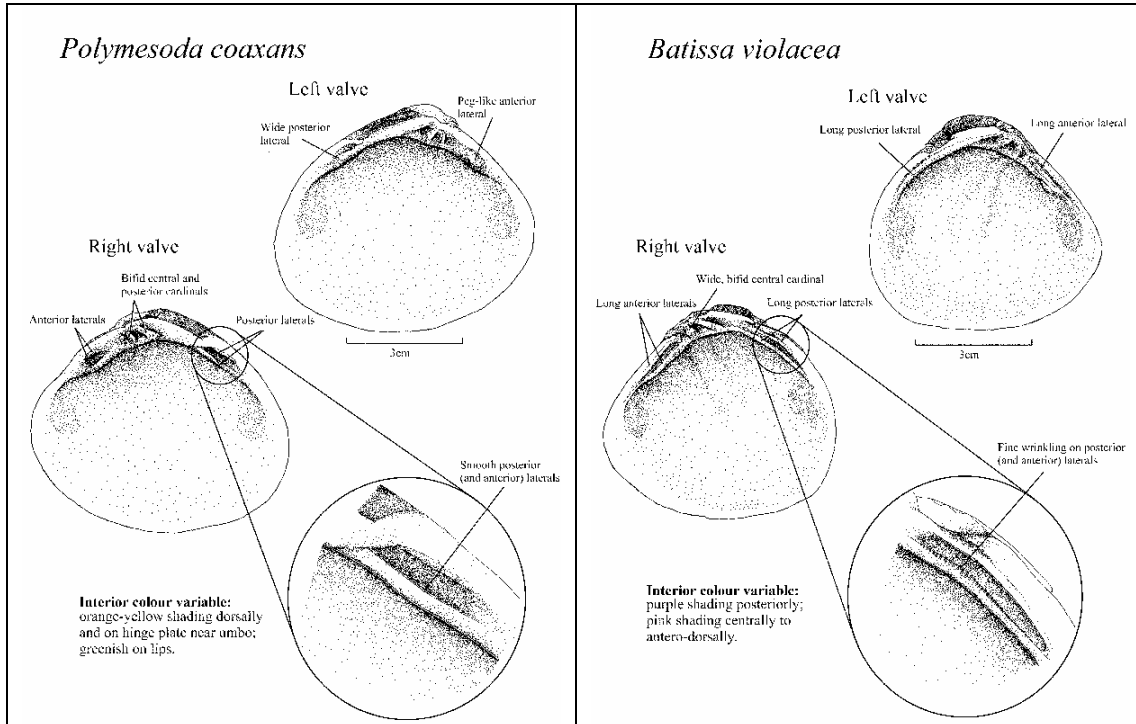


Metal Levels in Mud Clams from Estuarine Sites in the Fly River System



for

Ok Tedi Mining Limited

Prepared by

Wetland Research & Management

August 2003

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Frontispiece: Line drawings of the two species of mud clam found in the Fly Estuary, *Polymesoda coaxans* and *Batissa violacea*, showing distinguishing features.

EXECUTIVE SUMMARY

Metal levels (Cu, Cd, Pb & Zn) were determined for whole animal tissue samples from mud clams collected from sites in the Fly River estuary and control estuaries to the east (Bamu) and west (Oriomo, Pahoturi and Binaturi Rivers). Differences were examined between areas (inner, middle and outer Fly Estuary and control sites) and reporting periods (1994/95, 1998/99, 2000/01 & 2001/02). In addition, the dietary intake of metals by local villagers consuming shell fish was assessed.

Analyses detected significant between Area and Reporting period differences in metal levels in mud clams. Within the inner Fly estuary, mud clams from the most recent reporting period (2001/02) had significantly higher levels of all metals compared with earlier reporting periods. For the remainder of the system, levels of Cu, Cd and Zn in mud clams in the middle and outer Fly estuary were higher than adjacent control sites. There was also a trend for higher levels in the inner estuary compared with the middle and outer estuary, however this gradient was confounded by the likely difference in species composition between these areas. Finally, levels of Cu, Pb and Zn were highest in the 2001/02 Reporting period compared with the earlier reporting periods.

The gradient of metals in mud clams along the length of the estuary was consistent with the known gradient of dissolved and particulate Cu in the estuary. The current pattern of Cu concentrations in mud clams shows elevated Cu in the whole estuary compared with control sites, and with a gradient from the inner to the outer estuary.

Analysis of the current data were limited by the under-representation of sampling sites in the inner and middle estuary, with most sites located in the outer estuary. Even so, data showed a statistically significant increase in metal levels in mud clams in 2001/02 reporting period compared with earlier reporting periods. Also, the increase in metal levels was more pronounced at inner and middle Fly River estuary sites compared with levels at the outer estuary sites. Assuming there has been no change to the analytical technique, then it would appear that in recent years there has been a significant increase in metals levels in mud clams at estuarine sites, with these increases greatest at inner and middle estuarine locations. This may indicate an increased influence of mine waste on the estuary.

It is recommended that monitoring is continued, with all sites re-sampled in the 2003/2004 Reporting period. These data should be analysed to assess whether the same pattern as seen in this report still exists, if the trend has become more pronounced or if the trend was an anomaly.

Human dietary intake of metals in mud clams was estimated using data from inner Fly estuary sites and related to the recommended Provisional Maximum Tolerable Daily Intake for Cu, Cd and Pb (PMTDI); inner estuarine sites exhibited the highest tissue metal levels. Metal levels determined from mud clams with gut contents were used because specimens with gut contents reflect the tissues ingested by villagers in the estuary. Based on an estimated average diet, mud clams formed approximately 4 %, 19% and 5% of the PMTDI for Cu, Cd and Pb respectively. At these levels they do not pose a health threat, although at approximately 20% of the maximum intake, the highest exposure was to Cd. Comparison of metal levels against the Australian New Zealand Food Authority guidelines for maximum levels of metal

contamination in foods determined that the only exceedances were for Cu at the two inner Fly Estuary sites.

INTRODUCTION

BACKGROUND

This report presents results and interpretation of the analysis of data on tissue metal levels in mud clams collected from sites in the Fly estuary and control locations in rivers to the east (Bamu) and west (Oriomo, Binaturi and Pahoturi) of the mouth of the Fly River.

Prior to this report, data on tissue metal levels in mud clams were most recently reported by OTML (1997), in which metal levels in samples collected in 1993/94 were compared with samples collected in 1994/95. However there have been no analyses on samples collected subsequently. Additional specimens have since been collected in 1998/99, 2000/01 and 2001/02, and metal concentrations determined. Therefore, the current analyses were undertaken to assess long-term trends in concentrations of metals in mud clams, comparing earlier data with more recent samples. Analyses by OTML (1997) and subsequent investigations have established new protocols for analysis of mud clams, which are summarised below, as they influence sample collection and statistical analyses.

MUD CLAMS AS BIOMONITORS

Little is known of the ecology of mud clams in the Fly Estuary, although there is a vast literature on the use of bivalves (i.e. mussels and oysters) as biomagnifiers of trace metals (see review by Powell & White, 1990). Mud clams are widespread throughout the Gulf of Papua, and they have been utilised in monitoring programs by Ok Tedi Mining Limited, Porgera Joint Venture and the Purari Basin hydroelectric scheme. Mud clams are also utilised as a subsistence food source, although they do not appear to be a major component of the diet of villagers in the Fly River estuary.

It is recognised that some bivalves are able to regulate body metal levels and, therefore, not all would be suitable biomonitors. To assess the suitability of mud clams for biomonitoring, laboratory trials were conducted by Ok Tedi Mining Ltd to examine uptake and loss of Cu by *Polymesoda coaxans* (previously identified and referred to by OTML as *Geloina coaxans*) exposed to a range of dissolved Cu concentrations (OTML, 1995a). The species demonstrated significant uptake of Cu over a 12 day period, at high nominal concentrations (1000 ppb), with no significant loss of the accumulated Cu during a subsequent 12 day depuration period. Although this was a short duration trial at high exposures, it demonstrated that *P. coaxans* was not able to readily regulate tissue Cu concentrations and therefore, potentially may be a good biomonitor. Uptake and loss of other metals (Cd, Pb and Zn) were not tested, but it is assumed that this species of mud clam is also unable to regulate these metals. Based on this laboratory trial, the current biomonitoring program using mud clams was established in the Fly Estuary.

INFLUENCE OF GUT CONTENTS

It is known that residual matter in the guts of bivalves can have a significant effect on whole body metal concentrations if guts are included in analyses (Robinson *et al.*, 1993), and that specimens should be depurated in clean sea water for at least 36 hrs prior to dissection to eliminate ‘contamination’ by gut contents. This aspect was investigated by OTML (1997), whereby metal levels were compared between specimens containing gut contents with

specimens which had been kept in ‘clean’ seawater for 48 hrs to void their guts. This comparison established that specimens with gut contents (containing sediment potentially with a proportion of mine-derived particulates), had significantly higher metal concentrations than those with depurated guts. It was therefore recommended that when assessing bioaccumulation of mine-derived metals in mud clams, specimens should be depurated prior to preservation. However, it was also acknowledged that villagers living in the Fly Estuary, who consume mud clams as a subsistence food item, do not depurate mud clams prior to cooking and eating. Therefore, mud clams with gut contents should be analysed to reflect metal burdens in dietary intake by villagers. OTML (1997) therefore recommended that specimens should be depurated prior to analysis to determine extent of metal bioaccumulation, but non-depurated specimens should be used to establish potential dietary intake of metals by villagers. Subsequent to OTML (1997), mud clams have been collected from sites, with depurated and non-depurated replicates from each site.

MUD CLAM TAXONOMY

OTML (1997) also established that there were significant differences in the body size of mud clams between the Inner and the Middle/Outer Fly estuary sites, and that the smaller mud clams from the inner estuary contained greater concentrations of metals. Allowing for the size differences using analysis of covariance (OTML, 1997), analyses detected a gradient in metal concentrations from the inner to the outer estuary, consistent with there being a gradient in metals along the length of the estuary. However, the between-area size differences, and a cursory examination of the shells of some specimens suggested a potential taxonomic difference. Therefore, specimens from inner, middle and outer estuarine sites were taken to Dr Shirley Slack-Smith, Curator of Molluscs at the Western Australian Museum for taxonomic examination. This examination established that the samples contained two species of mud clam, with specimens from the inner estuary sites being identified as *Batissa violacea* and those from the Middle/Outer estuary sites being *Polymesoda coaxans* (previously called *Geloina coaxans*). The reported gradient in metal concentration was consistent with a gradient in exposure along the length of the estuary, reflecting dilution of mine derived sediment and Fly River water (OTML, 1997). However, the spatial difference in species composition may also account for the observed difference in metal concentrations, as between-species differences in rates of metal uptake and depuration are known from the literature. Without laboratory trials to compare the uptake and loss rates of both species under comparable exposures, it is not possible to differentiate the mine effects from the taxonomic influence. Therefore, it is considered that it is no longer appropriate to compare Inner estuary sites with Middle/Outer estuary and Control sites. Future analysis of Inner estuary data will therefore need to be restricted to temporal comparisons only, unless specimens of *Polymesoda coaxans* can be identified from inner estuary sites.

METAL LEVELS IN BARNACLES

As well as analysing mud clams, OTML (1997) reported results of analyses of data on metal levels in barnacles (*Balanus patelliformis* Bruguière) taken from the Fly Estuary and adjacent control sites. However, no additional barnacle data were available for analysis as part of this current report. Some archived samples of barnacles collected since 1993/94 are being processed, and these data will be reported subsequently.

This report presents analysis of mud clam tissue metals data for specimens collected on various occasions from selected sites from 1993/94 to the 2001/02 reporting periods.

METHODS

Mud clams were collected from sites in the Fly River estuary and control estuaries to the east and west of the Fly River as indicated in Table 1 and in Figure 1. Specimens were collected by searching through soft inter-tidal mud, either with a bush knife or by hand. In 1993/94, specimens were placed directly into plastic bags, frozen and returned to the laboratory. In subsequent years, ten specimens were collected as per 1993/94, but then an additional ten specimens were taken and these were kept in 'clean' water for 48 hrs to void their gut contents prior to being frozen and returned to the laboratory. Clean water was obtained by collecting estuarine water from the vicinity of the sampling site, allowing it to stand for sufficient time for any suspended material to settle. The clean water was then decanted from the containers and used for voiding the mud clams.

On returning to the laboratory, mud clam shells were washed with deionised water and then dissected using clean, stainless steel scalpels and plastic tweezers. Dissected tissues were placed in individual clean, labelled plastic bags, frozen and passed to the Environmental Chemistry Section for determination of dry weight concentrations of Cd, Cu, Zn and Pb. Prior to dissecting, the height, length, width and wet weight of each intact mud clam was recorded. Prior to metal determinations, tissues were freeze-dried, dry weight recorded and material then acid digested by microwave. Metal concentrations were then determined by AA spectrophotometer.

Table 1. Number of replicate mud clam samples available for analysis summarised by Area (Control, Inner, Middle and Outer Fly Estuary), Site, Gut content status (WO = without gut contents, and WG = with gut contents) for each reporting period. The number of replicate specimens taken from each area is indicated.

Area	Site	Status	00/01	01/02	93/94	94/95	98/99	
Control	BIN01	WG		10	10		10	
		WO		10			10	
	BMU01	WG			10	10		
		WO				10		
	BMU03	WG			10	9		
		WO				10		
	ORI01	WG	10	10	10	10	10	
		WO	10	10		10	10	
	PAH01	WG		10	10	10	8	
		WO		10		10	10	
	Inner	EST28	WG		10	10		10
			WO		10			8
EST29		WG		8	10	10	5	
		WO				10	6	
Middle	EST30	WG				10		
		WO		10		10		
Outer	EST09	WG		10	10		10	
		WO		10			10	
	EST18	WG		10	10	10	9	
		WO		10		10	9	
	EST19	WG		10	10	10	9	
		WO		10		10	9	
	EST22	WG	10	10	10	10	10	
		WO	10	10		10	10	
	EST24	WG	10				10	
		WO	7				10	
	EST26	WG	10		10		9	
		WO	10				10	

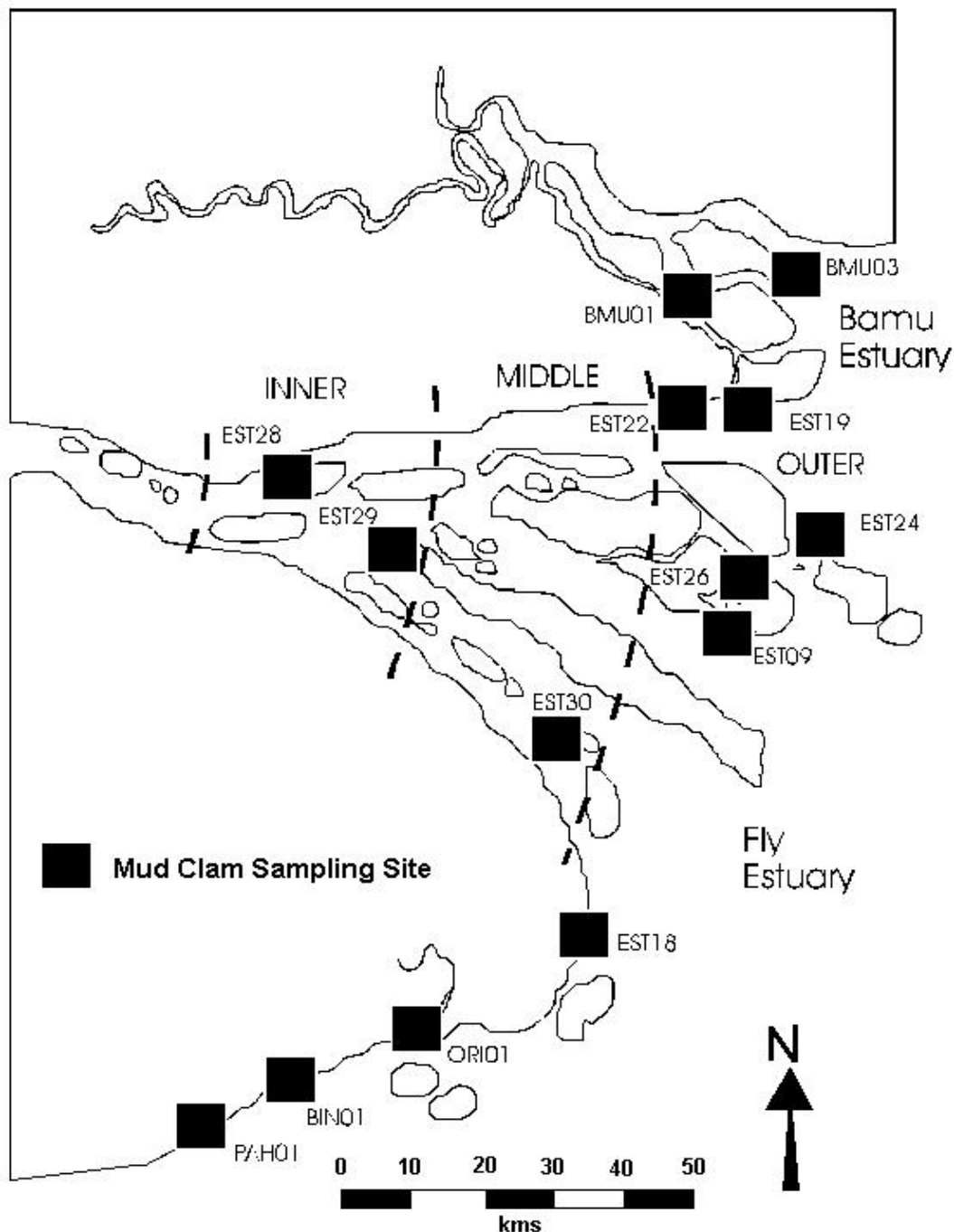


Figure 1. Location of sampling sites in the Fly River estuary and adjacent control locations, showing position of boundaries for Inner, Middle and Outer estuary sites.

DATA ANALYSIS

Analysis of variance (ANOVA) was used to test for between-site and reporting period differences in the concentration of each metal (Cu, Cd, Pb & Zn) in tissues from mud clams using the General Linear Model (GLM) procedure in the SAS statistical package. Analyses were performed on depurated specimens to avoid gut contents giving an erroneous indication of metal bioaccumulation due to any effects of contaminated sediments in non-depurated specimens (see above and also OTML, 1997).

To identify possible mine effects, estuarine sites were classified as being either from Control locations (BMU01, BMU03, BIN01, ORI01, PAH01) or downstream of the mine. Depending on position, downstream sites were subdivided into Inner estuary (EST28 & EST29), Middle estuary (EST30) and Outer estuary (EST09, EST18, EST19, EST22, EST24 & EST26). Based on the taxonomy of samples taken to Australia, and without specific identifications for all specimens collected, it was assumed that all Inner estuary sites contained *B. violacea* and all other locations contained *P. coaxans*, and therefore the two species were analysed separately.

Temporal differences in metal concentrations in *B. violacea* were tested to determine if levels in mud clams from the Inner Estuary varied over time (NB. no spatial comparisons were possible for Inner estuary specimens due to absence of the same species from different areas). Spatial and temporal effects were analysed for *P. coaxans*, comparing metal concentrations between Middle and Outer Fly River estuary sites with Control sites over time.

Equality of sample variances were tested with Brown-Forsyth and Levenes tests of homogeneity. Where necessary, data were $\log_{10}(x+1)$ transformed to achieve equality of variances. Tukey's (HSD) Multiple Range tests were applied to locate differences where there were significant main effects.

Ideally, a nested design would be applied for these analyses, with replicate mud clams from a site nested within sites which are used as replicate within areas, however, lack of replication of sites within each area prevented this approach. Therefore, ANOVAs were applied using all replicate mud clams within each area, providing more degrees of freedom and greater statistical power to detect differences, although the location of individual replicate mud clams would not have been randomly distributed within each area. The number of samples in each area, by gut content status and sampling period are indicated in Table 2.

Table 2. Summary of mud clam samples available for analysis indicating reporting period in which collected (Year), gut status (WO = without gut contents; WG = with gut contents), and Area from which collected (Control sites, and Inner, Middle or Outer Fly Estuary sites). The number of replicate specimens taken from each area is indicated.

Year	Status	Control	Fly River Estuary			Total
			Inner	Middle	Outer	
93/94	WG	50	20	-	50	120
94/95	WG	40	10	10	30	120
	WO	40	10	10	30	120
98/99	WG	28	15	-	57	100
	WO	30	14	-	58	102
00/01	WG	10	-	-	30	40
	WO	10	-	-	27	37
01/02	WG	30	18	-	40	88
	WO	30	10	10	40	90
Total		268	97	30	362	817

Previously OTML have reported the estimated dietary intake of copper by humans consuming locally caught fish. For estimating dietary intake, calculations were based on the Provisional Maximum Tolerable Daily Intake (PMTDI) of copper of 0.5 mg Cu kg⁻¹ body weight (WHO 1993). Assuming an average body weight of 70 kg for a villager on the Fly River, the recommended maximum daily intake of copper is 35 mg (or 35 000 µg). Equivalent PMTDIs for Cd and Pb are 71 and 429 µg per day respectively (NB there is no recommended maximum daily intake for Zn). These recommended maximum intakes were used in

conjunction with observed concentrations of these metals in mud clams with gut contents to estimate the potential dietary intake by villagers in the estuary consuming shell fish.

From past conversations with OTML employees who live in the Fly estuary (Greg Bani and Mabi Dukawa, Environment Dept.) and with villagers from the estuary, it has been established that mud clams form a small part of the diet of villagers in the Fly estuary. Mud clams tend to be eaten occasionally (once or twice a week), usually with sago and usually when fish were not available (i.e. during the rainy season when the seas were too rough to go fishing). Based on these discussions, a conservative estimate of 20 mud clams consumed by any one person per week was determined (approx. 3 per day).

The average wet weight of tissue in mud clams was reported by OTML (1997) to be 5.6g (n = 121, sd = 3.95). This equates to approximately 112 g wet weight of mud clam tissue consumed per person per week, or 16 g wet weight per day. Daily metal burdens were then estimated using the wet weight of tissue in mud clams, the number consumed and the mean metal content.

Mean concentrations of metals in mud clam specimens collected with gut contents were calculated for each site within the Fly estuary in the 2001/2002 reporting period. The site with the maximum mean concentrations in metal concentrations were then used to determine dietary intake and therefore the greatest potential risk from dietary metal exposure in Fly Estuary villagers consuming mud clams.

The OTML Regime stipulates that ‘Condition 1b – Fish Edibility’ is to monitor the edibility of freshwater fish by comparing metal concentrations in fish tissues to the 1996 Australian New Zealand Food Authority Food Standards Code (ANZFA, 1996). This approach was applied to assess edibility of mud clams from the estuary using the ANZFA standards for metals in molluscs. The ANZFA (1996) guidelines have since been replaced by the updated and revised ANZFA (2000) guidelines, however, the revised guidelines no longer consider Cu or Zn as metals of concern, and therefore do not provide a maximum level. For the purposes of this report the ANZFA (2000) guidelines were used for Cd and Pb, and the ANZFA (1996) guidelines were used for Cu and Zn (Table 3). For all sites monitored, the mean concentration of each metal at each site was determined, using mud clams with gut contents, and the mean values compared against the relevant standard.

Table 3. ANZFA (1996) and ANZFA (2000) guidelines for maximum levels of metal contamination in molluscs (mg/kg wet weight) (N/A – no guideline provided), highlighting values used as guidelines.

Metal	ANZFA (1996)	ANZFA (2000)
Cd	2.0	2.0
Cu	10.0	N/A
Pb	1.5	2.0
Zn	150.0	N/A

RESULTS

BETWEEN SITE COMPARISONS – 2001/2002

Initially, plots of mean concentrations (\pm 95% CI) of each metal at each site sampled in the 2001/2002 reporting period were prepared to identify gross patterns in metal concentrations across sites (Figure 2). Statistical analysis of these trends were not undertaken, as specimens from the Inner estuary site (EST29) were likely *B. violacea*, whilst all other sites contained *P. coaxans*, making comparisons invalid. From the plots, it is evident that all four metals show higher concentrations at sites closest to the head of the estuary (Inner and Middle and some Outer Estuary sites), compared with the Control sites. Pb and to a lesser extent Cd were elevated in specimens from the Oriomo River control site (ORI01).

BATISSA VIOLACEA – TEMPORAL CHANGES

One-way ANOVAs on metal concentrations in mud clams taken from Inner Fly Estuary sites (Table 1, 2 & Figure 1) detected significant temporal changes in Cu, Cd, Pb and Zn (Table 4, Figure 3).

All metals showed a trend of increasing concentrations in mud clams over time. For Cu and Zn, levels in mud clams in 2001/02 were significantly greater than in all preceding years, with no differences between preceding years. For Cd, levels in mud clams collected in 2001/02 and 1998/99 were not significantly different from each other, but both periods were significantly greater than 1994/95. For Pb, there was a trend of increasing concentrations over time, with levels in mud clams collected in 2001/02 greater than in 1994/95, but with intermediate levels for 1998/99 (Table 4, Figure 3).

Table 4. One-way ANOVAs on metal concentrations in mud clams from Inner Fly estuary sites by each reporting period. 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. Mean (+ 1 SE) concentrations are presented in Appendix 1.

Metal	df	F	P	Tukeys HSD Range Test		
Cu	2,33	47.75	<0.0001	2001/02 (412.5)	1998/99 (140.2)	1994/95 (122.6)
Cd	2,33	12.94	<0.0001	2001/02 (4.28)	1998/99 (3.39)	1994/95 (2.13)
Pb	2,33	3.46	0.0439	2001/02 (2.97)	1998/99 (1.31)	1994/95 (0.10)
Zn	2,33	49.51	<0.0001	2001/02 (669.3)	1998/99 (268.2)	1994/95 (152.4)

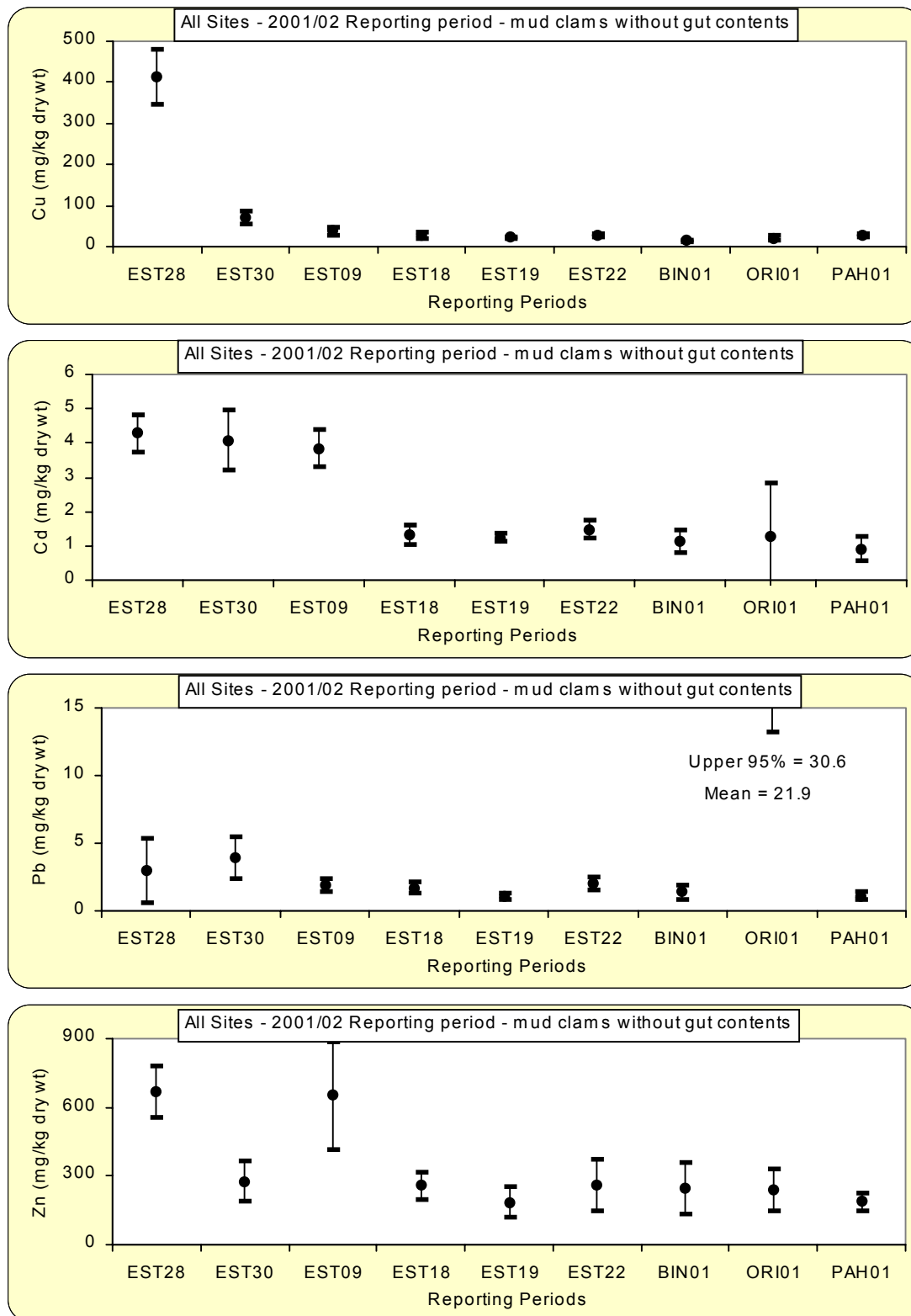


Figure 2. Mean (+ 95% CI) concentration of each metal in mud clams from each site sampled in the 2001/2002 reporting period. Data are for mud clams without gut contents, and sites are arranged in increasing distance from the mine (EST28 = Inner Estuary; EST30 = Middle Estuary; EST09, EST18, EST19 & EST22 = Outer Estuary; BIN01, ORI01 & PAH01 = Controls).

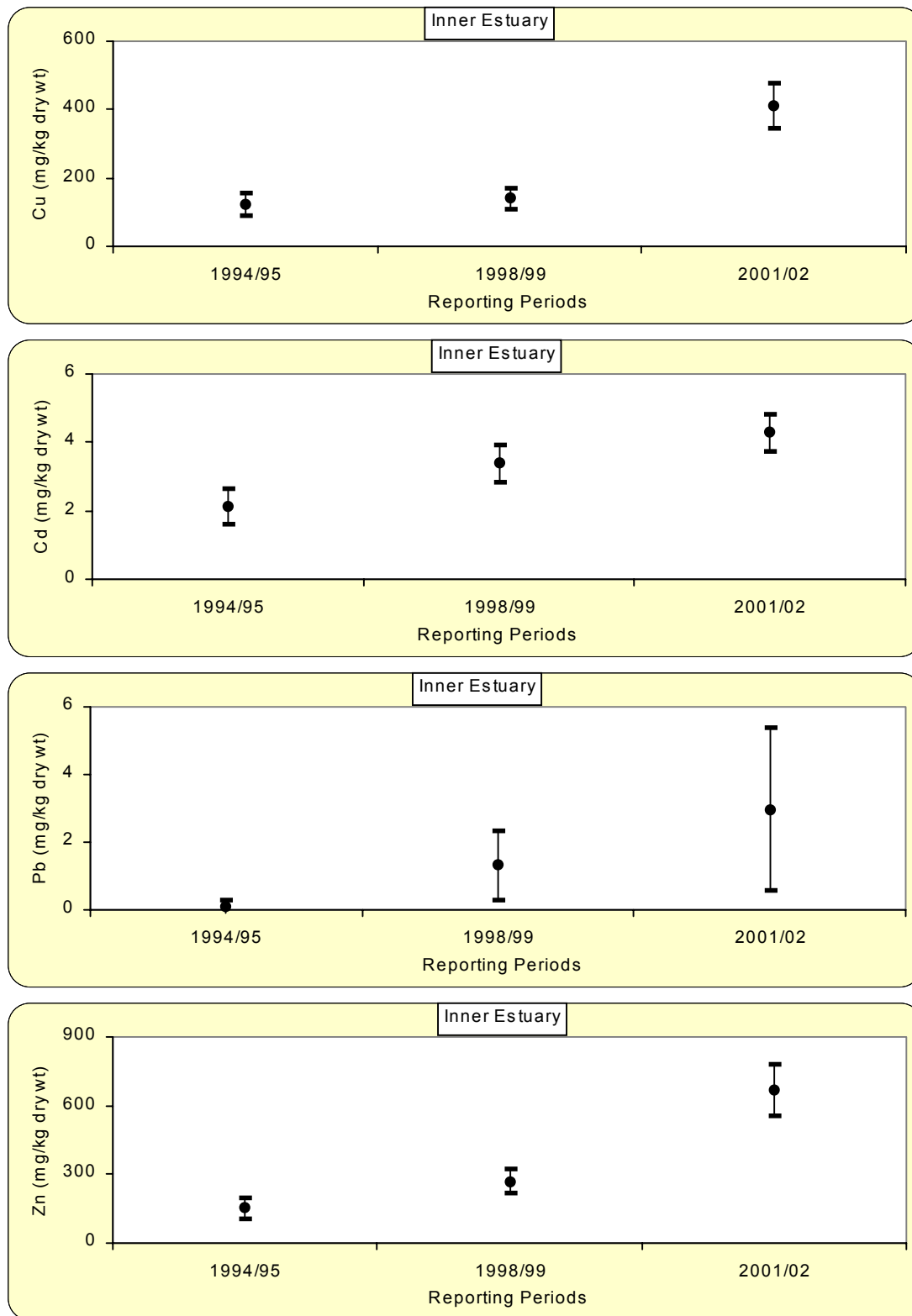


Figure 3. Temporal changes in concentrations of each metal (mean \pm 95% confidence intervals) in mud clams without gut contents collected from the Inner Fly estuary.

POLYMESODA COAXANS – SPATIAL & TEMPORAL CHANGES

Analysis of between-area and Reporting period differences in metal levels in mud clams from the middle and outer Fly estuary and the control locations detected significant between area and between sampling period differences for most metals (Table 5 & Figure 4).

Cu in mud clams

Cu showed a significant gradient in concentration in mud clams along the estuary, with specimens from the Middle Fly significantly higher than the Outer Fly which were higher than the Control sites. Cu concentration in mud clams was significantly higher in 2001/02 reporting period (mean = 26.77 mg/kg dry wt) than earlier reporting periods, which were not different to each other (Table 5). There was a significant interaction term, the reason for which is visible in Figure 4, whereby a ‘cross-over’ occurs for Cu levels in 1998/99 at Outer Fly and Control locations. This interaction term did not overly influence the dominant trends of a gradient along the estuary and higher concentrations in the most recent samples.

Cd in mud clams

Cd also showed a significant gradient in concentration in mud clams along the estuary, with specimens from the Middle Fly significantly higher than the Outer Fly which were higher than the Control sites. Temporal changes in Cd concentration in mud clams were less clear, with samples collected in 2000/01 significantly less than in all other reporting periods, and with no differences amongst other periods (Table 5.). There was a significant interaction term, the reason for which is visible in Figure 4, whereby a ‘cross-over’ occurs for Cd levels in 1994/95 at Outer Fly and Control locations.

Pb in mud clams

Pb concentrations in mud clams were not significantly different between areas, with similar concentrations in specimens from the Middle Fly, Outer Fly and Control sites. There was a significant temporal difference, with samples collected in 2001/02 significantly higher than samples collected in 1998/99, which were significantly greater than samples collected in 2000/01 and 1994/95. (Table 5.). Again, there was a significant interaction term, the reason for which is visible in Figure 4, whereby there are several ‘cross-overs’ amongst areas and reporting periods.

Zn in mud clams

Zn in mud clams showed no significant difference between Middle and Outer Fly estuary samples, but both areas were significantly higher than mud clams from control areas. There was a significant temporal difference, with a trend for highest concentrations in the most recently collected samples (viz. 2001/02 and 2000/01), and lower concentrations in samples collected in 1994/95 and 1998/99 (Table 5.). The interaction term was non-significant, indicating that these trends were consistent across Areas and Reporting periods (Figure 4).

Table 5 Two-way ANOVA on each metal (Cu, Cd, Pb & Zn) by areas (Middle Fly estuary, Outer Fly estuary and Control locations), and reporting periods. 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 in parentheses for metal concentrations in each sample, with geometric means presented for log₁₀ transformed data. Mean (+ 1 SE) concentration of each metal are presented in Appendix 1.

Effect	df	F	p	Tukeys HSD			
<u>Cu [log₁₀(x+1)]</u>							
Area	2	26.95	<0.0001	Middle (46.55)	Outer (22.68)	Control (18.69)	
Year	3	8.43	<0.0001	01/02 (26.77)	94/95 (21.45)	98/99 (21.28)	00/01 (17.35)
Area * Year	4	7.45	<0.0001				
<u>Cd [log₁₀(x+1)]</u>							
Area	2	23.22	<0.0001	Middle (3.07)	Outer (1.65)	Control (1.12)	
Year	3	12.00	<0.0001	94/95 (1.74)	98/99 (1.68)	01/02 (1.58)	00/01 (0.65)
Area * Year	4	3.53	0.0079				
<u>Pb [log₁₀(x+1)]</u>							
Area	2	0.34	ns	Middle (1.80)	Outer (1.40)	Control (1.30)	
Year	3	27.14	<0.0001	01/02 (2.42)	98/99 (1.69)	94/95 (0.82)	00/01 (0.48)
Area * Year	4	9.86	<0.0001				
<u>Zn [log₁₀(x+1)]</u>							
Area	2	3.73	0.0253	Middle (206.71)	Outer (183.98)	Control (147.31)	
Year	3	11.27	<0.0001	01/02 (232.63)	00/01 (180.53)	98/99 (174.58)	94/95 (117.87)
Area * Year	4	1.27	ns				

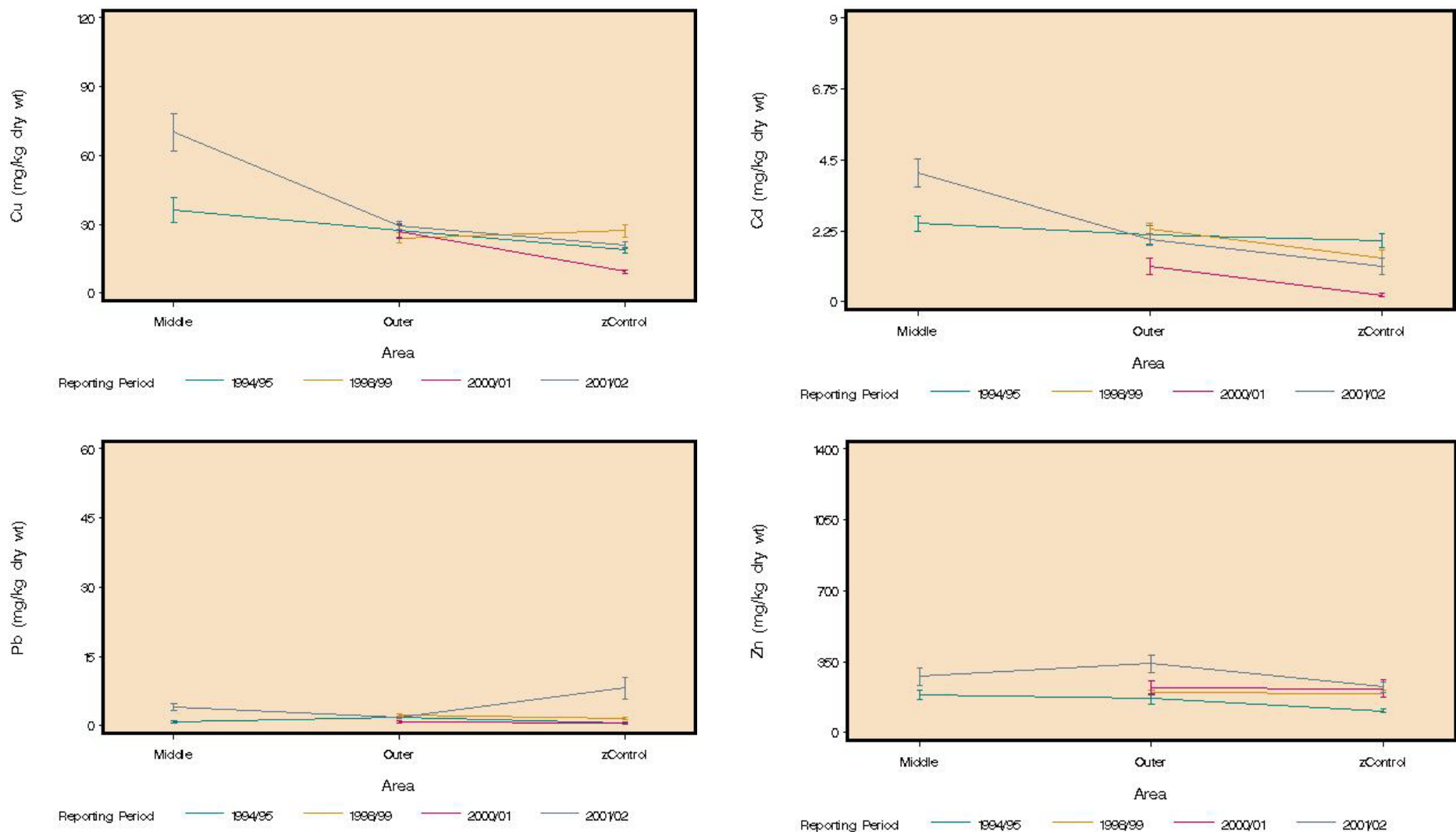


Figure 4. Changes in mean (\pm 1 SE) concentration of each metal in mud clams from the middle and outer Fly estuary and adjacent control sites showing two-way interactions by Area and Reporting Period.

ESTIMATED DIETARY INTAKE OF METALS BY HUMANS

Using the PMTDI for Cu, Cd and Pb of 35 000, 71 and 429 $\mu\text{g}/\text{kg}$ wet wt respectively, and the daily intake of approximately 16 g wet weight of mud clam tissue, the daily dietary intake of each metal was calculated as a percentage of the PMTDI, using the maximum mean concentration of each metal observed at a site within the Fly Estuary in the 2001/2002 reporting period (Table 6). The maximum mean values used were 452.1 mg Cu/kg dry wt at EST28, 4.16 mg Cd/kg dry wt at EST29, and 7.18 mg Pb/kg dry wt at EST28 (Table 6).

Table 6. Mean concentration (mg/kg dry wt) of each metal in mud clams (with gut contents) at each site within the Fly River estuary for specimens collected in 2001/2002 reporting period. The mean concentration used for determining dietary exposure for each metal is in bold.

Reporting period	Gut status	Site	Cu (mg/kg dry wt)	Cd (mg/kg dry wt)	Pb (mg/kg dry wt)	Zn (mg/kg dry wt)
2001/02	WG	EST09	35.1	4.09	3.15	592.0
2001/02	WG	EST18	28.7	0.97	1.82	212.4
2001/02	WG	EST19	24.1	1.49	2.24	208.0
2001/02	WG	EST22	25.8	1.63	2.66	434.5
2001/02	WG	EST28	452.1	3.62	3.14	624.8
2001/02	WG	EST29	138.0	4.16	7.18	628.5

For a daily intake of 16g of mud clam tissue with a concentration of 90.4 $\mu\text{g}/\text{g}$ copper wet wt the total intake would be 1446.7 μg copper per day. This is approximately 4.1 % of the PMTDI. To equal the PMTDI a villager in the inner Fly estuary would have to consume approximately 387 g of mud clam flesh each day, which equates to approximately 64 mud clams.

For a daily intake of 16g of mud clam tissue with a concentration of 0.832 $\mu\text{g}/\text{g}$ cadmium wet wt the total intake would be 13.3 μg cadmium per day. This is approximately 18.7 % of the PMTDI. To equal the PMTDI a villager in the inner Fly estuary would have to consume approximately 85 g of mud clam flesh each day, which equates to approximately 14 mud clams.

For a daily intake of 16g of mud clam tissue with a concentration of 1.44 $\mu\text{g}/\text{g}$ lead wet wt the total intake would be 23.0 μg lead per day. This is approximately 5.4 % of the PMTDI. To equal the PMTDI a villager in the inner Fly estuary would have to consume approximately 299 g of mud clam flesh each day, which equates to approximately 50 mud clams.

A component not considered in these estimates would be the ingestion of copper-enriched sediment present on the surface or insides of the shell of the mud clams if not well washed before cooking. However, particulate metal concentrations in the Fly estuary are close to background and not likely to be a problem. Also, there is the potential for loss of metals from the tissue during the cooking process, either by loss of fluids, volatilisation over an open fire or passing into solution when boiled/steamed. Given these possible sources of variance, the estimates indicate that consumption of mud clams is unlikely to cause an exceedance of the PMTDI by itself. The only metal of potential concern is Cd, with average weekly intake of mud clams providing approximately 20% of the maximum recommended exposure.

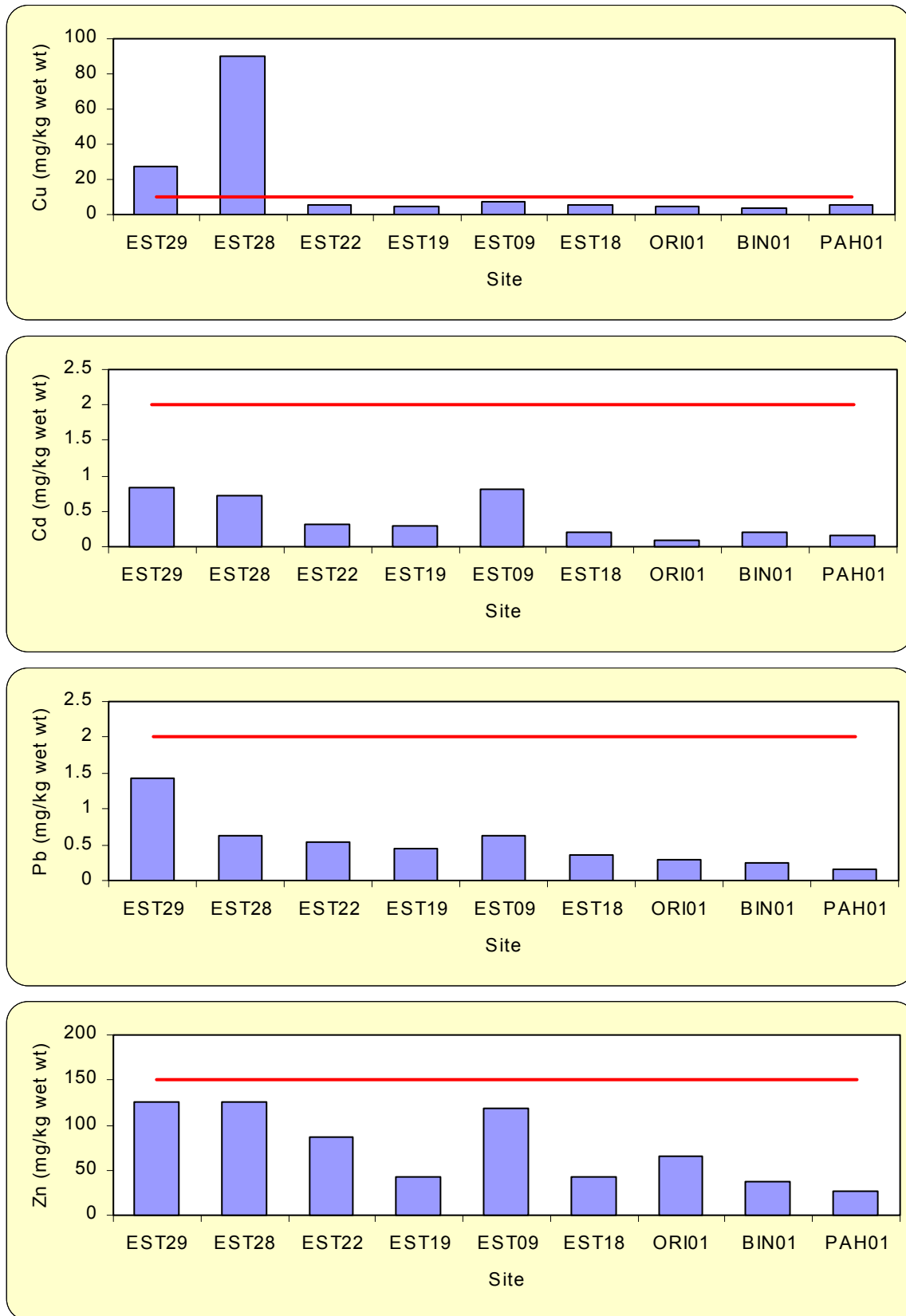


Figure 5. Mean concentration of each metal in mud clams with gut contents at each site in 2001/2002, indicating (red line) the ANZFA (1996; for Cu and Zn) and ANZFA (2000; for Cd and Pb) maximum levels for metal contaminants in molluscs.

Comparison to the relevant food standards codes for each metal from ANZFA (1996) and ANZFA (2000), using the mean metal concentration in mud clams with gut contents at each site in 2001/2002, determined that the only exceedances for any metals were for Cu at the Inner Estuary sites EST28 and EST29 (Figure 5). All other mean metal concentrations were below the relevant guidelines.

DISCUSSION

MUD CLAM TAXONOMY

Taxonomic examination by the Western Australian Museum of mud clam shells taken from various locations within the Fly Estuary identified two species of mud clam; *Polymesoda coaxans* and *Batissa violacea*. From the samples examined, the smaller species, *B. violacea* only occurred at the inner Fly Estuary sites, with samples from all other locations containing *P. coaxans*. This supports the known life history of *B. violacea* compared with *P. coaxans*, with the former preferring lower salinities than the latter (Dr Shirley Slack-Smith, Western Australian Museum, pers. com.).

Based on this information, and given the absence of species level identifications for all specimens collected by OTML, it was conservatively assumed that all mud clam specimens collected from the Inner Fly estuary were *B. violacea*, and all other mud clams were *P. coaxans*. This spatial difference in species composition prevented direct comparison of Inner Fly to Outer/Middle Fly estuary sites/samples. Therefore, it is not known if the gradient in concentrations of metals from the Inner to Outer estuary reflects declining exposure to Cu along the length of the estuary, or is related to differences in metal accumulation rates between the two species.

Dr Shirley Slack-Smith (The Western Australian Museum) provided taxonomic descriptions and line drawings of *B. violacea* and *P. coaxans* (Figures 6 & 7). It is recommended that these drawings and descriptions are used in future sample processing to identify specimens to species level, and the database be modified to include a species code, so that species-specific metals data are available. This will assist discrimination of the effects of species differences versus mine effects in the longitudinal gradient in metal concentrations along the Fly estuary. In addition, historically, the shells of all mud clam specimens used for metals determinations have been labelled and archived in the Biology Laboratory/ Store. It is recommended that these shells are examined and each specimen is identified to species level and the data on the database revised to reflect which species the samples (*viz.* metals data) are from. Finally, attempts should be made to collect specimens of *P. coaxans* from inner estuary sites, and these specimens then used for Inner, Middle and Outer estuary comparisons.

SPATIAL & TEMPORAL PATTERNS

Analyses detected significant between Area and Reporting period differences in metal levels in mud clams. Within the inner Fly estuary, mud clams from the most recent reporting period (2001/02) had significantly higher levels compared with earlier reporting periods. For the remainder of the system, levels of Cu, Cd and Zn in mud clams in the middle and outer Fly estuary were higher than adjacent control sites. There was also a trend for higher levels in the

inner estuary compared with the middle and outer estuary, however this gradient was confounded by the likely difference in species composition between these areas. Finally, levels of Cu, Pb and Zn were highest in the 2001/02 Reporting period compared with the earlier reporting periods.

Polymesoda coaxans

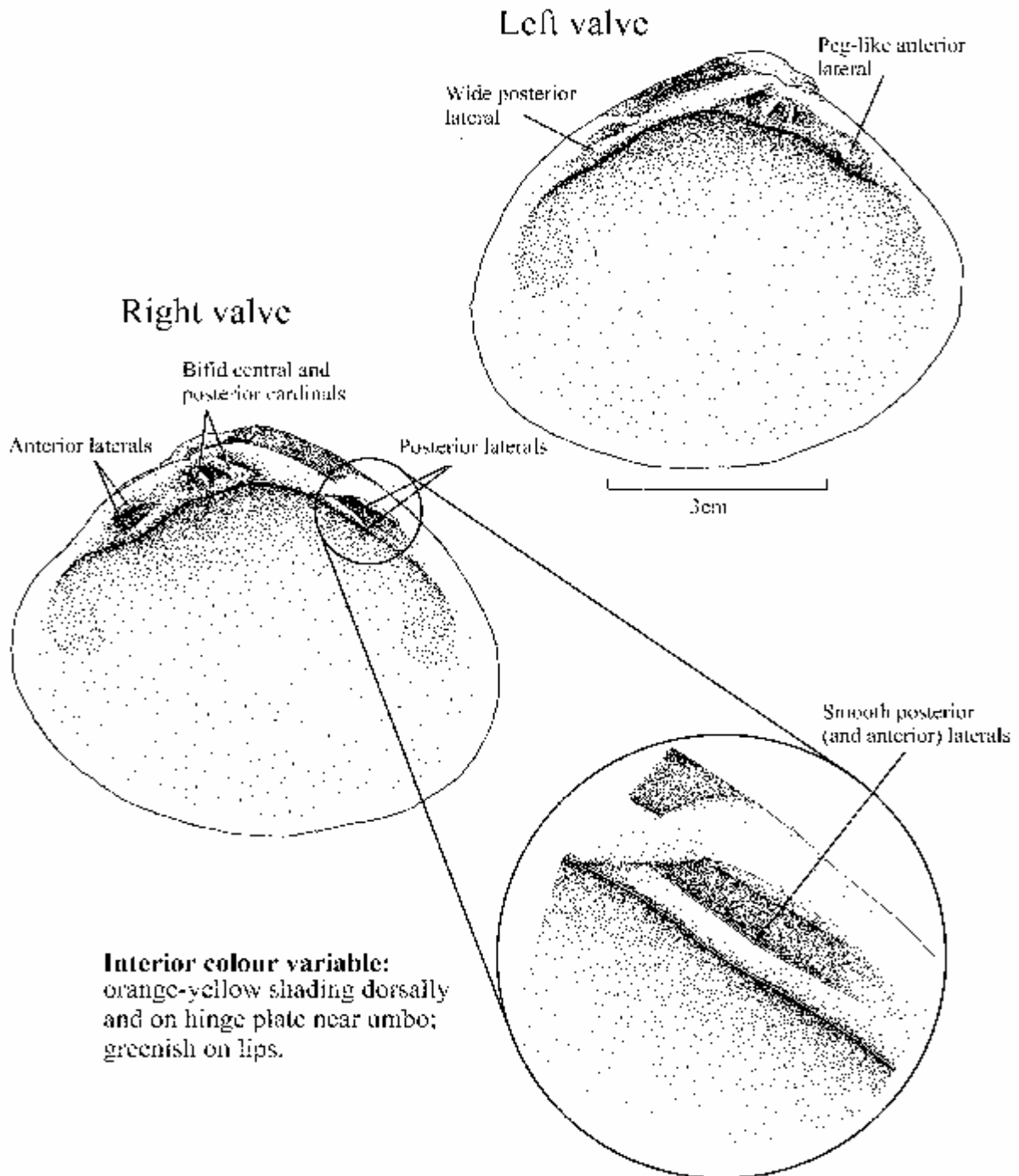


Figure 6. Morphological characteristics of *Polymesoda coaxans*, illustrating key features to use to differentiate this species from *Batissa violacea*.

Batissa violacea

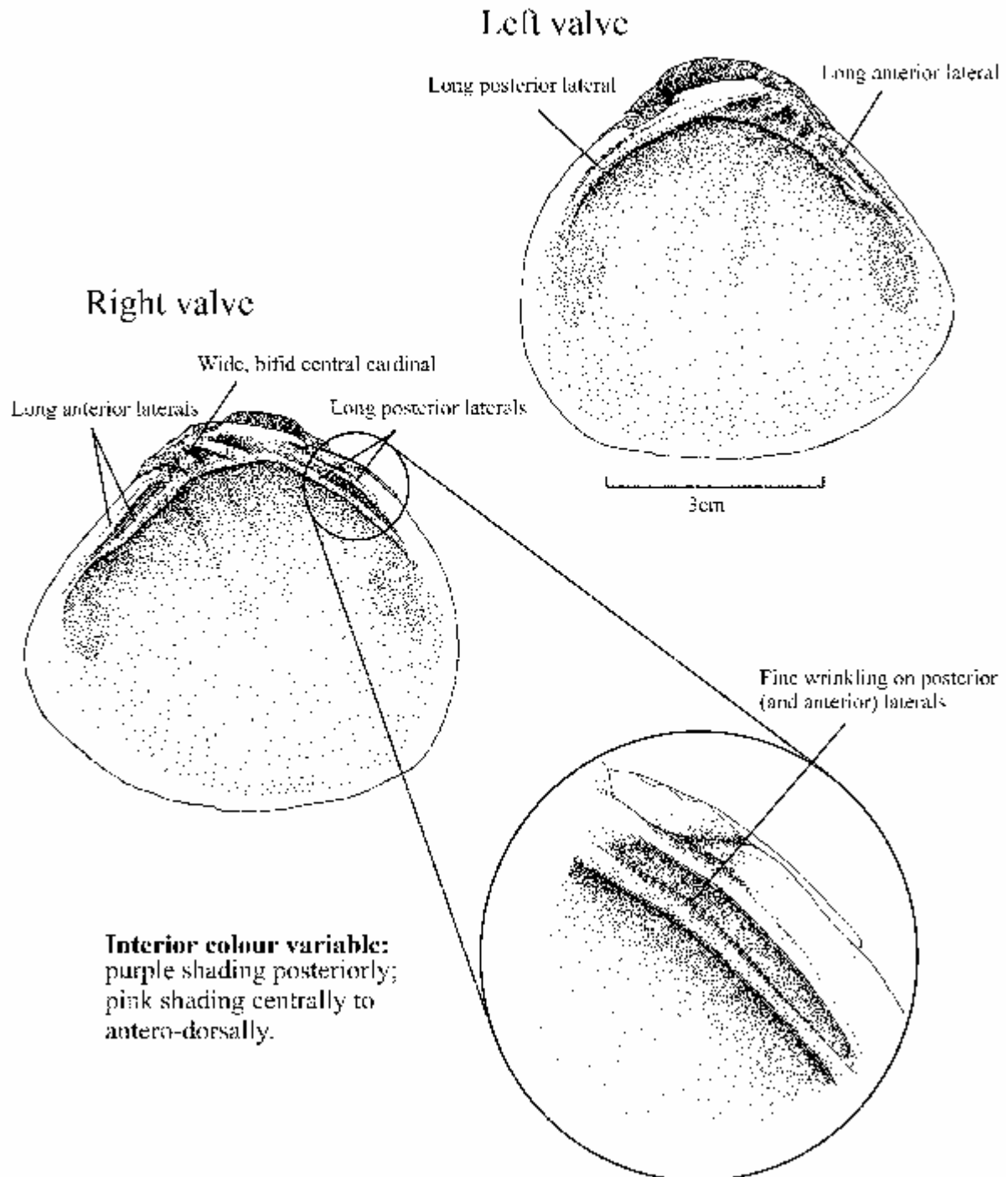


Figure 7. Morphological characteristics of *Batissa violacea*, illustrating key features to use to differentiate this species from *Polymesoda coxans*.

This pattern of a gradient of declining metal concentrations in mud clams, particularly Cu, with increasing distance from the head of the estuary was consistent with patterns of dissolved and particulate Cu in the estuary. Dissolved Cu concentrations show a rapid decrease as Fly River water elevated in dCu meets the estuary. This is due to dCu being absorbed onto clean particulates in the fluidised mud bath. After this rapid decline in dCu concentration, there is then a gradual decline to background levels towards the mouth of the estuary, reflecting conservative dilution by sea water on a salinity gradient along the long axis of the estuary. Particulate copper demonstrates a slightly different pattern. There is rapid dilution of pCu to background levels in the turbidity maxima zone, in the inner estuary, as the riverine sediment elevated in pCu is mixed with the estuarine mud (OTML, 1995b). Beyond the inner estuary, there is no detectable mine signature in the particulate phase.

OTML (1997) noted the same pattern described here of elevated Cu in the inner estuary but not at outer estuarine sites, and suggested that the diluting abilities of the fluidised mud bath currently exceed the inputs from the Fly River. It was recommended that monitoring should continue to look for any increases in Cu concentrations in mud clams in the middle and outer estuary which may indicate that the buffering capacity of the mud bath is being exceeded. OTML (1997) also noted that the current distribution of sites for mud clam collection was biased towards the outer Fly estuary, with few sites in the middle and inner estuary, and recommended that additional sites be established in the middle Fly estuary as an early warning monitor of increases that may suggest that the buffering capacity of the fluidised mud bath is being exceeded.

Analysis of the current data are still restricted by the under-representation of sampling sites in the inner and middle estuary, with sampling still biased towards the outer estuary. Even so, data show a statistically significant increase in metal levels in the most recent data compared with earlier reporting periods, and there is also an increase in metal levels at inner and middle Fly River estuary sites in the most recent data (ref. Figure 4), with levels at the outer estuary sites still relatively low.

Assuming the data set and analyses are not confounded by taxonomic differences, and that there has been no change to the analytical technique, then it would appear that in recent years there has been a significant increase in metals levels in mud clams at estuarine sites, with these increases greatest at inner and middle estuarine locations. This is cause for concern, as it suggests an increased influence of mine waste on the estuary. It is recommended that monitoring be continued, with all sites re-sampled in the 2003/04 Reporting period. These data should be analysed to assess whether the same pattern as seen in this report still exist (i.e. 2003/04 data elevated compared with earlier samples), or if the trend has worsened (i.e. mud clam metal levels in the 2003/04 reporting period are higher than 2001/02) or if the current trend was an anomaly (i.e. levels in 2003/04 are lower than the 2001/02 data). If the same trend exists, then the rate of increase should be calculated to determine at what point the relevant health guidelines (WHO, 1993 & ANZFA, 1996, 2000) will be exceeded. Currently there are insufficient data to confidently model the trend of increasing concentrations with time.

ESTIMATED DIETARY INTAKE OF COPPER BY HUMANS

Cu, Cd and Pb all demonstrated higher levels in mud clams from the Fly estuary compared with control sites indicating a possible mine-related signature. As a worst case scenario, the dietary intake of metals by villagers living at these locations and consuming mud clams was estimated, using samples which included gut contents and had the highest mean concentrations. Calculations demonstrated that using the maximum mean concentrations of Cu, Cd and Pb in mud clams, they formed approximately 4%, 19% and 5% of the respective recommended Provisional Maximum Tolerable Daily Intake. In all instances, even for the worst case scenario, concentrations did not exceed the PMTDI threshold and therefore did not pose an immediate health threat. Of the three metals, Cd posed the highest risk, comprising 19% of the PMTDI.

Metal concentrations in mud clams with gut contents were also compared against the ANZFA (1996) and ANZFA (2000) guidelines for maximum levels of metal contamination in food (molluscs). There were only two instances where the guidelines were exceeded, and these were for Cu at the inner Fly Estuary locations of EST 28 and EST29.

The literature reports instances of metals in bivalves exceeding health standards. For example, Han *et al.* (1994) recorded a mean concentration of Cu in oysters from the coast of Taiwan of 2194 ± 212 $\mu\text{g/g}$. The average daily intake of oysters by the population was estimated at 4 g/day (compared to 16 g/day of mud clams in present study) and Han *et al.* (*op. cit.*) estimated that the daily intake of Cu was up to 14 times the recommended maximum for people ingesting the oysters. Extreme values of 4401 ± 79 $\mu\text{g/g}$ were recorded from oysters from the Charting coastal area of Taiwan in 1986 when the flesh of the oysters was observed to be green in colour from Cu contamination! Oysters are recognised as particularly good bioaccumulators of trace metals. In comparison to levels in oysters, Han *et al.* (*op. cit.*), reported other bivalves from the same location exhibiting much lower Cu levels (clams, 39.3 ± 24.9 $\mu\text{g/g}$; blue mussels, 24.5 ± 4.61 $\mu\text{g/g}$; and mollusca (*Anadara subcrenata*) 3.95 ± 0.93 $\mu\text{g/g}$ Cu). Cu levels in mud clams from the inner Fly estuary were intermediate between the oysters and other bivalves collected along the coast of Taiwan, suggesting that mud clams in the Fly Estuary may be moderate bioaccumulators of Cu.

This report highlights a need to revise the approach for assessing metal concentrations in biota (estuarine and freshwater) against relevant guidelines, as per Regime Condition 1b – Fish Edibility. A range of guidelines are available, which are continually being revised and updated. The PMTDI approach is based on WHO (1993) guidelines, which are now 10 years old and may have been revised. This needs to be determined. Also, the PMTDI assumes intake of a metal from all sources. Analyses by OTML only consider intake from one food source (fish), and will therefore only indicate a proportion of risk (i.e. is intake from fish approaching the threshold) and not total risk. Condition 1b of the Regime stipulates use of the ANZFA (1996) guidelines, however, these have been superseded by the ANZFA (2000) guidelines. Adoption of the revised guidelines would normally be recommended, however, ANZFA (2000) no longer includes guidelines for Cu or Zn as these metals are no longer considered a health risk as food contaminants. This issue needs to be resolved.

Finally, the method of calculating the appropriate metal concentration to compare against the relevant guideline needs further consideration. The approach to date has been to take the mean concentration of each metal at a site and compare this value against the guideline. There is a rationale for using the median concentration, as this avoids the overbearing influence of

one or several exceptionally high or low ‘outlier’ values when calculating the mean for a site. The past use of the mean for a site was justified as it represents a mixed diet of individual fish/shell fish. However, an alternative argument is that the concentration within individual specimens should be compared against the guideline, and the rate of exceedance of the critical value reported. These issues need to be resolved and a standard protocol developed.

RECOMMENDATIONS

- Future samples, and all archived shells need to be examined and identified to species level to remove confounding effects of species differences in metal accumulation. The database should be modified to incorporate a “species” field to allow metals data to be recorded under the appropriate species.
- Currently the distribution of sites for mud clam collection is biased towards the outer Fly estuary. It is recommended that additional sites are established in the inner and middle Fly estuary to provide better coverage of the system and facilitate a more comprehensive statistical analysis.
- Sampling should be repeated in the 2003/04 Reporting period and data analysed to look for any further increases in Cu concentrations in mud clams in the inner, middle and outer estuary which may indicate that the buffering capacity of the mud bath is being exceeded.
- In all future sampling, mud clams should continue to be voided in ‘clean’ sea water for 48 hrs before processing to minimise variability in metal determinations due to gut contents. However, samples with gut contents also should be collected to demonstrate human exposure to metals ingested as part of the diet.
- Following re-sampling of estuarine sites in FY04, and if the trend of increasing concentrations still exists, then the rate of increase should be calculated to determine at what point the relevant health guidelines will be exceeded.
- The method for comparing tissue metal levels in estuarine and freshwater biota should be revised, to standardise the guidelines against which comparisons should be made (i.e. WHO, 1993; ANZFA, 1996; ANZFA, 2000), and the method for comparison (i.e. site mean, site median or individual specimens).

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APPENDICES

APPENDIX 1. MEAN METAL CONCENTRATIONS

Appendix 1. Mean (+ 1 SE) concentration of each metal at each site, by reporting period and gut status (WG = with guts; WO = without guts).

Reporting period	Gut status	Site	n	Cu (\pm 1 SE) (mg/kg dry wt)	Cd (\pm 1 SE) (mg/kg dry wt)	Pb (\pm 1 SE) (mg/kg dry wt)	Zn (\pm 1 SE) (mg/kg dry wt)
1993/94	WG	BIN01	10	42.72 (6.84)	0.43 (0.09)	0.98 (0.18)	163.48 (18.74)
1993/94	WG	BMU01	10	34.02 (3.82)	3.4 (0.61)	0.73 (0.18)	391.92 (61.6)
1993/94	WG	BMU03	10	18.5 (1.52)	1.18 (0.33)	0.59 (0.11)	105.12 (12.03)
1993/94	WG	EST09	10	65.76 (7.66)	5.5 (1.02)	1.66 (0.33)	280.83 (47.58)
1993/94	WG	EST18	10	25.06 (3.49)	1.53 (1.15)	1.7 (0.8)	208.42 (30.5)
1993/94	WG	EST19	10	19.98 (2.16)	2.53 (0.51)	1.81 (0.72)	153.53 (15.32)
1993/94	WG	EST22	10	44.96 (8.06)	1.57 (0.23)	2.45 (0.67)	176.84 (23.4)
1993/94	WG	EST26	10	43.41 (5.58)	3.58 (0.41)	2.81 (0.77)	169.37 (19.89)
1993/94	WG	EST28	10	313.16 (40.64)	11.11 (1.34)	6.55 (1.14)	657.27 (78.25)
1993/94	WG	EST29	10	115.25 (30.76)	4.68 (0.97)	2.57 (0.65)	1036.31 (156.96)
1993/94	WG	ORI01	10	62.29 (8.94)	1.29 (0.28)	2.18 (0.46)	237.37 (43.47)
1993/94	WG	PAH01	10	48.14 (7.57)	0.62 (0.13)	2.12 (0.43)	321.06 (51.71)
1994/95	WG	BMU01	10	20.1 (1.57)	2.02 (0.42)	0.96 (0.35)	106.5 (27.21)
1994/95	WG	BMU03	9	18 (1.75)	3.37 (0.42)	1.1 (0.1)	118 (14.23)
1994/95	WG	EST18	10	17.8 (2.51)	0.43 (0.09)	2.24 (1.01)	231.7 (45.57)
1994/95	WG	EST19	10	23.7 (1.84)	3.79 (0.61)	2.14 (0.6)	88.9 (14.42)
1994/95	WG	EST22	10	42 (4.71)	4.27 (0.84)	2.25 (0.52)	130.3 (14.3)
1994/95	WG	EST29	10	147.9 (23.44)	3.38 (0.37)	1 (0.33)	233.6 (19.05)
1994/95	WG	EST30	10	96.6 (21.53)	10.27 (1.58)	3.76 (1.39)	220.4 (19.71)
1994/95	WG	ORI01	10	23.7 (2.86)	0.99 (0.28)	1.12 (0.15)	125.8 (40.3)
1994/95	WG	PAH01	10	33.1 (3.69)	2.56 (0.65)	1.15 (0.36)	129.1 (24.48)
1994/95	WO	BMU01	10	19.9 (1.62)	2.65 (0.48)	0.2 (0.03)	109.4 (14.61)
1994/95	WO	BMU03	10	15.1 (0.99)	2.94 (0.28)	0.42 (0.08)	102.3 (9.16)
1994/95	WO	EST18	10	16.1 (1.09)	0.44 (0.17)	2.02 (0.64)	294.2 (49.21)
1994/95	WO	EST19	10	25.3 (3.09)	2.71 (0.31)	2.47 (0.37)	86.5 (9.59)
1994/95	WO	EST22	10	40.9 (6.42)	3.18 (0.45)	0.84 (0.24)	115.9 (23.61)
1994/95	WO	EST29	10	122.6 (17.07)	2.13 (0.27)	0.1 (0.1)	152.4 (22.32)
1994/95	WO	EST30	10	36.3 (5.32)	2.48 (0.23)	0.78 (0.15)	183.9 (23.82)
1994/95	WO	ORI01	10	16.9 (2.11)	0.46 (0.09)	0.94 (0.32)	123.9 (18.79)
1994/95	WO	PAH01	10	22.9 (3.3)	1.65 (0.27)	0.87 (0.44)	95.5 (7.62)
1998/99	WG	BIN01	10	19.8 (2.21)	1.41 (0.18)	2.15 (0.24)	450.1 (159.79)
1998/99	WG	EST09	10	26.5 (2.37)	3.37 (0.37)	1.65 (0.22)	231.4 (23.88)
1998/99	WG	EST18	9	29.56 (5.17)	1.14 (0.32)	2.09 (0.37)	194.44 (25.8)
1998/99	WG	EST19	9	25.44 (1.64)	2.98 (0.21)	4.22 (0.44)	135.44 (12.64)
1998/99	WG	EST22	10	40.7 (3.59)	2.96 (0.34)	2.9 (0.48)	276.7 (33.79)
1998/99	WG	EST24	10	19.22 (2.37)	0.72 (0.08)	1.53 (0.26)	93 (6.74)
1998/99	WG	EST26	9	41.89 (4.9)	6.5 (0.73)	3.48 (0.36)	303.22 (39.71)
1998/99	WG	EST28	10	71.7 (6.71)	2.45 (0.17)	2.12 (0.31)	433.1 (24.56)
1998/99	WG	EST29	5	125.4 (46.08)	2.92 (0.22)	2.64 (0.51)	306.6 (33.56)
1998/99	WG	ORI01	10	17.9 (1.37)	0.36 (0.06)	0.91 (0.15)	205.5 (54.91)
1998/99	WG	PAH01	8	41 (6.25)	4.43 (1.07)	2.32 (0.24)	234.63 (37.86)
1998/99	WO	BIN01	10	33.8 (7.46)	1.5 (0.21)	1.89 (0.29)	189.3 (26.09)
1998/99	WO	EST09	10	23 (2.05)	3.79 (0.36)	1.57 (0.26)	277.1 (34.87)
1998/99	WO	EST18	9	24.78 (3.09)	1.6 (0.27)	2.83 (0.4)	210 (39.64)
1998/99	WO	EST19	9	10.98 (2.58)	1.76 (0.5)	2.44 (0.88)	129.33 (17.25)
1998/99	WO	EST22	10	39.8 (9.54)	2.27 (0.16)	2.07 (0.51)	229.2 (40.94)

Reporting period	Gut status	Site	n	Cu (\pm 1 SE) (mg/kg dry wt)	Cd (\pm 1 SE) (mg/kg dry wt)	Pb (\pm 1 SE) (mg/kg dry wt)	Zn (\pm 1 SE) (mg/kg dry wt)
1998/99	WO	EST24	10	18 (2.22)	0.81 (0.17)	2.88 (1.16)	155.7 (28.15)
1998/99	WO	EST26	10	26.4 (3.3)	3.48 (0.32)	1.38 (0.13)	179.1 (14.69)
1998/99	WO	EST28	8	135.5 (18.78)	3.2 (0.3)	1.06 (0.31)	299.38 (31.69)
1998/99	WO	EST29	6	146.5 (30.89)	3.63 (0.53)	1.64 (1.21)	226.67 (43.47)
1998/99	WO	ORI01	10	21.4 (2.19)	0.55 (0.07)	1.17 (0.27)	208 (39.23)
1998/99	WO	PAH01	10	26.3 (2.57)	2.06 (0.66)	1.61 (0.22)	181.7 (29.87)
2000/01	WG	EST22	10	15.66 (1.9)	0.95 (0.14)	0.48 (0.12)	115 (9.51)
2000/01	WG	EST24	10	19.6 (3.09)	0.26 (0.06)	1.84 (0.94)	322.5 (80.4)
2000/01	WG	EST26	10	48.4 (5.01)	4.34 (0.64)	1.18 (0.65)	349.3 (55.69)
2000/01	WG	ORI01	10	19.21 (4.29)	0.4 (0.06)	0.99 (0.32)	393.8 (87.82)
2000/01	WO	EST22	10	26.3 (2.31)	1.18 (0.24)	0.33 (0.13)	152 (13.45)
2000/01	WO	EST24	7	20.32 (7.23)	2.36 (0.71)	0.72 (0.46)	296 (72.74)
2000/01	WO	EST26	10	31.7 (5.85)	0.19 (0.06)	1.15 (0.55)	232.9 (68.93)
2000/01	WO	ORI01	10	9.27 (0.94)	0.21 (0.04)	0.52 (0.14)	216 (44.74)
2001/02	WG	BIN01	10	17.7 (1.28)	1.03 (0.15)	1.27 (0.19)	186.1 (51.32)
2001/02	WG	EST09	10	35.1 (4.43)	4.09 (0.55)	3.15 (0.23)	592 (87.16)
2001/02	WG	EST18	10	28.7 (2.7)	0.97 (0.11)	1.82 (0.35)	212.4 (33.32)
2001/02	WG	EST19	10	24.1 (2.66)	1.49 (0.23)	2.24 (0.29)	208 (34.79)
2001/02	WG	EST22	10	25.8 (4.35)	1.63 (0.28)	2.66 (0.67)	434.5 (116.43)
2001/02	WG	EST28	10	452.1 (44.25)	3.62 (0.49)	3.14 (0.51)	624.8 (49.32)
2001/02	WG	EST29	8	138 (28.81)	4.16 (0.35)	7.18 (1.32)	628.5 (108.87)
2001/02	WG	ORI01	10	20.64 (2.93)	0.5 (0.08)	1.44 (0.26)	331.7 (80.78)
2001/02	WG	PAH01	10	25.4 (2.92)	0.78 (0.11)	0.8 (0.13)	128.5 (9.63)
2001/02	WO	BIN01	10	14.7 (1.09)	1.13 (0.18)	1.4 (0.27)	247.8 (57.19)
2001/02	WO	EST09	10	39.08 (5.02)	3.84 (0.28)	1.88 (0.25)	652 (120.79)
2001/02	WO	EST18	10	27.6 (3.21)	1.33 (0.15)	1.69 (0.21)	257.8 (29.94)
2001/02	WO	EST19	10	22.8 (1.27)	1.23 (0.06)	1.08 (0.11)	186.2 (33.75)
2001/02	WO	EST22	10	28.4 (2.33)	1.47 (0.13)	2.03 (0.24)	262.4 (58)
2001/02	WO	EST28	10	412.5 (34.11)	4.28 (0.28)	2.97 (1.22)	669.3 (57.23)
2001/02	WO	EST30	10	70.1 (8.19)	4.07 (0.45)	3.94 (0.77)	276.7 (43.92)
2001/02	WO	ORI01	10	21.4 (2.21)	1.29 (0.78)	21.9 (4.42)	238.6 (47.43)
2001/02	WO	PAH01	10	26.8 (2.3)	0.92 (0.18)	1.12 (0.16)	187 (19.61)