4	0.80 1	2.69 2	3.84 3
Metapograspus	3,16	5.598	0.008	218 4	228 3	229 1	382 2
Uca coarctata	3,24	0.939	ns	1785 4	2042 2	2142 1	2371 3
Mud crabs	3,16	0.661	ns	0.46 4	0.56 2	0.67 1	0.71 3

## Aluminium

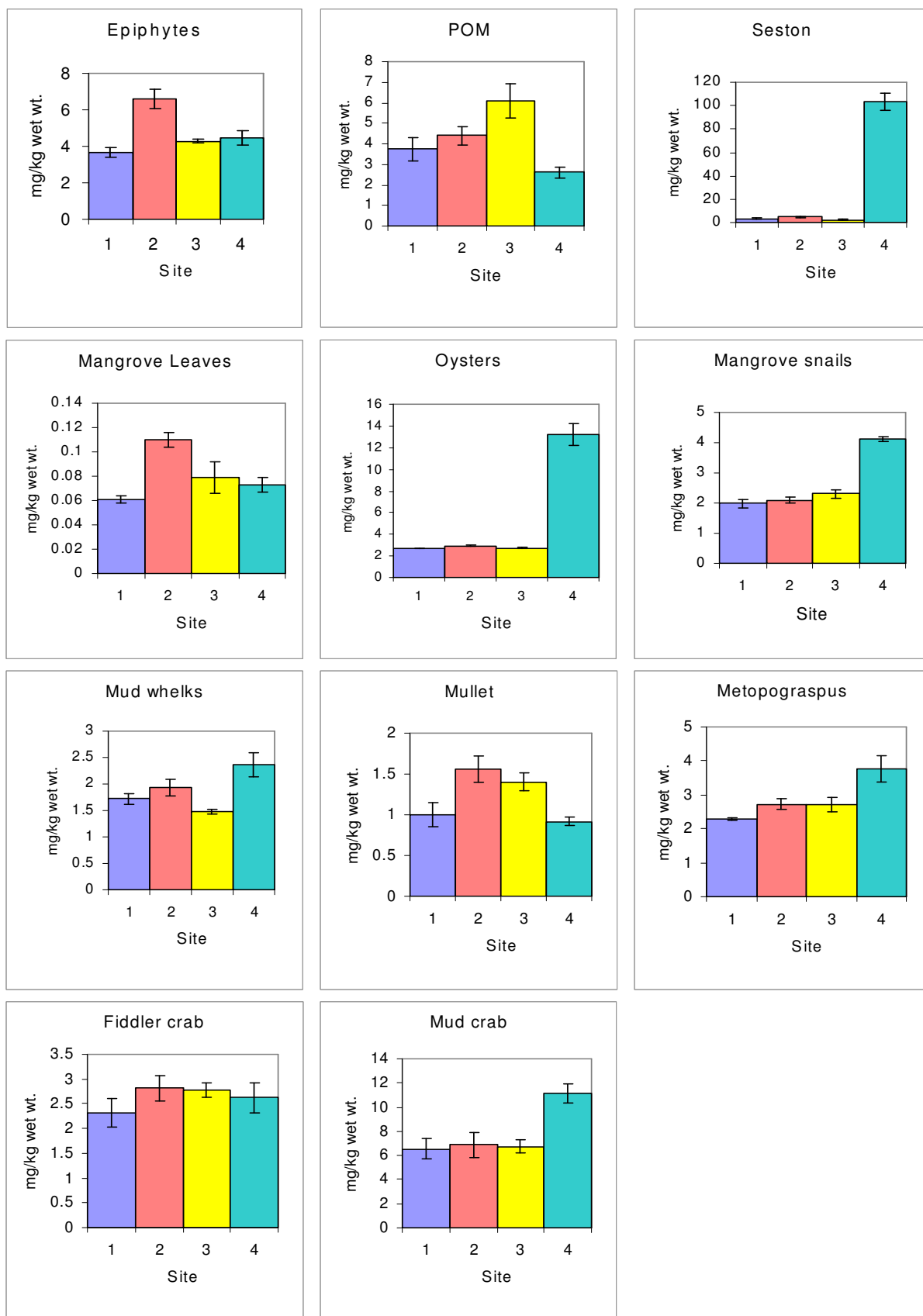


**Figure 12.** Mean (untransformed) aluminium concentrations (mg/kg wet wt.)( $\pm$  1SE) in biota at three inner harbour sites (1-3) in Port Curtis and an outer harbour reference site (4).

**Table 6.** One-way ANOVA comparing arsenic concentrations in biota from three inner harbour sites in Port Curtis (1-3) and an outer reference site (4). Data were transformed where indicated and a *posteriori* Tukeys range test applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean concentration (shown above). \*Where equality of variances could not be achieved through sqrt, log<sub>2</sub> or log<sub>10</sub>(x+1) transformation, then untransformed data are presented.

Specimen	df	F	p	Arsenic			
				Tukey's Multiple Range test			
POM	3,8	6.742	0.014	2.60 4	3.73 1	4.40 2	6.10 3
Epiphytes	3,8	12.146	0.002	3.67 1	4.30 3	4.47 4	6.60 2
Seston (log <sub>10</sub> )	3,16	85.364	<0.0001	2.60 3	3.54 1	4.72 2	103.60 4
Mangrove leaves	3,16	7.188	*0.003	0.061 1	0.073 4	0.079 3	0.110 2
Oysters	3,16	114.01	*<0.0001	2.68 1	2.74 3	2.94 2	13.2 4
Mangrove snails	3,16	77.063	<0.0001	2.00 1	2.10 2	2.32 3	4.12 4
Mud whelks	3,16	6.147	0.006	1.48 3	1.72 1	1.94 2	2.36 4
Mullet	3,16	6.005	0.006	0.92 4	1.00 1	1.40 3	1.56 2
Metapograspus	3,16	7.274	0.003	2.28 1	2.70 3	2.72 2	3.76 4
Uca coarctata	3,24	0.723	ns	2.33 1	2.63 4	2.77 3	2.81 2
Mud crabs	3,16	7.226	0.003	6.54 1	6.72 3	6.86 2	11.12 4

## Arsenic

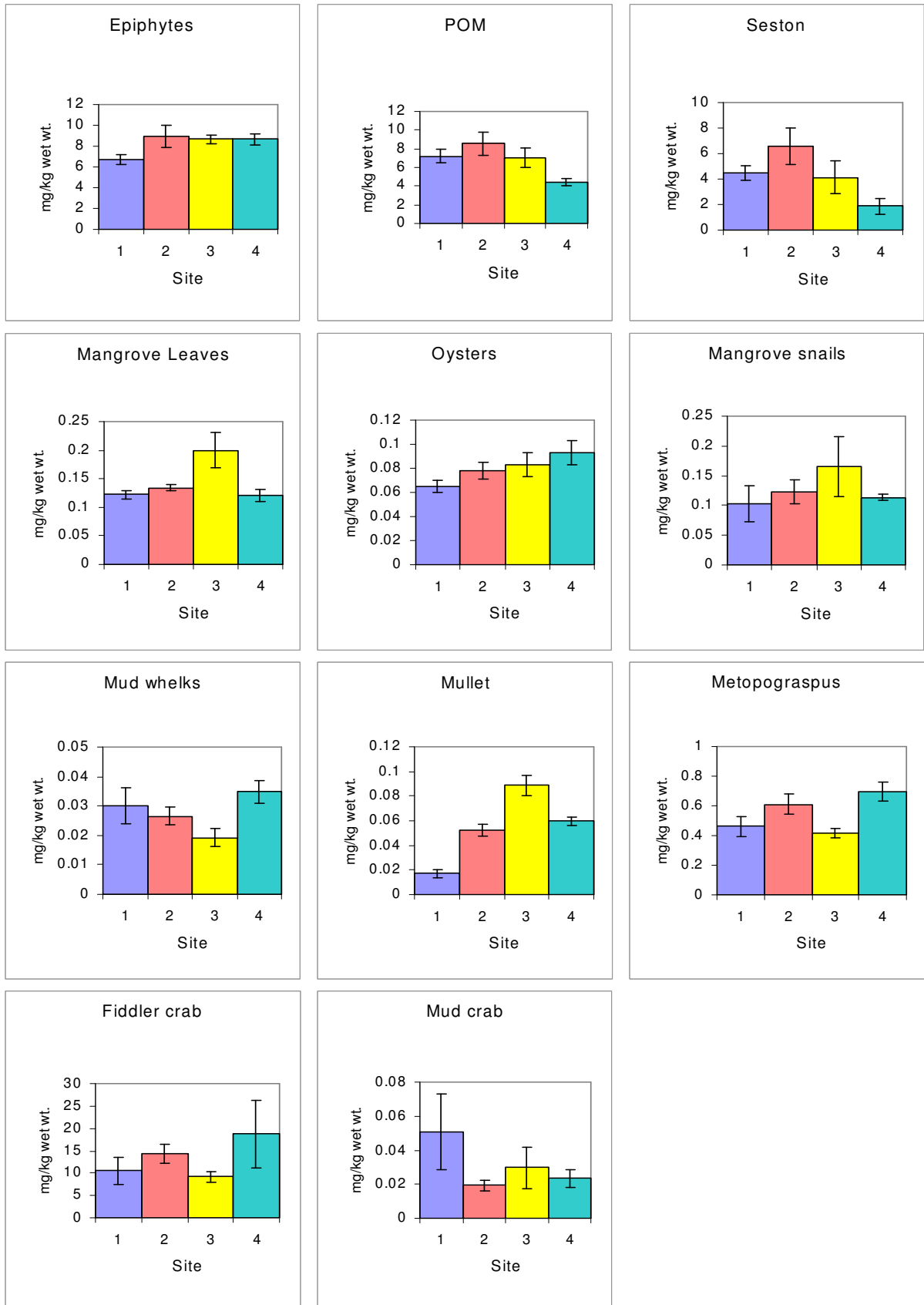


**Figure 13.** Mean (untransformed) arsenic concentrations (mg/kg wet wt.)( $\pm$  1SE) in biota at three inner harbour sites (1-3) in Port Curtis and an outer harbour reference site (4).

**Table 7.** One-way ANOVA comparing chromium concentrations in biota from three inner harbour sites in Port Curtis (1-3) and an outer reference site (4). Data were transformed where indicated and *a posteriori* Tukeys range test applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean concentration (shown above). \*Where equality of variances could not be achieved through sqrt, log<sub>2</sub> or log<sub>(10+1)</sub> transformation, then untransformed data are presented.

Specimen	df	F	p	Chromium			
				Tukey's Multiple Range test			
POM	3,8	3.615	0.065	4.47 4	7.03 1	7.23 2	8.57 3
Epiphytes	3,8	2.514	ns	6.67 1	8.67 3	8.67 4	8.93 2
Seston	3,16	3.495	0.04	1.87 4	4.12 3	4.48 1	6.58 2
Mangrove leaves	3,16	4.440	*0.019	0.12 4	0.12 1	0.13 2	0.20 3
Oysters	3,16	1.506	ns	0.07 1	0.78 2	0.83 3	0.93 4
Mangrove snails	3,16	0.692	ns	0.10 1	0.11 4	0.12 2	0.17 3
Mud whelks	3,16	2.316	ns	0.02 3	0.03 2	0.03 1	0.03 4
Mullet	3,16	2.112	*ns	0.02 1	0.05 2	0.06 4	0.09 3
Metapograspus	3,16	4.848	0.014	0.42 3	0.46 1	0.61 2	0.70 4
<i>Uca coarctata</i> (log <sub>10</sub> )	3,24	1.028	ns	9.17 3	10.51 1	14.31 2	18.80 4
Mud crabs	3,16	1.155	ns	0.02 2	0.02 4	0.03 3	0.05 1

## Chromium

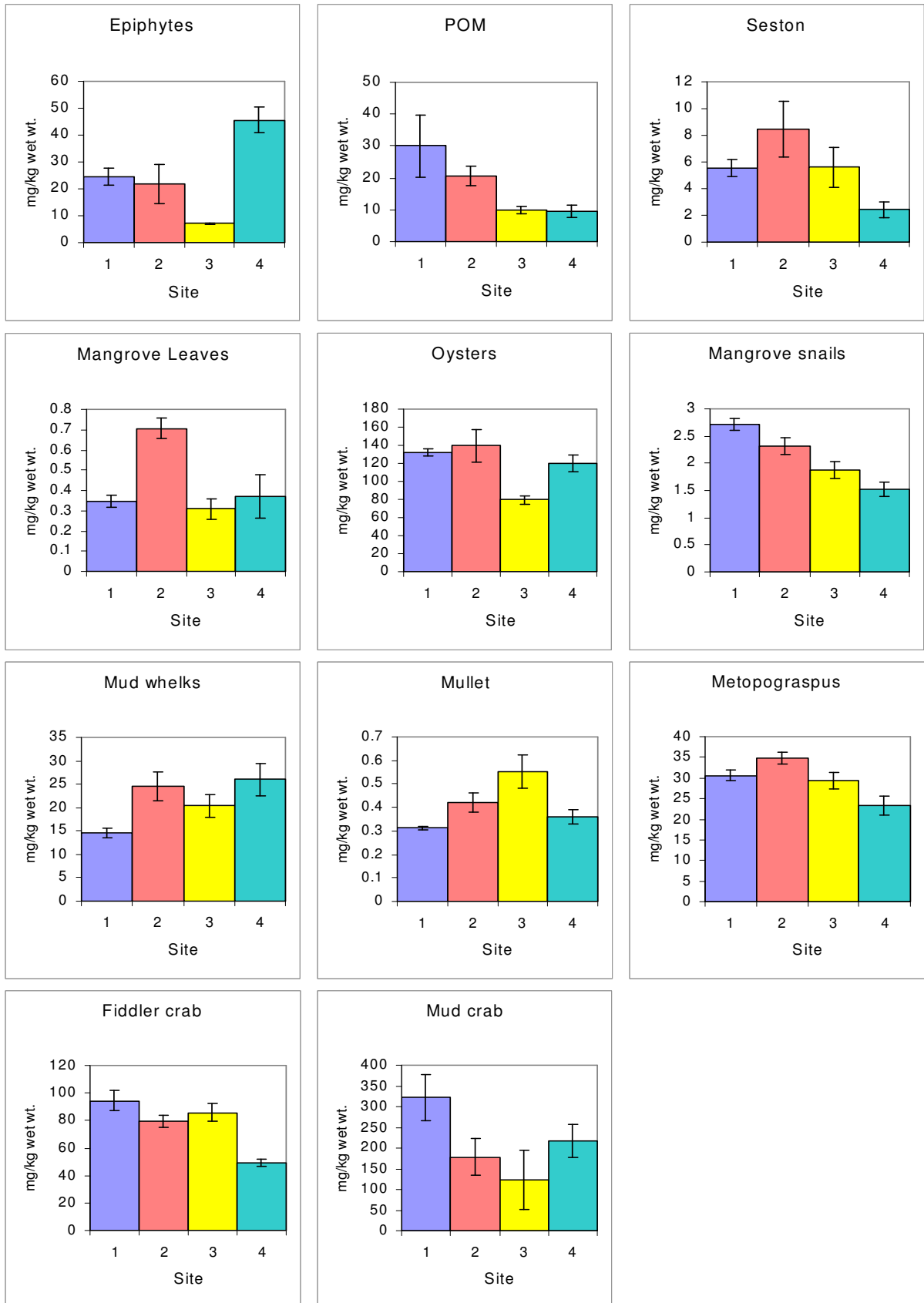


**Figure 14.** Mean (untransformed) chromium concentrations (mg/kg wet wt.)( $\pm$  1SE) in biota at three inner harbour sites (1-3) in Port Curtis and an outer harbour reference site (4).

**Table 8.** One-way ANOVA comparing copper concentrations in biota from three inner harbour sites in Port Curtis (1-3) and an outer reference site (4). Data were transformed where indicated and a *posteriori* Tukeys range test applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean concentration (shown above). \*Where equality of variances could not be achieved through sqrt, log<sub>2</sub> or log<sub>10</sub>(*x*+1) transformation, then untransformed data are presented.

Specimen	df	F	p	Copper			
				Tukey's Multiple Range test			
POM (sqrt)	3,8	4.790	0.34	9.47 4	10.07 3	20.67 2	30.00 1
Epiphytes (sqrt)	3,8	11.319	0.003	7.20 3	21.83 2	24.67 1	45.67 4
Seston	3,16	4.205	0.023	2.42 4	5.58 1	5.60 3	8.42 2
Mangrove leaves	3,16	7.363	*0.003	0.31 3	0.35 1	0.37 4	0.71 2
Oysters	3,16	6.244	0.005	79.60 3	119.40 4	132.00 1	139.60 2
Mangrove snails	3,16	13.863	<0.0001	1.52 4	1.88 3	2.32 2	2.72 1
Mud whelks	3,16	3.788	0.032	14.60 1	20.40 3	24.60 2	26.00 4
Mullet	3,16	6.066	0.006	0.31 1	0.36 4	0.42 2	0.55 3
Metapograspus	3,16	7.023	0.003	23.20 4	29.40 3	30.60 1	34.80 2
Uca coarctata	3,24	13.013	<0.0001	49.43 4	79.57 2	85.86 3	94.29 1
Mud crabs	3,16	2.423	ns	123.20 3	177.80 2	216.40 4	322.00 1

## Copper

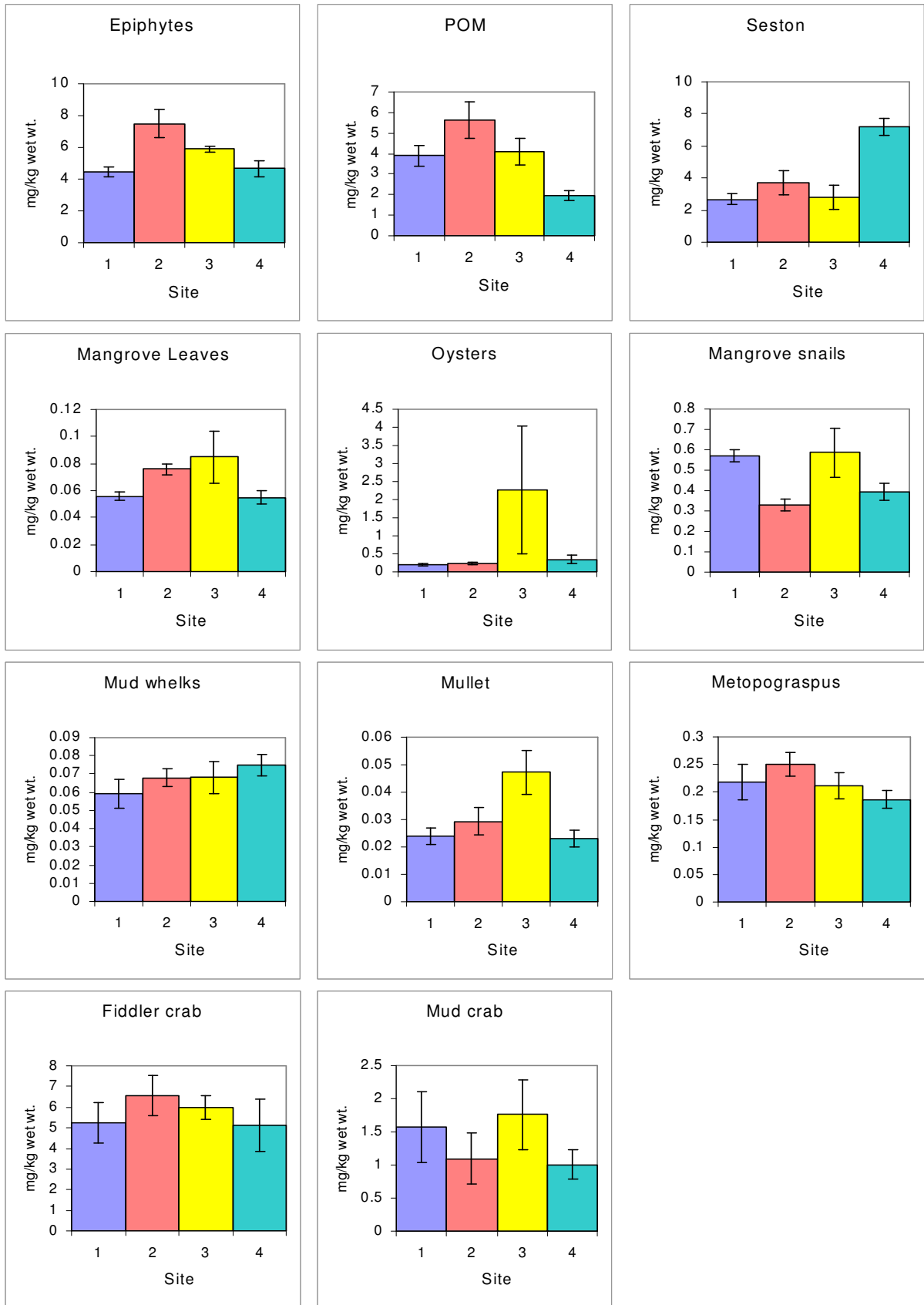


**Figure 15.** Mean (untransformed) copper concentrations (mg/kg wet wt.) ( $\pm$  1 SE) in biota at three inner harbour sites (1-3) in Port Curtis and an outer harbour reference site (4).

**Table 9.** One-way ANOVA comparing nickel concentrations in biota from three inner harbour sites in Port Curtis (1-3) and an outer reference site (4). Data were transformed where indicated and a *posteriori* Tukeys range test applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean concentration (shown above). \*Where equality of variances could not be achieved through sqrt, log<sub>2</sub> or log<sub>10</sub>(x+1) transformation, then untransformed data are presented.

Specimen	df	F	p	Nickel			
				Tukey's Multiple Range test			
POM	3,8	6.044	0.019	1.97 4	3.90 1	4.07 3	5.63 2
Epiphytes (sqrt)	3,8	6.655	0.014	4.47 1	4.67 4	5.90 3	7.50 2
Seston	3,16	11.910	<0.0001	2.68 1	2.80 3	3.70 2	7.18 4
Mangrove leaves	3,16	2.198	*ns	0.55 4	0.56 1	0.76 2	0.85 3
Oysters	3,16	10279	*ns	0.20 1	0.23 2	0.35 4	2.26 3
Mangrove snails	3,16	3.664	0.35	0.33 2	0.39 4	0.57 1	0.59 3
Mud whelks	3,16	0.811	ns	0.06 1	0.07 2	0.07 3	0.08 4
Mullet	3,16	4.805	0.014	0.02 4	0.02 1	0.03 2	0.05 3
Metapograspus	3,16	10190	ns	0.19 4	0.21 3	0.22 1	0.25 2
Uca coarctata	3,24	0.481	ns	5.10 4	5.24 1	5.99 3	6.56 2
Mud crabs	3,16	0.682	ns	1.01 4	1.10 2	1.57 1	1.76 3

## Nickel

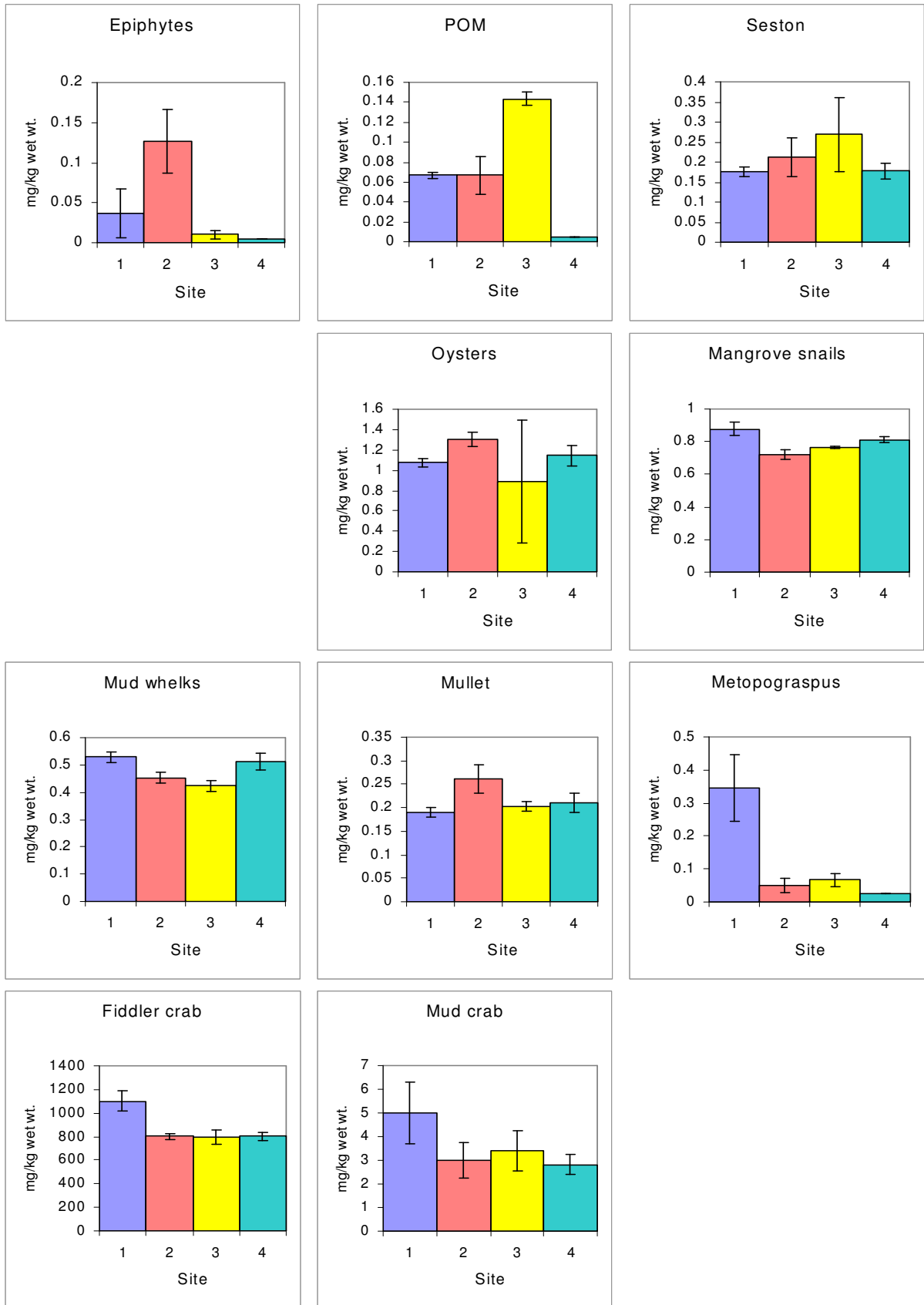


**Figure 16.** Mean (untransformed) nickel concentrations (mg/kg wet wt.) ( $\pm$  1SE) in biota at three inner harbour sites (1-3) in Port Curtis and an outer harbour reference site (4).

**Table 10.** One-way ANOVA comparing selenium concentrations in biota from three inner harbour sites in Port Curtis (1-3) and an outer reference site (4). Data were transformed where indicated and *a posteriori* Tukeys range test applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean concentration (shown above). \*Where equality of variances could not be achieved through sqrt, log<sub>2</sub> or log<sub>10</sub>(<sub>x+1</sub>) transformation, then untransformed data are presented.

Specimen	df	F	p	Selenium			
				Tukey's Multiple Range test			
POM	3,8	32.081	*<0.0001	0.01 4	0.07 1	0.07 2	0.14 3
Epiphytes	3,8	5.612	*0.23	0.005 4	0.01 3	0.04 1	0.13 2
Seston	3,16	0.652	*ns	0.18 1	0.18 4	0.21 2	0.27 3
Mangrove leaves	3,16			LOD			
Oysters	3,16	5.811	0.007	0.89 3	1.07 1	1.14 4	1.30 2
Mangrove snails	3,16	6.275	0.005	0.72 2	0.76 3	0.81 4	0.88 1
Mud whelks	3,16	5.010	0.012	0.42 3	0.45 2	0.51 4	0.53 1
Mullet	3,16	3.394	0.044	0.19 1	0.20 3	0.21 4	0.26 2
Metapograspus	3,16	8.585	*0.001	0.03 4	0.05 2	0.07 3	0.35 1
Uca coarctata	3,24	7.445	0.001	798 3	801 2	802 4	1102 1
Mud crabs	3,16	1.240	ns	2.82 4	3.00 2	3.40 3	4.98 1

## Selenium

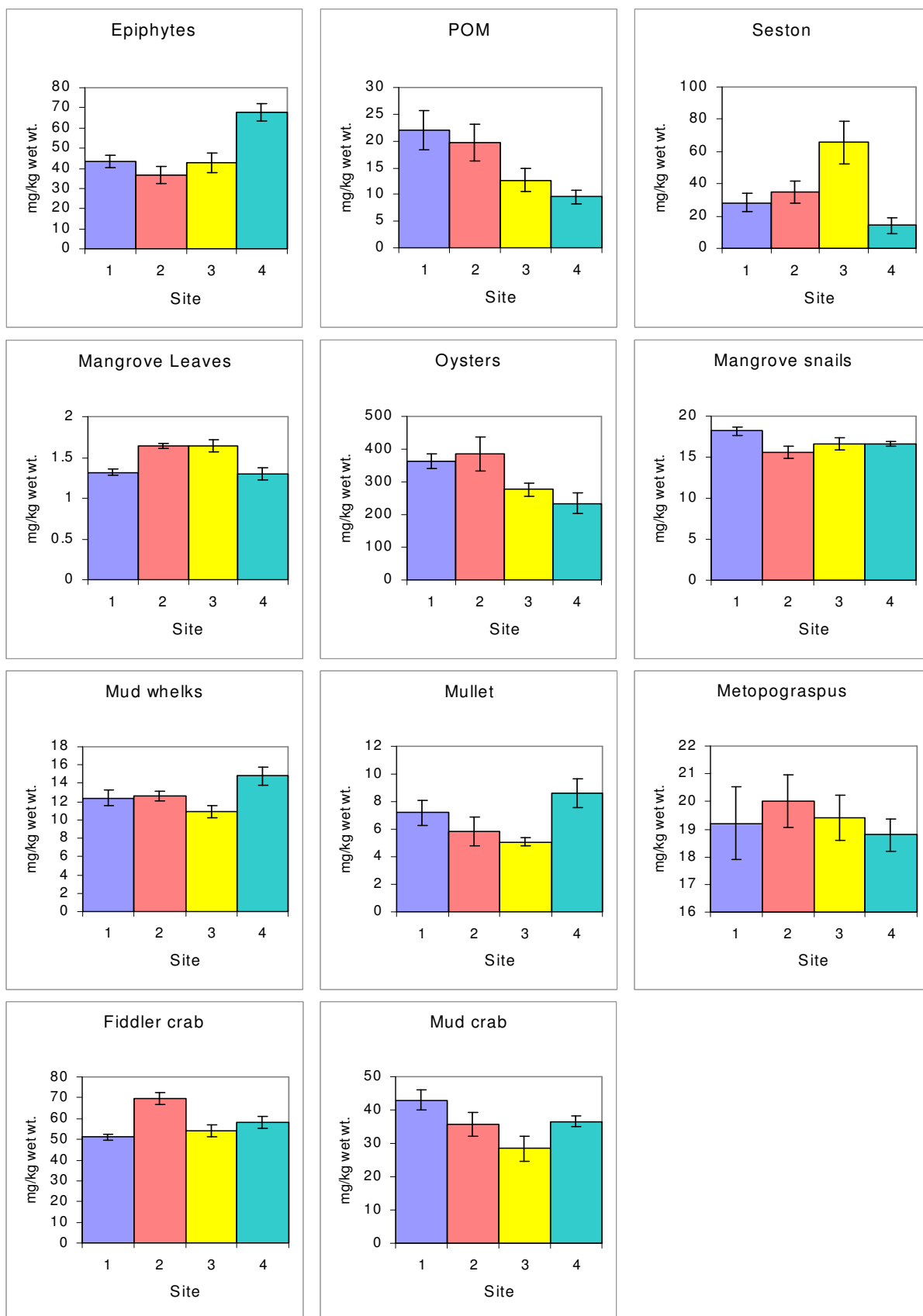


**Figure 17.** Mean (untransformed) selenium concentrations (mg/kg wet wt.) ( $\pm$  1 SE) in biota at three inner harbour sites (1-3) in Port Curtis and an outer harbour reference site (4). Concentrations in mangrove leaves were LOD.

**Table 11.** One-way ANOVA comparing zinc concentrations in biota from three inner harbour sites in Port Curtis (1-3) and an outer reference site (4). Data were transformed where indicated and a *posteriori* Tukeys range test applied to locate differences between sites; sites not significantly different from each other are joined by a common line and are arranged in ascending order of arithmetic mean concentration (shown above). \*Where equality of variances could not be achieved through sqrt, log<sub>2</sub> or log<sub>10</sub>(x+1) transformation, then untransformed data are presented.

Specimen	df	F	p	Zinc			
				Tukey's Multiple Range test			
POM	3,8	4.345	0.043	9.53 4	12.67 3	19.67 2	22.00 1
Epiphytes	3,8	10.843	0.003	36.67 2	42.67 3	43.33 1	67.67 4
Seston	3,16	6.819	0.004	14.08 4	28.20 1	34.80 2	65.60 3
Mangrove leaves	3,16	10.696	<0.0001	1.30 4	1.32 1	1.64 2	1.64 3
Oysters	3,16	4.329	0.21	234 4	276 3	362 1	386 2
Mangrove snails	3,16	3.505	0.040	15.60 2	16.60 3	16.60 4	18.20 1
Mud whelks	3,16	4.184	0.23	10.92 3	12.40 1	12.60 2	14.80 4
Mullet	3,16	3.006	ns	5.08 3	5.84 2	7.18 1	8.60 4
Metapograspus	3,16	0.275	ns	18.80 4	19.20 1	19.40 3	20.00 2
Uca coarctata	3,24	9.447	<0.0001	51.00 1	54.14 3	58.14 4	69.57 2
Mud crabs	3,16	3.639	0.36	28.40 3	35.80 2	36.60 4	43.00 1

## Zinc



**Figure 18.** Mean (untransformed) zinc concentrations (mg/kg wet wt.) ( $\pm$  1SE) in biota at three inner harbour sites (1-3) in Port Curtis and an outer harbour reference site (4).

### 3.3. FOOD WEB - BIOACCUMULATION RELATIONSHIPS

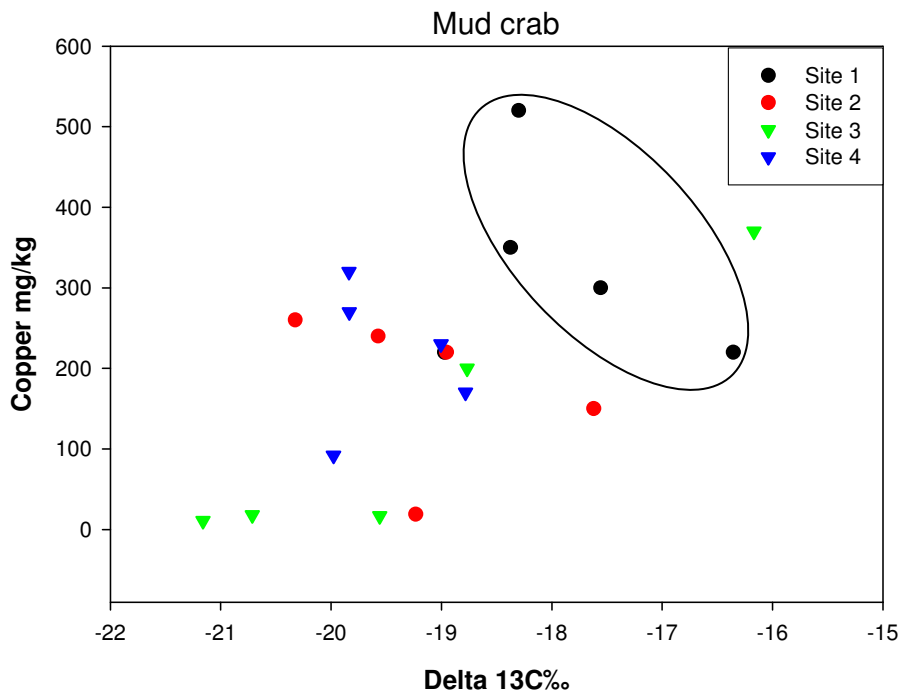
#### 3.3.1. Mud crab site differences

An attempt was made to explore the possibility that different dietary sources of carbon could explain some of the differences in metal accumulations in mud crabs. Ideally when comparing carbon signatures of specimens from different sites, the data should be standardised across sites by calculating the contribution of the dominant carbon sources to the assimilated carbon in each higher consumer level using a two end-point mixing model (see methods section 2.3.3). This approach reduces the likelihood of spatial differences in signatures of primary sources (algae) giving the appearance of site differences in carbon signatures of the consumers (mud crabs). Standardisation would require knowledge of the carbon signatures of not only mud crabs at each site, but signatures of the main dominant, carbon sources (algae>seston>mangroves) to allow valid between site comparisons. Although carbon signatures were established for mud crabs at all sites, they were not obtained for other specimens at all sites. Therefore standardisation was not undertaken on mud crab carbon signatures.

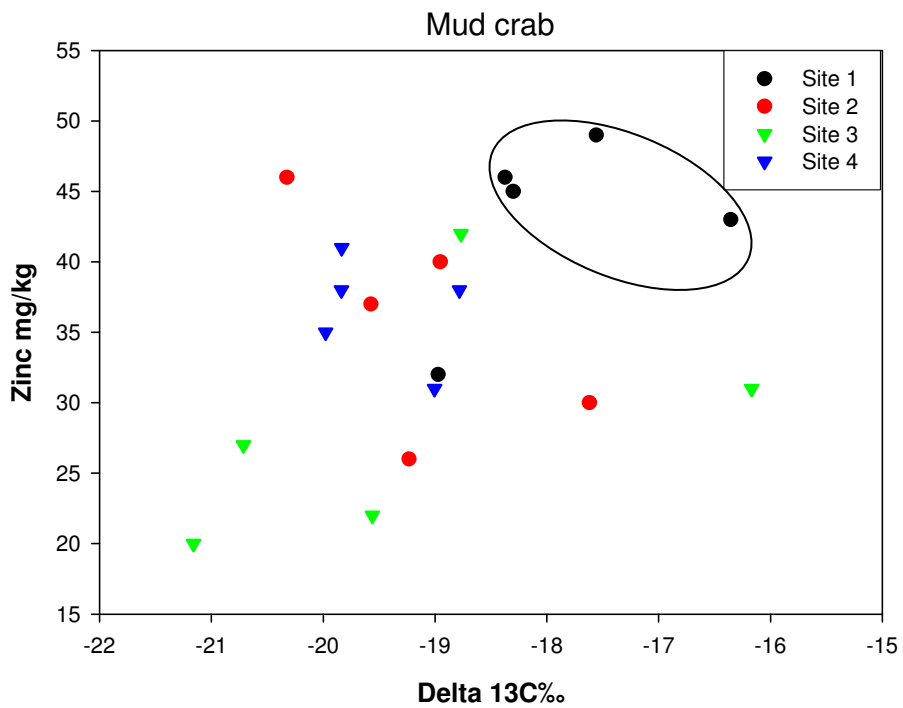
Never the less carbon signatures of individual male mud crabs from all sites were plotted against their individual accumulated metal concentrations to determine if there was commonality between metal accumulations and carbons signatures at each site. There was clustering of some crabs from each site in respect to  $\delta^{13}\text{C}$  and Cu, As and Zn concentrations respectively, suggesting a site relationship between metal accumulations and carbon signatures. Four of the five Site 1 crabs with more enriched carbon signatures (circled) tended to have the more elevated copper concentrations, whereas three out of the five crabs from Site 3 with some of the most depleted carbon signatures have the lowest copper concentrations (Figure 19). A similar pattern was observed for Zn (Figure 20). As mud crabs feed on the most available prey at any particular site it is possible that one or more particular food items are the source of copper and zinc to mud crabs at Site 1. Perhaps the predominant organism in the diets of these crabs is different at each site; crabs from each different site are ingesting more of one type of organism that is either enriched (Site 1) or depleted (Site 3) in copper (and zinc).

Similarly for arsenic, dietary differences among sites may be influencing arsenic accumulation at those sites. Crabs at Site 4 had elevated arsenic concentrations but more depleted carbon signatures (circled) compared to Site 1 crabs with lower arsenic accumulations but more enriched  $\delta^{13}\text{C}$  (Figure 21).

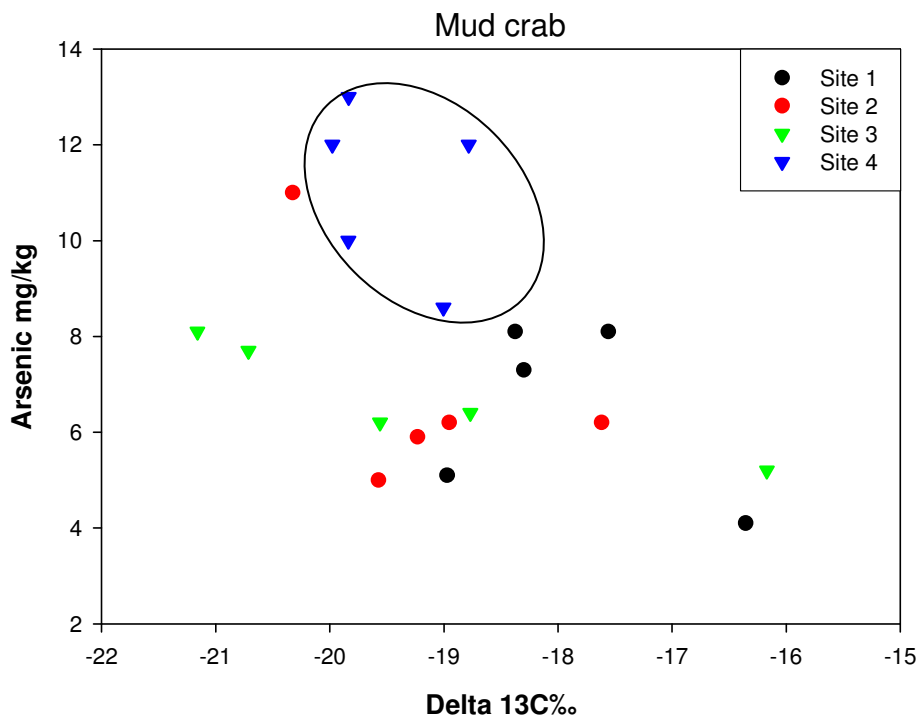
A significant correlation between carbon and Se and Zn assimilation was determined for mussels indicating that these elements follow the same digestive pathways as organic carbon in mussels (Wang & Fisher, 1996). Although likely to be more complex in higher-level consumers, perhaps similar pathways exist for some metals and carbon in mud crabs. Alternatively the crabs may all have the same diets, which may either be enriched or depleted in metals and the apparent difference is a shift in signal of the source (algae) between sites. Determination of the likely carbon sources at each site and the percentage contribution to the consumer of the dominant carbon source using end point mixing models in addition to the metal concentrations of that source at each site would clarify if valid metal food web pathways exist.



**Figure 19.** Mud crab hepatopancreas copper concentrations (mg/kg) against  $\delta^{13}\text{C}\text{‰}$  in mud crab muscle at Sites 1-4 (n=20).



**Figure 20.** Mud crab hepatopancreas zinc concentrations (mg/kg) against  $\delta^{13}\text{C}\text{‰}$  in mud crab muscle at Sites 1-4 (n=20).



**Figure 21.** Mud crab hepatopancreas arsenic concentrations (mg/kg) against  $\delta^{13}\text{C}\text{‰}$  in mud crab muscle at Sites 1-4 (n=20).

### 3.3.2. Trophic position and biomagnification

A food web of the mud crab in Port Curtis was defined and an attempt made to identify relationships between trophic position and metal accumulation. Timmermans et al., (1989) examined the distribution of trace metals in a littoral macro-invertebrate food web, sediments and water from two freshwater lakes in the Netherlands. Trophic level, feeding habits (deposit or filter feeder), body weight, and association with the substrate, physiological equipment and abiotic parameters were examined in order to elucidate why some organisms living in the same habitat accumulate more metals than others. They concluded that all the above parameters were likely to have an effect to some extent and that the specific factors that cause differences in accumulations were hard to discern.

Difficulties arise when trying to compare metal concentrations across different species. Elevated metal concentrations in seston may not be relative or equitable to what could be considered elevated metal accumulations in crustaceans. A number of abiotic factors such as dissolved metal concentration, metal speciation, salinity and water hardness influence uptake from the dissolved phase in addition to other physiological processes involved in trace element trophic transfer (Reinfelder et al., 1998). Factors such as food quantity, quality, feeding selectivity as well as gut passage time (Wang & Wong, 2003) can influence metal accumulation. Ultimately the accumulation strategy of an organism i.e. whether a net accumulator or a regulator or somewhere in between these two strategies, will affect metal bioaccumulation in that organism (Rainbow, 1990). Although some predator-prey relationships in metal accumulations were identified in this study (oyster – seston), a direct link with trophic

position was not ascertained. The metal regulatory ability of each organism will have also influenced the outcome of these findings.

Trace element biomagnification occurs when concentrations in the tissues of one organism exceed those in its food or in an adjacent trophic level (Reinfelder et al., 1998). Biomagnification of Hg in aquatic food webs has been well documented with observations of highest concentrations in the largest and long-lived high order consumers (Cabanna & Rasmussen, 1994, Bowles et al., 2001, Power et al., 2002). For most metals biomagnification into higher trophic levels is thought not to occur (Fisher & Reinfelder, 1995, Miramand et al., 1998) with the concentration at each trophic level being determined by the accumulation pattern (or strategy) of the particular species at each trophic level (Rainbow, 2002) but at the same time it is an ambiguous and largely understudied area (Reinfelder et al., 1998).

A recent study suggests that Cd and Zn may be biomagnified during trophic transfer from bivalves to two species of predatory gastropods and that dietary exposure dominated >90% of the accumulation (Wang & Ke, 2002). Interspecies difference in metal biokinetics was also highlighted. Timmermans et al., (1989) identified biomagnification of Zn in through one trophic level in a study of littoral macro-invertebrates. Although some patterns of metal accumulation were demonstrated among organisms in this study, relationships are obviously more complex higher up the food chain. Most assimilation efficiency studies have focused on chemical transfer in organisms at the bottom of the food chain but additional studies are required to examine more complex chemical transfers in higher trophic organisms (Wang & Fisher, 1999). Although true biomagnification per se was not observed in this study, bioamplification of some metals was evident.

## **CONCLUSIONS**

Since the completion of this study new evidence has come to light regarding both the dissolved metal concentrations and the hydrodynamics of the water body within and outside Port Curtis. Phase 2 of the CRC Contaminant Pathways Project has determined that dissolved concentrations of metals within Port Curtis remain elevated (in comparison to oceanic background concentrations) until the harbour oceanic interface where concentrations rapidly decline to background. The Narrows may be a sink or conversely contributing to the elevated metal concentrations in the inner harbour (Brad Angel pers. com.). The CRC hydrodynamic model for Port Curtis (Herzfeld et al., 2004) also suggests reduced flushing and a longer retention time of the water body of approximately 19 days in comparison to the rapid exchange that was previously thought to occur. A combination of these factors could lead to a greater load of metals being available for uptake into organisms inhabiting the harbour, in particular the autotrophic level supporting the food chain. Non pelagic type organisms such as the more sedentary, sessile species would be subjected to a longer exposure than the more mobile species. The two factors: elevated dissolved metals and reduced flushing, could contribute to the anomalous bioaccumulation of metals in biota in inner harbour sites compared to outer harbour sites recorded in this and previous studies.

There are consequences for management authorities of these findings. It was assumed that due to the rapid flushing of the harbour (2 to 3 days) suggested by a previous model that contaminants released into Port Curtis would be rapidly diluted and

dispersed. Therefore should bioaccumulation be caused by reduced flushing of the water body, a need arises for greater consideration to be given to the total load of metal being released into the harbour. This may have ramifications for regulatory bodies especially when considering the establishment and location of new industries in Port Curtis. Continual monitoring of biota in the harbour to ensure that a status quo is maintained is warranted.

In summary this study highlights the complexity of interactions that are likely to occur in metal pathways in estuarine food webs. Although uptake of metals from the dissolved phase is still important consideration, many studies are highlighting the significance of trophic transfer in metal accumulation by aquatic invertebrates. Advances in kinetic modelling will no doubt help to define many metal pathways through various food web interactions in the future. However, the findings of this study indicate that for the majority of organisms the uptake of metals through food pathways is likely to be complex and integrated, particularly for those in higher trophic positions and those that have the ability to regulate metal accumulations. The adage 'you are what you eat' may hold true for carbon sources, but not necessarily for metals accumulated by consumers in complex mangrove ecosystems.

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