REPORT SERIES 5b

Heavy Metals in Commercial Prawn and Crayfish Species in the Torres Strait

GREAT BARRIER REEF
MARINE PARK AUTHORITY

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Heavy Metals in Commercial Prawn and Crayfish Species in Torres Strait

Prepared by

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for the
Great Barrier Reef Marine Park Authority

1997

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SUMMARY

- The Torres Strait Baseline Study comprised four component programmes, each addressing a specific important aspect of the Torres Strait marine environment. This document reports results of the Commercial Fisheries component. Results of the other three components, Community Fisheries, Marine Sediments and Indicator Organisms, are reported separately in Dight and Gladstone (1993) and Gladstone (1996).

- This report provides information on the following heavy metals in commercial species of prawns and crayfish in the Torres Strait: silver, aluminium, arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, nickel, lead, selenium, strontium, uranium and zinc.

PRAWNS

- Three species of prawns were studied: *Penaeus esculentus* (brown tiger prawn) and *Metapenaeus endeavouri* (Endeavour prawn), which together comprise around 90% of prawns landed in Torres Strait and *Penaeus longistylus* (red spot king prawn) which makes up the remainder.

- Prawns were collected from two main sites in June and October/November 1992.

- Generally, metal levels reported were either significantly less than those reported previously from polluted environments, or additionally, in agreement with or less than those from representative unpolluted areas, including previous studies in Torres Strait.

- This was not the case for cadmium. Cadmium levels in the present study were comparable to those reported from elsewhere in Australia and a polluted coastal site in the Arabian Sea and higher than those reported from some unpolluted sites.

- While this indicates a high cadmium bioavailability in Torres Strait, it is clearly not due to pollution. Cadmium is not a metal associated with Fly River runoff but rather a naturally occurring element in marine carbonate sediments. Such sediments are prevalent in Torres Strait.

Factors affecting metal levels in prawns

- Prawn heads contained highest levels of most metals measured. This was not surprising, as the head contains the hepatopancreas, an organ known to be an important metal storage site in decapod crustaceans.

- Tail flesh contained among the lowest concentrations of all metals except arsenic and mercury. Thus, except in the case of these two metals, tail flesh does not contain important metal storage sites and is not a good indicator tissue for metal monitoring.

- While not a good indicator tissue, tail flesh is the most important tissue with respect to trade and human health. This is because it is the tissue that most people eat and thus it is generally accepted as the tissue to which trade and health limits apply. This study examined the influence of various 'manageable' factors on the levels of metals in this tissue.

- Most results of this study support the expectation that prawns regulate essential elements and accumulate non-essential elements.

- Two exceptions were copper and zinc, which are essential metals associated with Fly River runoff and thus expected to occur in higher concentrations in sediments at the northern site. Prawns from the northern site contained elevated levels of these two metals in head tissues (the principal storage tissue), while levels of these metals in tail flesh remained constant.

- Seasonal variation (between pre-and post- monsoon) in metal content of prawn tail flesh was minimal. However, the monsoon season encountered during this study was abnormal, with lower than average rainfall.

- There was no difference in tail flesh metal levels between small and medium sized prawns. However, large prawn tails contain slightly higher levels of cadmium and mercury (both non-essential elements).

- There were no substantial differences in tail flesh metal levels between sexes or prawns from different moult stages.

- *M. endeavouri* tail flesh contained higher levels of cadmium, zinc, mercury and arsenic when compared with the other two species.

- Metal levels in tail flesh were measured for prawns which were handled according to treatments approximating various industry practices. Levels of copper, strontium, iron and cadmium were elevated in at least one of these 'industry' treatments.

- It is suggested that this elevation is due to leakage of high level tissues in the head (e.g. the hepatopancreas) into lower level tail flesh. The hepatopancreas readily breaks down and turns to 'mush' after prawn death and particularly after freezing and thawing. It is noted that head tissues of *M. endeavouri* are particularly fragile and that head 'leakage' may account for elevated levels of some metals in tail flesh of that species.

Metal levels in prawns with respect to trade and health

- Concentrations of most metals in prawns reported in the is study complied with trade and health limits. This i true regardless of whether whole prawn or tail flesh is considered (ie taken as the 'edible portion')

- One exception was cadmium. Average concentrations of cadmium in tail flesh were close to the Maximum Permissible Concentration (MPC) of 0.2 mg/kg, while some samples analysed exceeded it. Sustained consumption of prawn tails does not appear to present a health risk with respect to the World Health Organisation's Provisional Tolerable Weekly Intake (PTWI) for cadmium. However a recent medical review indicates that the current PTWI for cadmium may be too high.

- Given the close relationship between cadmium levels in tail flesh and the MPC and the ambiguity over safe consumption limits, any factor which influences cadmium levels in this tissue, even slightly, is important.

- Results of this study show that such minor elevation of cadmium in prawn tail flesh occurs with size, after some forms of industry processing and that *M. endeavouri* tails generally contain higher levels.

- If whole prawn is taken as the 'edible portion' for the context of legal limits, both cadmium and copper concentrations exceeded the MPC.

- With respect to health limits however, copper load in 10 whole prawns would contribute less than 5% towards the PTWI.

- This is not the case for cadmium. A single whole prawn may contribute 20-25% of the PTWI, and thus regular consumption of many whole prawns may constitute a health risk depending on how much cadmium consumed is bioavailable in the human gut.

Recommendations for further research

- It is recommended that further work be undertaken on the potential for handling methods to influence tail flesh levels of cadmium.

- It is also recommended that clarification of safe sustainable cadmium intake levels be sought from the medical community.

- It is further recommended that research be undertaken on the bioavailability to humans of cadmium in whole prawns.

CRAYFISH

- This study reports levels of metals in the Tropical Rock lobster *Panulirus ornatus* in Torres Strait. The commercial crayfish catch is almost exclusively made up of this species.

- Crayfish were collected from three reefs in June and October/November 1992.

- Levels of most metals reported here agree with those reported for the same species in the Community Fisheries component of the Torres Strait Baseline Study (Gladstone 1995). They also generally agree with levels found in comparable tissues in Torres Strait prawns, as reported in this study, with some exceptions.

- Arsenic occurred in higher concentrations in crayfish tail flesh when compared to prawn tail flesh.

- Copper also occurred in higher concentrations in crayfish tail flesh when compared to prawn tail flesh. This may be due to more residual haemocyanin in crayfish tails, as they have a larger volume:surface area ratio. Haemocyanin is a copper based blood pigment common to all crustaceans.

- Cadmium levels were much lower in crayfish tails when compared to prawn tails. This may be because crayfish heads are removed immediately after capture and prior to freezing, hence eliminating the potential for 'leakage' of high level head tissues.

- The scope of this study was restricted to metal levels in crayfish tails only, as commercial product consisted almost exclusively of frozen tails at the time. Since then a market in whole live animals has developed, and hence levels in crayfish heads have also become relevant with respect to commercial product. See Gladstone 1996 for a discussion on metal levels in crayfish heads.

Factors affecting metal levels in crayfish

- Crayfish tails from a site within Fly River influence (Kakope Reef) did not contain elevated levels of metals, when compared with other sites.

- Crayfish tails from Dungeness Reef contained higher levels of iron and cadmium, when compared with crayfish from the Cape York area. While iron is a metal associated with the Fly River, Dungeness Reef is outside its expected zone of influence. Cadmium is not a metal associated with the Fly River but is known to be associated with carbonate sediments of marine origin. This sediment type is probably more prevalent at Dungeness Reef than at reefs near Cape York.

- Samples collected from Dungeness Reef in June 1992 contained the highest copper levels recorded in this study. It is unlikely that this trend reflects environmental gradient in copper availability, because copper is an essential element which is regulated by decapod crustaceans, at least in tail flesh.

- There were no significant differences in levels of any metal between sexes.

- Levels of arsenic, cadmium, iron, manganese and nickel were higher in smaller crayfish tails, possibly due to the dilution effect of rapid growth. Zinc was higher in larger crayfish tails, possibly due to higher excretion rates in smaller crayfish.

- Crayfish tails collected from an industry source contained elevated levels of lead, probably due to contact with petrol and oil residues in the fishing boat and during processing. This elevation was not sufficient to cause concerns with respect to trade and health, as levels were still well below respective limits.

Metal levels in crayfish with respect to trade and health

- Tail flesh levels of all metals for which there is an MPC, with the exception of arsenic and copper, easily conformed to it. With respect to health limits, levels of all metals including arsenic and copper were such that crayfish tails could be safely sustainably consumed.

- When total arsenic was converted to inorganic arsenic only (which is the component to which the MPC applies), levels in some samples approached the MPC, while only three individuals exceeded it.

- This indicates there may be only a small margin of safety between the MPC and arsenic in crayfish tails. The conversion factor used was based on a 'worst case scenario', thus the result is pessimistically biased.

- Copper levels in some crayfish samples also approached the MPC and three individuals (independent of those that exceeded the arsenic MPC) exceeded it.

- This indicates there may be only a small margin of safety between the MPC and copper in crayfish tails. The source of this copper is likely to be residual haemocyanin and not environmental.

- Cadmium levels in crayfish tails were consistently well below the MPC.

Recommendations for further research

- Further work is recommended to clarify levels of inorganic arsenic in crayfish tails. Inorganic arsenic should be measured directly, rather than relying on conversion estimates based on total arsenic.

- Further work is also recommended to identify factors which influence residual copper levels in crayfish tails. Since it is likely that copper exists as residual haemocyanin, emphasis should be placed on handling methods which promote its removal (e.g. rinsing methods).

- Further work should examine the impact of including head tissues on total metal concentrations in crayfish with respect to trade and health limits. The potential for head tissues to contaminate tail flesh should be examined.

INTRODUCTION

The Torres Strait Baseline Study (TSBS) was instigated in response to concerns that discharge from mining developments in Papua New Guinea was impacting levels of heavy metals in the Torres Strait marine environment. Besides providing an assessment of levels of metals present, an overriding objective of the TSBS was to provide sound baseline data as a foundation for ongoing monitoring. More specific objectives of the TSBS scientific program are detailed in Dight (1991). The TSBS comprised four component programmes, each addressing metal levels in a discrete and important aspect of the Torres Strait marine environment. The results of three of these (Community Fisheries, Marine Sediments, Indicator Organisms) were reported in Dight and Gladstone (1993) and Gladstone (1996). This report presents the results of the fourth component - Torres Strait commercial fisheries.

Prawns and crayfish are the two most important fisheries in Torres Strait, and hence are the focus of this study. Of the $20–30 M worth of fishery products harvested from the region annually, the prawn catch accounts for $17–20 M and is caught by commercial trawlers which are based at ports outside of Torres Strait. The crayfishery is worth $4–5 M annually (Elmer and Coles 1991), but is socially and economically more important to the region as most of this is caught by locally based commercial or community fishermen. Both fisheries are economically important on a national scale, as much of the product is exported. Other commercial fishery species in the area include mackerel, barramundi, reef fish and mud crabs. Due to the lower economic and socio-economic importance to the region of these minor fisheries however, they are excluded from the present study. Some of these species were included in the community fishery component of the TSBS (Dight and Gladstone 1993, Gladstone 1996).

A pre-requisite of any investigation into metal levels in organisms is a consideration of how those organisms deal with metals. Levels of heavy metals observed are the net result of that organism's accumulation strategy - that is, the difference between rates of uptake and storage, and excretion (Depledge and Rainbow 1990, Rainbow 1990, Rainbow 1991). Uptake can either be passive, whereby the rate of uptake is governed by the bioavailability of the dissolved metal in the environment, or via active transport pumps (Rainbow 1991, Depledge and Rainbow 1990, Rainbow et al 1990).

Iron, zinc, selenium, copper, manganese, nickel, cobalt, chromium and possibly arsenic, are all metals thought to be essential in at least trace concentrations to at least some organisms (Rainbow and Furness 1990). Decapod crustaceans are generally thought to regulate the concentration of essential elements in a reserve 'pool', available for metabolic requirements and accumulate the non essential ones (Rainbow 1990, Rainbow et al 1990). Regulation of zinc, copper, manganese and iron, and accumulation of cadmium, has been demonstrated or supported by many workers with evidence of either strategies observed in laboratory studies, or comparative studies of organisms collected from a range of sites of suspected differing metal bioavailabilities (Bryan 1968, White and Rainbow 1982, 1987, Nugegoda and Rainbow 1988, Martin 1974, Ray 1984, Ieely and Nott 1980, Rainbow 1985, Darmono 1990). However, all metals, including the essential ones, are toxic beyond a threshold concentration. To avoid toxic effects, excess levels of these elements must be bound in metabolically inert complexes such as metallothieneins or other high metal affinity ligands, for either long-term storage or excretion (Depledge and Rainbow 1990, Engel 1987, Rainbow 1991, Rainbow et al 1990).

The Torres Strait Baseline Study's pilot results (Dight and Gladstone 1993, Gladstone 1996) indicate that the Fly River is an important source of aluminium, arsenic, cobalt, chromium, copper, iron, mercury, manganese, nickel, lead and zinc in Torres Strait surface sediments.

Sediment sampling sites close to the Papua New Guinea mainland contain higher levels of these metals and were characterised by a higher proportion of fine organic carbon sediment of terrigenous origin. Conversely, cadmium and selenium appear to not be associated with Fly River runoff. Cadmium was associated with carbonate sediments of marine origin, while there was no particular association with sediment type in selenium levels.

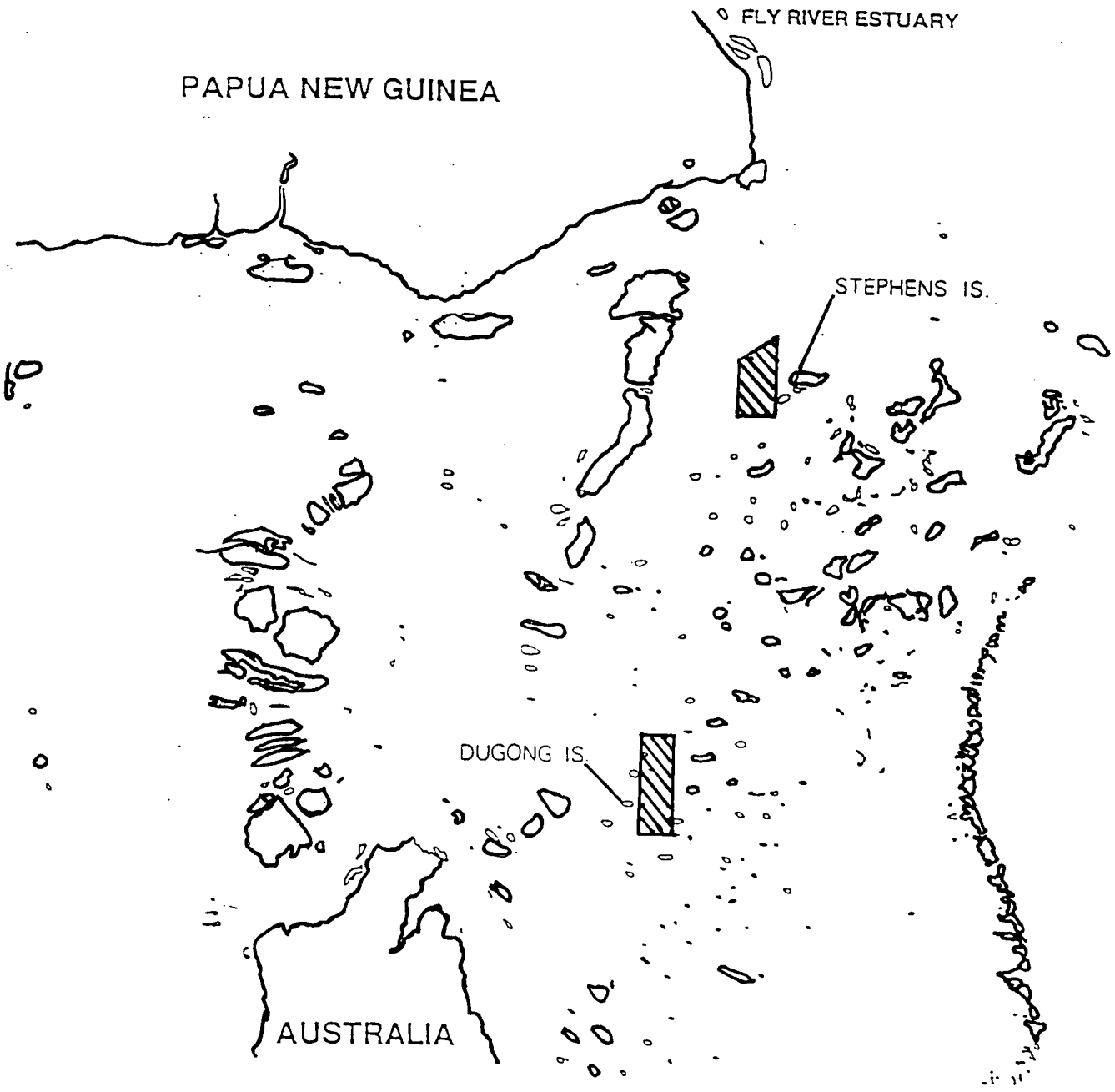
Gladstone (1996) concluded that Fly River sediments extend southward into Torres Strait to a line between latitude 9°12'S and 9°08'S, although the influence of smaller coastal rivers to the west may extend further south and occasional events of more extensive intrusion may occur.

This study approaches the issue of metal levels in prawns and crayfish in Torres Strait from two angles. Firstly, under the umbrella of the TSBS, a major objective was to quantify levels of trace metals in commercially important prawn and crayfish species in Torres Strait. In this regard, an aim was to understand the influence of environmental and biotic factors on metal levels. Secondly, in response to previous evidence of unacceptably elevated levels of some metals in one prawn species in Torres Strait (Mariarth 1991, Schneider 1990), a further objective was to assess metal levels with respect to health and trade issues. Hence, some factors investigated were chosen for their accessibility to management control and an emphasis was placed on edible tissues.

PART 1

METAL LEVELS IN TORRES STRAIT COMMERCIAL PRAWN SPECIES

Figure 1 Map of Torres Strait, showing prawn sampling sites (shaded)



METAL LEVELS IN TORRES STRAIT COMMERCIAL PRAWN SPECIES

1.1 Background

The Torres Strait commercial prawn catch is primarily made up of three species. *Penaeus esculentus* (the brown tiger prawn) and *Metapenaeus endeavouri* (the endeavour prawn) together comprise around 90% of prawns landed and *Penaeus longistylus* (the red spot king prawn) makes up the remainder (Watson et al 1990). As a result of permanent and seasonal closures, the commercial prawn grounds of Torres Strait are essentially confined to the Great North-East channel area east of Warrior Reefs (Elmer and Coles 1991, Watson and Mellors 1990). The present study examines metal levels in all three prawn species and sampling occurred within the commercial prawn grounds.

Life history details of prawns in Torres Strait have been published only for *P. esculentus* (Watson et al 1990). Spawning occurs all year round, with up to three peaks and the extensive seagrass beds on Warrior Reef form a major larval settlement and juvenile nursery ground for much of the Torres Strait fishing grounds (Blythe et al 1990, Turnbull and Mellors 1990, Turnbull 1991). Some prawns recruit directly from here into the fishery to the east, while the rest migrate to the west where they remain for several months before emigrating east and into the fishery. Thus prawns recruit into the fishery at a range of ages and sizes (Blythe et al 1990, Turnbull and Mellors 1990, Turnbull 1991). Further south, fishery recruits probably originate from alternative coastal nursery grounds (Rob Coles, pers com).

At least *P. esculentus* is known to be highly mobile. Tagging studies by Derbyshire et al (1990) recorded average movements of 40 kilometres over 70 days. Such mobility had important implications for the selection of sampling scales in the present study. A small scale was chosen to enable replication of the sampling design within populations, whilst a larger scale north-south comparison sampled separate populations.

For a comprehensive picture of metal levels in Torres Strait prawns, biotic and environmental factors which may influence metal levels were examined. A major limiting factor was the high cost of metal analysis and analytical resources were rationalised carefully. For the effects of biotic factors size, sex and moult stage, as well as different post-capture handling procedures, only one species, *P. esculentus,* was investigated. All three species were examined for spatial and seasonal patterns. As tail flesh only is deemed to be the edible portion and thus the target of health and trade limits, it was the main tissue investigated.

Two previous studies of metal levels in *P. esculentus* from Torres Strait provided evidence that cadmium levels in this species may be unacceptably high with respect to Australian standards. Schneider (1990) and Mariath (1991) both reported cadmium levels in this species in excess of the National Health and Medical Research Council's (NHMRC) Maximum Permissible Concentration (MPC) of 0.2 ppm for crustaceans (NHMRC, 1990). However, conclusions from both studies were limited by either small sample sizes, and/or large variability. An important aim of the present study is to further support or discount concerns arising from these earlier studies.

1.2 Methods

Samples of *Penaeus esculentus*, *P. longistylus* and *Metapenaeus endeavouri*, were collected from two sites (figure 1). The southern site was in the Dugong island area, in a rectangle bounded by the coordinates 10°24'S 143°13'E; 10°37'S 143°13'E; 10°37'S 143°7'E; and 10°24'S 143°7'E. The northern site was in the region of Stephens Island, in an area bounded by the coordinates 9°26'S 143°28'E; 9°30's 143°24'E; 9°38'S 143°24'E; 9°38'S 143°28'E.

Based on the high mobility of *P. esculentus*, as described earlier, these locations were selected to be as far apart as possible within the study area to maximise the chance of sampling different populations, while also being representative of important commercial fishing grounds in Torres Strait.

Prawns for analysis of spatial and biotic factors were collected in October/November 1992. Seasonal comparisons were made possible with further collections from the northern site only in March 1993. All sampling was done aboard the commercial trawler FV 'Smithy' and actual sites were determined by fishing patterns at the time. The wide spread of actual sampling locations within each site is not inappropriate, given the high mobility of the resource and the fishing fleet. The southern site was omitted from the final sampling period because no fishermen were operating in that area at that time.

As recommended in the pilot study (see appendix 1), 3 nights and 7 replicates per night were used as the lowest level sampling units, where possible. However, only 2 nights of samples from the October/November 1992 northern site were useable due to a freezer breakdown. Also, where numbers of some categories were scarce on a particular night, as few as 5 replicates were used. Appendix 4 lists actual sample sizes and analytical models for each ANOVA undertaken.

1.2.1 Sample Handling Protocols

Collection

Collection protocols were designed to give samples as much protection from post-capture metal contamination as possible, within the practical limitations of sampling aboard a working commercial prawn trawler. Major potential sources of contamination on a standard commercial prawn trawler were identified as the sorting/processing environment (e.g. exhaust fumes), brine tanks (e.g. galvanised cooling coils), and breakdown of prawn head tissue post-death. Prawn heads are known to contain high levels of some metals especially in the hepatopancreas (Schneider 1990, Rainbow 1988, Ray 1984, Darmono and Denton 1990, Peerzada et al 1992).

Samples were selected and removed from the sorting tray as soon as possible after the catch was landed to minimise contact with potentially contaminating exhaust fumes, machinery or body fluids and excretions from other biota in the catch. Prawns were then individually washed in clean seawater. A pleopod was removed (for subsequent moult staging) using stainless steel scissors, before samples were individually bagged and frozen.

Seawater was collected overboard at an up-wind and up-current position at the start of each night and stored in clean plastic containers. All buckets and containers used to hold or wash samples were plastic. They were acid washed at the start of each voyage, thoroughly rinsed in clean seawater after each shot and rinsed with Reverse Osmosis Polished (ROP) water at the end of each night's sampling. All personnel handling samples wore disposable vinyl surgical gloves. Plastic bags used for sample storage were supplied by the Animal Research Institute (Qld Department of Primary Industries), who had approved them for sample storage by analysing a subsample of each batch for metal content.

Sample Selection

Samples of *Penaeus longistylus* and *Metapenaeus endeavouri* were selected from the size class which was predominant in the catch. To enable a comparison of metal levels between sizes of *Penaeus esculentus*, two size classes were chosen for this species. Initially, samples from two pre-determined size classes were to be collected from both sites. However, this approach proved impractical due to different size class structures at each site, so the two

predominant size classes were sampled in each case. In the north, these were small (25 to 30 millimetres carapace length) and medium (30 to 38 millimetres carapace length) classes and in the south they were medium and large (40 to 45 millimetres carapace length) classes.

Moult Staging

Excised pleopods were examined under a light microscope for determination of moult stage, immediately after sampling. This assessment was based on extent of epidermal retraction, shape of epidermal line and setal development criteria described by Smith and Dall (1985). The categories used in this study were post-moult, where there is no evidence of epidermal retraction (= Smith and Dall's A - C); and pre-moult, where epidermal scalloping becomes disrupted by the tips of new setae and new setal nodes become increasingly developed so that pinpoints of light may be seen (= Smith and Dall's D_2 - D_4).

Dissections

Dissections took place in the NATA accredited Class 1 laminar flow hood at the Horn Island Research Station and instruments used were either high quality surgical stainless steel, or acid washed plastic. Between samples, instruments were scrubbed in Extran detergent and thoroughly rinsed with ROP water. Prawns were dissected while still par frozen, so that thawed fluid from individual tissues did not mix. Tissues separated were head, abdominal shell complete with pleopods, tail flesh minus alimentary tract and gonads, and in the case of females, ovaries. One tissue only was analysed from each prawn, so as to maintain independence of data for statistical procedures. After separation, tissues were thoroughly rinsed with ROP water before re-freezing for transport to the Animal Research Laboratory (DPI) in Brisbane, for analysis.

Prior to dissection, carapace length and gonad development stage were recorded and sex and species were confirmed. Ovary development was categorised in one of three stages: nil apparent-rudimentary development with a tinge of colour; early ripe, with moderate development, slight lobing, and a strong colour; and ripe, with strong lobing and colour.

Industry samples

To enable a preliminary comparison between prawns treated 'cleanly', as per the above protocols, and those treated according to routine commercial processing, cooked and uncooked samples of *P. esculentus* were concurrently collected from FV 'Smithys' commercial product after routine processing by the fishing crew. After gross sorting, these prawns were dipped in a sodium metabisulphate solution, further graded, then placed in brine tanks for three to twelve hours. From the brine tanks, some prawns were boiled in a large commercial stainless steel LPG operated prawn cooker. Cooked and uncooked prawns were snap frozen in an Individual Quick Freeze (IQF) tank, where super saturated ultra cold brine is rapidly circulated through the product, to bring its temperature to approximately -20°C in 15 minutes. After this initial IQF treatment, prawns were packed, then placed in the -35°C holding freezer.

This study includes three classes of 'industry' treatments. Green and cooked prawns were collected from uncooked and cooked product (respectively) processed on board as described above. These treatments were dissected while still par frozen, as described earlier for clean prawns. Chilled prawns were collected as per the green treatment, but prior to dissection they were allowed to fully thaw and remain chilled for 24 hours.

1.2.2 Laboratory Analysis

All metals analyses were conducted at the Queensland Department of Primary Industry's Animal Research Institute (QDPI-ARI). This is a NATA accredited laboratory which has

successfully participated in the US National Oceanic and Atmospheric Administration (NOAA) Inter-comparison for Trace Metals in Marine Sediments and Biological Tissues since 1991. This programme is designed to ensure that participating laboratories are able to produce accurate and repeatable results for a wide range of elements.

Samples were freeze-dried and microwave digested using double distilled nitric acid, prior to chemical analysis. All samples were weighed both wet and dry (after freeze-drying). The concentrations of the 16 elements silver, aluminium, arsenic (total), cadmium, cobalt, chromium, copper, iron, mercury, manganese, nickel, lead, selenium, strontium, uranium and zinc were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Appendix 3 describes protocols followed for sample preparation and analysis at QDPI - ARI. Details of quality assurance and inter-laboratory comparisons can be found in Gladstone 1996.

1.2.3 Statistical Analysis

General descriptive statistics and box and whisker plots were used to give an overview of results. Here, data were expressed on a wet weight basis, so that direct comparisons with trade and health limits (which are based on wet weight values) could be readily drawn. All other analyses, based on ANOVA, used dry weight data as they are less variable. A conversion ratio was calculated for all tissues and species examined, to enable easy conversion from wet to dry weights.

For any individual prawn only one tissue was analysed (to maintain data independance as discussed earlier). Thus, estimates of whole prawn metal concentrations were calculated as the sum of average tissue metal loads, divided by the sum of average weights of each tissue. The metal load for each tissue was calculated as the average metal concentration multiplied by the average weight of that tissue

A series of hierarchical ANOVA's, as described in appendix 4, were used to examine the factors site, size, sex, moult stage, tissue type, species, season and handling treatment. This uni-variate statistical tool was utilised in preference to multi-variate methods, because the fundamental questions in this study, particularly those relating to public health and trade, are uni-variate ones. Maximum Permissible Concentrations (MPC) are set for individual metals and not in multi-variate space. The model for each ANOVA was based on recommendations in Underwood (1981), and ANOVA's were performed using Statgraphics V5.

A sub-set of data was inspected for normality (see appendix 2). Although data distributions for some metals appeared non-normal, transformations were not considered necessary. Underwood (1981) gives a very useful discussion on the robustness of ANOVA to violations of the assumption of normality, citing various studies that have simulated the effects of non-normality on the outcomes of significance tests in ANOVA. In most cases, non-normality does not effect the outcome of the analysis, although problems may arise where the distribution of error terms differs markedly between treatments. As this was not the case in the present study (i.e. non-normality tends to be consistent for each metal regardless of the treatment), Underwood's recommendation to relax this assumption was followed.

Similarly, despite the presence of heterogeneous variances in some analyses, transformations were not considered appropriate. Firstly, they are only strictly necessary where results are significant and the error term in the F ratio is the residual. Where the null hypothesis is not rejected, heterogeneous variances will cause the value of F to be significant more often than should happen by chance alone, thus making a non-significant result reliable (Underwood 1981). Where the error term is a factor other than the residual, after the central limits theorem

(Zar 1984), the distribution of these means, which are estimates of the same population, will tend towards normality and homoscedasticity (Mapstone, pers com).

To avoid further complicating an already complex data set by transforming in some cases and not in others, transformations were avoided entirely. Any limitations of results are acknowledged and taken into account where data are interpreted and discussed.

ANOVA results are summarised in tables which list all tested factors and interactions. The error term in the F ratio is indicated at the heading in brackets. Significant results for a=0.05, pooled data (as per procedure outlined below) and tests for homoscedasticity as per Cochran's statistic (calculated with Statgraphics, a=0.05) are indicated.

Where a concentration result was below the minimum detection limit of 0.02 mg/kg, the value was taken to be the detection limit. Previous workers have dealt with these values in different ways, assigning them values between zero and the detection limit. A middle ground approach was taken elsewhere in the TSBS (Dight and Gladstone 1993, Gladstone 1996), with values being assigned as the midpoint between the detection limit and zero. At the risk of biasing some results upwards, the 'worst case scenario' approach was chosen for the present study to ensure that potential problem metals, with respect to trade and health limits, were highlighted.

Pooling and Power

Pooling procedures suggested by Winer (1971) Underwood (1981) and Andrew and Mapstone (1987), were followed for random factors and interactions in most situations. These procedures generally recommend pooling if the result is still non-significant at a=0.25 and, result in improving the power of non-significant tests by increasing the degrees of freedom in the denominator of the F ratio. However, pooling did not proceed if there was a combination of the following: variances were heterogeneous according to Cochran's statistic (at a=0.05); pooling would cause a non-significant F test to become significant; and pooling would cause the error term in that F test to be the residual. In these cases, it was considered that the disadvantage of a reduction in the reliability of a significant F test outweighed the advantages of pooling.

Where the null hypothesis was retained for fixed factors, primary and secondary interaction tests, power calculations were performed for the fixed factor only. Tables and formulae from Cohen (1977) were used to calculate the standard deviation of the means in the minimum detectable effect size with a=0.05 and power = 80%. This standard deviation was converted to the minimum detectable distance between any two means using a model with equally distributed means (Cohen 1977). The minimum detectable distance was also expressed as a percentage of the grand mean. To facilitate subsequent discussion, the following arbitrarily chosen categories of detectable effects were adopted: small (< 50% of mean); medium (50 - 100% of mean); large (100 - 150% of mean); and very large (>150% of mean).

1.2.4 Arsenic Conversion

Analytical methods employed in the present study measured total arsenic. Since trade and health limits are set for inorganic arsenic only, it was useful to convert total arsenic results in some instances.

Arsenic in seafood occurs mostly as the non-toxic, organic compound arsenobetaine (Edmonds and Francesconi 1988 and 1993), although highly toxic inorganic forms may also be present in small amounts. Edmonds and Francesconi (1993) collated results of numerous studies on arsenic in seafood and found a shallow sloped linear relationship between total and

inorganic arsenic. They suggest that the proportion of inorganic arsenic in marine organisms ranges from 1% at very low total arsenic concentrations, to 0.5% at total arsenic concentrations of around 20 mg/kg. Levels of total arsenic reported in the present study range across this spectrum, to levels greater than 20 mg/kg. Again following the worse case scenario strategy, a conversion of 1% was applied to total arsenic figures for the purpose of comparison with trade and health limits.

1.3 Results

1.3.1 Overview

In this general data overview, metal results from prawns collected in October/November 1992 are presented. Comparisons are made to previous studies, and trade and health limits and recommendations. All concentration data and references in this section are presented on a wet weight basis.

Table 1 sets out overall mean metal concentrations found in the present study, with standard errors. Tail flesh is the only tissue analysed in all three species studied and for most metals, the concentration in all three species is comparable. The exceptions are arsenic and cadmium, which are both substantially higher in tail flesh of *M. endeavouri*.

Table 2 collates comparative data from previous studies. Of the metals for which comparative data is available, levels reported in the present study are either less than those reported from the Arabian Sea, or additionally, in general agreement with those reported from the Adelaide River and Three Mile Creek. The exceptions are copper, zinc and cadmium.

Copper levels in table 1 are less than those reported from the Adelaide River and Three Mile Creek and similar to those reported from the Arabian Sea. Zinc levels in table 1 are similar to those reported from the Adelaide River and Three Mile Creek and greater than those reported from the Arabian Sea. Cadmium levels in table 1 are greater than those reported from the Adelaide River and Three Mile Creek, slightly less than, or in the case of *M. endeavouri*, similar to those reported from the Arabian Sea. When compared to previous Torres Strait data provided by Mariath (1991), tail flesh and head concentrations are similar, while levels in the shell are lower in the present study.

When comparing present study results for these three metals with those from Torres Strait provided by Schneider (1990), there is a common trend. Tail flesh and shell levels in the present study are less than those reported by Schneider, while other tissues, including estimates of whole prawn concentrations, are similar.

For metals for which a NHMRC MPC exists, figure 2 illustrates the distribution of the data summarised in table 1, with respect to the level of the MPC. Concentrations of mercury, lead and zinc are well below the MPC for all tissues and species combinations measured. The opposite is true for total arsenic, but once results are corrected to inorganic arsenic only, they conform easily.

In the case of copper, concentrations in tail flesh for all three species and ovary tissue of *P. esculentus* were below the MPC, but this was not the case for either shell or head tissue from this latter species. Both mean and median shell concentrations were close to the MPC, with almost 50% of measurements above it, while all measurements of head levels were way above it. The estimate for total body concentration was also way above the MPC.

Tail flesh in all species was again below the MPC for selenium, as was *P. esculentus* shell. However, head and ovary selenium levels in this species exceeded the MPC, and the estimate for total body concentration was approximately equal to it.

Cadmium was the only metal included in figure 2 for which tail flesh levels were not always below the MPC. In *P. esculentus* and *P. longistylus*, mean tail flesh levels and the majority of measurements were below the MPC, although some *P. esculentus* samples exceeded it. However in the case of *M. endeavouri*, mean and median cadmium tail flesh concentrations, as well as over 50% of measurements, exceeded the MPC. While concentrations in shell and ovary tissue of *P. esculentus* were mostly below or close to the MPC, head levels and the estimate for whole prawns exceeded it.

The pie diagrams in figure 3 illustrate the percentage contribution of each tissue to the total whole prawn metal load, for metals for which there is either an MPC or PTWI. It thus takes into account not only the concentration of each metal in the tissue, but also its relative biomass.

Clearly, head tissues are the major general contributor to total body metal load. Head contributions are exceeded only in the case of mercury, which is mostly provided by tail flesh, which is also a significant contributor of arsenic, selenium and zinc. Lead load is provided roughly evenly by head, tail flesh and ovary. Ovary also contributes substantially to the selenium, zinc and to a lesser extent arsenic burden. The shell is a relatively minor contributor of all metals examined.

Table 3 collates published human consumption limits, recommended dietary intakes and estimated intakes of metals from other sources, with metal loads reported in the present study. Let us assume that all metal in prawns occurs in a form which is bioavailable to humans. For most metals, 10 prawn tails or 10 whole prawns would contribute only a small fraction of the total weekly advisable intake, which would not be exceeded even if the content of these 10 prawns was added to the estimated intake from other sources. The only exception is cadmium. While 10 prawn tails could be safely consumed with respect to cadmium load, as described above, one single prawn head or whole prawn alone contains twice the advisable weekly intake.

Interestingly and again assuming total bioavailability to humans, prawn consumption may contribute substantially to the estimated dietary requirement for zinc.

Remaining prawn results are expressed on a dry weight basis. Table 4 presents the relationship between wet and dry weights for different tissues, as observed in this study. This relationship will allow the reader to convert concentration data presented in this report between wet and dry weight basis. To convert most tissues, a factor of four can be used, with the exception of shell tissue, which is closer to three. To convert from a wet to dry weight basis, multiply data by this factor and divide by it to convert from dry to wet weight basis.

Table 1. Overview of metal levels found in *P. esculentus, M. endeavouri* and *P. longistylus* in the present study. Data are arithmetic means, with standard errors in brackets, from all prawns 'cleanly' collected from both northern and southern sites in Oct/Nov 1992. Concentrations are in mg/kg on a wet weight basis.

	P. esculentus					*P. longistylus*	*M. endeavouri*
METAL	**Tail**	**Head**	**Shell**	**Ovary**	**Total**	**Tail**	**Tail**
Ag	0.01 (.003)	0.53 (0.04)	0.01 (0.00)	0.01 (0.00)	0.21	0.01 (0.001)	0.01 (0.01)
Al	0.87 (0.08)	31.52 (4.40)	15.36 (1.81)	0.53 (0.18)	12.55	0.56 (0.09)	0.79 (0.14)
As MPC=1.0	14.06 (0.49)	18.17 (1.54)	7.92 (0.55)	20.44 (1.02)	14.46	18.13 (0.95)	36.59 (0.95)
Cd MPC=0.2	0.16 (0.02)	5.79 (1.17)	0.06 (0.01)	0.24 (0.05)	2.30	0.08 (0.01)	0.44 (0.08)
Co	0.01 (0.00)	0.14 (0.01)	0.05 (.004)	0.02 (.001)	0.06	0.01 (0.00)	0.00 (0.00)
Cr	0.10 (.004)	0.19 (0.02)	0.12 (0.01)	0.18 (0.01)	0.13	0.11 (0.01)	0.10 (0.01)
Cu MPC=10	3.86 (0.14)	54.78 (3.55)	9.88 (0.69)	3.82 (0.22)	22.94	3.37 (0.15)	3.37 (0.15)
Fe	2.35 (0.12)	76.22 (8.93)	143.82 (11.4)	7.11 (0.36)	31.34	2.79 (0.18)	2.70 (0.13)
Hg MPC=0.5	0.04 (.002)	0.02 (.002)	0.03 (.003)	0.01 (.001)	0.02	0.03 (.003)	0 (0.01)
Mn	0.12 (0.01)	1.54 (0.16)	1.32 (0.07)	1.62 (0.06)	0.77	0.18 (0.01)	0.10 (0.003)
Ni	0.02 (.001)	0.70 (0.04)	0.92 (0.10)	0.04 (0.01)	0.28	0.02 (.002)	0.02 (.001)
Pb MPC=1.5	0.05 (.005)	0.05 (.005)	0.06 (0.01)	0.24 (0.10)	0.06	0.06 (0.01)	0.07 (0.01)
Se MPC=1.0	0.49 (0.01)	1.31 (0.05)	0.64 (0.07)	3.55 (0.25)	0.98	0.55 (0.02)	0.65 (0.02)
Sr	3.62 (0.17)	162.3 (7.55)	456.2 (27.8)	2.70 (0.35)	65.89	4.90 (0.61)	4.09 (0.22)
U	0.01 (0.00)	0.01 (.002)	0.01 (.001)	0.01 (0.00)	0.01	0.01 (0.00)	0 (0.00)
Zn MPC=150	14.49 (0.46)	56.45 (4.96)	4.74 (0.31)	61.89 (3.91)	32.41	13.92 (0.34)	15.32 (0.44)

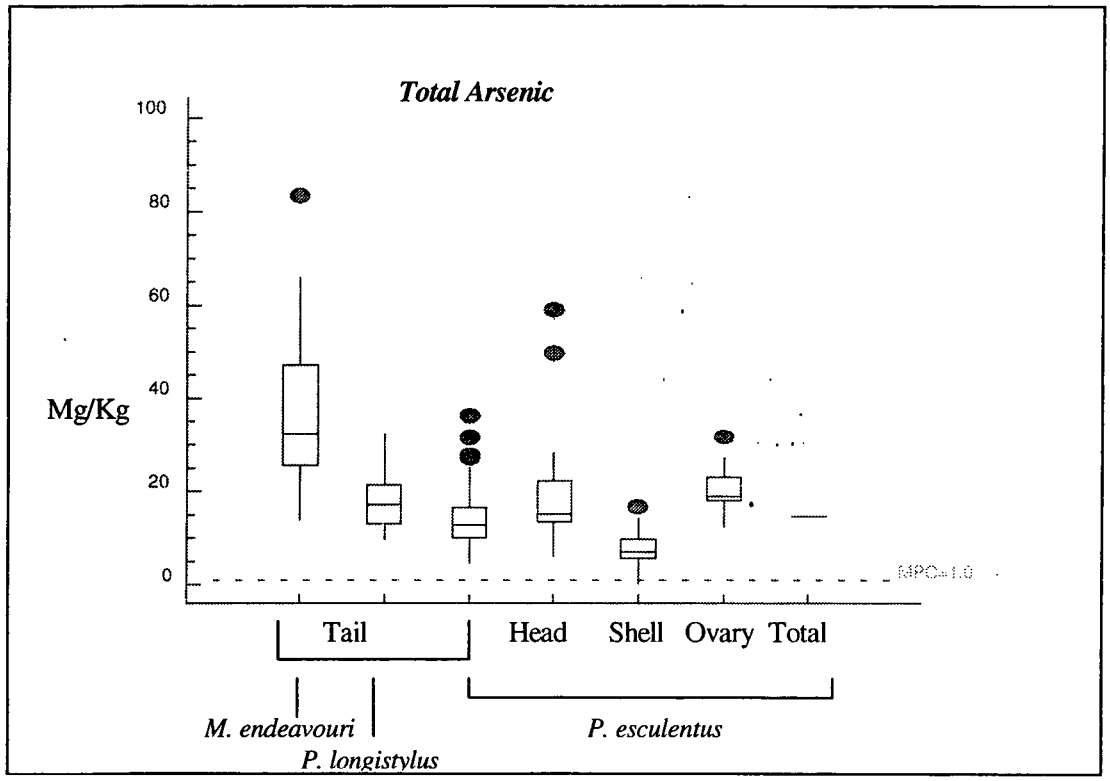
Table 2. Comparative data from previous studies of metal levels in related species of prawns. All data are in mg/kg on a wet weight basis. Source codes are as follows:

Code 1: **Mariath 1991.** *Penaeus esculentus* collected from **A)** Torres Strait and **B)** Western Australia, collected 1989-90.
Code 2: **Schneider 1990.** *Penaeus esculentus* from Torres Strait, collected **A)** 1986 and **B)** 1988.
Code 3: **Peerzada et al 1992.** *Macrobrachium novaehollandiae* from the relatively pristine estuary of Adelaide River, Northern Territory, collected 1990.
Code 4: **Darmono and Denton 1990.** *Penaeus merguiensis* from the intermediately clean environment of Three Mile Creek, near Townsville, collected 1986.
Code 5: **Tariq et al 1993. A)** *Penaeus japonicus* and **B)** *Metapenaeus monocerous* from the polluted coastal Arabian Sea, adjacent to Pakistan, collected 1987-88.

* Concentration in hepatopancreas only, not total head.
ND Below detection

METAL	TISSUE	SOURCE							
		Previous Torres St Studies				Adel.R	3Ml Ck	Arabian Sea	
		1A	1B	2A	2B	3	4	5A	5B
Ag	Tail Head						ND 9.91*	0.25	0.29
Cd	Tail Head Shell Ovary Total	0.17	0.15 4.00 0.24	0.80 5.59 1.59 1.86	0.36 2.19 0.75 0.78	ND 1.50* ND	ND 1.60*	0.47	0.47
Cr	Tail							0.18	5.90
Cu	Tail Head Shell Ovary Total			10.70 44.55 33.36 22.18	11.40 46.40 34.01 22.82	7.20 330.0* 113.0	9.10 199.1*	4.55	1.20
Fe	Tail Head Ovary					5.40 42.00* 19.00	0.61 39.31*	837	806
Hg	Tail Head						0.02 0.02*	0.13	0.15
Mn	Tail Head Ovary					0.90 5.30* 2.50	1.09 6.07*	3.14	6.42
Ni	Tail							13.10	12.64
Pb	Tail							1.60	1.71
Zn	Tail Head Shell Ovary Total			19.96 51.96 29.15 27.84	16.11 41.42 23.00 22.56	13.00 55.00* 68.00	12.60 51.68*	7.11	4.21

Figure 2. Box and whisker plots of levels of metals for which there is an Australian NHMRC MPC. Data are on a wet weight basis. The dotted lines represent MPC levels



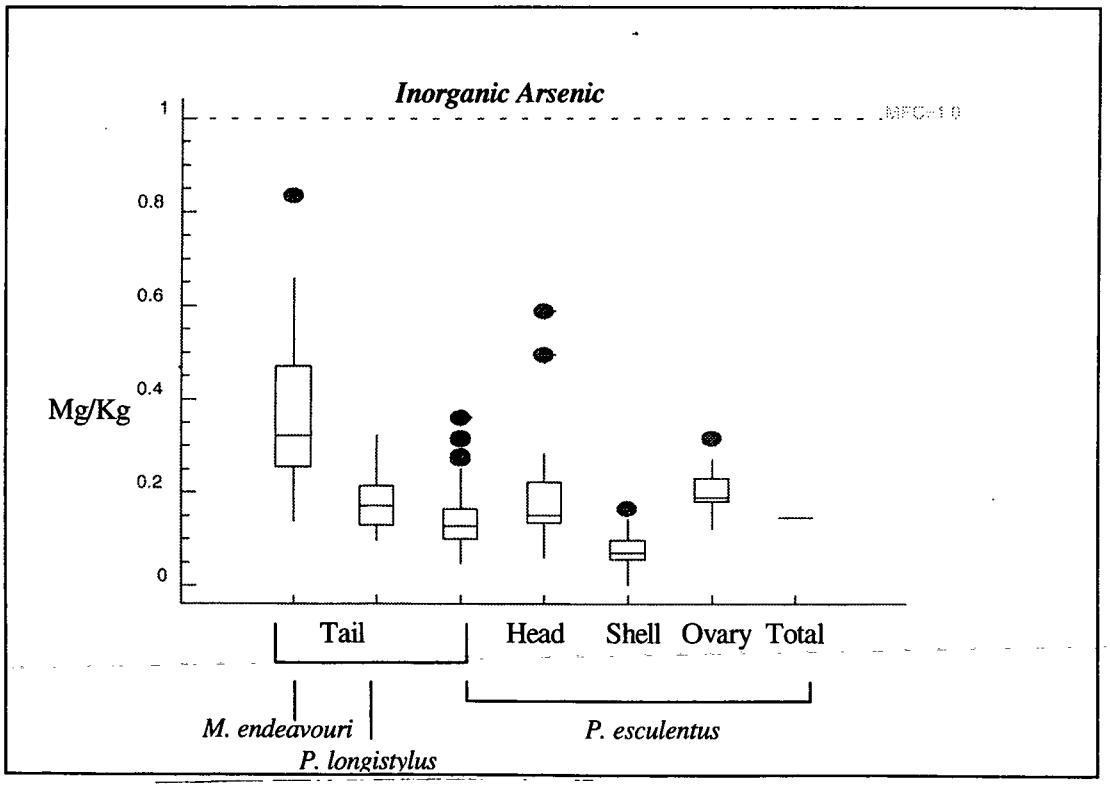
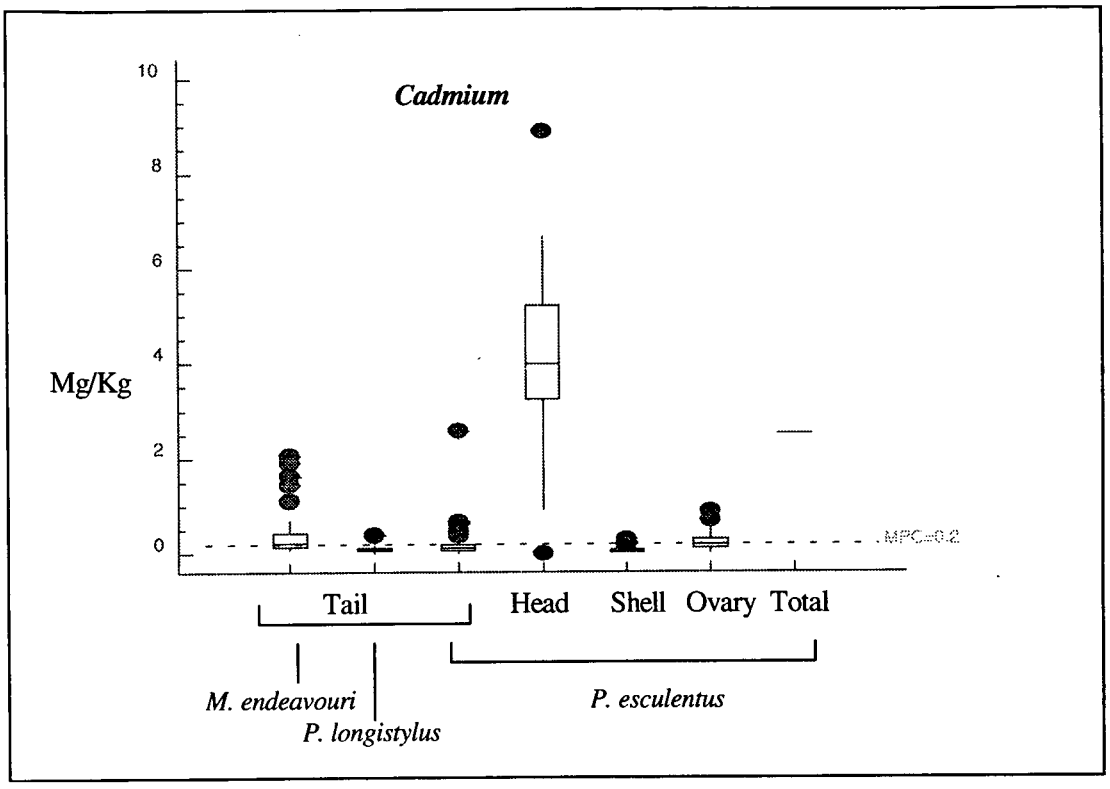


Figure 2. Cont...



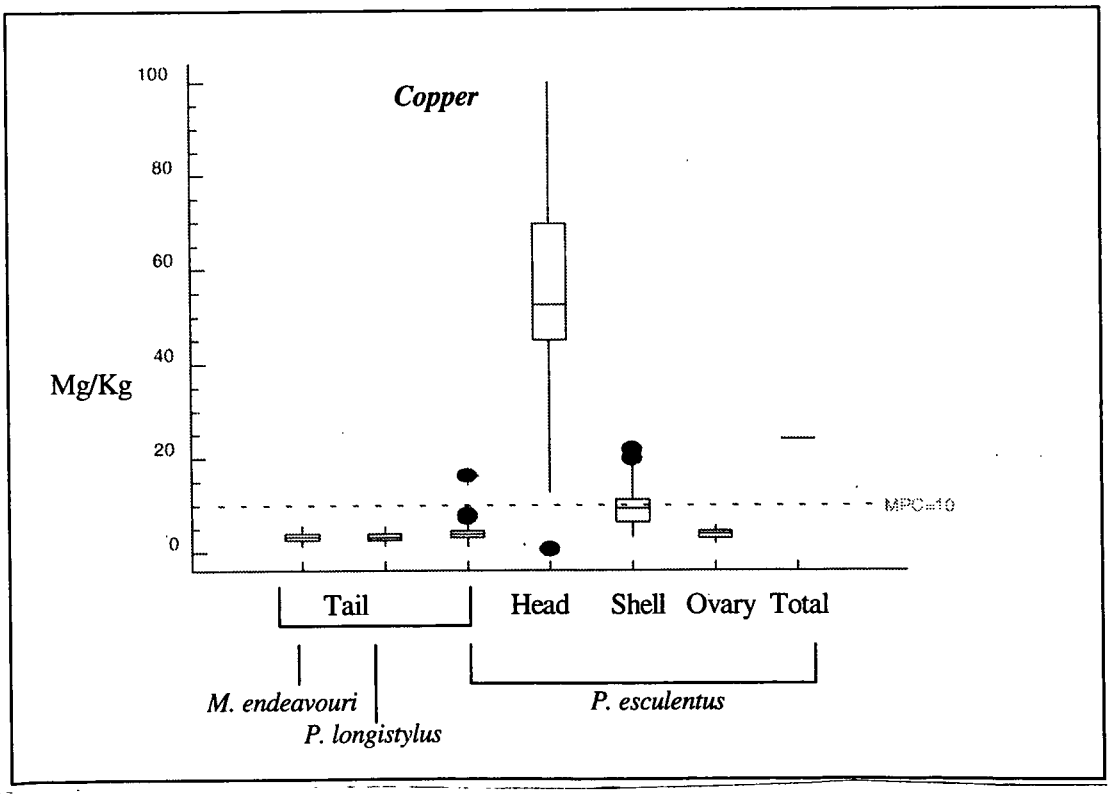
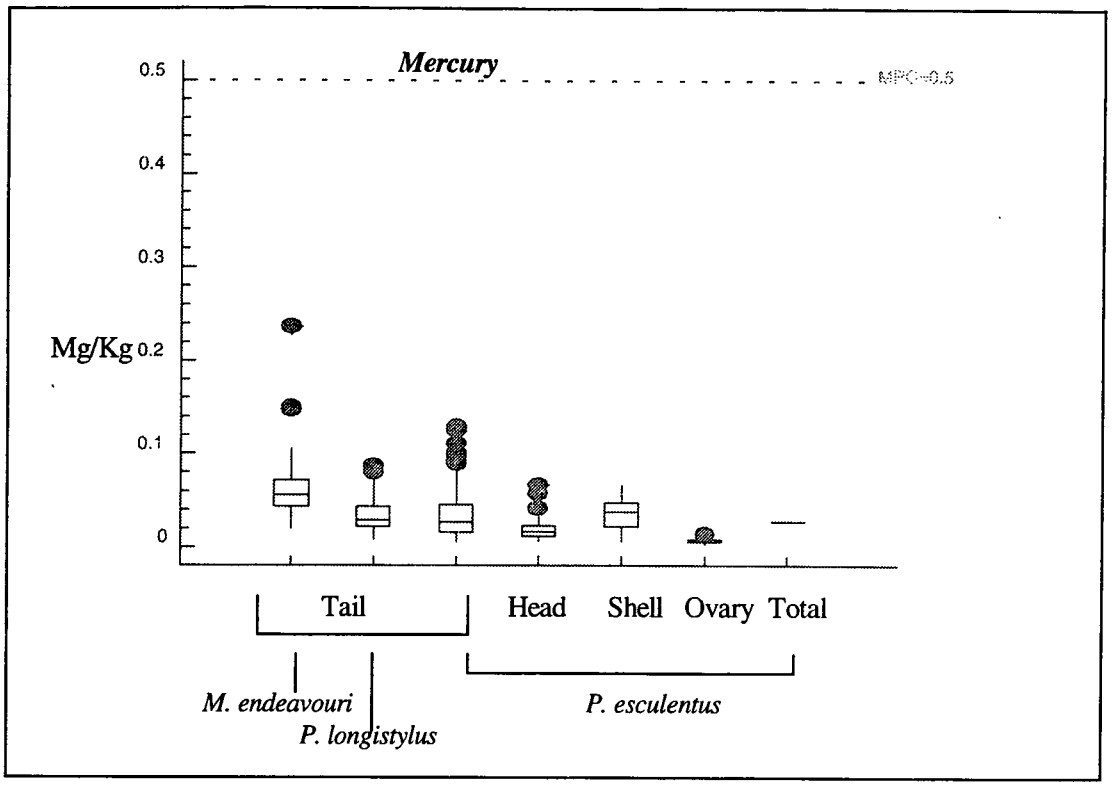


Figure 2. Cont...



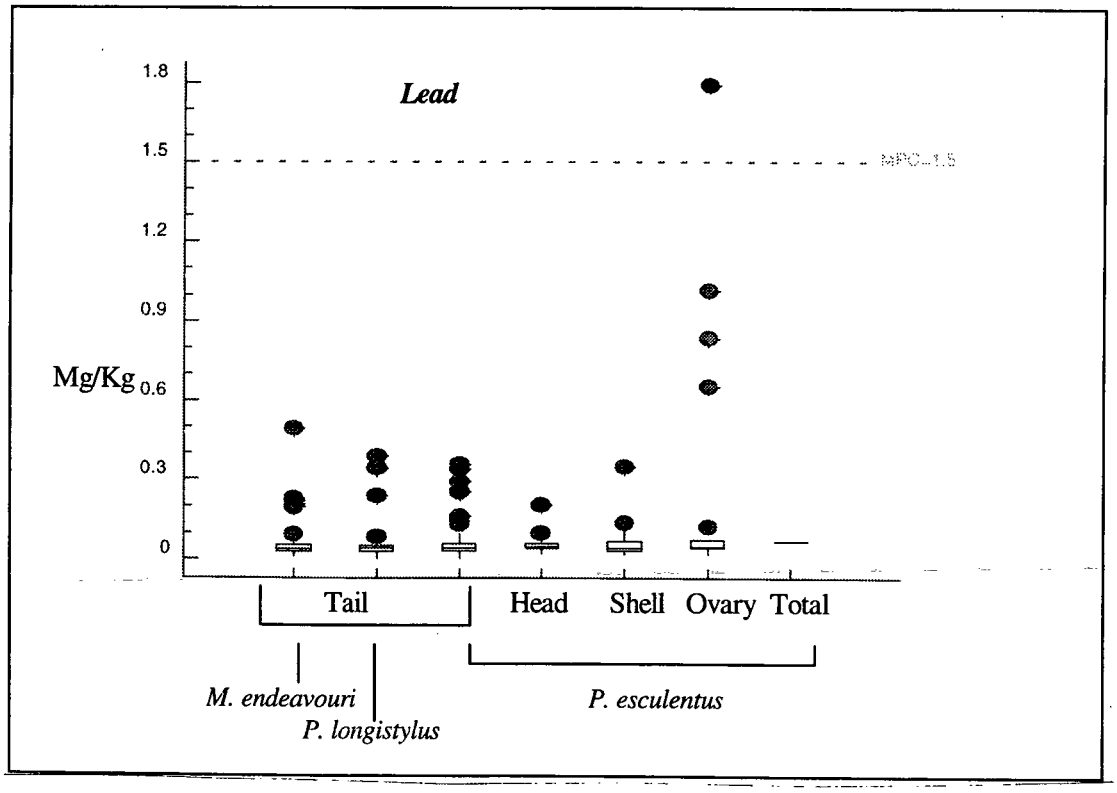
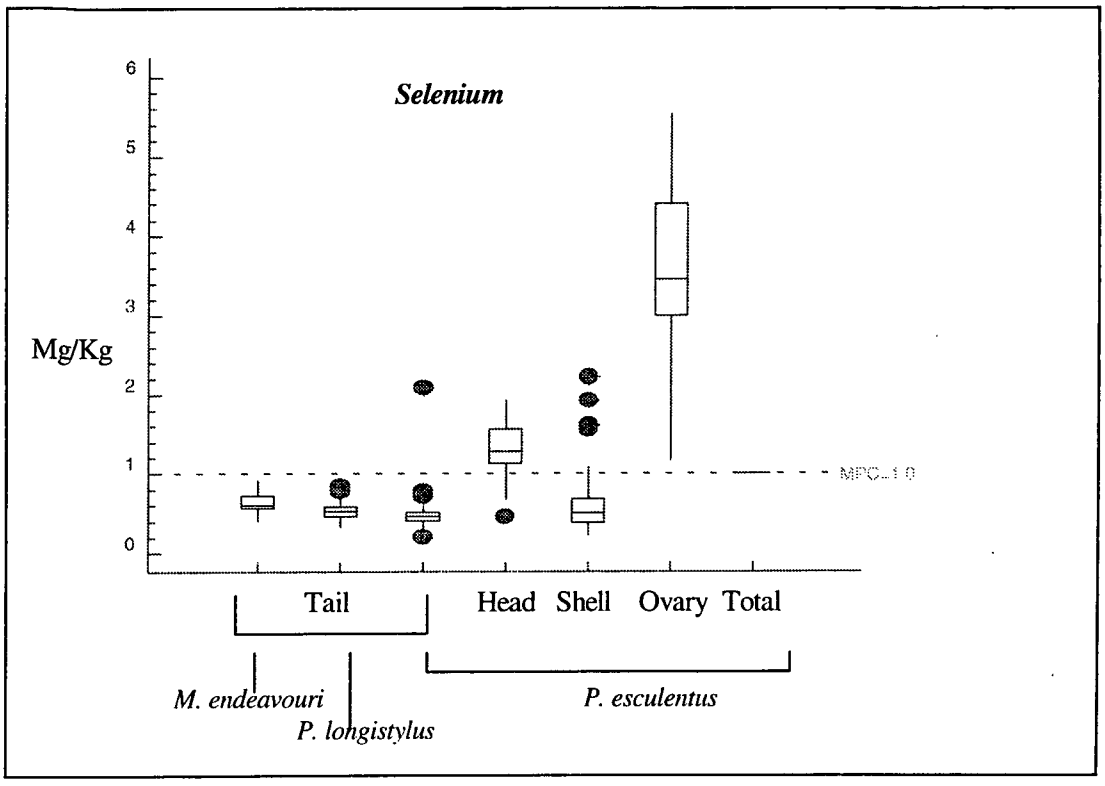
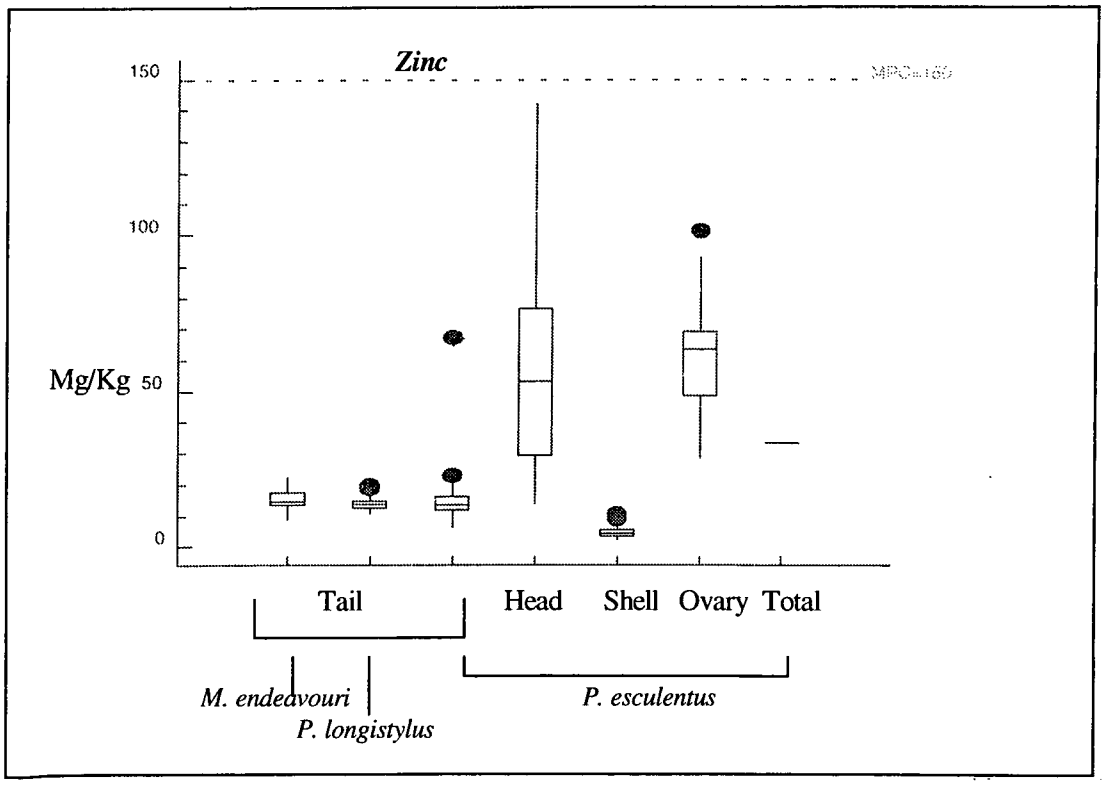


Figure 2. Cont...





Notes on interpreting Box and Whisker Plots:
The horizontal line inside the box is the median and the box encloses the middle 50% of observations. The vertical 'whiskers' extend to the range of the data, or 1.5 interquartile ranges from the box, which-ever is the smaller. Data points beyond this are marked individually.

Figure 3. Pie diagrams showing the relative contribution of different tissues to total metal load in *Penaeus esculentus*, for metals included in Table 3.

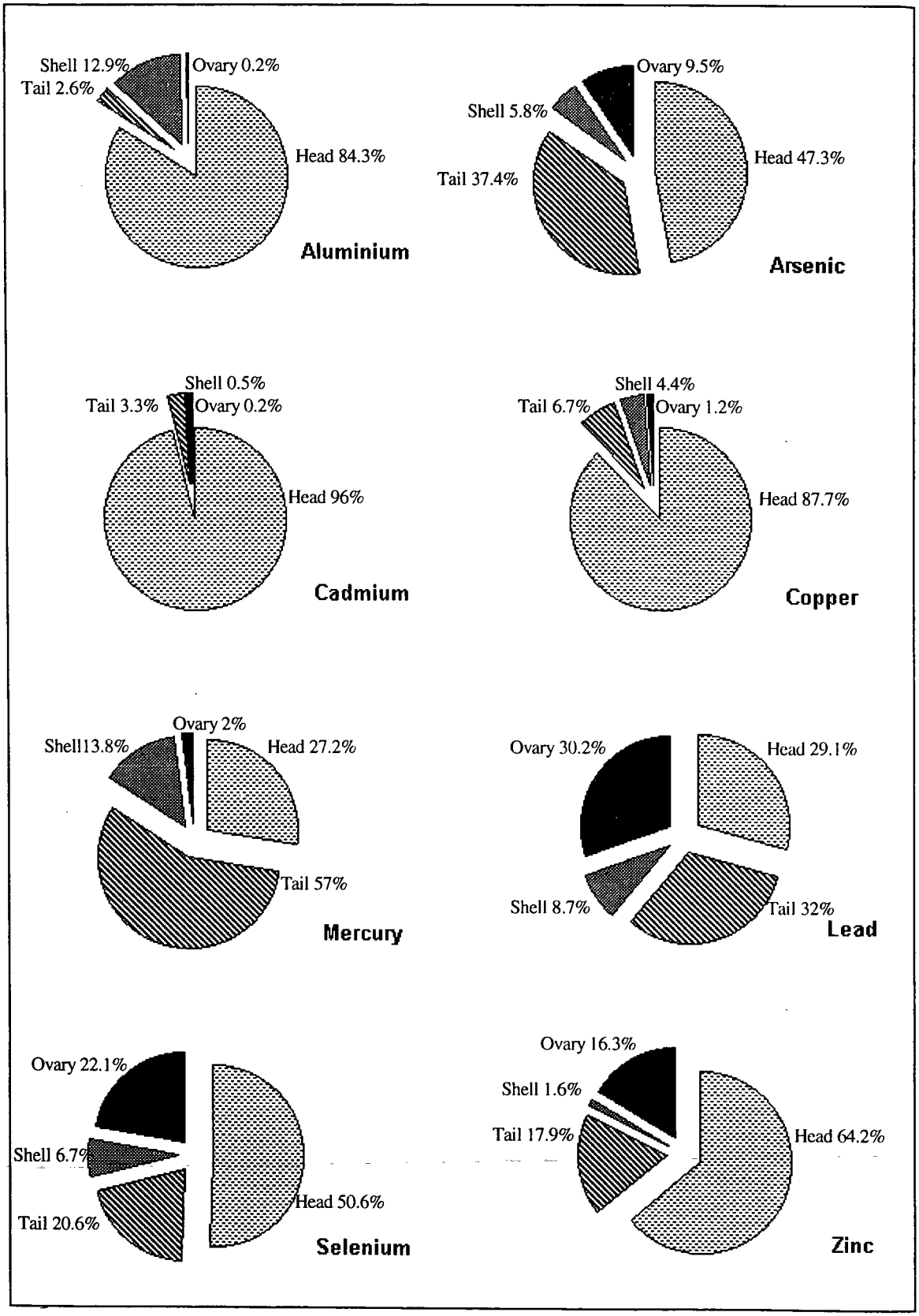


Table 3. Total burden of metals in different tissues and whole prawn for *P. esculentus*, published estimates of maximum advisable weekly intakes, dietary requirements and dietary intakes. Sources of published estimates follow. Where conversion from published values to a weekly figure was necessary to enable comparability, an assumed adult body weight of 60kg (female) - 75kg (male) was used.

#	1989 WHO (Provisional Tolerable Weekly Intake)
u	1973 WHO
n	1986 WHO (Provisional Tolerable Weekly Intake)
X	1972 WHO (Provisional Tolerable Weekly Intake)
*	1990 NHMRC
NEA	No Estimate Available

Note: Arsenic values from present study are converted to an estimate for inorganic arsenic only. Maximum advisable weekly intake is for inorganic arsenic. Estimated intake from other sources is a measure of total arsenic.

METAL	Max Advis Weekly Intake (mg)	Est Weekly Dietary needs (mg)	Intake from other sources (mg)	Metal load in P. esculentus (mg)				
				Tail	Head	Shell	Ovary	Whole
Al	42 - 43 #	NEA	NEA	0.01	0.51	0.08	0.001	0.60
As	0.9-1.13 #	Not Required	0.19-0.29 *	0.002	0.003	0.0004	0.0006	0.006
Cd	0.42-0.53 #	Not Required	0.17-0.23 *	0.004	0.11	0.0002	0.001	0.11
Cu	210 - 263 **u**	33.6-42 **u**	NEA	0.07	0.93	0.05	0.01	1.05
Hg	0.3 **X**	Not Required	0.24-0.03 *	0.001	0.0003	0.0002	.00002	0.001
Pb	1.5 - 1.9 **n**	Not Required	0.52-0.66 *	0.001	0.001	0.0003	0.001	0.003
Sc	NEA	NEA	NEA	0.009	0.022	0.003	0.01	0.04
Zn	>1400 **u**	15.4-38.2 **u**	NEA	0.26	0.94	0.02	0.24	1.46

Table 4. Relationship between wet and dry weights for different tissues of *Penaeus esculentus* and tail muscle in *Metapenaeus endeavouri* and *P. longistylus*. This relationship is expressed as the ratio of wet weight over dry weight

TISSUE	*P. esculentus*			*M. endeavouri*			*P. longistylus*		
	Mean	SE	n=	Mean	SE	n=	Mean	SE	n=
Tail	3.91	0.03	136	4.03	0.04	41	3.97	0.03	39
Head	3.81	0.16	41						
Shell	3.24	0.11	41						
Ovary	4.08	0.14	21						

1.3.2 Patterns With Prawn Size

Metal levels in female *Penaeus esculentus* from three size classes and both north and south sites are compared in the ANOVAs summarised in tables 5–8. The three size classes are small (25 to 30 millimetres CL), medium (30 to 38 millimetres CL) and large (40 to 45 millimetres CL) (see Methods section). Small and medium prawns are compared in the north (table 5 and 6) and medium and large prawns are compared in the south (table 7 and 8). Silver, cobalt and uranium data is not included in these analyses as most values were at the detection limit of 0.02 mg/kg

While variances were heterogeneous for data of some metals according to Cochran's scores, this did not effect the validity of results as no tests using the residual mean square as the denominator of the F ratio were significant in these instances.

In the north, the only significant size related results were size x night interactions for chromium and zinc (table 5). In neither case was there an overlying trend in metal levels with respect to size (figure 4). In the case of chromium, this result appears to be due to variability between nights in small prawns, while inter-night variability in zinc levels was only slight for both sizes (figure 4).

Where there were no significant size related tests in the north, power was generally good, with a small-medium minimum detectable distance (<100% of mean). The exceptions were lead and selenium tests which could only detect a large (100–150% of mean) distance with 80% power and, iron and mercury tests which were very insensitive with unacceptably large minimum distances (>150% of mean).

In the south, where the size comparison was between medium and large prawns, mercury, selenium, arsenic and cadmium showed significant results for size related tests (table 7). In the case of mercury and selenium, levels in large prawns were slightly higher (figure 5). If means are converted to wet weight concentrations using table 4, both size classes were well below the MPC for these metals.

Cadmium levels were generally substantially higher and more variable at both the individual and inter-night level in larger prawns (figure 6). When converted to wet weight concentrations, only the mean for large prawns was above the MPC of 0.2 mg/kg at 0.39 mg/kg, while the mean for medium prawns was below the MPC at 0.14 mg/kg. Arsenic levels were higher in medium prawns (figure 5) and, when means are converted to wet weight concentrations and corrected to estimate of inorganic arsenic only, both sizes were well below the MPC.

Where there was no significant size related result in the south, power was generally good with a small-medium minimum detectable distance for most metals. The exceptions were manganese, iron and nickel which were all insensitive tests with a large or very large minimum detectable distance at a power of 80%.

Table 5. ANOVA summary - analysis of differences between metal levels in tail flesh of medium and small *P. esculentus* at the northern site

METAL	SIZE (Size X Night)	NIGHT (Residual)	SIZE X NIGHT (Residual)	HET VARIANCES (COCHRANS)
Al		*Pooled*	*Pooled*	
As			*Pooled*	
Cd		*Pooled*	*Pooled*	
Cr			*Significant*	
Cu		*Pooled*	*Pooled*	
Fe				
Hg				*Yes*
Mn		*Pooled*	*Pooled*	
Ni		*Pooled*	*Pooled*	
Pb		*Pooled*	*Pooled*	
Se		*Pooled*	*Pooled*	
Sr		*Pooled*	*Pooled*	*Yes*
Zn			*Significant*	

Table 6. Power analysis for non-significant size effects in Table 5
MDD= Minimum Detectable Distance with a = 0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg), a=.05 b=.2	MDD % of Mean
Al	3.96	10.34	3.22	81.28
As	68.21	436.6	20.9	30.63
Cd	0.27	0.02	0.16	56.75
Cr	0.39	0.08	1.86	479.3
Cu	15.12	20.12	4.49	29.68
Fe	9.96	67.98	52.77	529.9
Hg	0.12	0.002	0.29	238.5
Mn	0.52	0.02	0.12	23.33
Ni	0.07	0.002	0.04	59.63
Pb	0.26	0.09	0.30	118.9
Se	1.68	0.14	2.39	142.5
Sr	14.25	71.22	8.44	59.21
Zn	57.18	697.9	169.1	295.7

Figure 4. Size x night interactions in chromium and zinc levels in *P.esculentus* tail flesh, between small and medium prawns at the northern site. Individual bars represent separate nights. Sample sizes: Small n=7,6 for nights 1,2. Medium n=10/night

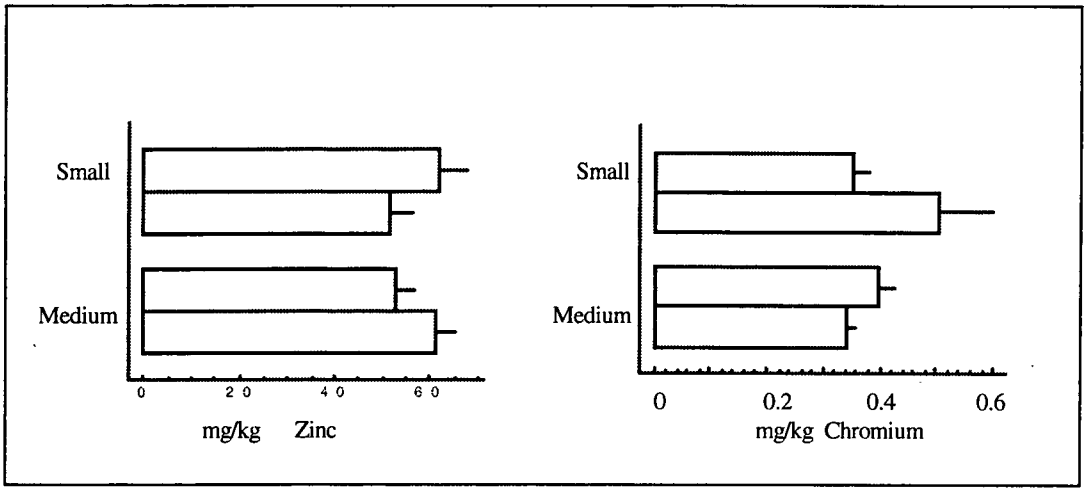


Table 7. ANOVA summary for comparison between metal levels in tail flesh of medium and large *P.esculentus* at the Southern site.

METAL	SIZE (Size X Night)	NIGHT (Residual)	SITE X NIGHT (Residual)	HET VARIANCES (COCHRANS)
Al		*Significant*	*Pooled*	
As	*Significant*	*Pooled*	*Pooled*	
Cd			*Significant*	
Cr		*Significant*	*Pooled*	
Cu		*Pooled*	*Pooled*	
Fe		*Significant*		
Hg	*Significant*			*Yes*
Mn				
Ni				
Pb			*Pooled*	*Yes*
Se	*Significant*	*Pooled*	*Pooled*	
Sr		*Significant*	*Pooled*	
Zn				

Table 8. Power analysis for non-significant size effect results in table 6. MDD= Minimum Detectable Distance with a = 0.05 and power = 80%

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg) a=.05 b=.2	MDD % of Mean
Al	3.01	7.19	1.88	62.32
Cr	0.38	0.01	0.05	14.23
Cu	14.29	18.27	2.99	20.95
Fe	8.13	8.58	9.38	115.3
Mn	0.41	0.02	0.43	104.2
Ni	0.06	0.001	0.10	168.7
Pb	0.19	0.03	0.12	61.01
Sr	12.37	15.27	2.74	22.12
Zn	53.18	197.1	44.92	84.47

Figure 5. Effect of size on mercury, selenium and arsenic levels in tailflesh from medium and large *P.esculentus*, from the southern site
Sample sizes: Large n=21 Medium n=45

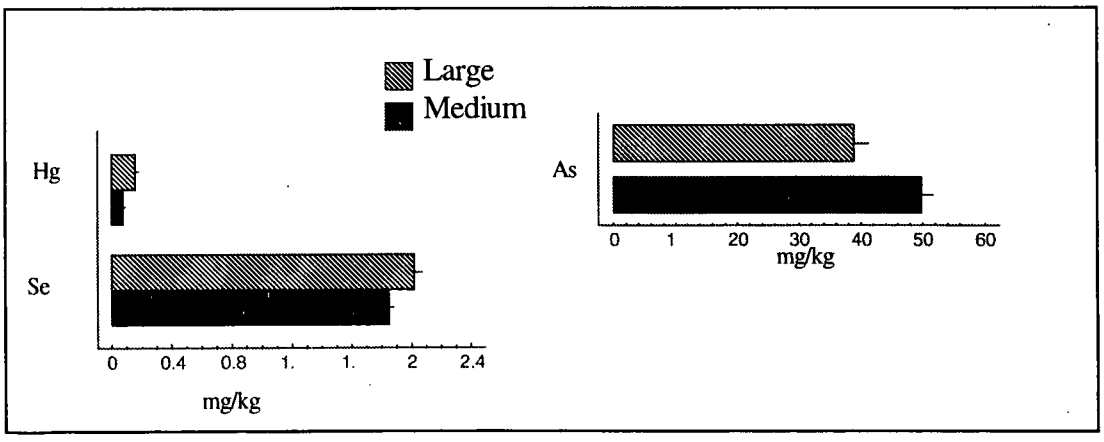
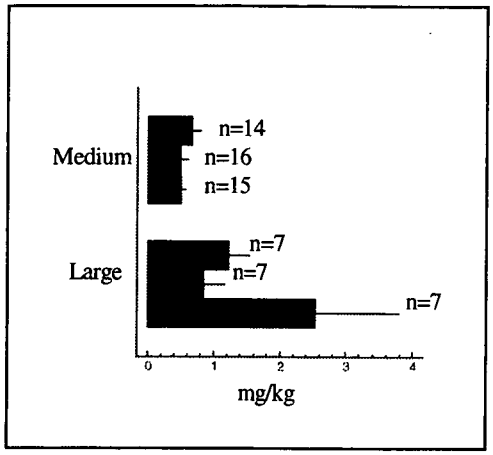


Figure 6. Interaction between size and night in levels of cadmium in tail flesh of medium and large *P.esculentus* from the southern site. Each bar represents a separate night



1.3.3 Patterns With Tissue Type

Metal levels in head, shell and tail flesh tissues from female, medium sized prawns were compared across both north and south sites, in the ANOVA's summarised in tables 9–11.

According to Cochran's statistic, variances were heterogeneous for all metals (table 9). Thus, any significant result for nights and the tissue x nights interaction must be interpreted with caution, as these tests used the residual mean square as the denominator in the F ratio.

Lead was the only metal for which there was no significant tissue type related result. However, the test for a tissue effect for this metal was in-sensitive, with a large (>100 % of mean) minimum detectable distance with 80% power (table 10). While there was a significant tissue x night interaction for mercury (table 9), there was no overall pattern of differences between tissues (figure 8).

The tissue difference pattern varied between remaining metals (figures 7–9), but a recurrent theme was that head levels contained among the highest metal levels and tail contained among the lowest, with shell levels being either high and similar to those found in head tissue (manganese, nickel chromium (figure 9)); somewhere in between those found in head tissue and tail flesh (aluminium, cobalt (figure 7)); or low and similar to those found in tail flesh (uranium (figure 7), silver, cadmium, copper (figure 8), selenium (figure 9)). The exceptions were iron (figure 7) and strontium (figure 9), which both occurred in much higher concentrations in the shell, although tail flesh still contained the lowest concentration. Also, while levels of arsenic (figure 7) and zinc (figure 9) were highest in head tissue, their concentration in tail flesh was above that in the shell.

There were no significant site related effects in levels of aluminium, cadmium, cobalt, iron, lead or uranium. However, these results cannot suggest an absence of an effect, as the tests for site were very weak, with an unacceptably large(>150% of mean) minimum detectable distance, with 80% power, in each case (table 11). Such low power was typically the result of large variability in metal levels from head and shell tissue.

Arsenic (figure 10) and mercury (figure 8) were the only two metals with an overall site effect and levels of both were higher in the north. In the case of mercury, this trend appeared to be clear only in tail flesh (figure 8), despite the absence of a tissue x site effect. Nickel, chromium and selenium showed a pattern in which head levels were higher in the south, shell levels were higher in the north, and tail levels showed no difference between sites (figure 9). Manganese and strontium also showed a similar pattern in which shell levels were higher in the north, while both head and tail levels showed no site differences (figure 9). Zinc (figure 9), silver and copper (figure 8) levels in head tissues were all higher in the north. There was also inter-night variability in levels of the latter two metals in head tissues.

Head cadmium levels were substantially higher on one night in the north (figure 8), but there was no significant tissue x site interaction. Poor power in site tests (table 11) has been discussed above.

Table 9. ANOVA summary for comparison between tissue types and sites for medium sized *P. esculentus*

METAL	TISSUE (TisXNit)	SITE (Night)	NIGHT (Residual)	TISxSITE (TisXNit)	TISxNIT (Residual)	HET.VAR (Cochran)
Ag	*Significant*		*Significant*	*Significant*	*Significant*	*Yes*
Al	*Significant*		*Significant*			*Yes*
As	*Significant*	*Significant*				*Yes*
Cd	*Significant*		*Significant*		*Significant*	*Yes*
Co	*Significant*					*Yes*
Cr	*Significant*			*Significant*		*Yes*
Cu	*Significant*	*Significant*		*Significant*	*Significant*	*Yes*
Fe	*Significant*					*Yes*
Hg		*Significant*			*Significant*	*Yes*
Mn	*Significant*			*Significant*		*Yes*
Ni	*Significant*			*Significant*		*Yes*
Pb						*Yes*
Se	*Significant*			*Significant*		*Yes*
Sr	*Significant*		*Significant*	*Significant*		*Yes*
U	*Significant*					*Yes*
Zn	*Significant*	*Significant*		*Significant*		*Yes*

Table 10. Power analysis for results of metals for which there were no significant results involving tissue type. MDD= Minimum Detectable Distance with a = 0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg)	MDD (% of mean)
Pb	0.19	0.03	0.24	130

Table 11. Power analysis for results of metals for which there were no significant results involving site. MDD= Minimum Detectable Distance with a = 0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg)	MDD (% of mean)
Al	46.78	9435	389	831
Cd	6.04	492	88.7	1470
Co	0.20	0.01	0.46	233
Fe	206	26652	653	317
Pb	0.19	0.01	0.31	164
U	0.03	0.001	0.13	436

Figure 7. Levels of aluminium (Al), arsenic (As) (Total), iron (Fe), cobalt (Co) and uranium (U) in different tissues of *P.esculentus*
Sample sizes: Head n=41; Shell n=41; Tail n=65.

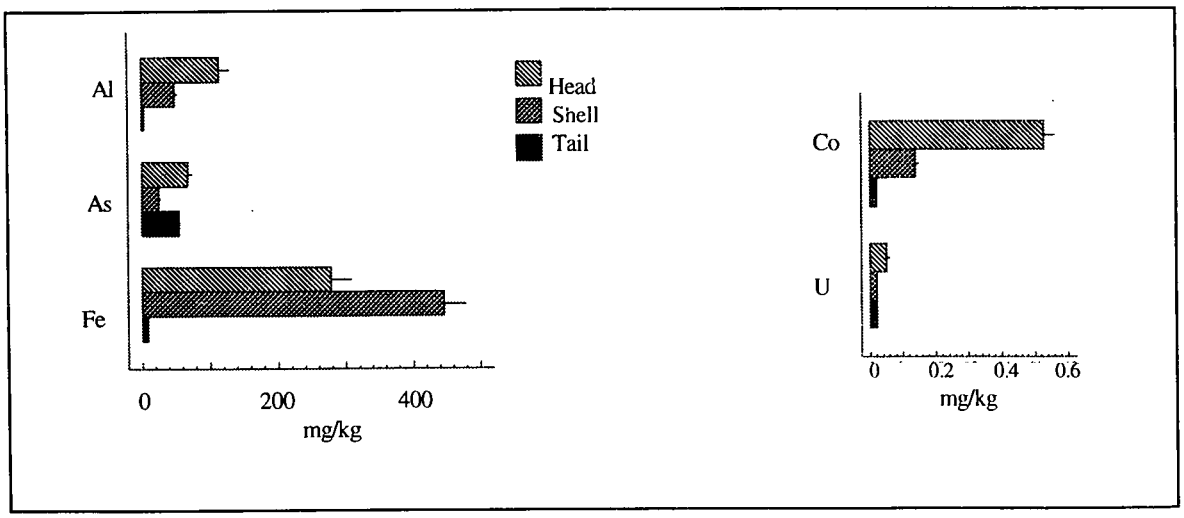


Figure 8. Levels of silver (Ag), cadmium (Cd), copper (Cu) and mercury (Hg) in different tissues of *P.esculentus* from different sampling nights. Individual bars represent different sampling nights.

Legend: North Sample sizes/night n=10 all tissues

South Sample sizes/night n=7, head and shell
n=14,16,15(nights 1,2,3) Tail

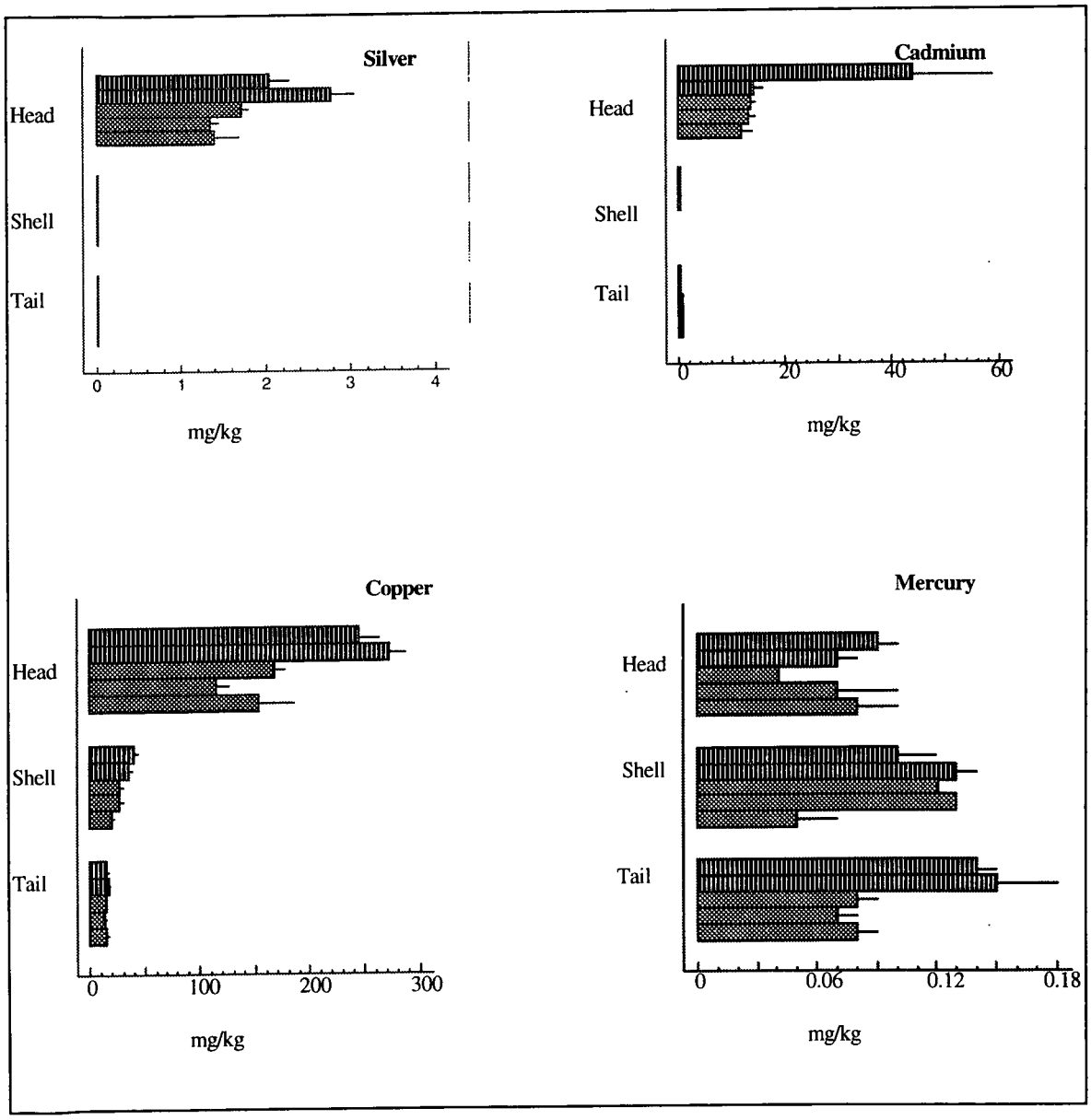


Figure 9. Levels of chromium (Cr), manganese (Mn), nickel (Ni), selenium (Se), strontium (Sr) and zinc (Zn) in different tissues of *P.esculentus* from the north and south sites

Legend: North Sample size/tissue n=20

South Sample sizes/tissue n=21 head and shell
n=45 Tail

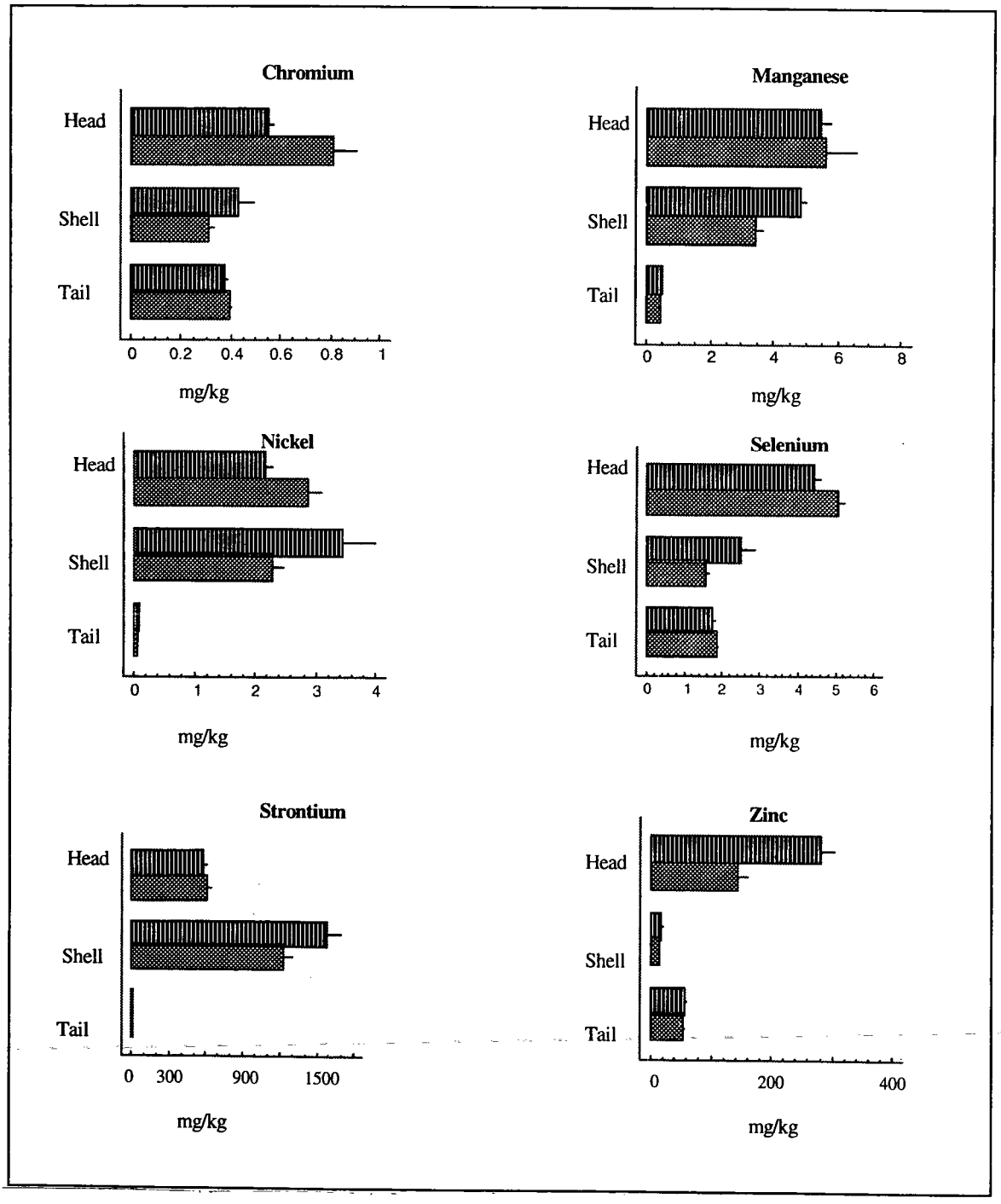
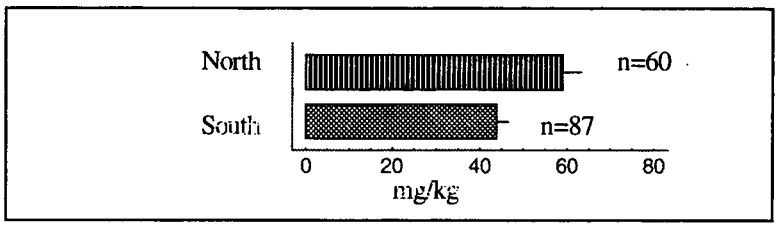


Figure 10. Levels of arsenic (As) in *P.esculentus* tissues from the north and south sites



1.3.4 Patterns With Sex

Metal levels in tail flesh of male and female, medium sized prawns from the southern site only were compared in the ANOVA's summarised in tables 12 and 13. Data for metals silver, cobalt and uranium were excluded from the analyses as >90% of values were at the detection limit of 0.02 mg/kg.

Where Cochran's statistic indicated heterogeneous variances, this was only of consequence to the significant night result for nickel. Inter-night differences in levels of all metals for which there was a significant night result, including nickel, were small.

Where there was no significant sex related effect, power was generally good, with a small to medium minimum detectable distance with 80% power in all cases except arsenic and copper, which allowed only a large distance with the same power (table 13).

Levels of strontium and mercury were higher in males (figure 11,12). The significant sex x night interaction for selenium was due to slightly higher levels in males from one sampling night only. When converted to wet weight concentrations, all mercury and selenium means were below the MPC.

Figure 11. Levels of strontium across sex, for tail flesh from medium sized *P.esculentus* from the south site. Sample sizes: Male n=21 Female n=45

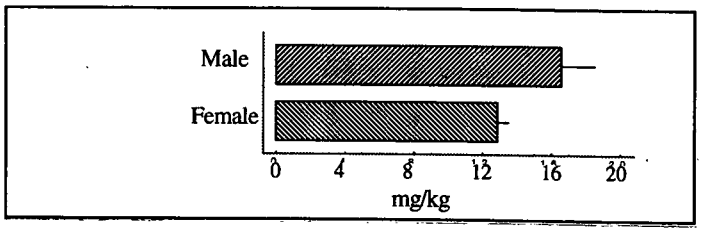


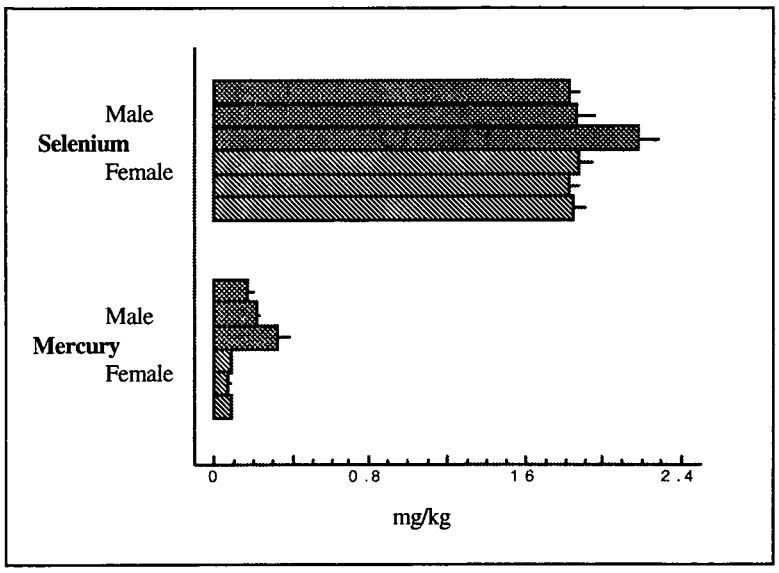
Table 12. ANOVA summary for comparison between male and female medium sized *P. esculentus* tail flesh from the southern site

METAL	SEX (sex X ngt)	NIGHT (residual)	SEX X NIGHT (residual)	HET. VAR (Cochrans)
Al			*Pooled*	
As				
Cd		*Pooled*	*Pooled*	
Cr		*Significant*	*Pooled*	
Cu		*Significant*		
Fe		*Significant*	*Pooled*	
Hg			*Significant*	
Mn		*Significant*		
Ni		*Significant*	*Pooled*	*Yes*
Pb			*Pooled*	*Yes*
Se			*Significant*	
Sr	*Significant*	*Pooled*	*Pooled*	
Zn				

Table 13. Power analysis for non-significant results involving the fixed factor sex. MDD= Minimum Detectable Distance with a = 0.05 and power = 80%

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg) a=0.05;b=0.2	MDD (% of mean)
Al	2.97	9.57	2.17	73
As	46.99	273.8	52.95	113
Cd	0.55	0.13	0.25	46
Cr	0.38	0.01	0.06	15
Cu	14.63	32.69	18.30	125
Fe	8.61	10.05	2.22	26
Mn	0.42	0.02	0.39	93
Ni	0.06	0.001	0.02	31
Pb	0.20	0.03	0.11	58
Zn	54.32	139.63	37.81	70

Figure 12. Levels of selenium (Se) and mercury (Hg) across sex and night, for tail flesh of medium sized *P.esculentus* from the south site
Sample sizes/night: n=7 all nights male
n=14,16,15 nights 1,2,3 female



1.3.5 Patterns With Moult Stage

Metal levels in tail flesh from pre- and post- moult female, medium sized prawns from the southern site only were compared in the ANOVA's summarised in tables 14 and 15. Data for metals silver, cobalt and uranium were excluded from the analyses as most values were at the detection limit of 0.02 mg/kg.

While variances were heterogeneous for data from some metals according to Cochran's values, this did not effect the validity of results as no tests using the residual mean square as the denominator in the F ratio were significant in these instances.

Where there was no significant moult stage related effect, the power of the test for moult stage was generally good, with a small to medium (<100% of mean) minimum detectable distance with 80% power in all cases except arsenic, which allowed only a large (100–150% of mean) distance with the same power (table 15).

Moult stage was a significant factor for levels of strontium, zinc and copper. However, in all three cases, the differences between means was small (figure 13). When converted to wet weight concentrations, means for zinc and copper were below the MPC.

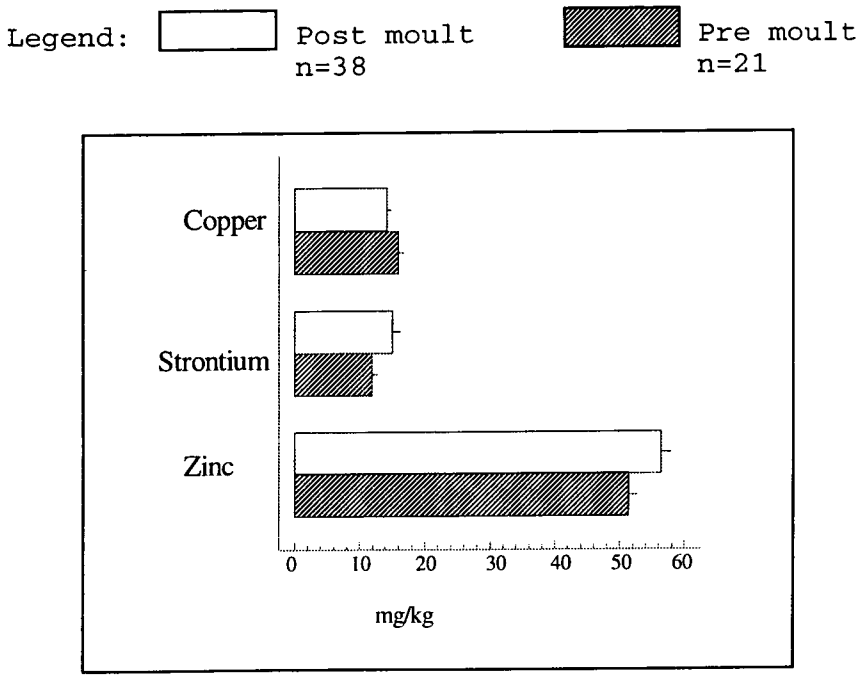
Table 14. ANOVA summary for comparison between metal levels in tail flesh from pre- and post-moult medium sized female *P. esculentus* from the southern site

METAL	MOULT STG (MS x night)	NIGHT (residual)	MS X NIGHT (residual)	HET VAR (cochrans)
Al			*Pooled*	
As				
Cd		*Pooled*	*Pooled*	
Cr		*Significant*	*Pooled*	
Cu	*Significant*	*Significant*	*Pooled*	
Fe		*Pooled*	*Pooled*	
Hg			*Pooled*	*Yes*
Mn			*Pooled*	
Ni		*Significant*	*Pooled*	
Pb			*Pooled*	*Yes*
Se			*Pooled*	
Sr	*Significant*			*Yes*
Zn	*Significant*		*Pooled*	

Table 15. Power analysis for moult stage F tests, where all tests involving moult stage were non-significant. MDD= Minimum Detectable Distance with a = 0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD mg/kg a=.05,b=.2	MDD % of mean
Al	3.10	10.28	2.57	83
As	47.22	328	57.93	123
Cd	0.55	0.13	0.29	53
Cr	0.38	0.01	0.07	18
Fe	8.17	7.94	2.25	28
Hg	0.12	0.01	0.08	62
Mn	0.42	0.01	0.08	18
Ni	0.06	0.001	0.03	42
Pb	0.20	0.02	0.10	55
Se	1.90	0.05	0.18	10

Figure 13. Levels of strontium, copper and zinc in tail flesh of female medium *P.esculentus* from the south site, across moult stage



1.3.6 Patterns With Processing And Handling

Metal levels in tail flesh of medium sized southern prawns were compared across different handling methods in the ANOVA's summarised in tables 16 and 17. Data for metals silver, cobalt and uranium were excluded from the analyses most values were at the detection limit of 0.02 mg/kg. The handling treatments (also described in methods section) were routine 'clean' contamination controlled methods, plus three industry related methods. These were 'green' and 'cooked', where prawns were processed on board the trawler by the crew as normal commercial product and 'chilled', where prawns were collected as per the 'green' treatment and allowed to fully thaw and remain chilled for 24 hours before dissection.

While variances were heterogeneous for data from some metals according to Cochran's scores, this was only of consequence in the case of lead, which showed a significant night effect.

Where there was no significant effect of handling method, power was mostly good with a minimum detectable distance of <50% of the grand mean, with 80% power, in most cases. Only the test for an effect of handling method in aluminium levels had a larger minimum detectable distance with the same power (table 17).

Copper, cadmium, strontium and iron levels all showed an overall effect of handling method and in all cases one of the industry related methods had the highest level (figure 14). Tail flesh from chilled prawns had highest levels of strontium and iron, green and cooked prawns had highest levels of cadmium and cooked prawns had highest levels of copper. Levels in tail flesh from cleanly collected prawns were always among the lowest levels (figure 14).

In the case of copper, the difference between the 'clean' mean and the highest 'industry' mean (cooked), converted to a wet weight basis, was 1.82 mg/kg. This increase, when added to the overall mean of 3.86 mg/kg for copper in *P.esculentus* tail flesh (table 1), is of no

consequence with respect to the MPC of 10 mg/kg. Means for copper in all treatments, when converted to wet weight concentrations, were below the MPC.

This was not the case for cadmium. The difference between the 'clean' mean and the highest 'industry' mean (green), converted to a wet weight basis, was 0.06 mg/kg. This increase would be sufficient to push the overall mean for *P.esculentus* tail flesh of 0.16 mg/kg (table 1) above the MPC of 0.2 mg/kg. When converted to wet weight concentrations, means for clean and chilled prawns were below the MPC at 0.15 mg/kg and 0.14 mg/kg respectively and close to or above it for cooked and green prawns at 0.19 mg/kg and 0.21 mg/kg respectively.

Selenium, chromium, zinc and manganese levels all showed a significant interaction between nights and handling method (table 7), in complex patterns which showed no overlying trend across handling method (figure 15). When converted to wet weight concentrations, all selenium and zinc means were below their respective MPC's.

Figure 14. Level of cadmium (Cd), copper (Cu), iron (Fe) and strontium (Sr) in tail flesh of medium sized *P.esculentus* from the south site, across handling methods. n=15/treatment

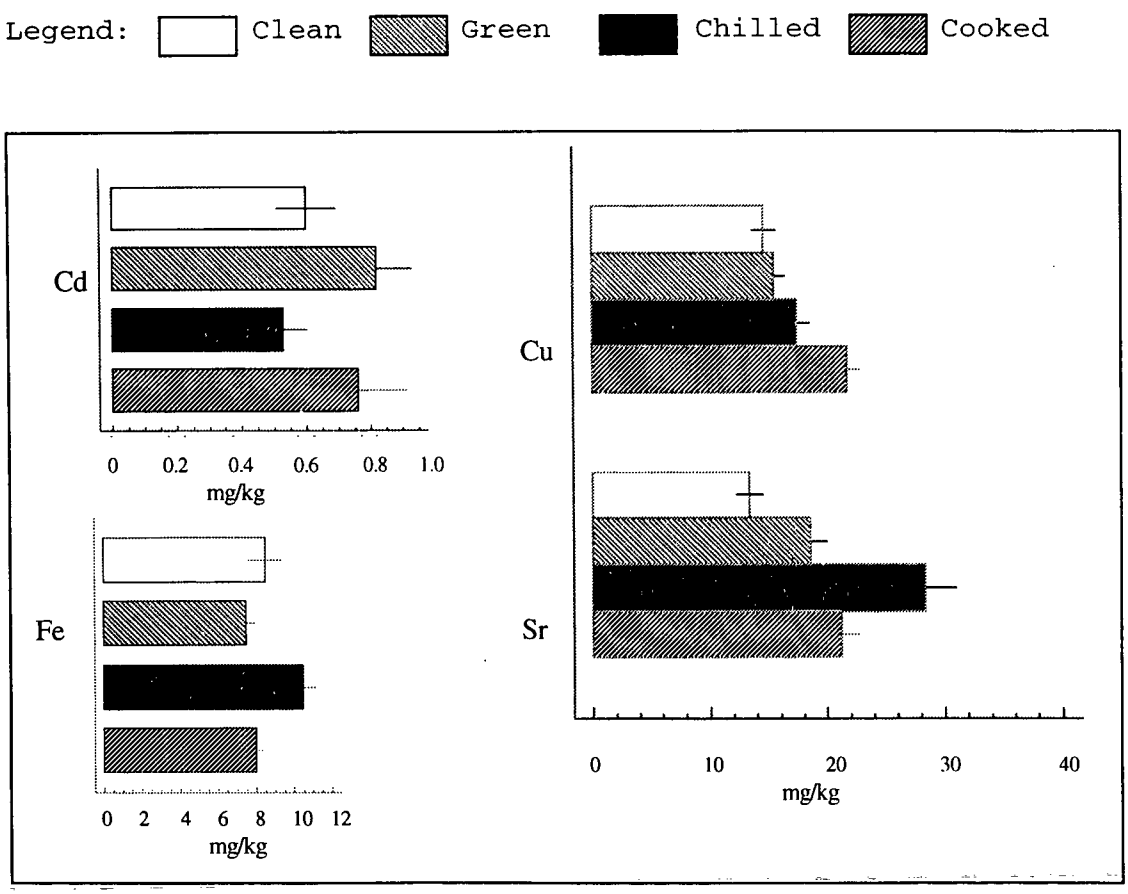


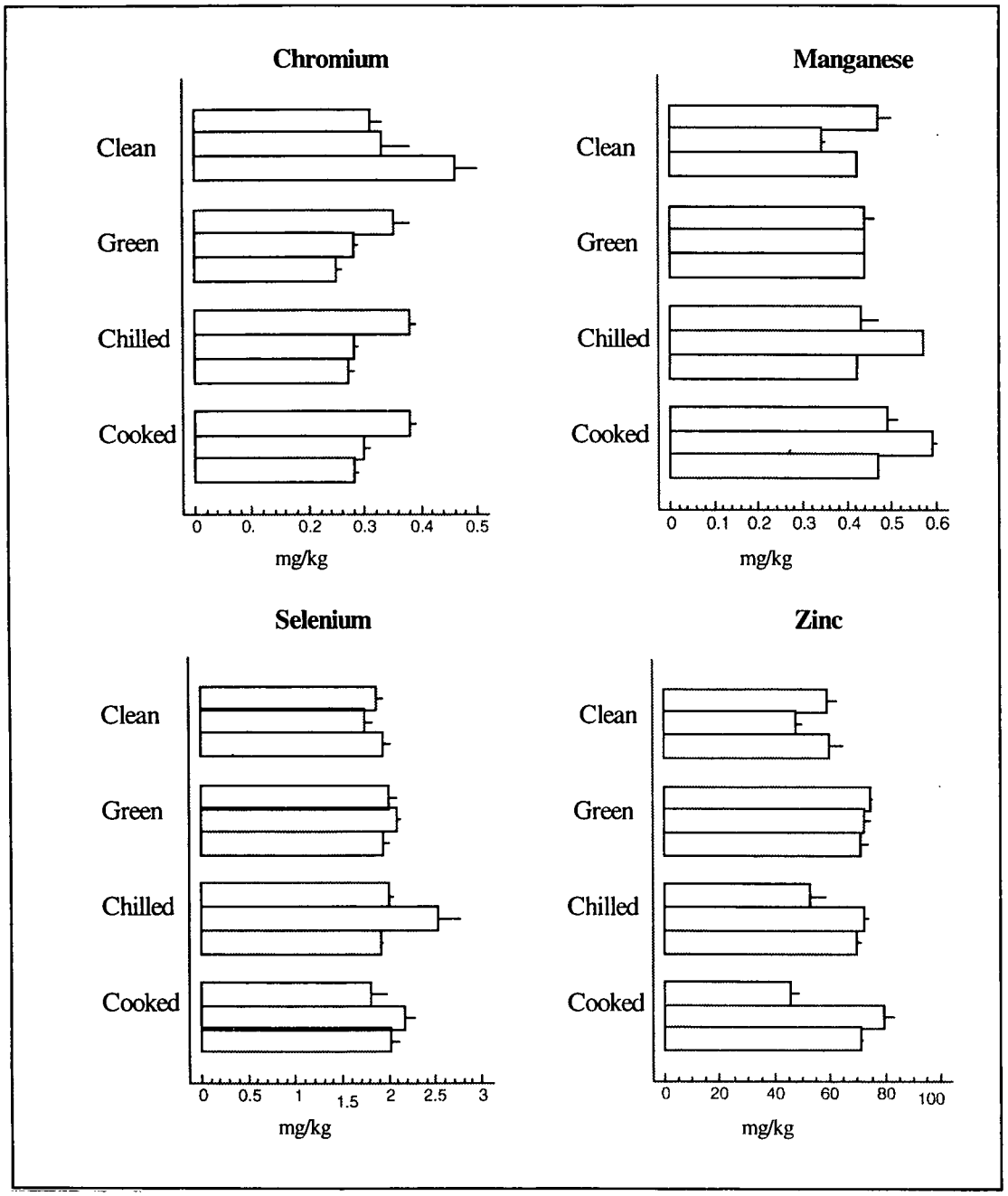
Table 16. ANOVA summary for tests of the effect of different handling methods on metal levels in tail flesh of medium sized *P. esculentus* from the southern site

METAL	TREATMENT (treatXngt)	NIGHT (residual)	TREAT X NIGHT (residual)	HET VAR (Cochran)
Ag *				
Al				*Yes*
As		*Pooled*	*Pooled*	*Yes*
Cd	*Significant*			*Yes*
Co *				
Cr		*Significant*	*Significant*	
Cu	*Significant*			
Fe	*Significant*		*Pooled*	
Hg			*Pooled*	*Yes*
Mn			*Significant*	
Ni		*Significant*		
Pb		*Significant*	*Pooled*	*Yes*
Se		*Significant*	*Significant*	
Sr	*Significant*		*Pooled*	
U *				
Zn		*Significant*	*Significant*	

Table 17. Power analysis for F tests for the effect of handling treatment, where all tests including this factor were non-significant. MDD= Minimum Detectable Distance with a = 0.05 and power = 80%

METAL	GRAND MEAN	MS (F ratio error)	MDD mg/kg a=0.05;b=0.2	MDD % of mean
Al	1.70	2.43	1.81	107
As	45.08	156.57	5.60	12
Hg	0.13	0.01	0.04	28
Ni	0.08	0.001	0.04	46
Pb	0.17	0.01	0.05	31

Figure 15. Levels of selenium, chromium, zinc and manganese in tail flesh of medium sized *P.esculentus* from the south site, across handling method and sampling night. n=5/night/treatment



1.3.7 Spatial Patterns

Metal levels in tail flesh of *P. esculentus*, *P. longistylus* and *M. endeavouri* are compared across sites in the ANOVA's summarised in tables 18–20. Data on the metals silver, cobalt and uranium were excluded from the analyses as most values were at the minimum detection limit of 0.02 mg/kg.

There were no significant species or related results for levels of aluminium and copper. While the inter-species test for copper was reliable with a small minimum detectable distance at 80% power, the test for aluminium differences was insensitive. It accommodated only a very large minimum detectable distance at the same power (table 20).

Levels of zinc, mercury (figure 16), arsenic (figure 17) and cadmium (figure 18) were higher in *M. endeavouri* than the other two species, although in the case of zinc this difference was only slight (figure 16). Arsenic levels were higher in the north in all three species, an effect which was most marked in *M. endeavouri* (figure 17). Conversely, cadmium levels in all species were generally higher in the south, again most markedly in *M. endeavouri* (figure 18).

While cadmium levels in *M. endeavouri* appear to be more variable between nights than the other species (figure 18), the significant night related result for this metal should be treated with caution due to variance heterogeneity (table 8).

When converted to wet weight concentrations, all zinc and mercury means were below the MPC. The same was true for arsenic, once figures for total arsenic are corrected to an estimate of inorganic arsenic only. Mean concentration of cadmium in *M. endeavouri*, at 0.43 mg/kg, was above the MPC, while mean levels of this metal in *P. esculentus* and *P. longistylus*, at 0.11 mg/kg and 0.08 mg/kg respectively, were below it.

Manganese levels were highest and most variable between nights in *P. longistylus* (figure 18). Iron, strontium (figure 17), lead and selenium (figure 18) levels all tended to be slightly higher in *P. longistylus* and *M. endeavouri* than in *P. esculentus*. Iron and strontium levels displayed higher inter-site variability in *P. longistylus* (figure 17), while lead and selenium levels were more variable between nights in this species and *M. endeavouri* (figure 18). There were no consistent inter-species trends in chromium levels, which were dominated by species-specific inter-night variability.

Table 18. ANOVA summary for comparison between metal levels in tail flesh of *P. esculentus*, *M. endeavouri* and *P. longistylus* from both the northern and southern sites

METAL	SPECIES (sp x ngt)	SITE (night)	NIGHT (Residual)	SPECxSIT (sp x ngt)	SPECxNGT (residual)	HET.VAR (cochran)
Al			*Significant*	*Pooled*	*Pooled*	
As	*Significant*	*Significant*		*Significant*		*Yes*
Cd	*Significant*		*Significant*		*Significant*	*Yes*
Cr			*Significant*		*Significant*	
Cu				*Pooled*	*Pooled*	
Fe	*Significant*			*Significant*		*Yes*
Hg	*Significant*					
Mn	*Significant*				*Significant*	
Ni			*Significant*		*Significant*	
Pb					*Significant*	
Se	*Significant*		*Significant*		*Significant*	
Sr	*Significant*			*Significant*		*Yes*
Zn	*Significant*		*Pooled*	*Pooled*	*Pooled*	

Table 19. Power analysis of F tests for site, where all tests including this factor were non-significant. MDD = Minimum Detectable Distance, where a=0.05 and power = 80%

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg) a=0.05;b=0.2	MDD (% of mean)
Al	2.76	37.04	24.34	883
Cd	0.85	3.25	7.21	848
Cr	0.40	0.06	0.96	241
Cu	13.93	26.18	20.47	147
Hg	0.19	0.02	0.62	333
Mn	0.51	0.02	0.62	122
Ni	0.07	0.01	0.40	563
Pb	0.23	0.09	1.22	540
Se	2.20	0.49	2.81	128
Zn	56.78	86.95	4.66	8

Table 20. Power analysis of F tests for Species, where all tests including this factor were non-significant. MDD = Minimum Detectable Distance, where a=0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg) a=0.05;b=0.2	MDD (% of mean)
Al	2.76	8.04	1.04	38
Cu	13.93	14.38	1.39	10

Figure 16. Mean levels of zinc and mercury in tailflesh of *P.esculentus* (n=41), *M.endeavouri* (n=41) and *P.longistylus*(n=38)

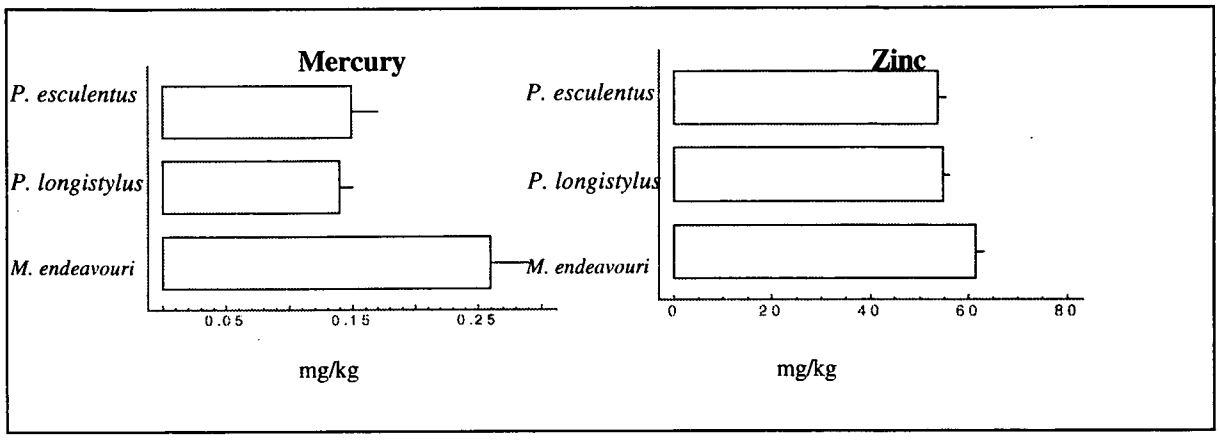


Figure 17. Mean levels of arsenic, iron, and strontium in tailflesh of *P. esculentus* (n=20 north, n=21 south), *M. endeavouri* (n=20 north, n=21 south) and *P. longistylus* (n=19 north and south), across sites

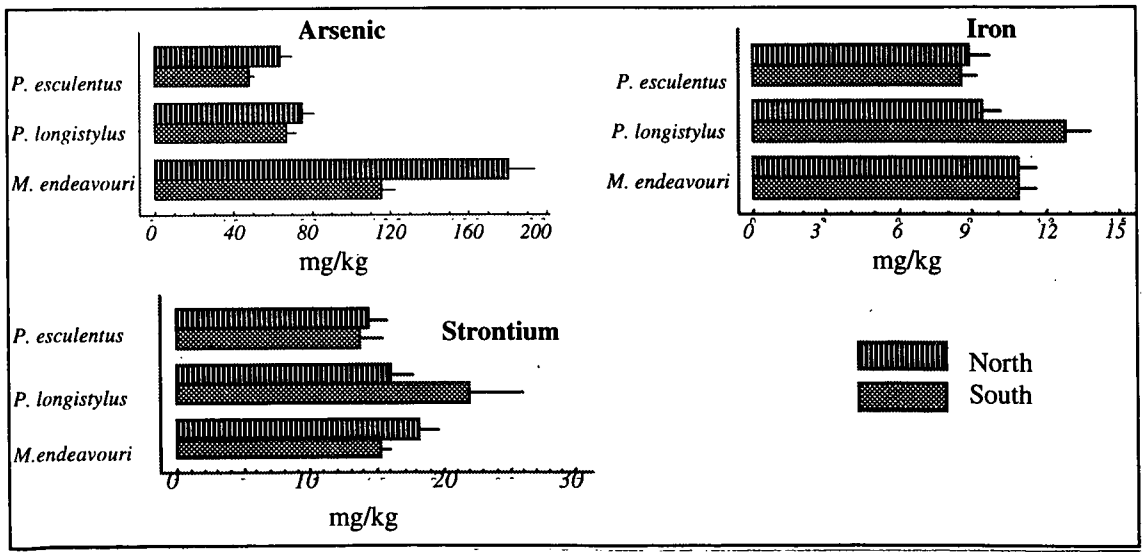
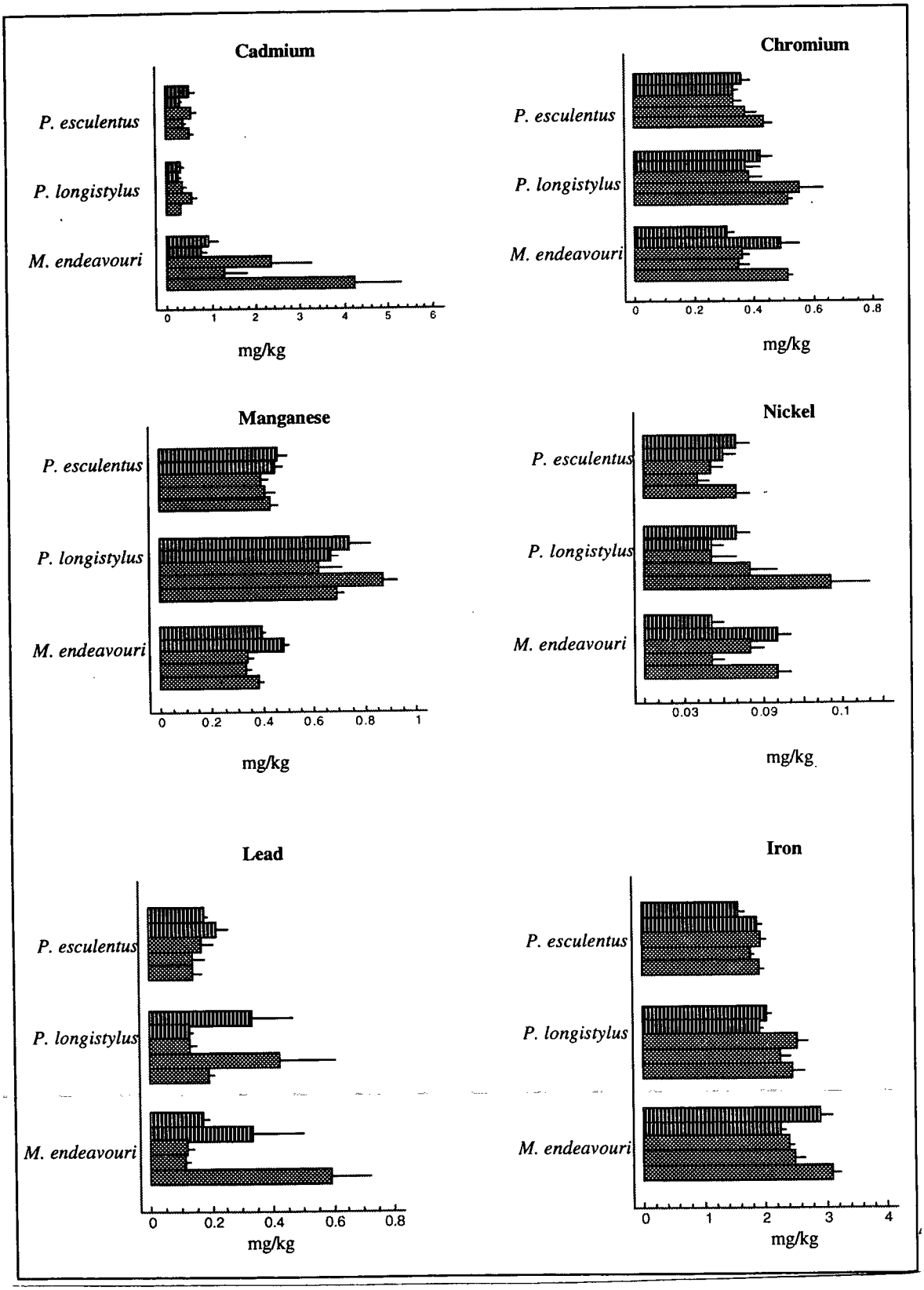


Figure 18. Mean levels of cadmium, chromium, manganese, nickel, lead and selenium in tailflesh of *P.esculentus*, *M.endeavouri* and *P.longistylus*, across sampling nights

North n=10/night (2 nights)
South n=7/night (3 nights)



1.3.8 Seasonal Patterns

Tables 21–23 summarise the results of an interseasonal comparison of metal levels in prawn tail flesh of all three species, between pre- and post-wet season of 1992/93. Prawns in this analysis were from the northern site only. Data for metals silver, cobalt and uranium were excluded from the analyses as all values were at the minimum detection limit of 0.02 mg/kg. There were significant differences in metal levels between species (or related interactions) for all metals except aluminium, chromium and lead. These non-significant results are reliable, as the minimum detectable distance with 80% power was small (table 23).

There were no season related effects on levels of arsenic, cadmium, mercury, copper, selenium and zinc (table 21). These non-significant season results were reliable, with a small or medium minimum detectable distance with 80% power in all cases (table 22). *M. endeavouri* contained much higher levels of cadmium and mercury (figure 19) and slightly higher levels of selenium (figure 20), than the other two species. *P. longistylus* contained slightly higher levels of arsenic, while *P. esculentus* contained slightly higher levels of copper (figure 19).

The lack of a significant season effect on levels of aluminium and lead is only associated with a large minimum detectable distance with 80% power (table 22).

Iron, manganese, nickel, selenium and zinc levels displayed either significant species and night differences, or an interaction between the two. This was overlaid with a slight general trend for pre-wet season levels of iron and manganese to be higher than in the post-wet season. Manganese levels were also slightly higher in *P. longistylus* than in the other two species (figure 20). There were no significant seasonal influences on nickel or zinc levels and while this nickel result had a large minimum detectable distance at 80% power, the zinc result had a small minimum detectable distance at the same power (table 22).

The seasonal effect on levels of strontium and chromium is clearer and both metals occurred in higher concentrations in the pre-wet season, in all three species (figures 21 and 22). Strontium levels were slightly lower in *P. esculentus* than in the other two species (figure 21).

Table 21. ANOVA summary for the inter-season comparison of metal levels in tail flesh of *P. esculentus*, *P. longistylus* and *M. endeavouri* from the northern site

METAL	SPECIES (sp x ngt)	SEASON (night)	NIGHT (Residual)	SPECxSEAS (sp x ngt)	SPECxNGT (residual)	HET.VAR (cochran)
Al			*Significant*	*Pooled*	*Pooled*	*Yes*
As	*Significant*					*Yes*
Cd	*Significant*			*Pooled*	*Pooled*	
Cr		*Significant*				*Yes*
Cu	*Significant*			*Pooled*	*Pooled*	
Fe	*Significant*	*Significant*	*Significant*			*Yes*
Hg	*Significant*		*Pooled*	*Pooled*	*Pooled*	
Mn	*Significant*	*Significant*			*Significant*	*Yes*
Ni					*Significant*	*Yes*
Pb					*Pooled*	*Yes*
Se	*Significant*				*Pooled*	*Yes*
Sr	*Significant*	*Significant*				*Yes*
Zn			*Significant*		*Significant*	*Yes*

Table 22. Power analysis of F tests for season, where all tests including this factor were non-significant. MDD = Minimum Detectable Distance, where a=0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg) a=0.05;b=0.2	MDD (% of mean)
Al	2.27		2.21	97.2
As	94.00		58.46	62.2
Cd	0.48		0.28	58.9
Cu	13.13		2.90	22.1
Hg	0.21		0.07	33.7
Ni	0.06		0.08	140.6
Pb	0.14		0.10	71.4
Se	1.98		0.69	34.8
Zn	54.99		25.25	45.90

Figure 19. Levels of arsenic, cadmium, copper and mercury in tail flesh of *P.esculentus*, *P. longistylus* and *M. endeavouri*, from the northern site and both pre- and post- wet season sampling

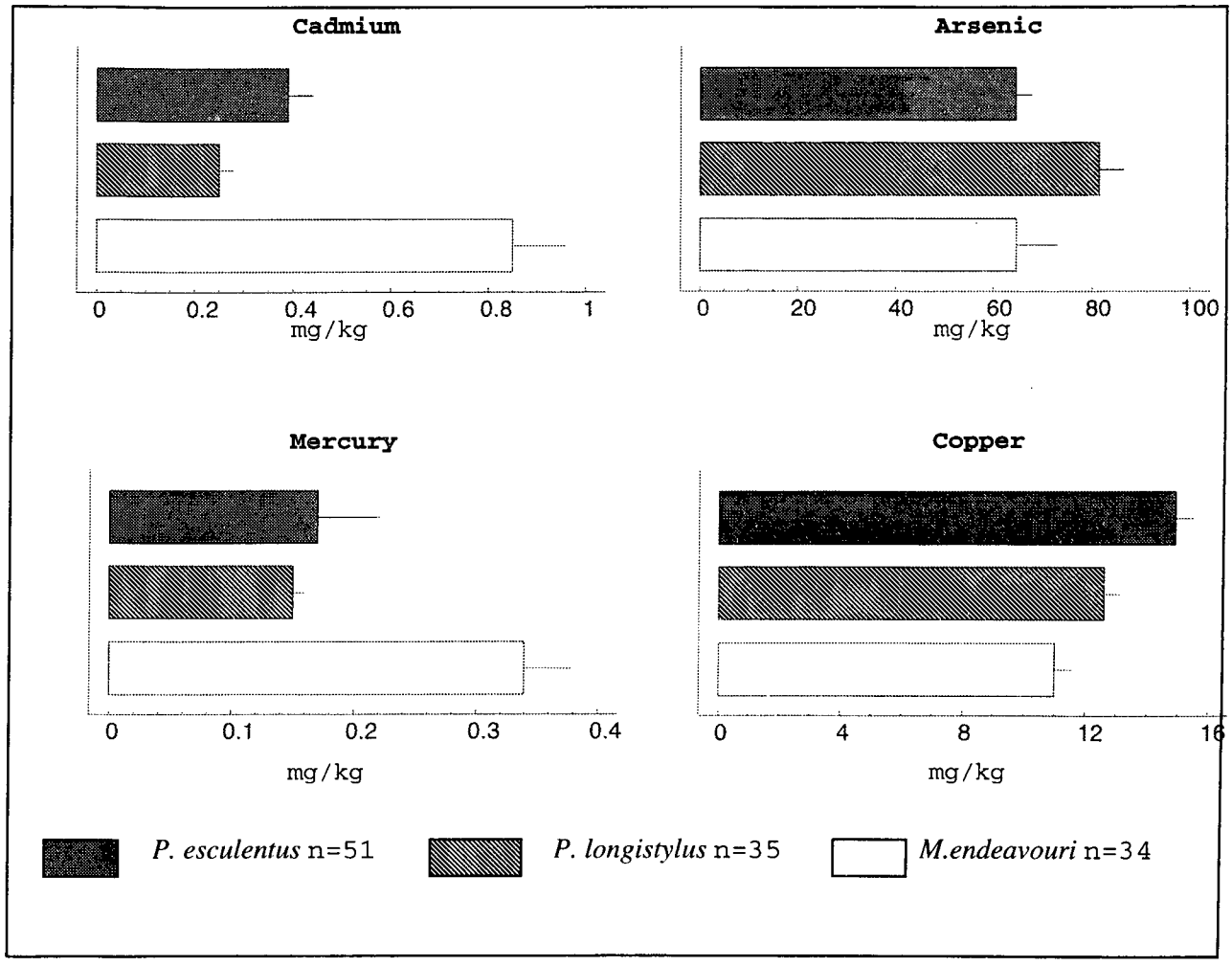


Table 23. Power analysis of F tests for Species, where all tests including this factor were non-significant. MDD = Minimum Detectable Distance, where a=0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD (mg/kg) a=0.05;b=0.2	MDD (% of mean)
Al	2.27		0.78	34.5
Cr	0.34		0.11	32.4
Lead	0.14		0.11	32.4

Figure 20. Interseasonal differences in levels of iron, manganese, nickel, selenium and zinc in tail flesh of *P.esculentus*, *P.longistylus* and *M.endeavouri*, from the northern site. Individual bars represent sampling nights

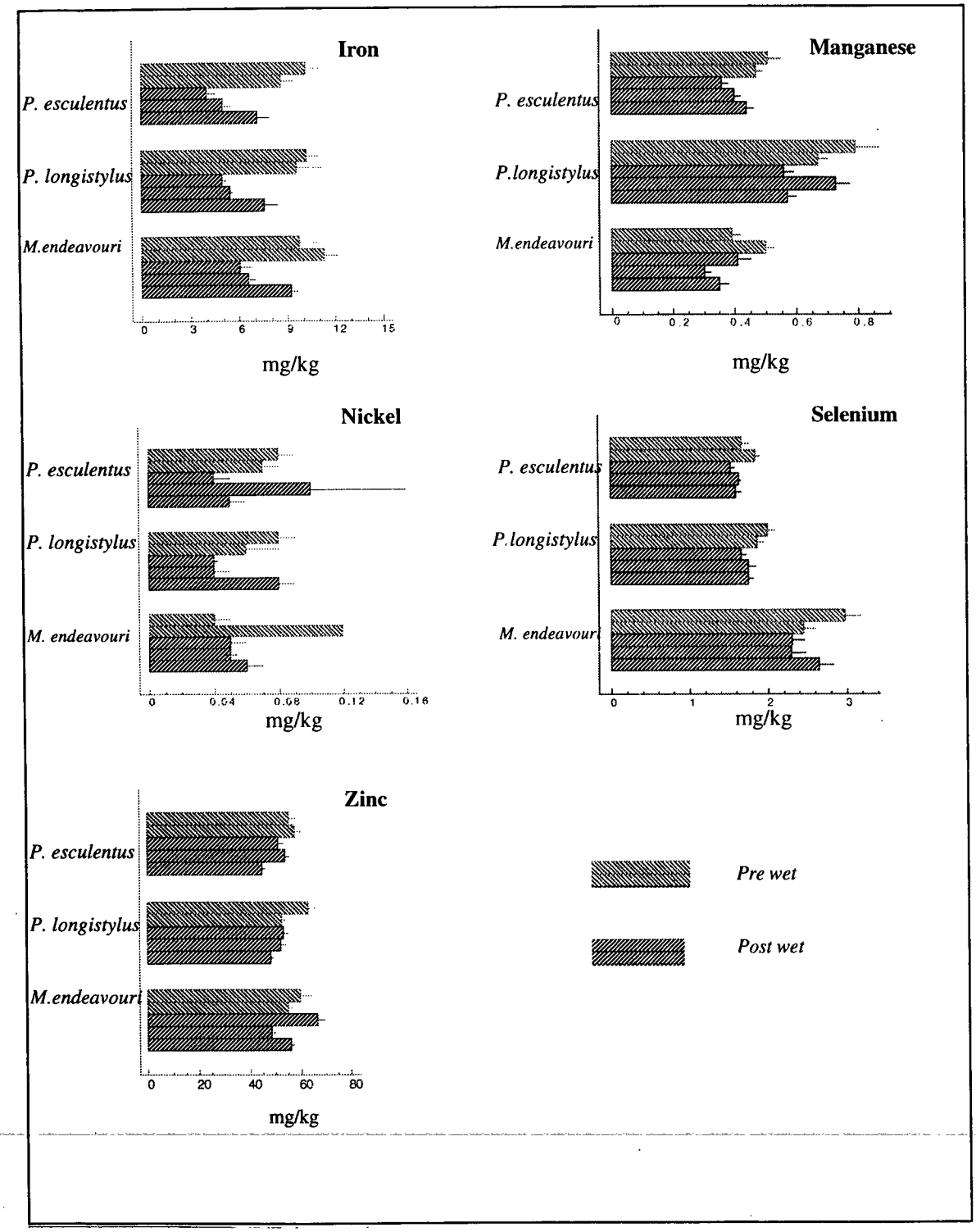


Figure 21. Seasonal differences in strontium levels in tailflesh of *P.esculentus* (n=30 pre-wet, n=21 post wet), *P.longistylus* (n=14 pre-wet, n=21 post wet) and *M.endeavouri* (n=13 pre-wet, n=21 post wet), from the northern site

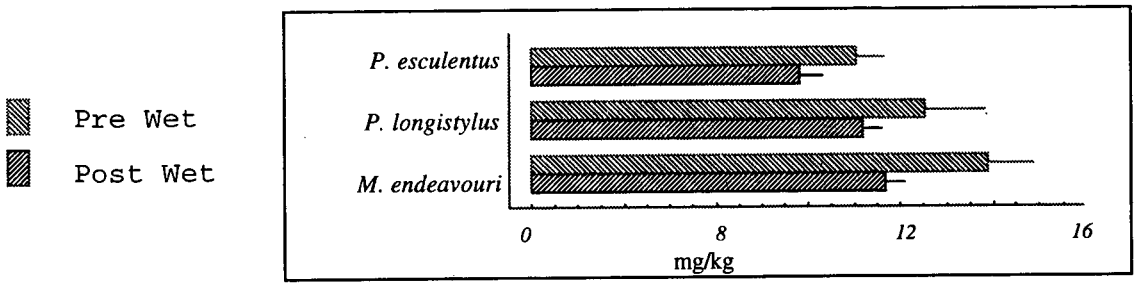
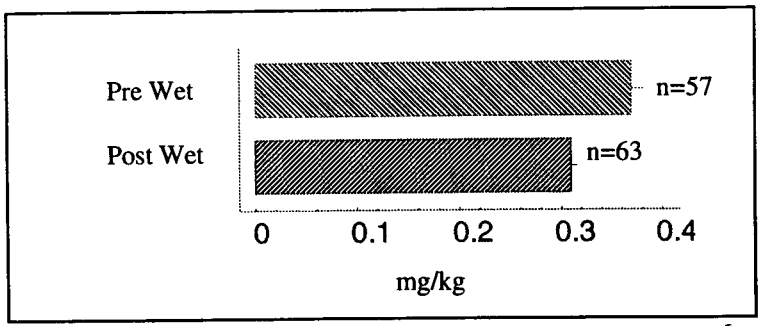


Figure 22. Seasonal differences in chromium levels in tailflesh of prawns from the northern site. Data are means, with standard error bars, of concentrations based on dry weights.



1.4 Discussion

1.4.1 Prawn Metal Levels In The Context Of Previous Studies

Comparisons between metal levels in the present study and those from previous reports are restricted, because a large metal suite such as that presented here is not common in the literature. However, a limited comparison was possible with selected studies of metal levels in related prawn species, as summarised in table 2.

Between the work of Schneider (1990) and Mariath (1991), previous results are available for *P. esculentus* in Torres Strait, for three metals. Further comparative data for levels of a wider range of metals in related prawn species were selected from the literature, to represent sites subject to a range of anthropogenic influences. Tariq et al (1993) provide data from the heavily polluted Pakistan coast in the Arabian Sea, Darmono and Denton (1990) provide data from Three Mile Creek, near the small coastal city of Townsville and subject to a moderate level of urban runoff and a pristine comparison is presented in Peerzada et al's (1992) studies in the Adelaide River, Northern Territory.

These comparisons must only be made in light of several limitations. Firstly, the latter three studies involve different species. Many workers have demonstrated that even closely related species can show markedly different metal uptake and accumulation strategies (Rainbow et al 1990, Rainbow 1988, Rainbow 1991). Also, prawns in the latter two studies are from estuarine environments. Lower salinities generally increase metal uptake rates in marine invertebrates, including prawns (McLusky et al 1986, Denton and Burdon-Jones 1982), so all else being equal, levels of some metals would be expected to be higher in estuarine prawns than in their fully marine counterparts. Finally, different pre-analysis sampling protocols were practised in these different studies and this may effect metal concentrations in some tissues. This issue is discussed later.

Where comparative data were available, metal levels from the present study were either significantly less than those reported from the heavily polluted Arabian Sea, or additionally, in agreement with or less than those from the pristine Adelaide River and moderately influenced Three Mile Creek sites. Thus, in the context of these comparisons, metal levels reported from Torres Strait prawns in the present study appear to be typical of those from clean, coastal marine environments. The exceptions were copper, zinc and cadmium.

The departure from the above pattern for zinc and copper levels in some tissues is probably due to cross-tissue contamination. This concept will be discussed fully later. It is important to note however that estimates of whole body load of these metals in *P. esculentus* in the present study did agree with previous whole body estimates for the same species in Torres Strait (tables 1 and 2). This agreement is consistent with the expectation that, being essential elements, copper and zinc are regulated by prawns. A logical inference from this is that these consistent total body loads may approximate the total metabolic requirement of these elements in this species. Corroborative data is provided by White and Rainbow (1985 & 1987), in their estimates of the enzymatic requirements of decapods for some essential elements. Those authors acknowledge the many assumptions in their calculations and that they provide at best an estimation of the right order of magnitude. Nevertheless, their values for copper (26.3 mg/kg) and zinc (34.5 mg/kg), iron (27.4 mg/kg) and manganese (3.9 mg/kg) closely match whole prawn concentrations reported here (tables 1 and 2).

Cadmium levels reported from Torres Strait prawns (present and previous studies) are similar to levels found in prawns from the Arabian Sea and much higher than those from Three Mile Ck or the Adelaide River (tables 1 and 2). These results probably reflect a similar cadmium bioavailability pattern and support earlier concerns (Schneider 1990, Mariath 1991) that cadmium levels in some Australian prawns, including those from Torres Strait, are elevated. As cadmium is

non essential and is not regulated but accumulated by decapods (Rainbow 1988, Ray 1984), body concentrations reflect available environmental levels. Increases in cadmium content of decapod tissue with increasing environmental concentrations has been demonstrated or supported by many workers and while accumulated cadmium is primarily concentrated in the hepatopancreas, environmental gradients are also reflected in tail muscle (Alliot and Frenet-Piron 1990, Bjerregaard 1990, Darmono 1990, Rainbow 1985, White and Rainbow 1982, 1986).

While the bioavailability of cadmium may be similar in the Arabian Sea and Torres Strait, the source of cadmium is likely to be very different. The prawns analysed by Tariq et al (1993) were collected in the vicinity of major rivers draining heavily industrial and urbanised parts of Pakistan, including the Hub, Malir and Indus Rivers. The conclusion in that study was that these adjacent areas were sources of anthropogenic metal contamination into the Arabian Sea. In the case of Torres Strait however, there are no adjacent cadmium pollution sources and observed elevated cadmium levels are likely to be from entirely natural causes. This will be discussed later with respect to seasonal and spatial patterns.

1.4.2 Metal Distribution Among Tissues

Metal accumulation does not occur uniformly across tissues, because certain organs and tissues perform specific roles in metal uptake, retention and excretion. Thus, metal content in different tissues varies according to function in and physical access to uptake and excretion processes. Of particular importance is the specific role of some tissues as sites of selective accumulation of detoxified metals (Rainbow 1991).

With the exception of mercury and lead, the head had elevated concentrations of all metals measured (table 1, figures 2, 8, 9) and in the case of metals with trade or health limits, the head contained a major proportion of total body load (figure 3). This result is consistent with many studies of other decapods including closely related species (Whyte and Boutillier 1991, Bryan 1968, Schneider 1990, Mariath 1990, Darmono and Denton 1990) and is not surprising as the head contains the hepatopancreas or digestive gland, which is known to accumulate some metals principally as metallothionein complexes (Rainbow 1990, Bryan 1968, Ieely and Nott 1980, Engel 1987, Bjerregaard 1990). Also, the gills, which are a permeable interruption in the head cuticle, are probably a site of uptake and excretion (Bryan and Ward 1965, Bryan 1968, Rainbow 1990) and may contribute significantly to total head metal loads.

Some workers have also highlighted exoskeleton as an important sink for metals in decapods, principally via adsorption (Bryan and Ward 1965, Wright and Brewer 1979, Depledge 1989, Rainbow 1990), but the present study did not generally support this. It is likely that routine thorough washing of all samples with Reverse Osmosis Polished (ROP) water would have removed any free ions superficially adhered to the cuticle. Thus, where levels of aluminium, iron, cobalt, copper, manganese, nickel and strontium were elevated in abdominal cuticle (table 1, figures 2,7–9), this was probably due to the internal content of exoskeleton cells. Incorporation of iron in the exoskeleton matrix has been supported by previous workers and may promote shell hardening (White and Rainbow 1987, Depledge 1989). Strontium is also an important component in cuticle structure and its strong representation in decapod shells has been noted previously (Martin 1974). Bryan and Ward (1965) demonstrated that manganese is distributed throughout the calcified part of the shell and that the outer part contains more than the inner part. They suggested that as manganese is able to substitute for calcium in calcium carbonate, it may to some extent replace calcium in the cuticle. For metals with a health or trade limit, abdominal shell tissue was never an important source of total body metal loads, due to the relatively small biomass of this tissue (figure 3).

The significance of elevated levels of arsenic, manganese, lead, selenium and zinc in ovaries of ripe females (table 1) is unclear, although similar trends in zinc and manganese levels were found in ovary tissue of *Macrobrachium sp* from the Adelaide River (Peerzada et al 1992). Ripe prawn ovaries are not insubstantial tissues and so have the potential to contribute significantly towards total metal load. In the present study ripe ovary tissue accounted for 30% of lead, 22% of selenium and 16% of zinc load (figure 3). These figures do not appear to present concern with respect to trade or health (figure 2, table 3) and while levels of selenium in ovary are above the MPC for that metal (figure 2), the resulting net whole prawn concentration of this metal was below the MPC. An early WHO report (1973) states that no deleterious effects of excessive selenium intake have been identified for humans.

While tail flesh generally contained the lowest levels of most metals, this tissue did appear to be an important storage site for arsenic and mercury. Tail muscle concentrations of these metals approached or exceeded levels in the head and due to the high relative volume of tail tissue, it contributed 37% and 57% (respectively) of the total body load of these metals (table 1, figure 3,8,9). Tail flesh also carried substantial (17–32%) proportions of the total body load of lead, selenium and zinc (figure 3). Concentration data for these metals reported here did not exceed trade or human health limits (figure 2, table 3).

1.4.3 Spatial Variation

Actual strategies for metal accumulation and storage aside, it is important to consider what metals were available for uptake in the first place - that is, metal levels in the organism's immediate environment. Penaeid prawns live in close association with sediment. They bury in surface sediment layers for part of the day and night and, periodically emerge to feed on small benthic epifauna (Dall et al 1990, Hill 1985). While the northern site in this study is south of the general zone of Fly River sediment influence, it is reasonable to expect that sediments at the northern, Stephens Island site would be under relatively more influence from Papua New Guinea terrigenous sources than the southern site.

If essential metals are regulated by prawns, we would expect levels of these elements in prawns to be comparable between sites, independent of gradients in sediment. The present study's results for most essential metals were consistent with this principal. Cobalt, iron, aluminium, manganese and nickel (table 9, figure 9) are all Fly River associated metals which should have occurred in higher concentrations in northern sediment, yet in prawns they occurred in generally consistent levels between sites, at least in the major storage tissue, usually the head (table 1, figure 3).

However, copper, zinc and chromium are essential elements also associated with the Fly River which did vary between sites. Copper and zinc levels in the major storage tissue, the head (figure 3), followed the expected sediment gradient, with higher levels in the north (figure 8,9). Curiously, chromium in head tissues displayed the reverse, with higher levels in the south (figure 9). It is possible that either metabolic requirements varied between sites, or *P. esculentus* is less adept at regulating these metals than species studied by previous workers (White and Rainbow 1982 and 1986).

As discussed earlier, non-essential metals are generally thought to be accumulated by decapod crustaceans and levels of these elements in the major storage tissues of prawns would be expected to follow environmental gradients. Mercury is one such Fly River metal (Gladstone 1996) that was higher in the major storage tissue (tail flesh) at the northern site (figure 8). While the present study cannot report significant spatial trends in other non-essential metals, it cannot be assumed that such trends are absent, as the relevant F tests were insensitive to all be very large effect sizes (table 11). Salinity, temperature and pH are known to affect bioavailability of some metals (McLusky et al 1986, Denton and Burdon-Jones 1982) and inter-site variability in these physio-chemical

properties is expected in Torres Strait. A plume of slightly brackish water protruding from the Fly River into northern Torres Strait via the Great North East Channel, is thought to be a permanent feature of the region (Wolanski 1991). As the northern site lies in the path of this plume, it is likely that at some times salinities there are slightly lower than at the southern site. Grain sizes and organic carbon content and carbonate content would also have varied between sites and, these are all factors which may affect metal bioavailability (Luoma 1990).

It is interesting to note that where the main storage tissue, usually the head, showed inter-site trends, tail flesh levels remained constant (figures 8, 9). This re-asserts the general lack of metal storage sites in tail muscle and indicates that muscle levels of most metals are regulated.

1.4.4 Seasonal Variation

Post-wet season samples were available from the northern site only, where a major environmental seasonal difference should have been the presence of the monsoon and the more pronounced intrusion of the brackish tongue from the Fly River (Wolanski 1991). Thus, bio-availability of many metals should have been higher post-wet season. However the wet season of 1992/93 was abnormal, with less than average rainfall. Physical data gathered in the area at that time for the Torres Strait Baseline Study indicate that surface salinities were actually less pre-monsoon than during the monsoon (Gladstone 1996). Further, inter-seasonal comparisons in the present study are limited, because tail flesh only was analysed. We have already seen that except for metals which are stored there, levels in tail flesh are generally regulated.

Nevertheless, minor seasonal variation in tail flesh levels was apparent for four metals - chromium, iron, manganese and strontium, with slightly higher levels pre-monsoon (figures 20,21,22). With the exception of strontium, these metals are essential and as such should be regulated at the organism, let alone tail flesh level. It is possible that there was inter-seasonal variation in the metabolic requirement for these metals.

1.4.5 Manageable Factors And Their Affect On Metal Levels In Tail Flesh

Although tail flesh is not an important storage site for most metals and as such is not a good analytical tissue of choice for the assessment of environmental gradients in metal availability, it does happen to be the tissue that most people eat. Despite some ethnic groups preference for consumption of other tissues, tail muscle is generally accepted as the 'edible portion' for the context of trade limits. It is thus the tissue of most relevance to a discussion on trade and human health concerns. Because such concerns were the impetus for the present study and in light of the high cost of metal analysis and the need to rationalise resources, the present study's address of the following factors was restricted to the net effect on tail muscle only.

1.4.6 Biotic Factors

Age and Growth

A prawn's age can be considered its period of exposure to environmental metal levels. Thus, tissue loads would tend to be greater in older prawns, simply because they have been around longer. However, in terms of concentration in tissues, an increase in metal load with age will be countered by an increase in biomass due to growth. Growth has the potential to dilute accumulated metals to an extent that tissue concentrations actually fall (Rainbow 1990). White and Rainbow (1987) demonstrated such a growth dilution trend in levels of zinc, iron, manganese and cadmium in a mesopelagic decapod. When growth rates are faster, as is often the case in younger organisms, this growth dilution effect will be greater.

In the present study, size was used as a relative measure of age. In the northern site, where small (<30 millimetres carapace length) prawns could be compared to medium (33–38 millimetres

carapace length) prawns, there were no significant differences in tail flesh concentrations of any metals (table 5). For essential metals, this result is consistent with the concept of regulation. However, concentrations of non essential metals, such as cadmium, mercury and lead, should be accumulated. The growth rate of *P. esculentus* between these two size classes is probably rapid enough to dilute and mask the increase in these metals.

When two larger size classes were compared at the southern site, cadmium and mercury levels in tail flesh were higher in larger prawns (figure 5,6). Site differences aside, the growth rate between these two larger site classes is probably slower, so that the accumulation of these metals was able to catch up with and exceed the dilution effect.

Sex

For most metals, the accumulation strategy in tail flesh appears to be the same for both sexes, as there were no differences between them in prawns of the same size from the south (table 7). The exceptions were strontium and mercury, which were both slightly higher in males. This may simply be due to differing growth rates between sexes. Female *P. esculentus* grow faster than males (Derbyshire et al 1990), so growth dilution of accumulated metals is probably more marked in females.

Moult Stage

The moult cycle is thought to play a role in metal uptake. The cuticle is the major membrane which separates internal tissues from environmental metal concentrations and its permeability varies throughout the moult cycle (Rainbow 1988). Higher rates of cadmium and zinc uptake and loss in a shrimp after moulting was attributed to higher permeability of the new, soft cuticle (White and Rainbow 1984 and 1986). Also, work by Engel (1987) suggests that the moult cycle interacts with the biochemical processes that control the turnover of metallothioneins and the translocation of essential metals Cu and Zn between heamocyanin and hepatopancreas. Other workers have linked moulting with excretion of Cu and Zn, via sloughing of membrane-bound intracellular granules (Almohanna and Nott, in Engel)

In the present study, the net effect of moult stage on metal levels in tail flesh was absent or slight (table 14, figure 13). This result does not imply that the moult cycle is not an important part of metal cycling in prawns but rather that this cycling does not involve tail muscle.

Other Species

The nature of metal accumulation strategies varies between species and may be quite different even in closely related species (Rainbow 1990). Observed significant inter species variability in levels of some metals in tail flesh (figures 16–19), is therefore not unexpected.

However, differing accumulation strategies may not be the only cause of observed variation. An interesting trend in tail concentrations of zinc, mercury, arsenic and cadmium is that levels in *M. endeavouri* are consistently higher than the other two species. A contributing factor may be inter-species differences in post-death integrity of some head tissues.

It is the authors observation that head tissues in *M. endeavouri* are much more fragile after death than the other two species. During processing, heads often break off and the hepatopancreas quickly begins to break down. It is possible, that in *M. endeavouri*, rapid deterioration of head tissues physically mobilises the high levels of metals contained in these tissues and facilitates their leakage into the otherwise lower level tail flesh. Not all metals which occur in elevated concentrations in head tissue (figure 2) are also elevated in tail flesh of *M. endeavouri*. Thus, other factors such as the specific organ of association and robustness of metallic bonds, may also influence metal release during post-death tissue breakdown.

1.4.7 Handling Methods

The possibility that metals may leak from high level head tissues such as the hepatopancreas, into the otherwise low level edible portion of prawns, poses important questions for the prawn industry with respect to product handling. Also, the processing environment has the potential to introduce metal contamination to the catch, from sources such as diesel exhaust fumes, blood and body fluids of the by-catch and corrosion on galvanised cooling coils. This study compared tail metal levels in carefully and cleanly collected prawns with the products of a variety of handling methods considered to be typical industry treatments.

There are three main conceivable mechanisms by which metals could be mobilised from head to tail. Firstly, as implied earlier in the case of fresh *M. endeavouri* samples, simple post-death proteolysis, or enzymatic tissue breakdown, should be considered. Gillespie et al (1983) have described this process as the cause of hepatopancreatic breakdown and contents release in mud crabs. Samples categorised as 'green' in the present study represent the effect of such post-death breakdown as well as extra-contamination during routine processing.

Secondly, ice crystal damage to cell membranes and contents may cause cells to rupture. This process may explain the elevated levels of cadmium, zinc and copper previously found in tail flesh of *P. esculentus* in Torres Strait, by Schneider (1990) (table 2). While Schneider's whole prawn concentration estimates were comparable with the present results, the distribution among tissues was different (table 1). Schneider's method of allowing prawns to fully thaw before dissection may have facilitated leakage from head tissues to tail tissues of metals released after cell rupture. 'Chilled' samples in the present study were 'green' prawns thawed fully and left chilled for 24 hours before dissection. This simulated the common practice in retail fish outlets of displaying thawed product for prolonged periods before sale and consumption and allowed an assessment of its impact on metal levels.

Thirdly, cooking may promote rupture of high level head tissues. Slattery et al (1992) suggest that while cooking had the benefit of inactivating digestive enzymes that cause proteolysis in spanner crabs, a range of cooking methods still contributes to leakage of the hepatopancreas. 'Cooked' fresh prawns were also compared in the present study.

One possible limitation of the comparative methods used here is that thorough ROP rinsing of tissues for analysis may have efficiently removed metal rich fluids and free ions adhered to tail flesh, thus masking some elevation in metal levels. However, this study did find that, when compared to 'clean' prawns, tail flesh levels of copper, strontium, iron and cadmium were elevated in at least one of the 'industry' treatments (figure 14).

Iron and strontium levels were most elevated after freezing and thawing, while copper levels were most affected by cooking. Neither of these metals were affected by immediate post-death contamination or leakage. Cadmium was most elevated in tail flesh under both 'green' and 'cooked' treatments, indicating redistribution of this metal from head tissues is probably promoted by simple post-death tissue breakdown and cooking. Also, corrosion on galvanised cooling coils within the brine and IQF tanks may have made extra cadmium available via passive absorption. Galvanised surface contact has been suggested as a potential source of cadmium in fisheries products by Mariarth (1991) and Hamdorf (1991).

1.4.8 The Context Of Trade Limits And Human Health

Of the metals for which a trade limit or health data exist, levels of most metals reported in the present study are of no cause for concern, whether tail muscle or whole prawn is used for comparison with relevant legal limits (figure 2, table 3). Indeed, prawns may be a worthwhile dietary source of zinc (table 3). The exceptions to this were copper and cadmium.

While copper levels in prawn tail flesh were generally well below the Maximum Permissible Concentration (MPC), the concentration in whole prawn was above it due to high levels in and relative volume contribution of the head. Thus, copper levels may be of concern with respect to trade regulations but only if the whole prawn is considered to be the edible portion. However, they pose no problem with respect to human health, as consumption of ten whole prawns per week would represent less than 5% of the World Health Organisation's (WHO) Provisional Tolerable Weekly Intake (PTWI) (WHO 1986, table 3).

Levels of cadmium in tail flesh were always below the MPC only in the case of *P. longistylus*. While levels in *P. esculentus* were generally close to the MPC of 0.2 ppm (wet weight basis), in *M. endeavouri* they frequently exceeded it (figure 2). Estimated cadmium concentration in a single whole *P. esculentus* was over ten times the MPC (figure 2). This was mainly due to the high concentration of cadmium in and the large contribution to total body volume of the head (figures 2 and 3). Clearly though, even if tail flesh only is considered, cadmium levels are either close to or exceed the MPC.

These same levels at first appear to be of lesser concern to human health, based on the PTWI for cadmium and estimates of total cadmium intake from other dietary sources in the average Australian diet (table 3). Ten prawn tails could be consumed each week with a considerable margin of safety below the PTWI (table 3). However, a single whole prawn contains 20–25% of the PTWI for cadmium, which when added to intakes from other sources would place total intake at close to the PTWI (table 3). Thus, regular consumption of several whole prawns may pose a health risk with respect to this metal.

However, the PTWI may not be a suitable guide to safe cadmium consumption. A recent review of medical research indicates that cadmium induced toxic effects in humans may occur at much lower intake levels than previously predicted, and that the current PTWI is far too high. It concludes that widespread cadmium intakes sustained at the PTWI would result in a prevalence of cadmium induced renal damage in humans, that there may be no 'safety margin' between current levels in human kidneys and levels at which early kidney effects may occur, and that any actions to decrease human exposure to cadmium would be justified (Elinder and Jarup 1996). Clearly, more direction from the medical community on safe cadmium consumption levels is required before levels reported in the present study can be assessed with respect to human health.

Given the precarious relationship between trade limits and levels in tail flesh and Elinder and Jarup's (1996) recommendations and conclusions, knowledge of any factor which influences cadmium levels, even slightly, is important. Results of this study indicate that such minor elevation occurs with prawn age, after some forms of industry related processing (figure 14) and that *M. endeavouri* tails generally contain higher levels (figure 18). The insensitivity of some F tests for inter site variation in cadmium levels in the present study (table 11, 19), inhibited the assessment of potential site related trends.

While these results provide insight into factors which both influence cadmium levels and may be amenable to management, they are neither conclusive or comprehensive enough to justify action. If indeed management steps are to be considered necessary, further work is needed. Due to the potential significance of even small elevations in cadmium, any further research must be designed

to allow sensitive significance testing. Also, further studies on the effect of processing treatments should include more species and a greater variety of treatments which vary the length of freezer storage, number of thaw-refreeze cycles and cooking/freezing combinations. The method of snap freezing may also influence the extent of ice crystal formation and hence all wall damage.

It is important to note that the above assessments are pessimistically biased, as they present the worst case scenario of all cadmium present occurring in a bioavailable form. The reality of this assumption is not clear, although some literature suggests that it may be accurate. According to Edmonds and Francesconi (1993), cadmium is unlikely to form stable bonds with carbon in the human gut and will therefore be present mostly as the Cd++ ion and thus highly available, although the rate of absorption will depend on other substances present. Slattery et al (1992) support this view. Other authors, however, suggest that, based on intake and excretion studies in humans, only 5–10% of ingested cadmium is absorbed (Fassett 1980, Friberg et al 1974). Clearly, further understanding of cadmium bioavailability is required before health implications of whole prawn consumption can be determined.

1.4.9 Recommendations For Further Work

1. The issue of levels of cadmium with respect to trade limits needs further work. The most important aspect is the potential of handling methods to influence breakdown and re-distribution of high level head tissues. Further experimental work on the effect of various handling methods should be carried out.

2. Clarification of safe sustainable cadmium intake levels should be sought from the medical community, with a possible review and reduction of the PTWI.

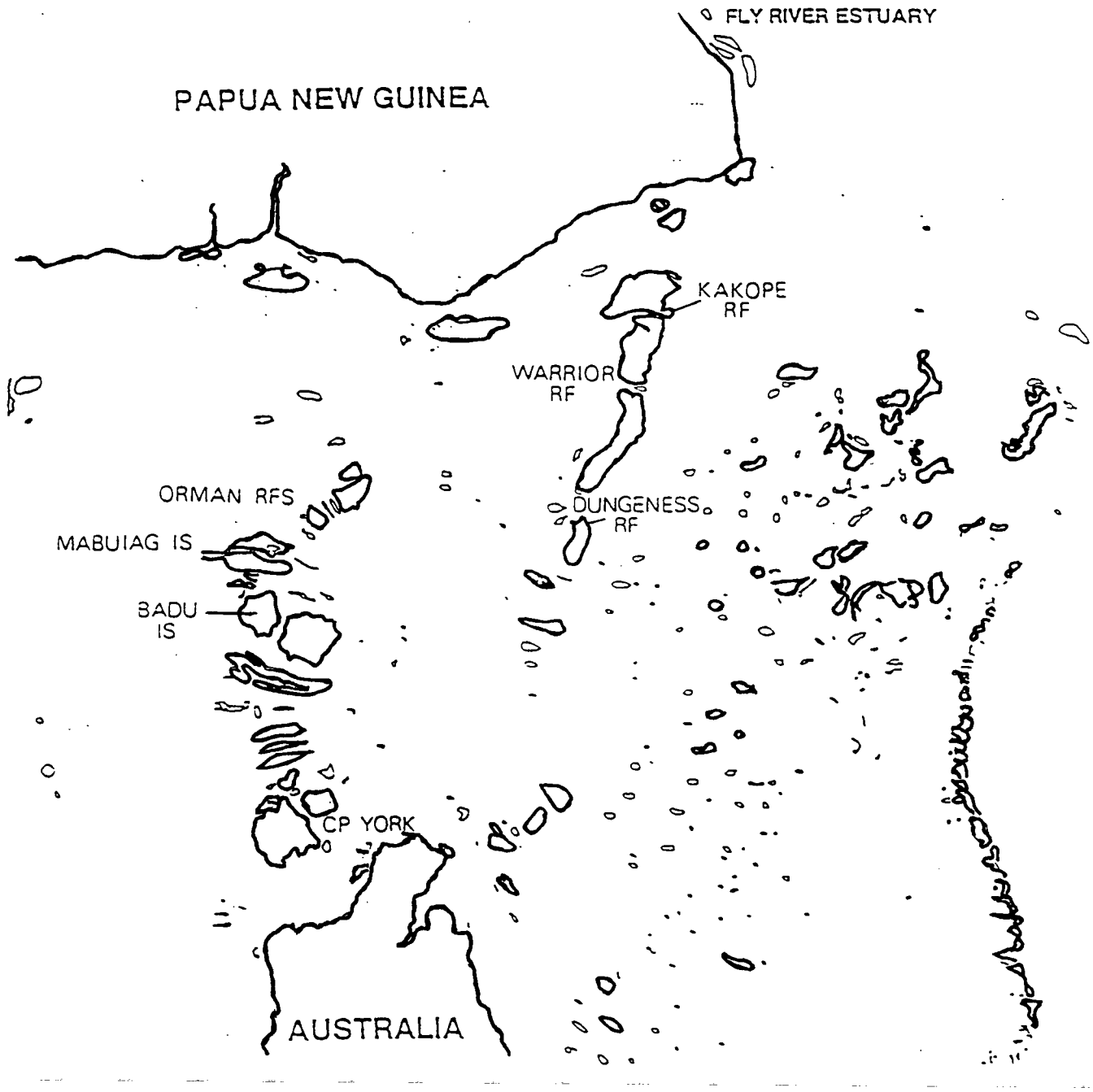
3. It is also recommended that research be undertaken on the bioavailability to humans of cadmium in whole prawns.

4. High variability between metal levels in individuals caused frequent insensitive significant tests in this study. Any future work must be accompanied by very sensitive significance tests. This is because levels reported in this study are close to the MPC, so anything which causes even a small change will be of importance. A cost efficient way to increase the power of these significance tests is to maximise the degrees of freedom in the denominator of the F ratio (for example, by sampling over many nights), rather than increasing the sample size of prawns analysed per se. Measures of variability obtained in the present study should be used to optimise the experimental design and sampling effort of further work.

PART 2

METAL LEVELS IN THE TORRES STRAIT COMMERCIAL CRAYFISH (*Panulirus ornatus)*

Figure 23. Map of Torres Strait, showing crayfish sampling sites (shaded)



METAL LEVELS IN THE TORRES STRAIT COMMERCIAL CRAYFISH *Panulirus ornatus*

2.1 Background

The commercial crayfish catch in Torres Strait is almost exclusively made up of the Tropical Rock Lobster *Panulirus ornatus*. Fishing activity is centred in four main areas, the Warrior Reef complex, Mabuiag Island area and Orman Reef complex, Badu/Moa Island area and reefs to the south and Thursday Island/Cape York area (figure 23).

In Torres Strait, the Crayfish fishery is a dive fishery and each crayfish is individually speared. Both breath-hold and Surface Supplied Breathing Apparatus ('Hookah'), diving methods are used. At the time of this study, crayfish heads were removed during processing before freezing and the commercial product consisted of tails only.

P. ornatus in Torres Strait is a relatively sedentary species, at least for their first two or so years of life. Crayfish in the fishery emigrate at sexual maturity, about 2.5 years old. Some move into the Gulf of Papua, where spawning occurs, probably followed by death (Pitcher 1991, Bell et al 1986). After a free swimming larval stage, currents return young crayfish to the Torres Strait (probably among other places) for settlement. Thus, all crayfish resident in the Torres Strait fishing grounds are juveniles or sub-adults (Pitcher 1991). Due to the minimum legal size limit, crayfish recruit into the fishery at 1+ year old and as there is only one annual migration and spawning event, distinct age classes (1+ and 2+ year olds) occur in Torres Strait. The commercial catch is mostly made up of 2+ year old individuals. Tagging studies have indicated that until the major once-in-a-lifetime migration, the species is not very mobile and only displays localised within-reef movements (Bell et al 1986).

No previous studies have been done on metal levels in crayfish from Torres Strait. This study will provide a preliminary assessment of metal levels and likely factors determining these in *P. ornatus*, as well as pilot estimators of variability in and among these factors. Spatial and seasonal patterns and the effects of sex, moult stage and size were examined. Tail flesh and abdominal shell are the only two tissues considered, as it is these tissues that make up the marketed product. Heads are removed prior to sale, but as they may be in part consumed by the fishermen, crayfish heads are included in the community fisheries component of the Torres Strait Baseline Study (Gladstone 1996).

2.2 Methods

Crayfish were sampled in the Cape York area and Dungeness Reef in June and November 1992. Samples were also collected from Southern reefs in the Orman complex in June 1992 by a CSIRO fisheries research team and Kakope Reef in October 1992 (figure 23). Where possible, 3 sites were haphazardly sampled at each location. The exceptions were Dungeness Reef and southern Orman reefs in June 1992, where only two sites could be sampled. Between two and twelve crayfish were collected from each site.

2.2.1 Sample Handling Protocols

Collection

Crayfish collection protocols were designed to minimise opportunities for post-capture metal contamination, within practical limitations. Collections were made by divers and processed in a small aluminium dinghy. Underwater, crayfish were speared through the head with a stainless steel spear. The head and gut was removed as soon as possible, using a stainless steel knife and discarded. The remaining tail with carapace intact, was scrubbed in clean

seawater. Tail width at the second abdominal segment, sex and moult stage were recorded and the sample was double bagged and immediately chilled on ice in an esky. Samples were frozen as soon as possible, usually after 1–3 hours.

Moult stage was assessed as pre-, post- or inter-moult based on the softness of outer cuticle and presence or absence of a second cuticle. However, as insufficient numbers of any category were collected within other factors, analyses for the effect of moult stage were not possible.

All buckets and containers used to hold or wash samples were plastic, acid washed at the start of each field trip, thoroughly rinsed in clean seawater between samples and in ROP water periodically. All personnel handling samples wore vinyl gloves. During sample handling, all engines in the sampling dinghy were turned off to eliminate sample exposure to exhaust fumes. Plastic bags were supplied by the Animal Research Institute (Department of Primary Industries) and were cleared for sample storage as already described.

Industry Samples

To enable a preliminary comparison between crayfish treated 'cleanly', as described above and regular commercial product, further samples of industry produced product were collected in parallel with 'clean' samples, from two sites on the southern Orman reefs in June 1992. These samples were purchased from a processing plant at Mabuiag Island and were product of two Mabuiag Island based fishermen who were known to have fished in the southern reefs of the Orman complex that day. Their crayfish were also speared through the head and left on the floor of the fishing dinghy at ambient temperature, with the damaged head intact, for 0.5–3 hours. Head and gut were then removed and tails were immersed in iced brine in an esky, for a further 1–3 hours, before being brought to the processing plant for washing in seawater and freezing.

Dissections

As with the prawn samples, dissections took place in the clean facility at the Horn Island Research Station while the samples were par frozen. Tails were separated into tail flesh and cuticle, and tissues were thoroughly rinsed with ROP water. All instruments and equipment were either surgical grade stainless steel or acid washed plastic and all equipment was thoroughly scrubbed with Extran detergent and rinsed with ROP water between samples.

2.2.2 Laboratory Analysis

All metals analyses were conducted at the Queensland Department of Primary Industry's Animal Research Institute (QDPI-ARI). This is a NATA accredited laboratory which has successfully participated in the US National Oceanic and Atmospheric Administration (NOAA) Inter-comparison for Trace Metals in Marine Sediments and Biological Tissues since 1991. This programme is designed to ensure that participating laboratories are able to produce accurate and repeatable results for a wide range of elements.

Samples were freeze-dried and microwave digested using double distilled nitric acid, prior to chemical analysis. All samples were weighed both wet and dry (after freeze-drying). The concentrations of the 16 elements silver, aluminium, arsenic (total), cadmium, cobalt, chromium, copper, iron, mercury, manganese, nickel, lead, selenium, strontium, uranium and zinc were measured using Inductively Coupled Plasma Mass Spectrometry. Appendix 3 describes detailed protocols for sample preparation and analysis as practised at QDPI-ARI.

2.2.3 Statistical Analysis

To provide an overview of results and comparisons with trade and health limits, means, descriptive statistics and box and whisker plots are presented using wet weight concentration data. A series of ANOVA's based on dry weight concentration data were then used to examine the factors season, location and sample treatment. Presentation of ANOVA results and pooling and power analysis procedures, were followed in analysis of crayfish data as already discussed in part 1. The effect of size on metal levels was assessed concurrently using covariance analysis. As was the case with prawns, univariate statistics were chosen over multivariate methods.

Conversion factors, to convert wet to dry weight data, of 4 and 3.2 were used for tail flesh and shell respectively. These were based on prawn data for the same tissue (table 4).

Estimates for whole tail concentration and load were made, based on the sum of average load in both tail flesh and shell and the sum of average weights of these tissues. To provide an estimated range which covered all size classes, whole tail calculations were done for small (40–50 millimetres tail width) and large (75–85 millimetres tail width) crayfish.

2.2.4 Arsenic Conversion

As was the case for prawns, analytical methods used in the analysis of crayfish tissue measured total arsenic. In order to allow direct comparisons with trade and health limits, which refer to inorganic arsenic only, a conversion factor of 0.01 was used to convert total arsenic measurements to an estimate of inorganic arsenic. Refer to the methods section of part 1 for details on the rationale behind this ratio.

2.3 RESULTS

2.3.1 Overview

In this general data overview, metal results from crayfish collected according to 'clean' protocols only are summarised. Concentration data are presented on a wet weight basis.

Table 24 sets out overall mean metal concentrations with standard errors and a calculation of the total metal load, for each tissue plus whole tale estimates.

Of the estimated total metal load in whole tails, the burden of silver, cadmium, cobalt, chromium, manganese, lead and uranium was born equally between the two tissues analysed. The aluminium, iron, nickel and strontium load was contained mostly in the shell, while arsenic, copper, mercury, selenium and zinc were contained mostly in tail flesh (table 24).

Relating metal loads to intake limits and recommendations summarised earlier in table 3 and again assuming complete bioavailability, two whole crayfish tails consumed per week easily falls within maximum advisable consumption limits of all metals considered, even when added to intakes from other sources. Zinc content in a single crayfish tail is up to half the recommended weekly dietary intake of this element.

For metals for which an MPC exists, figure 24 illustrates the distribution of the data summarised in table 24, with respect to the level of the MPC. Concentrations of all metals, including arsenic (converted to an estimate for inorganic arsenic only), were generally well below the respective MPC's in each tissue and estimates for whole tail. In the case of copper

and inorganic arsenic however, some samples approached the MPC and three individuals (independant in each case) exceeded it.

Table 24. Overview of metal levels found in *Panulirus ornatus* in the present study. Concentration data are arithmetic means of all crayfish collected according to the 'clean' protocols, expressed on a wet weight basis. Standard errors are shown in brackets. Total Load estimates were calculated based on average weights and mean concentrations of different tissues.

* Arsenic values: MPC applies to inorganic arsenic only, while reported concentrations are total arsenic. Multiply concentrations by 0.01 for estimate of inorganic arsenic only.

METAL	TAIL FLESH		SHELL		WHOLE TAIL	
	Conc'n (mg/kg)	Tot Load (mg)	Conc'n (mg/kg)	Tot Load (mg)	Conc'n (mg/kg)	Tot Load (mg)
Ag	0.008 (0.001)	0.0004–0.001	0.02 (0.004)	0.0004–0.001	0.01–0.02	0.001–0.002
Al	1.20 (0.17)	0.06 - 0.22	16.14 (1.36)	0.34–1.15	5.68–13.28	0.40–1.37
As * MPC=1.0	55.63 (2.51)	2.67–10.35	7.72 (0.60)	0.16–0.54	41.26–16.91	2.83–10.90
Cd MPC=0.2	0.01 (0.001)	0.0005–0.002	0.01 (0.001)	0.0003–0.001	0.01–0.01	0.001–0.003
Co	0.01 (0)	0.0003–0.001	0.13 (0.002)	0.003–0.01	0.05–0.11	0.003–0.01
Cr	0.14 (0.02)	0.007–0.03	0.11 (0.005)	0.002–0.008	0.13–0.12	0.01–0.03
Cu MPC=10	6.90 (0.35)	0.33–1.28	1.27 (0.09)	0.03–0.09	5.21–2.35	0.36–1.37
Fe	2.60 (0.21)	0.12–0.48	454.10 (7.19)	9.54–32.24	138.01–367.53	9.66–32.72
Hg MPC=0.5	0.03 (0.002)	0.001–0.005	0.01 (0.001)	0.0003–0.001	0.02–0.01	0.001–0.01
Mn	0.18 (0.004)	0.01–0.03	1.00 (0.07)	0.02–0.07	0.43–0.84	0.03–0.10
Ni	0.03 (0.002)	0.002–0.006	2.13 (0.02)	0.04–0.15	0.66–1.73	0.05–0.16
Pb MPC=1.5	0.07 (0.01)	0.004–0.01	0.13 (0.01)	0.003–0.01	0.09–0.12	0.01–0.02
Se MPC=1.0	0.53 (0.01)	0.03–0.10	0.33 (0.02)	0.01–0.02	0.47–0.37	0.03–0.12
Sr	5.08 (0.43)	0.24–0.94	1660 (18.52)	34.87–117.89	501.41–1342.7	35.11–118.83
U	0.006 (0.0001)	0.0003–0.001	0.02 (0.001)	0.0004–0.001	0.01–0.02	0.001–0.002
Zn	35.63 (0.92)	1.71–6.63	0.91 (0.04)	0.02–0.06	25.22–7.57	1.73–6.69

Figure 24. Box and whisker plots of levels of metals for which there is an Australian NHMRC MPC. Data is on a wet weight basis, from 'cleanly' collected crayfish. The dotted line represents the MPC.

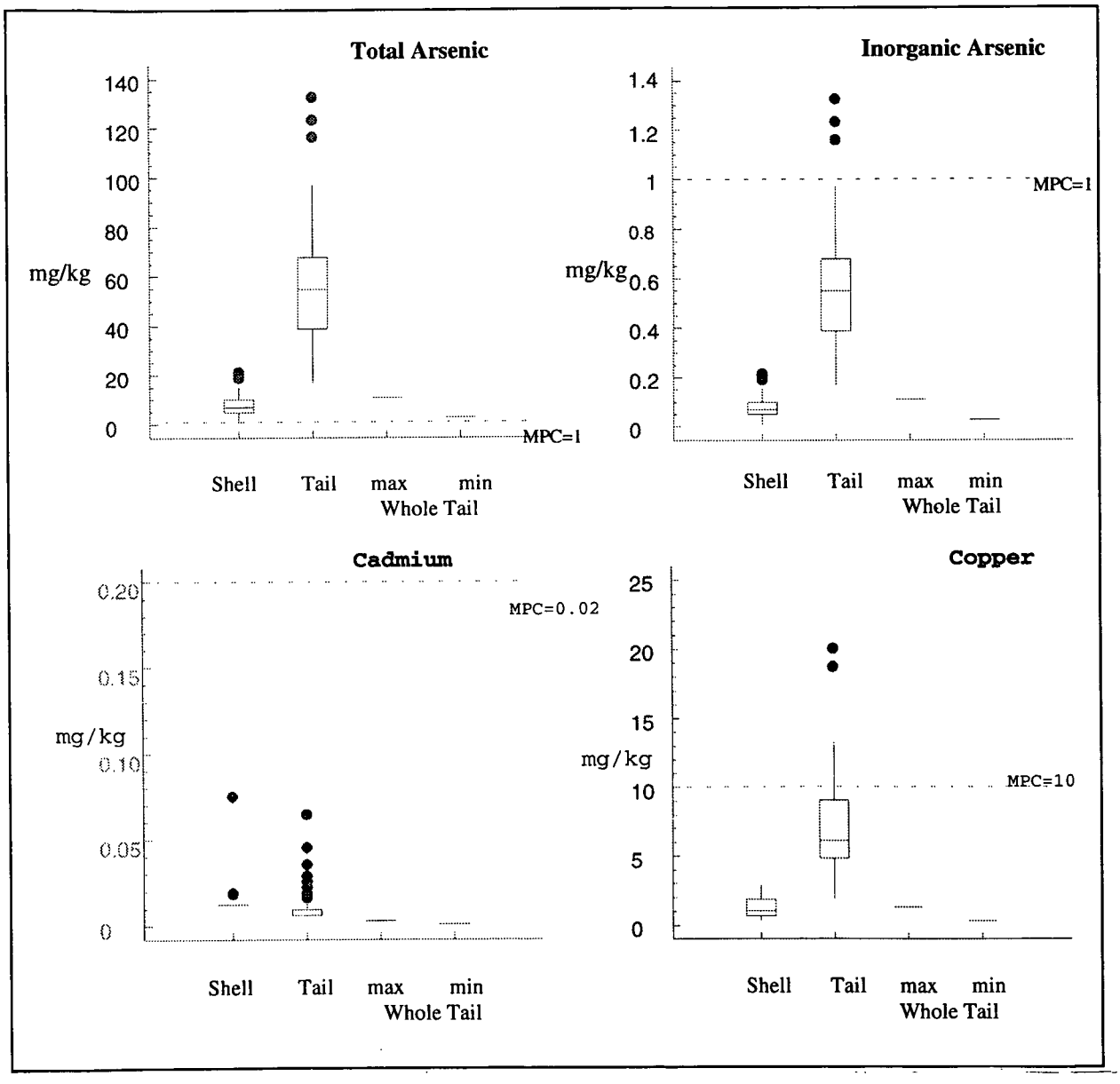
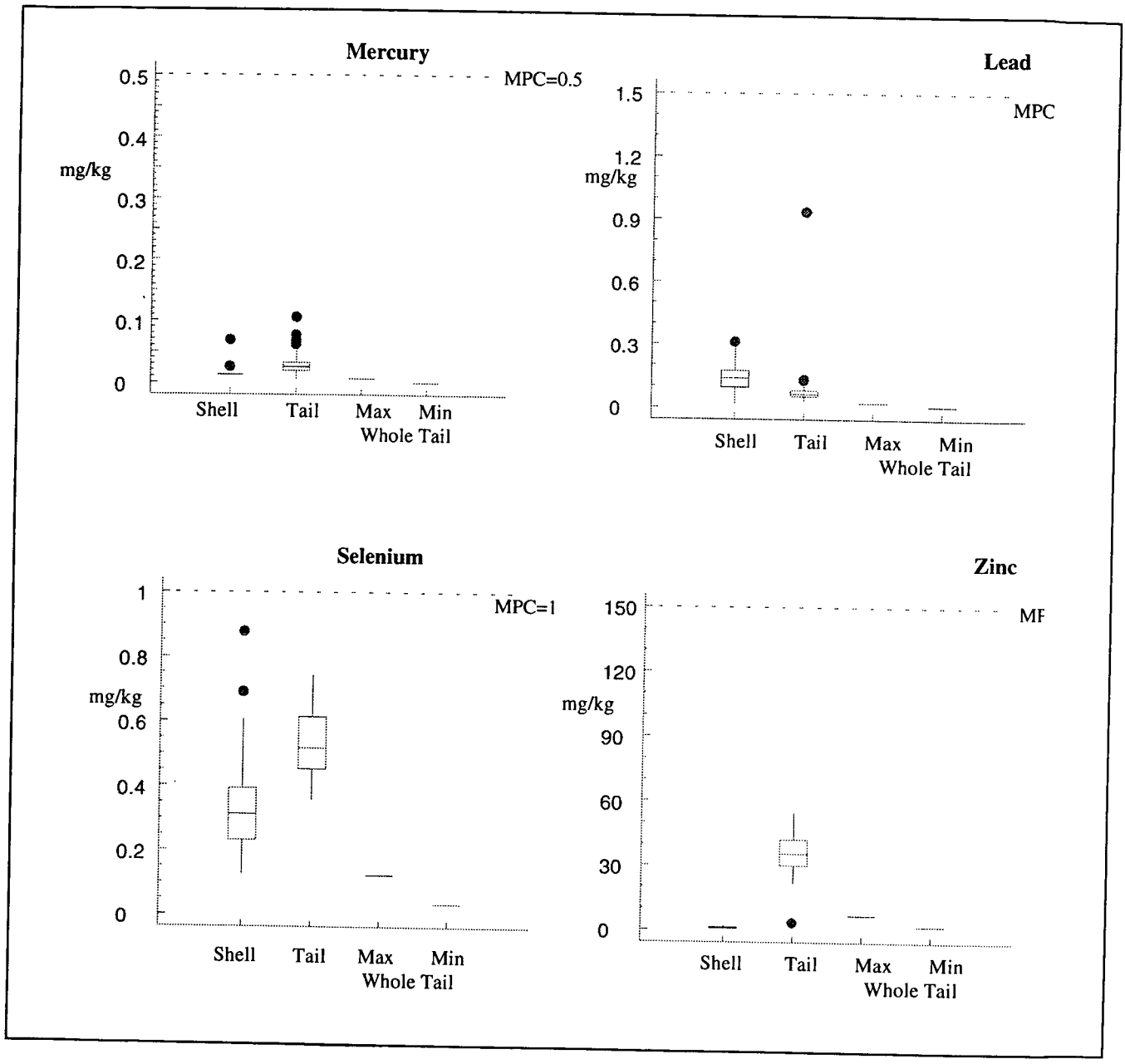


Figure 24. Cont...



2.3.2 Spatial And Seasonal Patterns

Metal levels in tail flesh of crayfish collected in June 1992 from the locations Cape York, Dungeness Reef and southern Orman Reefs, were compared in ANOVA's summarised in tables 26 and 27. Metal levels in tail flesh of crayfish collected in October 1992 from the locations Cape York, Dungeness Reef and Kakope Reef, were compared in ANOVA's summarised in tables 28 and 29. The two locations common to both periods, Cape York and Dungeness Reef, were compared from the two sampling periods in the ANOVA's summarised in tables 30 and 31. Tail width was a co-variate in all ANOVA's.

A large size range was represented in the collection, with tail widths ranging from 30–85 millimetres. Figures 25 and 26 illustrates the frequency of different classes of tail widths at different locations on both sampling periods. The distribution was bi-modal in June, with modes at 35–35 millimetres and 70–75 millimetres, while there was only one mode, at 55–60 millimetres, in October. Table 25 collates sample sizes across locations, sites and size classes.

Table 25. Sample sizes of collections from June and October 1992, across locations, sites and size categories

Location	Period	Site	Sample size per tail width (mm) category						
			30-40	40-50	50-60	60-70	70-80	80-90	Total
Cape York	June'92	1	0	0	0	1	3	1	5
		2	1	1	0	1	2	0	5
		3	2	0	1	1	1	0	5
	Oct/Nov '92	1	1	2	2	2	1	0	8
		2	0	3	3	0	0	0	6
		3	0	1	2	2	0	0	5
Dungeness	June'92	1	2	2	1	2	3	0	10
		2	0	0	0	1	3	1	5
	Oct/Nov '92	1	0	1	1	2	0	1	5
		2	0	1	3	1	0	0	5
		3	0	1	3	0	1	0	5
South Orman	June'92	1	0	0	2	1	2	0	5
		2	0	0	1	2	0	2	5
Kakope	Oct/Nov '92	1	0	0	2	0	0	1	3
		2	0	2	1	0	0	0	3
		3	0	1	1	0	1	0	3

Of samples collected in June 1992, there was no significant effect of location on levels of aluminium, arsenic, copper, iron, mercury, lead and strontium (table 26) but these results were accompanied by large or very large minimum detectable distances at 80% power (table 27). There was also no effect of location on chromium, manganese, nickel, selenium and zinc levels (table 26), but the tests for these metals were more sensitive, with a small or medium minimum detectable distances with 80% power in each case (table 27).

There were no significant effects of location on levels of any metal, in crayfish collected in October 1992 (table 28). While this result for arsenic, copper, manganese, selenium and zinc levels was accompanied by small or medium minimum detectable distances with 80% power, the tests for all other metals were much less sensitive, with large or very large minimum detectable distances (table 29).

When samples from Cape York and Dungeness locations were compared from both sampling periods, all metals except iron and cadmium showed no significant effect of location (table 30). These tests were generally sensitive, with a small or medium minimum detectable distance with 80% power; except for tests on aluminium and strontium levels, which were accompanied with a large or very large (respectively) minimum detectable distance with the same power (table 31).

There was significant inter-site variation in aluminium levels in June crayfish at all three locations sampled (table 26). Despite heterogeneous variances, the difference between sites was not trivial (figure 27). Aluminium levels were higher in samples collected in October, than those collected in June (figure 28).

Arsenic levels were significantly influenced by individual tail width in samples collected in June (table 28) and samples collected from Cape York and Dungeness Reefs in October (table 30). Levels of this metal tended to be higher in the smaller two or three size classes, with 40–50 millimetres crayfish in the June/October comparison containing the highest levels (figures 29 and 30). When the mean total arsenic level of 220 mg/kg for this group is corrected to an estimate of inorganic arsenic only and a wet weight concentration, the resulting value of 0.71 mg/kg falls below the MPC of 1.0 mg/kg.

Cadmium levels were significantly affected by location in June samples (table 28) and October samples from Cape York and Dungeness (table 30), with higher levels in crayfish from Dungeness Reef (figure 32). The mean concentration in samples from Dungeness of 0.05 mg/kg converts to a wet weight concentration of 0.02 mg/kg, which is well below the MPC of 0.2 mg/kg. Levels of cadmium were also influenced by individual size, with smaller crayfish tending to have higher levels (figure 31).

Within sampling locations, there were inter-site differences in chromium levels at South Orman (table 26, figure 33). Tail width was also a significant factor in both June and October samples (tables 27 and 28). While variation in chromium levels between size classes was not great in June samples, the 40–50 millimetres tail width group collected in October had much higher levels than other groups from either period (figure 34).

A location X season interaction significantly influenced levels of copper in crayfish tail flesh (table 30), with samples from Dungeness containing higher levels than Cape York samples from the June collection (figure 35), while the reverse was true for some size classes in the October samples (figure 36). Tail width also significantly influenced copper levels (table 30), although there was no overriding pattern to this (figures 35 and 36). The highest mean copper concentration was 40 mg/kg from 50–60 millimetres (1 individual) and 70–80 millimetres (6 individuals) tail width crayfish from Dungeness Reef in June. The wet weight conversion of 12.9 mg/kg exceeded the MPC of 10 mg/kg. All other size classes from both locations and both periods, when converted to wet weight concentrations, fell below the MPC.

Iron levels in October samples were significantly effected by tail width (table 28), with crayfish of up to 60 millimetres tail width tending to have higher levels (figure 37). When samples from the common locations from both sampling periods were combined, there was a significant location effect (table 30). Crayfish tail flesh from Dungeness Reef had higher iron levels than those from the Cape York area (figure 38).

Tail width was the only significant factor influencing manganese levels in crayfish tail flesh (tables 26, 28, 30), with the smallest size class of 30–40 millimetres containing highest levels, classes between 40–80 millimetres showing very little variability and the 80–90 millimetres size class containing the lowest levels (figure 39). While tail width was also a

significant influence on selenium levels (tables 26, 28, 30), inter-size class variation was slight (figure 40).

Nickel levels were also significantly influenced by tail width (tables 28 and 30) and smaller crayfish tended to contain higher levels (figures 41 and 42). When October samples only are considered, this trend is almost linear (figure 41). Although there was also a significant difference in nickel levels between seasons (table 30), the pattern across size classes is not consistent and any differences are only slight (figure 42).

There was significant inter site variability in lead levels (table 26) in tail flesh of crayfish from all three locations sampled during June (figure 43), with no clear trend discernible between locations. Lead levels were however, influenced by a season x location interaction (table 30), with Cape York crayfish containing higher levels in June 1992 than in October (figure 44). Mean lead levels in all categories were well below the MPC of 1.5 mg/kg even before conversion to wet weight concentrations.

Zinc levels in crayfish tail flesh collected in June 1992 were significantly influenced by inter-site variability (table 26) but differences between sights were only slight and there were no inter-location trends (figure 45). Tail width was again an important factor in determining metal levels and levels in larger crayfish tended to be higher (figures 46, 47, 48). This was also superimposed by significant seasonal variation in crayfish from Cape York and Dungeness Reefs combined, with samples collected in June generally containing higher levels than those collected in October, across all size classes (figure 48). As was the case for lead, all mean zinc levels were below the MPC of 150 mg/kg even before conversion to wet weight concentrations.

Table 26. ANOVA summary for differences in metal concentration in tail flesh of *P. ornatus* collected in June 1992 at locations Cape York, Dungeness Reef and southern Orman Reefs. Tail width is included as a co-variate.

METAL	LOCATION (site)	SITE (residual)	TAIL WIDTH (residual)	Hetero. Variances
Al		*Significant*		*Yes*
As			*Significant*	
Cd	*Significant*		*Significant*	*Yes*
Cr		*Significant*	*Significant*	
Cu				*Yes*
Fe				*Yes*
Hg				*Yes*
Mn			*Significant*	
Ni				
Pb		*Significant*		
Se			*Significant*	
Sr				*Yes*
Zn		*Significant*	*Significant*	

Table 27. Power analysis of non-significant location results in Table 25. MDD = Minimum Detectable Distance with a=0.05 and power = 80%

METAL	GRAND MEAN	MS (F ratio error)	MDD a=.05;b=.2	MDD (% of mean)
Al	2.17	23.71	10.74	495.75
As	140.90	4838.38	153.38	108.85
Cr	0.37	0.01	0.22	58.84
Cu	24.05	238.05	34.02	141.44
Fe	7.59	30.42	12.16	160.18
Hg	0.08	0.002	0.10	117.74
Mn	0.56	0.01	0.22	39.29
Ni	0.11	0.001	0.07	62.54
Pb	0.21	0.01	0.26	122.06
Se	1.71	0.20	0.99	57.84
Sr	17.45	268.78	36.15	207.13
Zn	123.40	617.76	54.80	44.41

Table 28. ANOVA summary for differences in metal concentration in tail flesh of *P. ornatus* collected in October/November 1992 at Cape York, Dungeness Reef and Kakope Reef. Tail width is included as a co-variate.

METAL	LOCATION (site)	SITE (residual)	TAIL WIDTH (residual)	Hetero. Variances
Al				*Yes*
As				
Cd				*Yes*
Cr			*Significant*	*Yes*
Cu				
Fe			*Significant*	*Yes*
Hg				*Yes*
Mn			*Significant*	*Yes*
Ni			*Significant*	*Yes*
Pb				*Yes*
Se				
Sr				
Zn			*Significant*	*Yes*

Table 29. Power analysis of fixed factor non-significant results in Table 27. MDD = Minimum Detectable Distance with a=0.05 and power = 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD a=.05,b=.2	MDD % of mean
Al	5.15	28.16	8.78	170.26
As	201.81	3758.71	101.39	50.24
Cd	0.03	0.0004	0.03	102.32
Cr	0.51	0.77	1.45	289.16
Cu	18.93	42.72	10.81	57.09
Fe	8.52	99.82	16.52	194.01
Hg	0.09	0.01	0.14	148.37
Mn	0.57	0.01	0.16	27.48
Ni	0.10	0.004	0.10	103.87
Pb	0.25	0.21	0.76	300.90
Se	1.60	0.15	0.64	40.09
Sr	14.13	123.34	18.37	129.96
Zn	98.40	193.21	22.99	23.36

Table 30. ANOVA summary for seasonal differences in metal concentration in tail flesh of *P. ornatus* collected locations Cape York and Dungeness Reef. Tail width is included as a co-variate.

METAL	LOCATION (Site)	SEASON (Site)	LOCxSEAS (Site)	SITE (Res)	TAIL WTH (Res)	HET. VAR Cochrans
Al		*Significant*	*Pooled*		*Pooled*	*Yes*
As				*Pooled*	*Significant*	
Cd	*Significant*		*Pooled*			*Yes*
Cr			*Pooled*	*Pooled*		
Cu			*Significant*	*Pooled*	*Significant*	
Fe	*Significant*			*Pooled*	*Pooled*	
Hg			*Pooled*	*Pooled*	*Pooled*	
Mn				*Pooled*	*Significant*	
Ni		*Significant*	*Pooled*	*Pooled*	*Significant*	
Pb		*Significant*	*Significant*	*Pooled*	*Pooled*	
Se			*Pooled*		*Pooled*	
Sr			*Pooled*		*Pooled*	*Yes*
Zn		*Significant*		*Pooled*	*Significant*	

Table 31. Power analysis for fixed factor non-significant results in Table 29. MDD=Minimum Detectable Distance with a=0.05 and power= 80%.

METAL	GRAND MEAN	MS (F ratio error)	MDD a=.05,b=.2	MDD % of mean
Al	3.22	4.29	3.94	122.07
As	178.81	3813.00	43.22	24.17
Cd	0.03	0.0003	0.03	100.29
Cr	0.46	0.28	0.37	80.39
Cu	21.36	78.93	6.22	29.12
Fe	7.73	35.76	4.19	54.18
Hg	0.09	0.003	0.04	43.74
Mn	0.56	0.01	0.08	14.84
Ni	0.11	0.002	0.03	27.32
Pb	0.20	0.004	0.04	21.88
Se	1.69	0.15	0.75	44.14
Sr	15.05	252.32	30.18	200.52
Zn	107.53	305.58	12.24	11.38

Figure 25. Frequency histogram of crayfish tail widths collected from different locations in June 1992

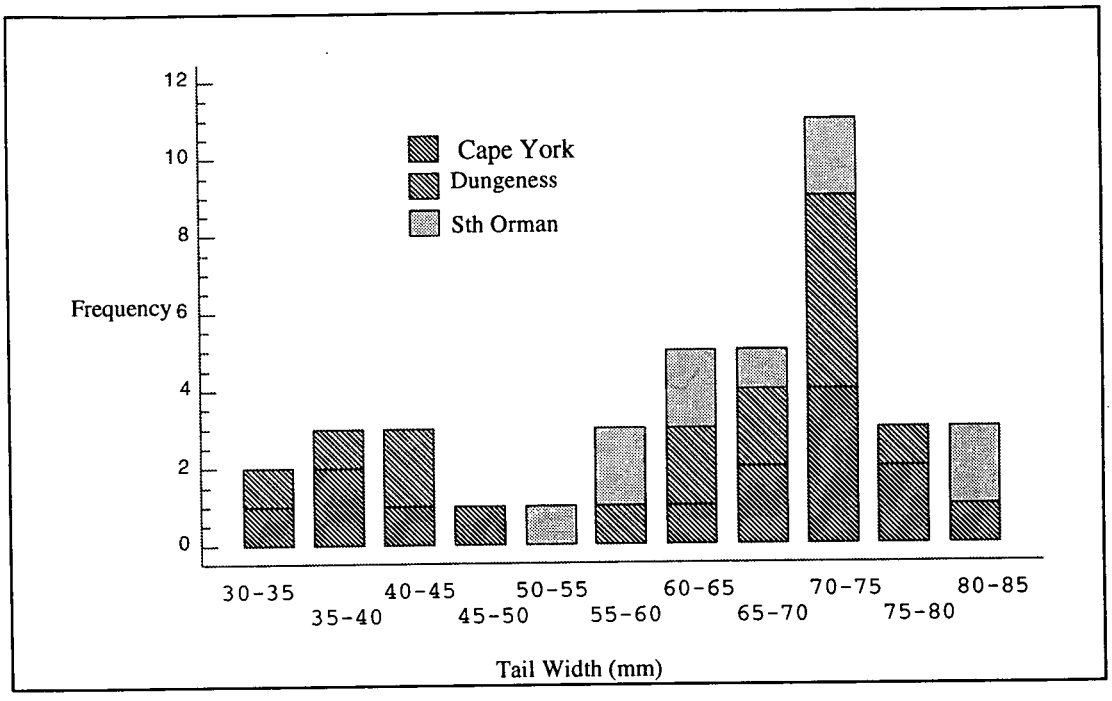


Figure 26. Frequency histogram of crayfish tail widths collected from different locations in October 1992

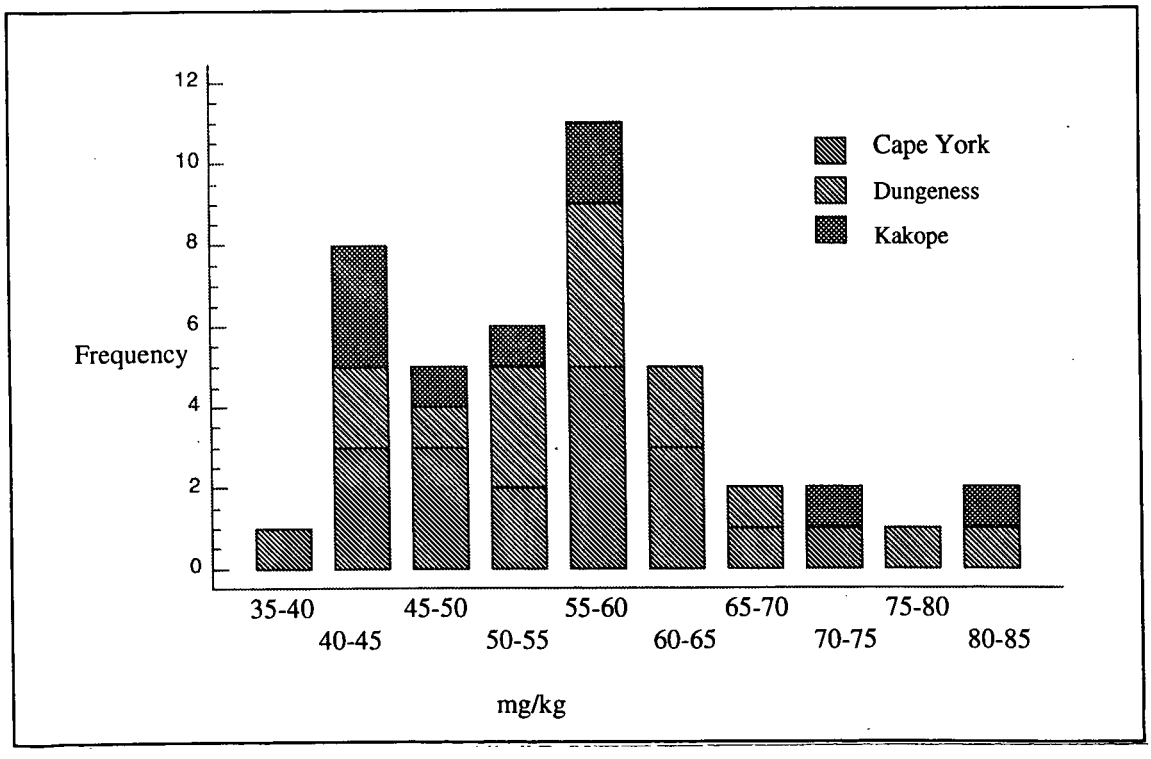


Figure 27. Aluminium levels in crayfish tails collected from different sites and locations in June 1992. Bars represent individual sites.

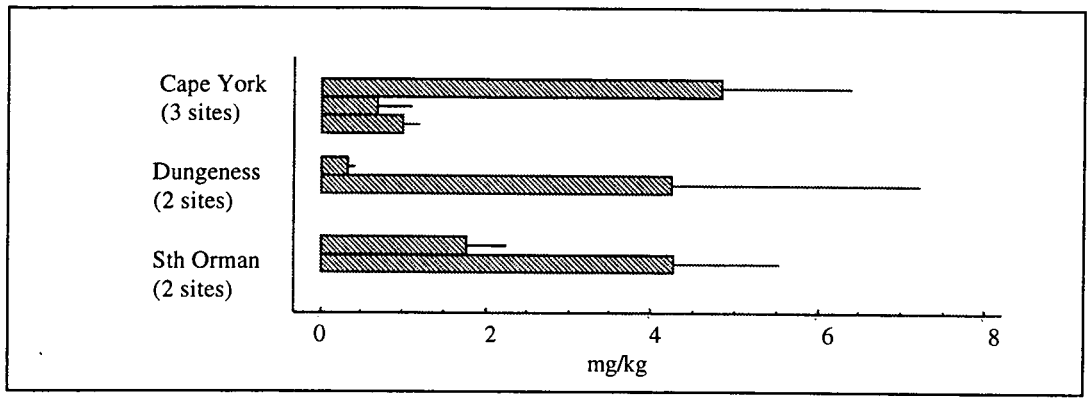


Figure 28. Seasonal differences in Aluminium levels (dry weight basis) in crayfish tails collected from Cape York and Dungeness Reefs

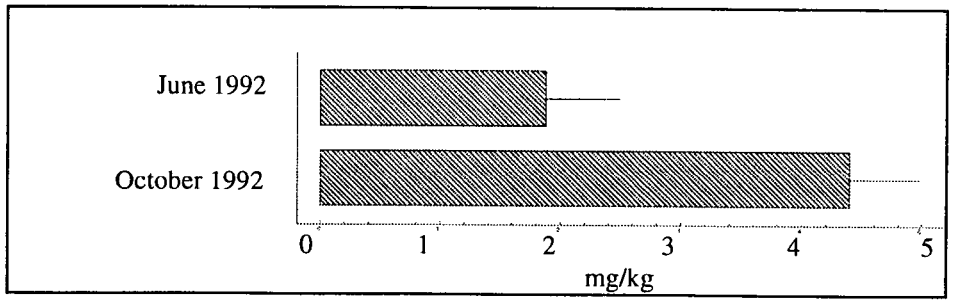


Figure 29. Tail width Vs mean arsenic levels, with standard error bars, in tail flesh of crayfish sampled at Cape York, Dungeness and South Orman sites in June 1992

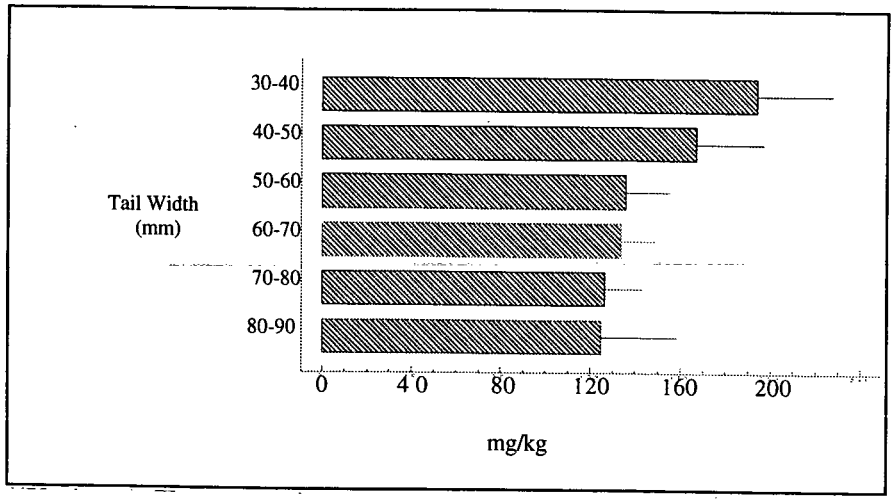


Figure 30. Tail width Vs mean arsenic levels, with standard error bars, in tail flesh of crayfish sampled at Cape York and Dungeness sites in June 1992 and October 1992

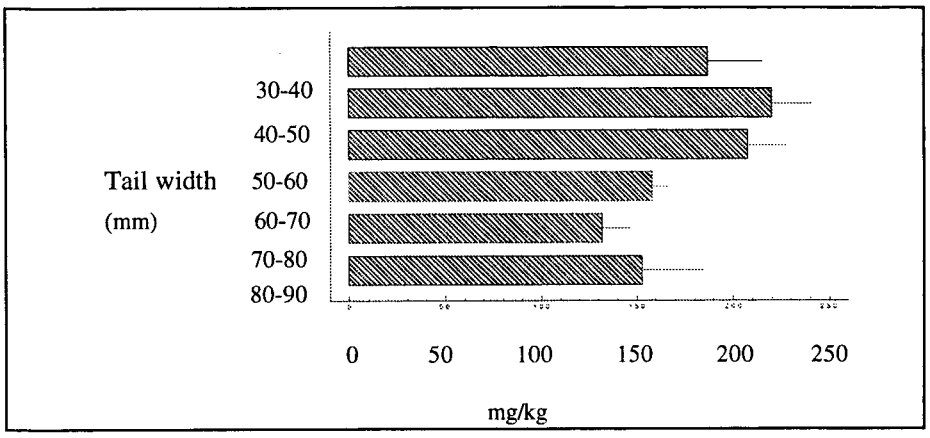


Figure 31. Tail width Vs mean cadmium levels, with standard error bars, in tail flesh of crayfish sampled in June 1992

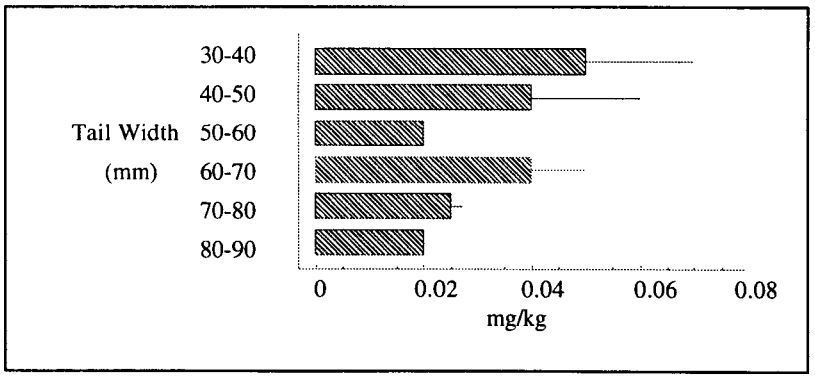


Figure 32. Overall locational differences in Cadmium levels (means with standard error bars) in crayfish tails collected from June and October 1992

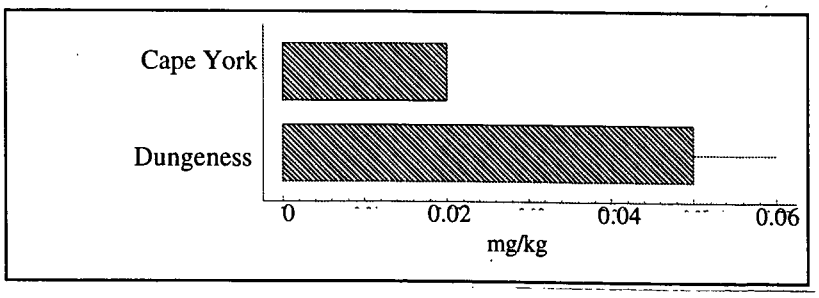


Figure 33. Site differences in Chromium levels (dry weight basis) in crayfish tails collected in June 1992. Bars represent individual sites.

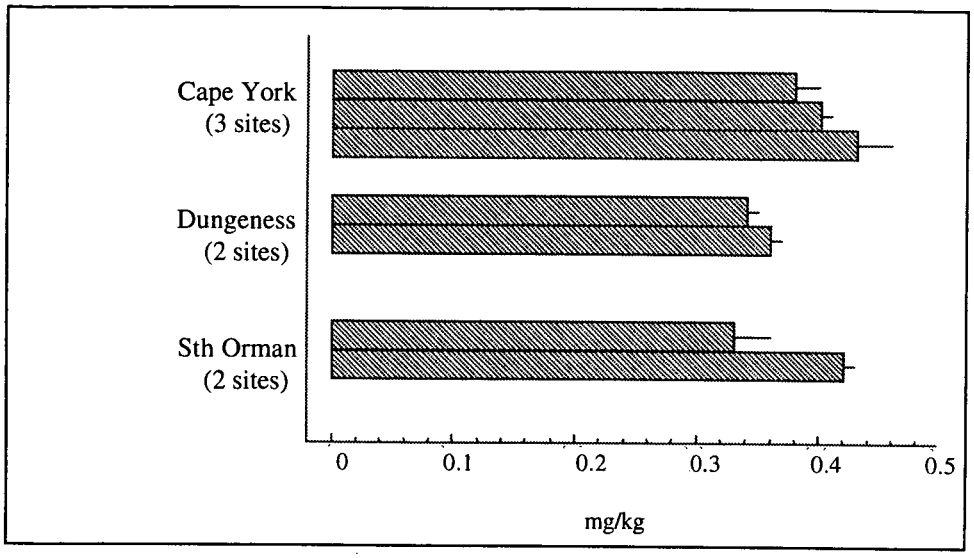


Figure 34. Chromium levels (means and standard error bars) in crayfish tails collected in June 1992 excluding South Orman samples, 2 sites at South Orman in June 1992 and all locations in October 1992

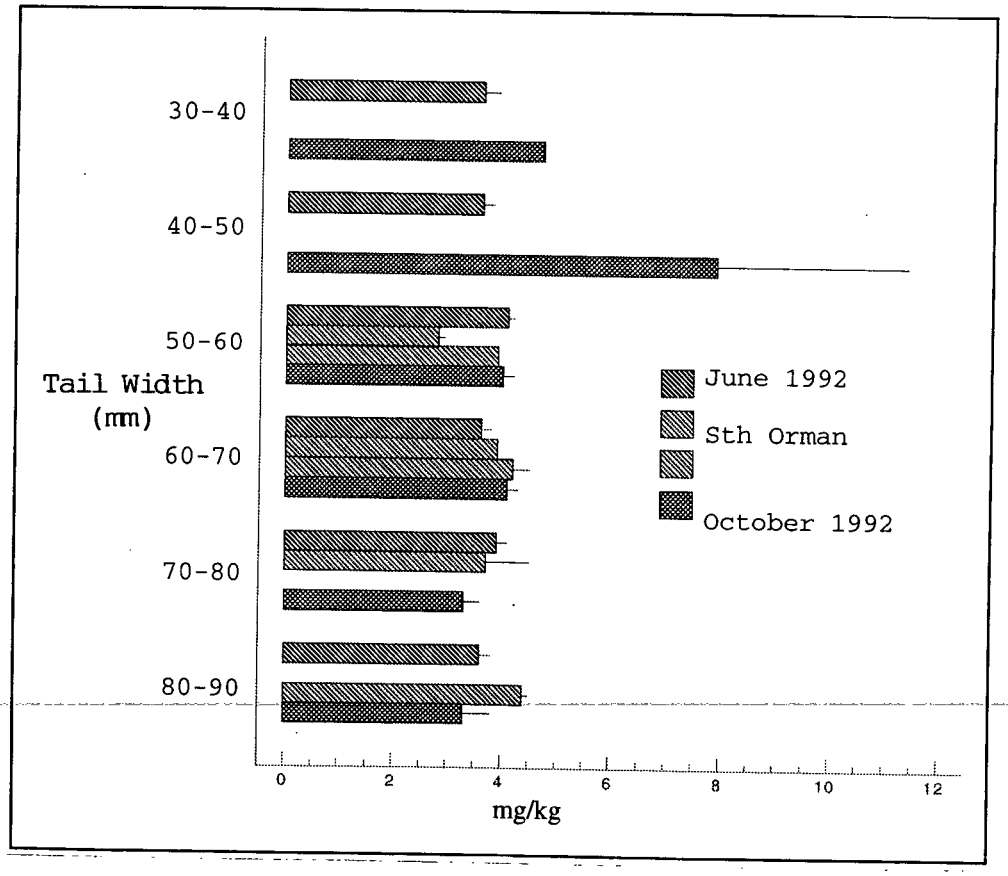


Figure 35. Tail width Vs Copper levels in tail flesh of crayfish collected from Cape York and Dungeness sites in June 1992

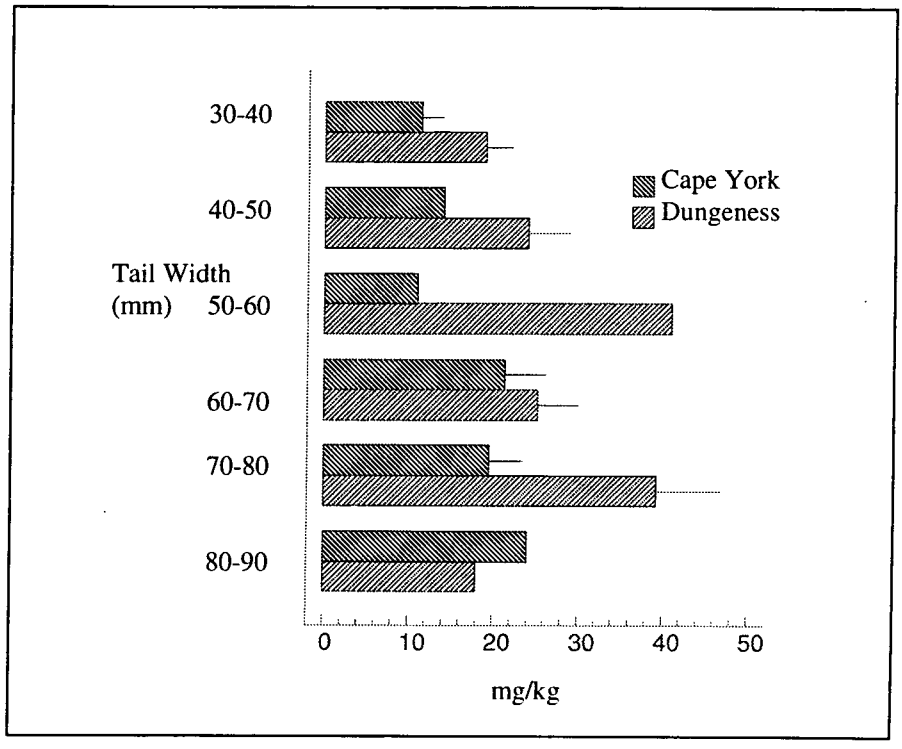


Figure 36. Tail width Vs Copper levels in tail flesh of crayfish collected from Cape York and Dungeness sites in October 1992

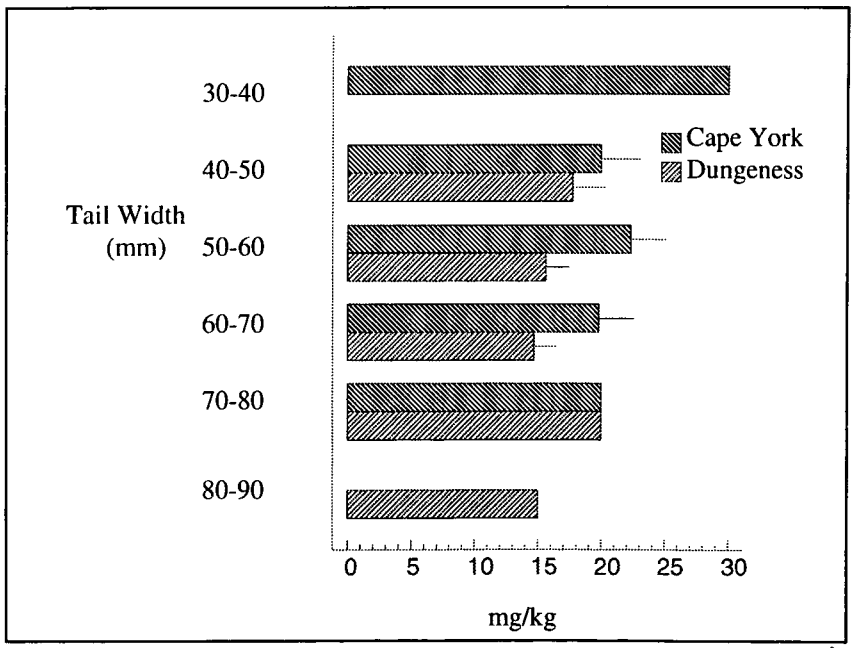


Figure 37. Tail width Vs mean iron levels, with standard error bars, in tail flesh of crayfish sampled from Cape York, Dungeness and Kakope sites in October 1992

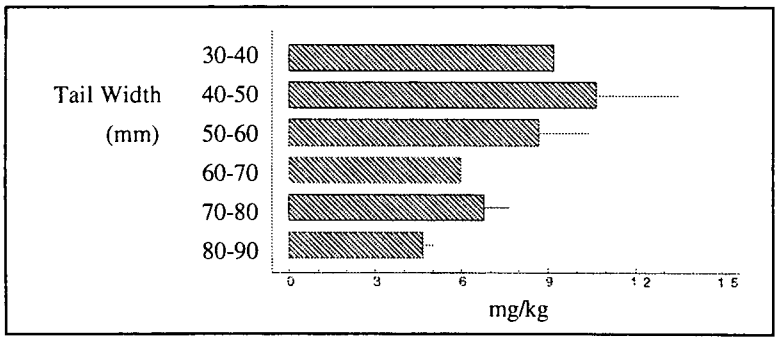


Figure 38. Iron levels in crayfish tails from Cape York and Dungeness sites, collected from both sampling periods combined

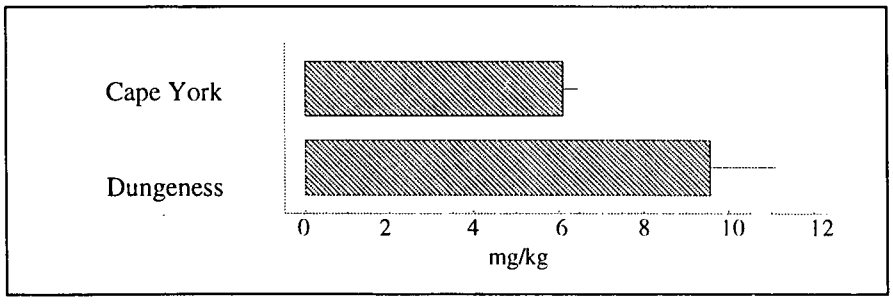


Figure 39. Tail width Vs mean manganese levels, with standard error bars, in tail flesh of crayfish collected from all sites in both June and October 1992

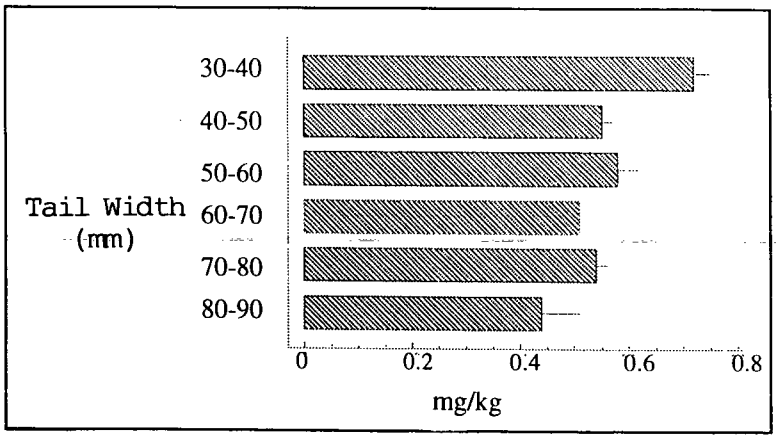


Figure 40. Tail width Vs mean selenium levels, with standard error bars, in tail flesh of crayfish collected from all sites in June 1992

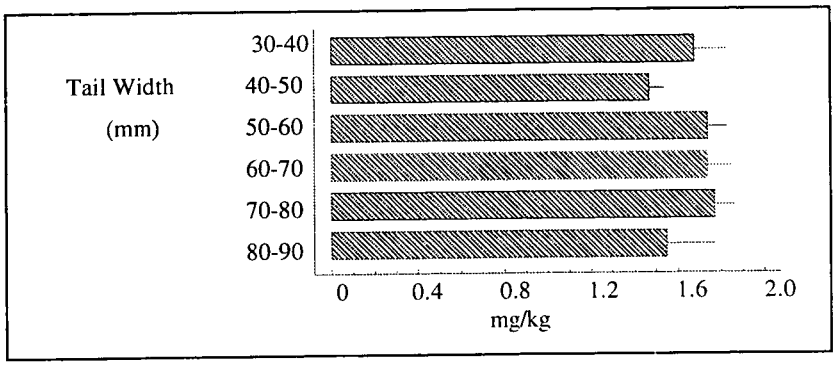


Figure 41. Tail width Vs mean nickel levels, with standard error bars, in tail flesh of crayfish collected from all sites in October 1992

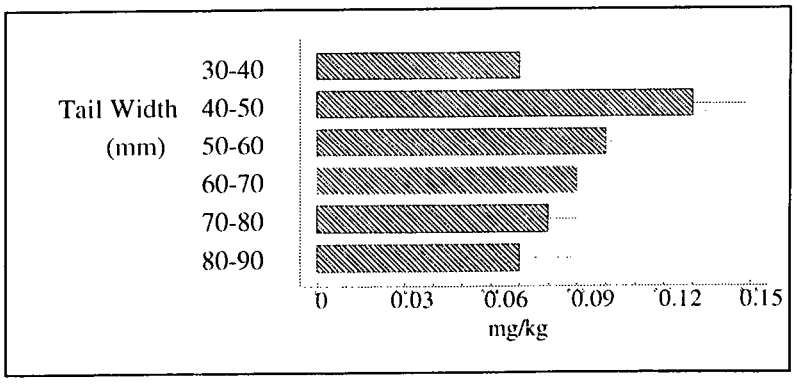


Figure 42. Tail Width Vs nickel levels in tail flesh of crayfish collected in June and October 1992 combined, from Cape York and Dungeness sites

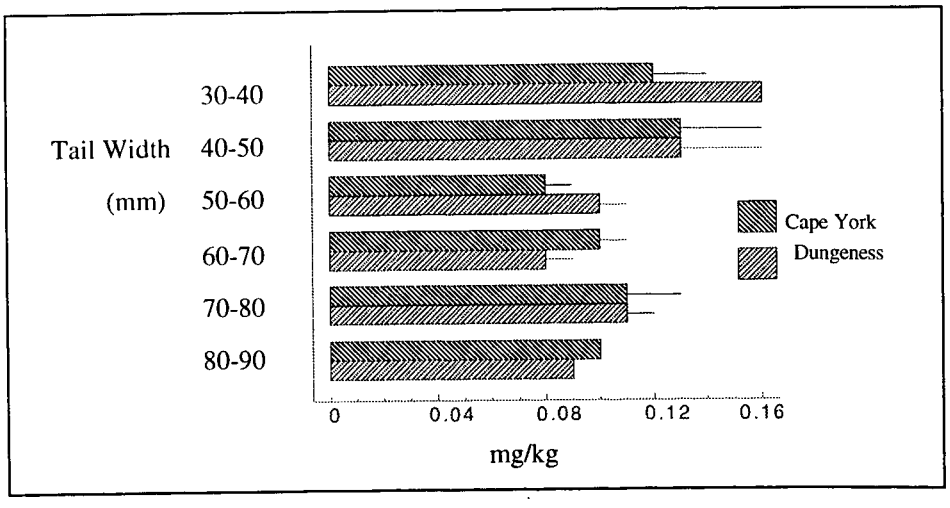


Figure 43. Inter site variation in lead levels in tail flesh of crayfish collected in June 1992

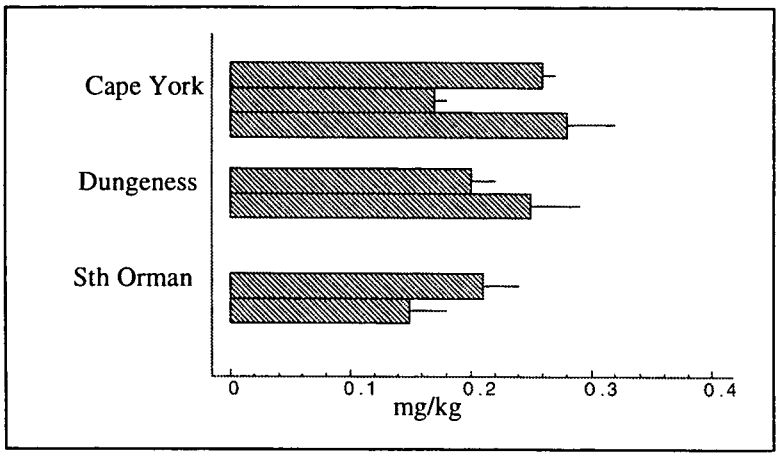


Figure 44. Seasonal variation in Lead levels in tail flesh of crayfish collected from Cape York and Dungeness

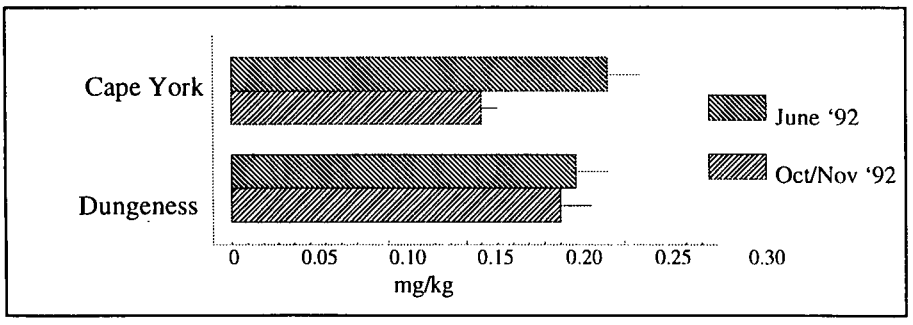


Figure 45. Inter site variation in zinc levels in tail flesh from crayfish collected in June 1992. Bars represent individual sites

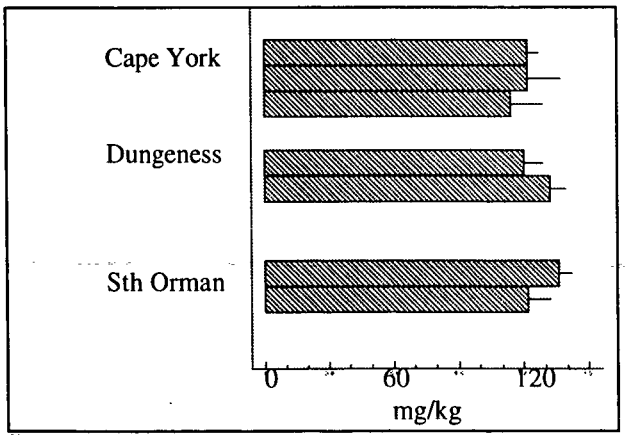


Figure 46. Tail width Vs mean zinc levels, with standard error bars, in tail flesh of crayfish collected from all sites in June 1992.

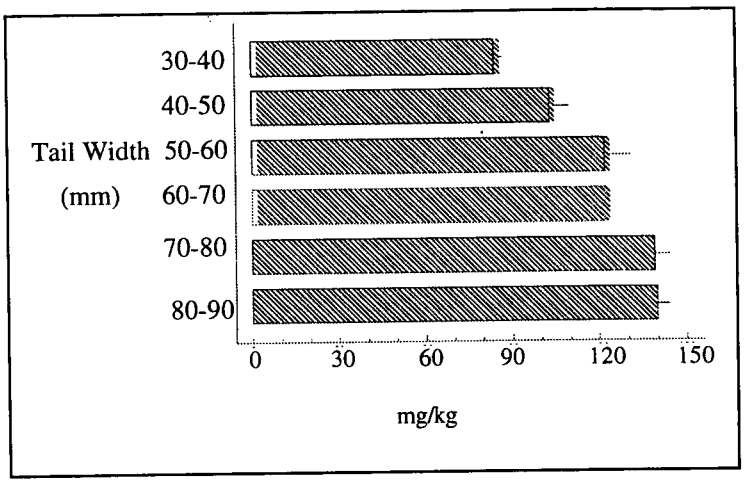


Figure 47. Tail width Vs mean zinc levels, with standard error bars, in tail flesh of crayfish collected from all sites in October 1992.

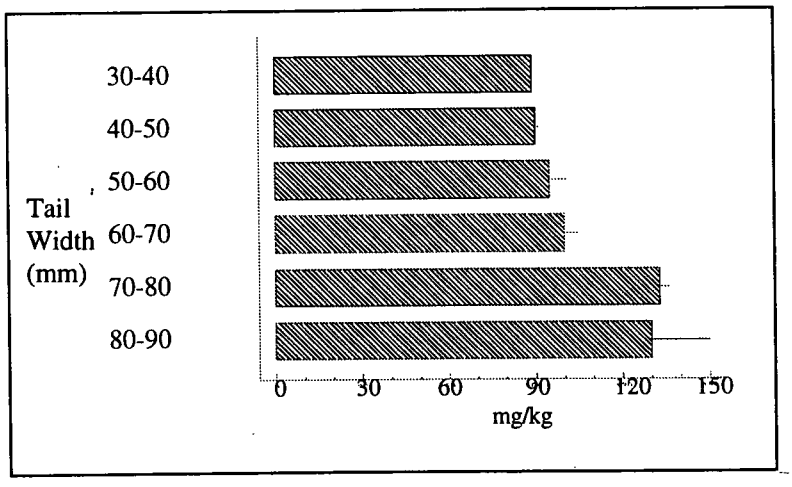
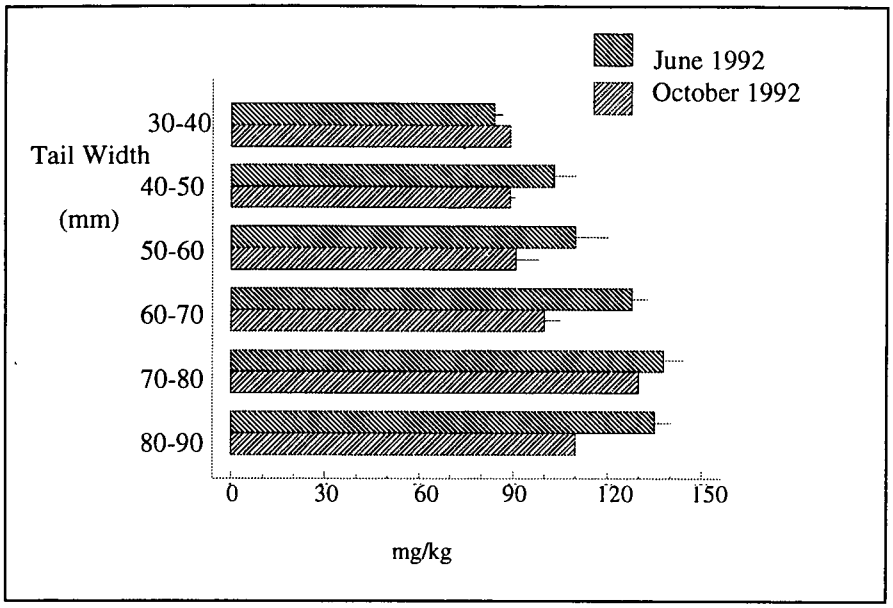


Figure 48. Seasonal variation in zinc levels in tail flesh of crayfish from Cape York and Dungeness sites combined



2.3.3 Patterns With Sex

To enable a comparison of metal levels in crayfish tail flesh between sexes, crayfish of between 40-60 millimetres tail width collected in October 1992 from all locations combined, were examined. This group was chosen because there were no significant inter-location or site results in table 28, the limited size range would minimise size related effects and the sex ration in this combined group was 50:50, with 15 individuals in each sex.

A one way analysis of variance was performed for each metal but there were no significant differences between sexes, at a = 0.05, in levels of any metal. Table 32 summarises the power of these non-significant tests. The tests which yielded these non-significant results were generally sensitive, with a small or medium minimum detectable distance in most cases. The exceptions were tests on aluminium, cadmium, chromium and lead levels, which offered only a large or very large minimum detectable distance.

Table 32. Power analysis for non-significant sex results. MDD = Minimum Detectable Distance with a=0.05, b=0.2(Power = 80%)

METAL	GRAND MEAN	MS Denom (F test error)	MDD mg/kg	MDD (% of mean)
Al	5.80	40.46	6.36	109.70
As	217.73	5162.42	71.85	33.00
Cd	0.03	0.001	0.03	102.89
Cr	0.55	0.60	0.77	139.52
Cu	18.77	46.61	6.83	36.37
Fe	9.50	68.75	8.29	87.25
Hg	0.10	0.004	0.06	64.76
Mn	0.59	0.02	0.15	25.05
Ni	0.11	0.004	0.06	57.85
Pb	0.28	0.25	0.50	181.60
Sc	1.57	0.07	0.26	16.82
Sr	14.41	91.72	9.58	66.48
Zn	92.80	369.94	19.23	20.73

2.3.4 Patterns With Handling Methods

Metal levels in tail flesh of crayfish from South Orman Reefs in June 1992, collected according to both the clean handling protocols practised in this study and commercial fishing practices, were compared in ANOVA's summarised in tables 33 and 34. Crayfish sizes were 58–84 millimetres and 73–80 millimetres (tail widths) in 'clean' and 'industry' crayfish respectively.

There was a significant handling method effect on levels of lead and strontium (table 33) and while lead levels were higher in tail flesh of 'industry' crayfish (figure 49), the reverse was true for strontium levels (figure 50). While handling method was not a significant factor in levels of any other metal tested (table 33), most of these non-significant tests were extremely insensitive, with a huge minimum detectable distance with 80% power of up to over 2500% of the mean value, in the case of nickel (table 34). The exceptions were tests on selenium and zinc levels, which were both accompanied by small or medium minimum detectable distances with the same power.

There were significant differences between different sites within a handling treatment in levels of arsenic, chromium, manganese and copper (table 33). While there were no overall inter-treatment trends in levels of these methods, highest arsenic levels were reported from one of the 'industry' sites (figure 51).

Figure 49. Differences in lead levels in tail flesh of *P.ornatus* between industry product and cleanly handled research samples

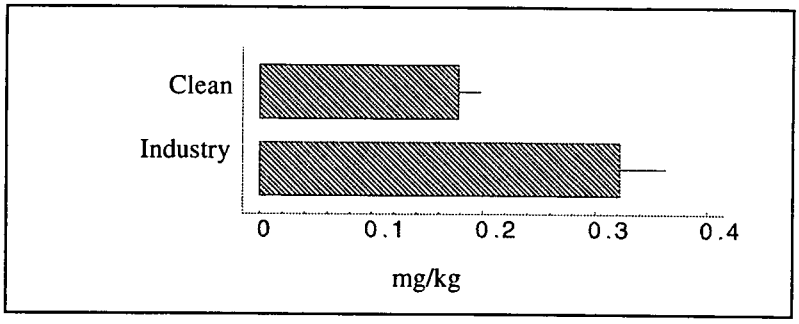


Figure 50. Differences in strontium levels in tail flesh of *P.ornatus* between industry product and cleanly handled research samples

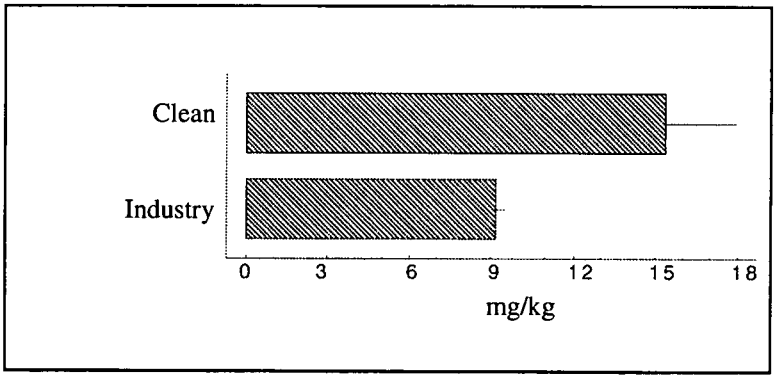


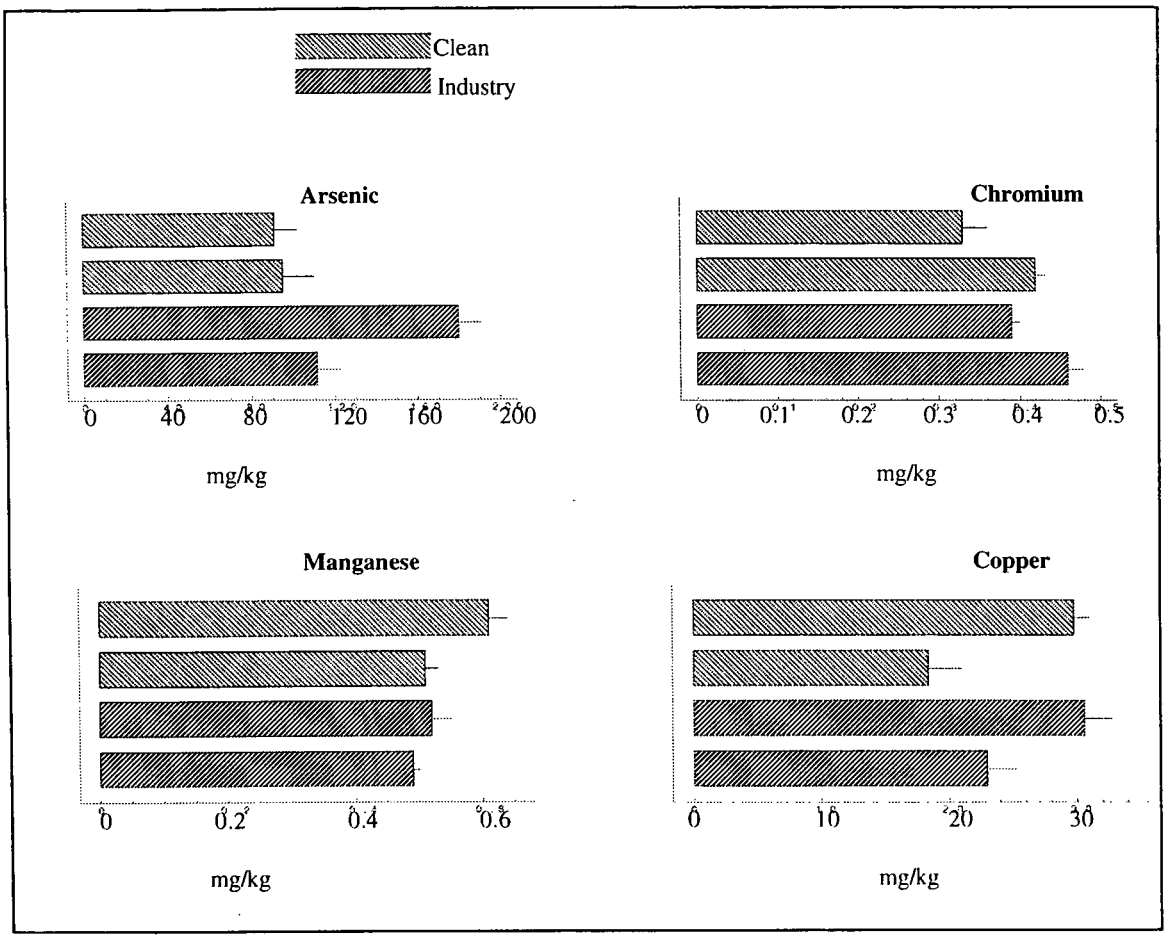
Table 33. ANOVA summary - analysis of the effect of handling, on metal levels in *P. ornatus* tail flesh, between sites at south at Orman Reef. Samples were collected in June 1992.

METAL	TREATMENT (Site)	SITE (Residual)	HETERO VAR (Cochrans)
Al			*Yes*
As		*Significant*	
Cd			*Yes*
Cr		*Significant*	
Cu		*Significant*	
Fe			
Hg			
Mn		*Significant*	
Ni		*Pooled*	
Pb	*Significant*	*Pooled*	
Se		*Pooled*	
Sr	*Significant*		*Yes*
Zn		*Pooled*	

Table 34. Power analysis for non-significant results for treatment F tests. MDD = Minimum Detectable Distance with a=0.05, b=0.2(Power = 80%)

METAL	GRAND MEAN	MS (F test Denom)	MDD mg/kg	MDD % of mean
Al	3.48	18.94	26.11	750.35
As	119.35	5902.45	460.96	386.23
Cd	0.03	0.0002	0.08	326.36
Cr	0.40	0.02	0.73	182.78
Cu	25.45	234.65	91.91	361.14
Fe	7.51	8.08	17.06	227.10
Hg	0.09	0.0005	0.13	155.10
Mn	0.53	0.02	0.73	138.13
Ni	0.09	2.96	2.24	2527.24
Se	1.60	0.31	0.73	45.46
Zn	126.40	4219.39	84.44	66.81

Figure 51. Inter site variation in *P.ornatus* tail flesh concentration, in 'clean' and 'industry' samples collected from southern Orman Reefs in June 1992. Bars represent separate sites



2.4 Discussion

Crayfish metal levels reported in the community fisheries component of the Torres Strait Baseline Study (Gladstone 1995), agree with those reported here. There is further agreement (table 24) with results for comparable tissues in prawns from the present study (tables 1, 24), with some exceptions.

Arsenic (converted to inorganic arsenic) and copper concentrations in crayfish tail flesh were higher than those reported for prawn tail flesh, to an extent that levels in some crayfish tail samples approached or exceeded the MPC (figure 24). From the perspective of health limits however, levels of metals in crayfish tails are not a cause for concern. Maximum total loads of all metals in whole crayfish tails (table 24), when summed with the estimated consumption of metals from other dietary sources and assuming total bioavailability, fall well below the respective intake limits (tables 3 and 24).

The results of this study indicate a small margin of safety between arsenic levels in some crayfish and the trade limit, especially in the case of small (<50-60 millimetres tail width) animals which tend to contain higher concentrations of this metal (figures 29 and 30). It is important to note that inorganic arsenic was merely estimated in this study from total arsenic measurements, using a worst case scenario conversion factor (see methods section in part 1).

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Copper present in prawn and crayfish tails is probably residual haemocyanin, the copper based blood pigment common to all crustaceans (White and Rainbow 1982, Rainbow 1985,1988). Higher levels in crayfish tissue may be due to their higher volume: surface area ratio when compared with prawns, which would lessen (relatively) the removal of residual haemocyanin during rinsing. As copper levels reported in this study indicate a small margin of safety between arsenic levels in some crayfish and the trade limit, the potential of rinsing to remove residual haemocyanin and hence lower tail copper levels should be investigated.

There was a significant interaction effect with season and location on copper levels in crayfish tails, where tails from Dungeness Reef in June contained the highest levels (figures 35 and 36). Although copper is a metal associated with the Fly River, Dungeness Reef is outside its expected zone of influence, although probably within the influence of western Papua New Guinea coastal rivers (Gladstone 1996). However, copper is an essential element which is regulated in decapod crustaceans (Rainbow 1990, Rainbow et al 1990), at least in the tail flesh (see discussion in part 1). Thus, the observed trend is unlikely to be a reflection of environmental patterns.

Crayfish zinc levels were also higher in tail flesh and strontium and iron levels were higher in shell, than levels of these metals in the same tissue in prawns. Clearly, tail flesh is an important storage site for arsenic and zinc and shell an important storage site for strontium and iron, in both prawns and crayfish. However, head tissues also contain at least equally important storage sites for these metals in prawns (table 1, figure 3).

Cadmium levels in crayfish tail flesh were lower than those reported for prawns and all samples were well below the MPC for this metal (table 32). This is consistent with the concept that head tissues are an important source of tail flesh cadmium contamination, as discussed with respect to prawns in detail in Part 1 of this report. As was the case for prawns, crayfish heads are also known to contain elevated levels of some metals including cadmium (Gladstone 1996). Under crayfish collecting and commercial fishing protocols practised at the time, the head was removed as soon as possible after crayfish death and prior to freezing. This early elimination of head tissues as a source of cadmium in tail flesh may explain the lower levels of this metal in crayfish tails.

2.4.1 Factors Effecting Metal Levels In Crayfish

It is important to note that, as was the case for many metals in prawns, metal regulation or accumulation strategies may not be reflected proportionally in the tail flesh. Crayfish probably cycle essential metals between body tissues during the course of metabolic processes. Adams et al (1982) present evidence that copper, zinc and iron are cycled between heamocyanin and other blood proteins, and the hepatopancreas, of the crayfish *Austropotamobius pallipes*. Except immediately after moulting, when the absence of a hard

cuticle may enable direct absorption (Adams et al 1982) tail flesh is unlikely to be a site of direct metal uptake or metabolism. It can thus be considered a controlled environment, except in the case of metals which are principally stored there, representing the net result of processes dependant on other tissues and organs, particularly those in the head. As tail flesh only was examined, an holistic discussion on metals in crayfish is outside the scope of this study. Nevertheless, as tails only are the marketed product, it is net levels occurring in tail flesh which is of most interest, from both a trade and human health view point.

Size

When considering inter-seasonal and inter-location differences in metal levels in crayfish tail flesh, size was a significant covariant for some metals. Crayfish from a range of sizes were included in this study and the size frequency distributions observed (figures 25,26) are consistent with previous population studies (Pitcher 1991, Pitcher unpublished reports). They feature two main size classes in June, which represent two age classes and one broader size class in October which also encompasses two age classes. The latter is characterised by a broader mode, representing the earlier smaller class with growth increments, plus those members of the larger June class which failed to depart from the area on the annual one way migration.

Levels of arsenic (figures 30,31), cadmium (figure 32), iron (figure 38), manganese (figure 40) and nickel (figure 42) all vary with size such that smaller size classes generally contain higher levels than larger crayfish, although in some cases the trend is less clear due to small sample sizes in some size classes from some sites (see table 25). A likely explanation for this trend is the diluting effect of tissue growth. While the uptake strategy practised for the non-essential metal cadmium is probably one of net, the rate of biomass increase during growth is sufficiently great to dilute that accumulation, such that the actual concentration of cadmium decreases with size. Similarly, while the overall strategy for essential metals is probably one of regulation, the rate of growth may be large enough to overcome attempts at regulation of iron, manganese and nickel, such that the concentration of these metals also decreases. This model is further supported by the existence of a threshold size class for the above essential metals, below which growth rates are probably more rapid and above which there is little variation in metal levels between size classes (figures 30, 31, 38, 40 and 42).

The above model does not explain the size related trends in zinc levels, which were higher in larger size classes (figures 48 and 49), but it is not surprising that different strategies are apparent for different metals. Being an essential element, crayfish probably adopt a regulation strategy for zinc, as has been demonstrated for other decapod crustaceans (White and Rainbow 1986, Nugegoda and Rainbow 1988, White and Rainbow 1982, Rainbow 1985). Bryan (1968) suggests that decapod crustaceans generally receive more zinc than they require and that excretion processes play a more important role than uptake processes. It is possible then, that the rate of excretion is higher in smaller size classes, due to their larger surface area: volume ratio.

Spatial and temporal patterns

Panulirus ornatus is a bottom dweller which lives in crevices and holes in benthic structures. It has a varied diet, including plants and benthic invertebrates (Joll and Phillips 1986). Metals available for uptake are likely to be dependant on dietary sources and dissolved metals in the water column. They would thus be influenced by levels in the sediment in indirect ways, as food species may feed in or on sediment and during periods of rougher weather when surface sediments are re-suspended, particulate and dissolved metals present in the water column probably increase to more closely reflect levels found in sediment.

There were no locational differences in tail flesh levels of Fly River associated metals in October 1992, when a far northern site was sampled. Kakope Reef is well within the extent of Fly River influence in Torres Strait (Gladstone 1996). It should be noted that these F tests, with the exception of those for copper, manganese and zinc, were often insensitive (table 29).

Crayfish tails from Dungeness Reef consistently contained higher levels of iron and cadmium than those from Cape York. As was the case for copper, the Fly River is unlikely to be an important source of iron at Dungeness Reef, as while it is a metal associated with Fly River runoff (Dight and Gladstone 1993, Gladstone 1996), Dungeness Reef is outside the expected zone of Fly River influence (Gladstone 1996). Also, as iron is an essential element, it is probably regulated by crayfish and thus observed spatial trends are unlikely to be the result of environmental gradients.

Cadmium is associated with carbonate sediments of marine origin (Dight and Gladstone 1993, Gladstone 1996). Higher levels of this metal in crayfish tails at Dungeness Reef may suggest that sediments there have a higher carbonate content than those at Cape York Reefs. While the Torres Strait Baseline Study sediment program did not collect samples from the Cape York area, it is conceivable that sediments in this area would have a higher terrigenous origin, from mainland Cape York. Further, crayfish in the Cape York area grow faster than their counterparts at Dungeness Reef (Skewes, pers comm) and thus a possible dilution effect of growth as discussed above, would be more pronounced there.

Sex

There were no significant differences between sexes in levels of any metal. This is consistent with the absence of a sex related effect on prawn tail flesh in the present study (see section 4.1). Further, since all crayfish in the areas of Torres Strait sampled were juveniles, functional sex related distinctions would not be developed.

Industry Handling

Industry practices in capture and processing of crayfish tails present very different potential sources of metal contamination to those previously discussed for prawns (see section 4.1). Firstly, although the crayfish head will contain high levels of most metals, it poses a lesser threat to tail flesh tissues via leakage. This is because the head is removed and discarded soon after crayfish death. Also, the smaller surface area : volume ratio makes crayfish tails less susceptible than prawns to the effects of head leakage. Secondly, the crayfish processing environment is quite different. Since the fishing operation generally takes place in a small dinghy, rather than on the back deck of a larger vessel, exhaust fumes and fuel residues are in closer proximity to sample handling.

It is likely, then, that elevated lead levels in industry tails (figure 50) were due to contact with petroleum products, either residues from previous spillage on the floor of the dinghy, or from exhaust fumes. When converted to its wet weight concentration equivalent of 0.1 mg/kg, the elevated mean level of lead in tail flesh was well below the MPC of 1.5 mg/kg and of minimal importance to health limit considerations (table 3). Thus, the scale of this lead contamination is small with respect to legal and health limits and should not be cause for concern.

The elevation of strontium in 'clean' crayfish tails is difficult to interpret with respect to handling. For metals for which there were no significant effects of industry processing, this preliminary investigation of the net effects of industry handling in this study was of limited benefit. While there were no significant differences between industry and 'clean' crayfish tails for most metals, the F tests in most cases were insensitive, only being capable of detecting extremely large effect sizes in most cases (table 34). However, except in the case of arsenic

and copper, this large detectable effect size may not be inappropriate, since levels of other metals were well within their respective trade and health limits.

With the increase in importance of whole live crayfish as the marketed product, further work is needed to address the potential for metals in crayfish heads to contaminate tail flesh during processing and handling. If head removal immediately after crayfish death and prior to further processing and consumption was practiced at the consumer end of the marketing process, this potential would probably be eliminated.

2.4.2 Recommendations For Further Research

1. Measure inorganic arsenic directly to elucidate whether or not levels in crayfish tail flesh are of concern to trade limits. Sampling design should include crayfish size as a factor.

2. Further examine copper levels in crayfish tails. Conduct sampling in the middle of the year, including Dungeness as a location and crayfish size as a factor. Examine the effects of different levels of rinsing on residual copper in tail flesh.

3. Since recommendations 1 and 2 come from a concern that levels of arsenic and copper may be close to their MPC's, further work must allow detection of small-scale trends. Thus, sampling must be designed, using estimates of variability found in this study, to minimise the detectable effect size with strong power. A cost efficient way to do this is to maximise the degrees of freedom in the denominator of the F ratio, for example, by sampling a larger number of sites within locations.

4. Asses the effect that the addition of head tissues to the marketed product will have on total metal concentrations in crayfish with respect to trade and health limits. Particularly examine the potential for industry handling methods to cause some metals in head tissues to contaminate tail flesh.

HEAVY METALS IN *PENAEUS ESCULENTUS* IN TORRES STRAIT PILOT STUDY

Prawns for the pilot study were collected from both the north and south site (see figure 1) in May/June 1992. Samples were collected aboard the commercial prawn trawlers FV 'Maggie Jo' (in the north), and FV 'Smithy' (in the south). Each site was sampled over 2 or 3 nights, with 3 or 4 separate shots being trawled each night. The objective of the pilot program was to assess variability in metal levels in prawns at various scales in order to choose the optimum sampling unit for the main study. In addition, the comparability and relative merits of various sample handling procedures was compared, in order to assess the poolability of samples collected under various regimes.

Initially, the intention was to remove the head from other tissues immediately after capture and prior to freezing, in a portable clean air 'glove-box' on board the trawler. In practice, this procedure was soon abandoned due to the space and time limitations on board the trawlers. Various 'next best' procedures were tried, until the optimum method, as adopted in the main study, was evolved. Treatment variations were further confounded by freezer and transport problems, which allowed some samples to defrost and re-freeze at least once. The resulting suite of different sample handling treatments raised the important question of whether or not samples collected under such different regimes should be treated separately.

Treatments can be categorised as follows:

1. Freeze immediately, dissect at later date while par frozen.
2. Keep samples chilled until 12–36 hours (with intention of dissecting ASAP), freeze and dissect at later date while par frozen.
3. Dissect immediately, within 4 hours (the original goal).
4. Dissect within 12–36 hours.

After considering previously published records of metal levels in prawns, high variability was anticipated. It was suggested that since the hepatopancreas is a major storage site for many metals, smaller variance may be achieved by analysis of this tissue alone. This idea was also trialed in this pilot study.

This pilot study was based on cadmium data, since this had been identified as the metal of most concern.

Pilot analysis of prawn data:

1 Tiger prawn samples were selected across nights and shots, in the following way. Only large females were selected, to eliminate variability due to sex and size. It was not possible to standardise gonad and moult stage.
Northern Site: 3 shots selected from all 3 nights, 3 reps/shot
Southern Site: 4 shots selected from both nights, 3 reps/shot

2 Dry weight concentrations are used because they are less variable.

3 In considering whether levels of a factor can be pooled, Winer's (1971) criteria (as described in Underwood 1981) of setting $a>0.25$ in an F- or t-test was used.

4. As a whole, variances in ANOVA's were heteroscedastic. However, this was not a concern because the aim of the analyses was to investigate the probability that the null hypothesis could explain the data. (see Underwood 1981 for a discussion on this.) Thus, transformations were not performed.

Can dissection codes be pooled?

5. Comparison 1:
The two main handling methods 1 and 3, were reproduced within nights in the south, for tiger prawns.
F tests did not support pooling these two codes at a>0.25 and on the whole levels were higher from dissection code 1 (see table 3).

Table 3 Cadmium levels in tissues for dissection codes 1 and 3. Samples were tiger prawns, from the southern site. In each cell, n=6, representing pooled samples from 2 shots. Values are on a dry weight basis in mg/kg (ppm).

Dissection Method		Head total night 1	night 2	Tail Muscle night 1	night 2
3	x	27.50	19.03	0.48	0.25
	SE	3.15	3.60	0.08	0.03
1	x	38.68	39.78	0.62	0.48
	SE	16.31	9.56	0.06	0.08

6. Comparison 2:
The tail flesh of 5 endeavour prawns each from handling methods 3 and 4 (reproduced in one shot) was analysed for the southern site only.
A t-test supported pooling these two methods at a>0.25.

7. Overall however, there was insufficient evidence to support pooling different handling methods. For this reason, samples collected during the pilot program were excluded from the main study.

Is it an advantage to analyse hepatopancreas instead of total head?

8. At dissection code 1 only (since it is the one that is standard for subsequent analyses), the precision was calculated for these two tissue types, for use as a comparable measure of variability.
n=6 per night (shots were dropped). Results were:

Night	Precision estimate Hepatopancreas	Head total
3	.26	.21
4	.21	.42
5	.20	.24

(Dissection code 1 was not used on nights 1 and 2)

Except on night 4, analysis of hepatopancreas did not improve the precision of estimates of levels in the head. Thus, the option of using it as an alternative to total head was not pursued.

Can shots be pooled?

9. In 6 ANOVAs performed using shots as a factor, 5 supported pooling shots at a>0.25. Also, in 5 out of 6 cases, the variance attributable to shots was 0. Thus, the factor shots will be dropped from future analyses.

10. After pooling shots however, there was no consistent evidence to support pooling nights.

11. Thus, the random factors that remain for the design of future analyses are nights and replicates.

How many nights and how many replicates?

12. To allow enough analyses to cover all prioritised factors (see proposal), the maximum total number of replicates per random unit (=nights x reps) should not exceed 20.

13. The effect of varying number of nights and replicates on overall variance was calculated according to formulae in Andrew and Mapstone (1987). In summary, increasing the number of nights had a much greater benefit to overall variance than increasing the number of replicates. The optimal combination was the maximum possible number of nights, i.e. three and seven replicates.

14. In the north, only two nights are available because samples from one night were included in the freezer problem already described. In these cases, two nights with 10 replicates will be used.

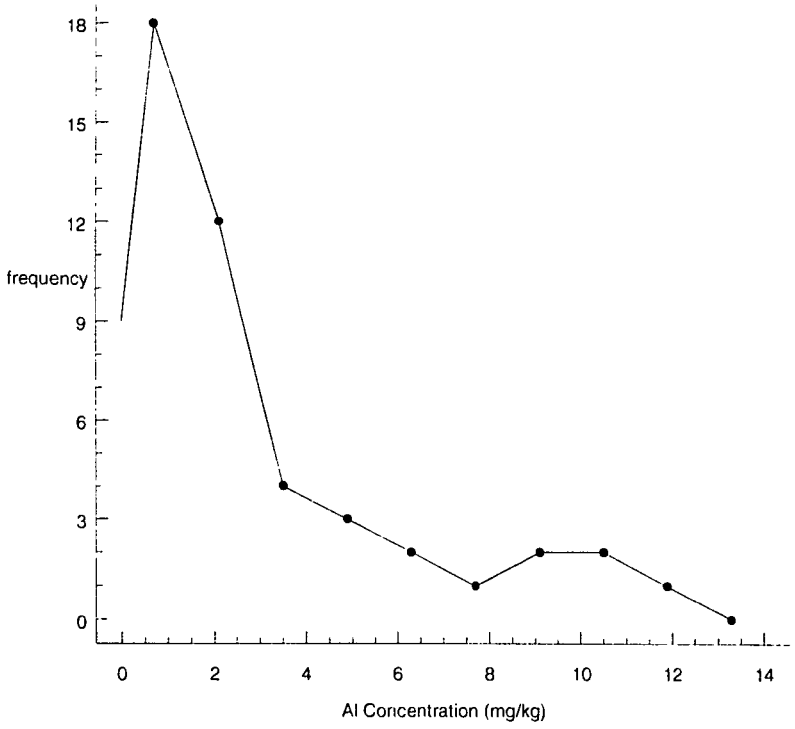
METAL LEVELS IN *PENAEUS ESCULENTUS* - NORMALITY OF DATA

The following table and frequency polygons illustrate typical data distributions for levels of some metals in tail flesh of *P. esculentus*. To avoid confounding factors, only medium sized prawns from one site (south) and season (October/November 1992), were selected. Only metals for which there are MPC or health standards have been included.

The standardised coefficients of skewness and kurtosis measure departure from symmetry and relative flatness or steepness respectively. For both statistics, when the values are outside the rang +2.0 to -2.0, the data may depart significantly from a normal distribution. Calculations were done in the statistical computer package 'statgraphics'.

	Mean	Standardised Skewness	Standardised Kurtosis
Aluminium	3.09	4.12	1.76
Arsenic	49.80	- 0.29	- 1.09
Cadmium	0.55	2.94	0.19
Copper	14.69	- 1.35	- 0.65
Mercury	0.08	3.94	2.55
Lead	0.19	10.32	27.36
Selenium	1.85	0.99	- 0.38
Zinc	53.87	2.88	0.11

While aluminium and lead data clearly do not follow a normal distribution, symmetry and kurtosis of other metals do not display gross departures from normality. Considering this, along with Underwood's (1981) discussion on the general robustness of ANOVA to violations of the normality assumption, data in the present study was not transformed prior to analysis.


18
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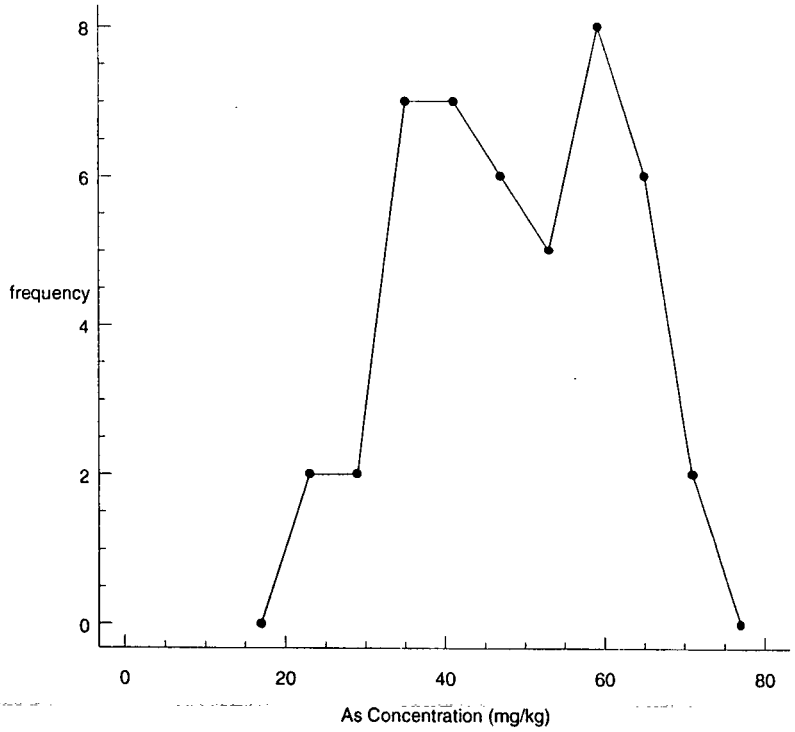
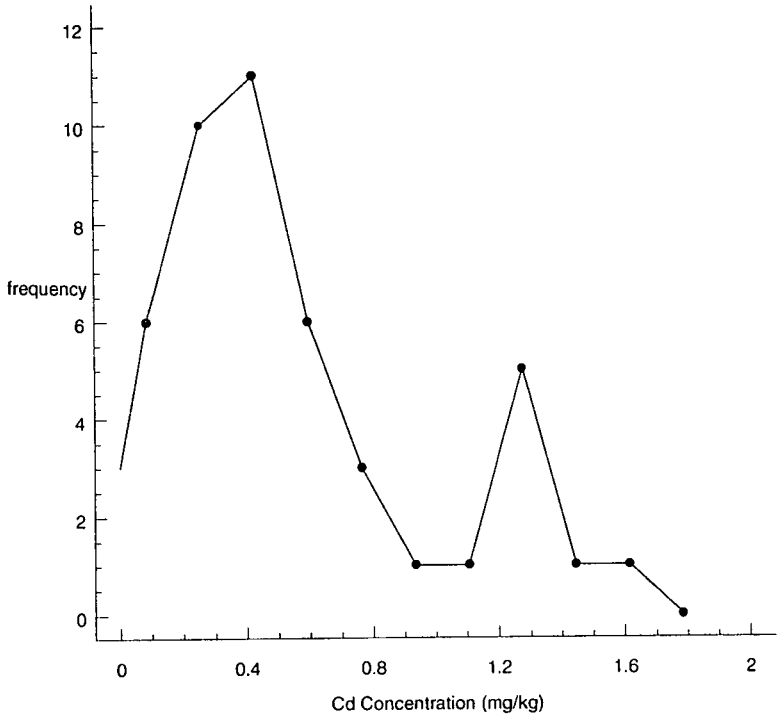
frequency
9
6
3
0
0
2
4
6
8
10
12
14
Al Concentration (mg/kg)


8
6
frequency
4
2
0
0
20
40
60
80
As Concentration (mg/kg)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8
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6
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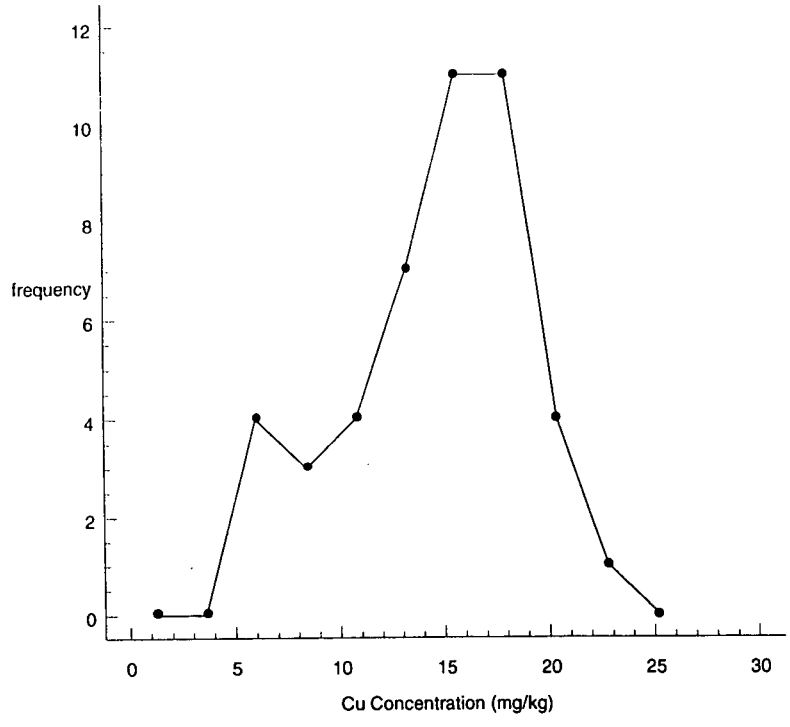
0
0.4
0.8
1.2
1.6
2
Cd Concentration (mg/kg)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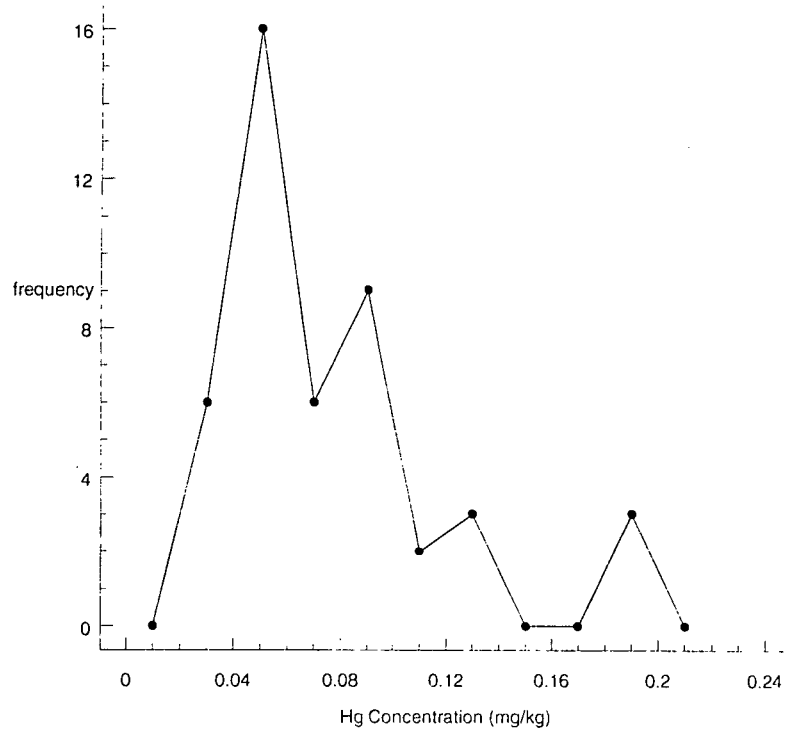
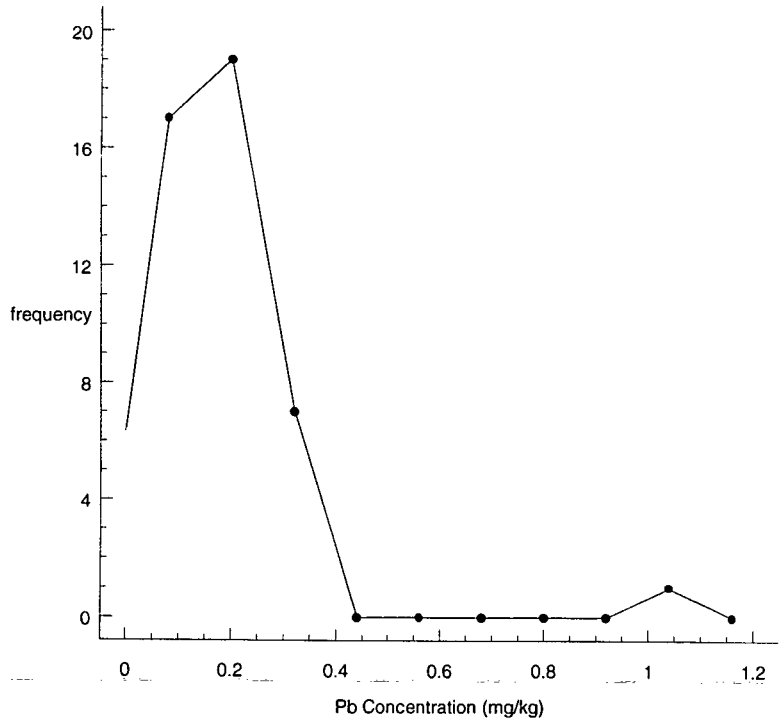
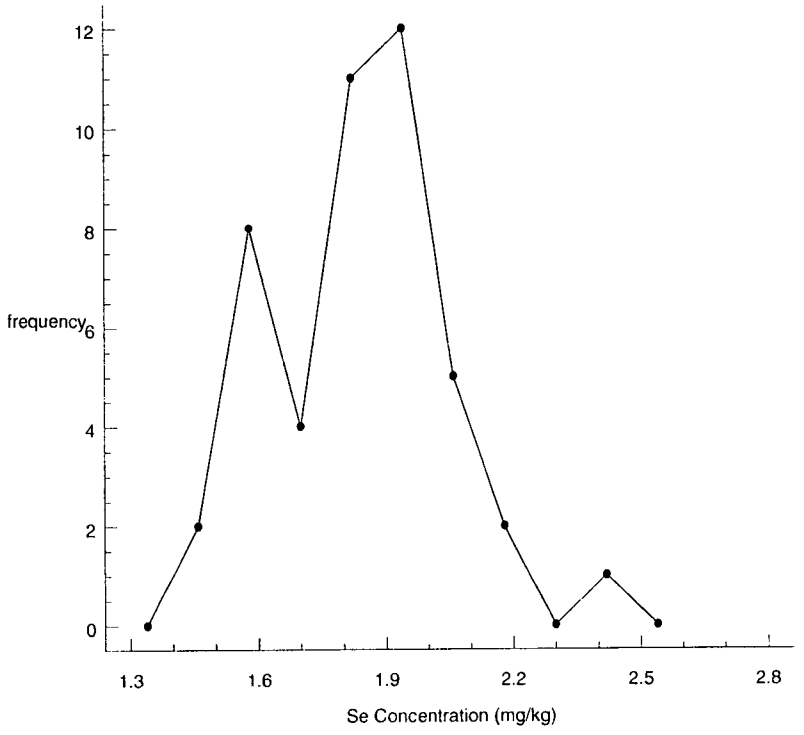
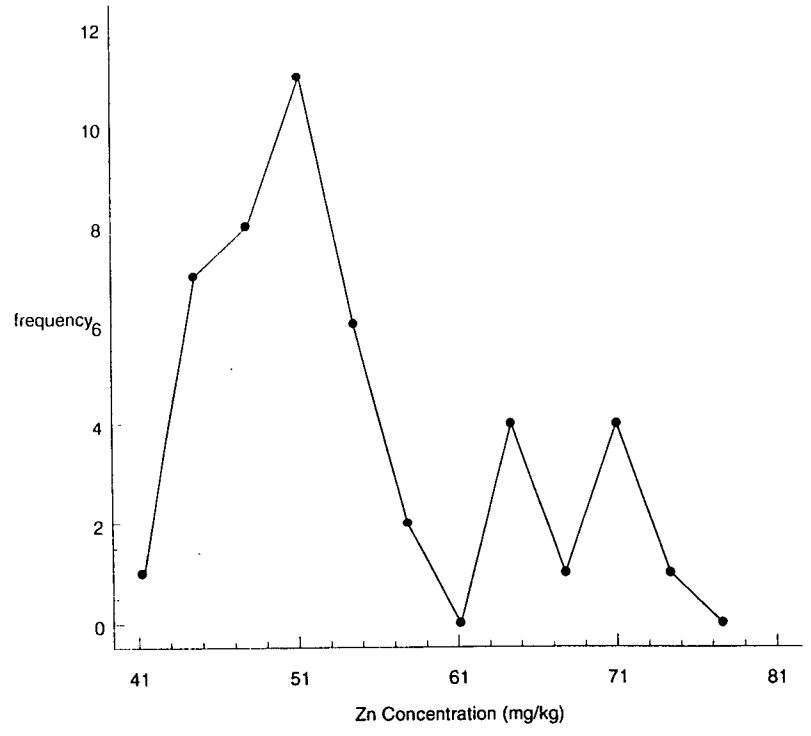
10
8
frequency
6
4
2
0
0
5
10
15
20
25
30
Cu Concentration (mg/kg)


16
12
frequency
8
4
0
0
0.04
0.08
0.12
0.16
0.2
0.24
Hg Concentration (mg/kg)


20
16
12
frequency
8
4
0
0
0.2
0.4
0.6
0.8
1
1.2
Pb Concentration (mg/kg)


12
10
8
frequency 6
4
2
0
1.3
1.6
1.9
2.2
2.5
2.8
Se Concentration (mg/kg)


12
10
8
frequency 6
4
2
0
41
51
61
71
81
Zn Concentration (mg/kg)

Procedures used by Queensland Department of Primary Industry (Animal Research Institute) for Trace Metal Analysis of Biological Samples

Prepared by H Mawhinney and E McElroy

Apparatus

All analyses were carried out on a standard Perkin-Elmer Sciex ELAN™ 5000 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

Materials and Methods

All plastic-ware used for the preparation of standard solutions and for dilution and storage of sample digests was soaked in nitric acid (10%) for a minimum of 48 hours. All items were then rinsed three times using reverse osmosis (RO) prepared water followed by three further rinsings with polished reverse osmosis (ROP) prepared water (18M Ω).

All borosilicate glass volumetric flasks were fitted with PTFE stoppers. These flasks and the PTFE beakers were refluxed with HNO_3 (conc.) for eight hours, allowed to cool, rinsed three times with RO water and then soaked and cleaned as per plastic-ware.

Mixed multi-elemental standard solutions were prepared from 1000 ng/L stock solutions. Aluminium standards were prepared separately in TPX (polymethylpentane) volumetric flasks. The adsorption/release equilibriums of glass with aluminium in solution make low level determinations of this element in glass highly inaccurate. Tin standards were also prepared separately in glass, daily from concentrate.

HNO_3

Nitric acid was purified by sub-boiling double distillation of reagent grade feedstocks in quartz stills.

Sample Preparation

Sample dissolution was achieved using a HNO_3 microwave assisted digestion. The system used a was a Microwave Laboratory Systems MLS 1200 manufactured by MILESTONE, Italy.

Nitric acid was specially prepared by double distillation of AR grade acid in sub-boiling point quartz stills.

All samples were freeze-dried and ground to a fine powder to achieve an homogeneous final product. One hundred to two hundred milligrams of this material was accurately weighed into a TFM insert of the microwave digestion system. HNO_3 (4 millimetres) was added and the vessels sealed and placed in the microwave oven. The oven program used was:-

Step (1)	250 watts for 8 minutes
Step (2)	400 watts for 4 minutes
Step (3)	250 watts for 4 minutes

It should be noted that 250 watts power with this system is a continuous energy output which results in more even and controlled heating producing a gradual pressure increase to a maximum of 30 bar.

The vessels were then removed from the oven and cooled in an ice-bath for a minimum of one hour. This step is necessary to avoid losses of sample as an aerosol upon opening of the vessels. The sample solution is then transferred to a PTFE beaker and made up to 10.0 grams with ROP water. 3.0 grams of this solution is transferred to a polypropylene tube. This tube is set aside for mercury and tin determinations.

The remaining 7.0 grams of sample solution in the beaker is taken to near dryness on a ceramic hotplate at 90°C. A further 2 millimetres of HNO_3 and 2 millimetres of H_2O_2 is added dropwise and again taken to near dryness. This step is included to ensure complete digestion and to remove volatile interfering matrix components. The digestion solution is washed into a 50 millimetres polypropylene tube using 1% HNO_3 and accurately made up to 20.0 grams. This tube is used for solution nebulization ICP-MS.

Running Procedures

Each set of digestions contain 8 samples, a standard reference material (SRM) and either a blank or a duplicate sample. This is maintained for all samples that are digested using the above procedure.

The samples are run on the ICP-MS using solution nebulization. A set of calibration standards are run and then the digest solutions including SRM's, blanks and duplicate samples. For the purposes of the method all readings must lie between the lowest and highest standard concentration on the calibration curve. If readings are below the reading of the lowest standard concentration then a more concentrated solution must be run and if the readings are above the highest concentration a dilution of the sample must be run.

All standard concentrations must be within the linear dynamic range on the calibration curve. Calibration check solutions and blanks are also analysed in the run sequence and the instrument is recalibrated by reanalysing all standard solutions every 3 hours.

This method of analysis exceeds the guidelines set out in our quality control manual accredited by the National Association of Testing Authorities, Australia (NATA).

MODELS FOR ANALYSIS OF VARIANCE

Nesting is indicated by brackets around the parent factor. R= Random factor, F = Fixed factor.

Part 1 Metal Levels in Prawns

1.1 The effect of size: Metal levels in tail flesh of medium and small *P. esculentus* at the northern site only. (tables 5 and 6 refer)

ANOVA Model:

Factor	R F	No of Levels	degrees of freedom	MS denominator in F ratio
Size	F	2	1	Size x Night
Night	R	2	1	Residual
Size x Night			1	Residual
Residual	R		29	

Sample Sizes:

Prawn size:	Night 1	Night 2
Medium	10	10
Small	7	6

1.2 The effect of size: Metal levels in tail flesh of medium and large *P. esculentus* at the southern site only. (tables 7 and 8 refer)

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Size	F	2	1	Size x Night
Night	R	3	2	Residual
Size x Night			2	Residual
Residual	R		60	

Sample Sizes:

Prawn size:	Night 1	Night 2	Night 3
Medium	14	16	15
Large	7	7	7

1.3 The effect of tissue type and site: Metal levels in different tissue types of medium sized *P. esculentus* from different sites (tables 9, 10 and 11 refer).

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Tissue	F	3	2	Tissue x Night
Site	F	2	1	Night
Night (Site)	R	3,2	3	Residual
Tissue x Site			2	Tissue x Night
Tissue x Night			6	Residual
Residual	R		132	

Sample Sizes:

Tissue	North (2 days)	South (3 days)
Tail	10,10	14,16,15
Shell	10,10	7,7,7
Head	10,10	7,7,7

1.4 The effect of sex: Metal levels in male and female medium sized *P. esculentus* tail flesh from the southern site (tables 12 and 13 refer).

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Sex	F	2	1	Sex x Night
Night	R	3	2	Residual
Sex x Night			2	Residual
Residual	R		60	

Sample Size:

Sex	Night 1	Night 2	Night 3
Male	7	7	7
Female	14	16	15

1.5 The effect of moult stage: Metal levels in tail flesh from pre- and post- moult medium sized female *P. esculentus* from the southern site (tables 14 and 15 refer).

Factor	RF	No Levels	degrees of freedom	MS denominator in F ratio
Moult Stage	F	2	1	Moult Stage x Night
Night	R	3	2	Residual
Moult Stage x Night			2	Residual
Residual	R		53	

Sample Sizes:

Moult Stage	Night 1	Night 2	Night 3
Pre-moult	8	9	4
Post-moult	12	13	13

1.6 The effect of handling method: Metal levels in tail flesh from medium sized *P. esculentus* from the southern site, subjected to four different handling treatments (tables 16 and 17 refer).

Factor	RF	No Levels	degrees of freedom	MS denominator in F ratio
Treatment	F	4	3	Treatment x Night
Night	R	3	2	Residual
Treatment x Night			6	Residual
Residual	R		48	

Sample Sizes:

Treatment	Night 1	Night 2	Night 3
Clean	5	5	5
Chilled	5	5	5
Green	5	5	5
Cooked	5	5	5

1.7 The effect of site, and differences between species: Metal levels in tail flesh of *P. esculentus*, *M. endeavouri* and *P. longistylus* from the northern and southern sites (tables 8, 9 and 20 refer).

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Species	F	3	2	Species x Night
Site	F	2	1	Night
Night (Site)	R	2,3	3	Residual
Species x Site			2	Species x Night
SpeciesxNight			6	Residual
Residual	R		105	

Sample Sizes:

Species:	North (2 nights)	South (3 nights)
Penaeus esculentus	10,10	7,7,7
Penaeus longistylus	9,10	5,7,7
Metapenaeus endeavouri	10,10	7,7,7

1.8 The effect of season and differences between species: Inter-seasonal comparison of metal levels in tail flesh of *P. esculentus*, *M. endeavouri* and *P. longistylus* from the northern site (tables 21, 22 and 23 refer).

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Species	F	3	2	Species x Night
Season	F	2	1	Night
Night(Season)	R	2,3	3	Residual
Species x Season			2	Species x Night
Species x Night			6	Residual
Residual	R		105	

Sample Sizes:

Species:	Pre Wet (2 nights)	Post Wet (3 nights)
Penaeus esculentus	15,15	7,7,7
Penaeus longistylus	8,6	7,7,7
Metapenaeus endeavouri	9,4	7,7,7

Part 2 Metal Levels in Crayfish

The following table (duplicate of table 31) denotes sample sizes of collections from June and October 1992, across locations, sites and size categories, as used for analyses 2.1–2.3 (below).

Location	Period	Site	Sample size per tail width (mm) category						
			30–40	40–50	50–60	60–70	70–80	80–90	Total
Cape York	June'92	1	0	0	0	1	3	1	5
		2	1	1	0	1	2	0	5
		3	2	0	1	1	1	0	5
	Oct/Nov '92	1	1	2	2	2	1	0	8
		2	0	3	3	0	0	0	6
		3	0	1	2	2	0	0	5
Dungeness	June'92	1	2	2	1	2	3	0	10
		2	0	0	0	1	3	1	5
	Oct/Nov '92	1	0	1	1	2	0	1	5
		2	0	1	3	1	0	0	5
		3	0	1	3	0	1	0	5
South Orman	June'92	1	0	0	2	1	2	0	5
		2	0	0	1	2	0	2	5
Kakope	Oct/Nov '92	1	0	0	2	0	0	1	3
		2	0	2	1	0	0	0	3
		3	0	1	1	0	1	0	3

2.1 The effect of location: Metal levels in tail flesh of *P. ornatus* collected in June 1992, at locations Cape York, Dungeness Reef and Sth Orman Reefs. Tail width is included as a co-variate. (tables 26 and 27 refer)

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Tail width		covariate	1	Residual
Location	F	3	2	Site
Site(Loc'n)	R	3,2,2	4	Residual
Residual			32	

2.2 The effect of location: Metal levels in tail flesh of *P. ornatus* collected in October/November 1992, at locations Cape York, Dungeness Reef and Kakope Reef. Tail width is included as a co-variate. (tables 28 and 29 refer)

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Tail width		covariate	1	Residual
Location	F	3	2	Site
Site(Loc'n)	R	3,3,3	2	Residual
Residual			37	

2.3 The effect of season: Metal levels in tail flesh of *P. ornatus* collected from Cape York and Dungeness Reef, in June and October/November 1992. (tables 30 and 31 refer)

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Tail Width		covariate	1	Residual
Location	F	2	1	Site
Season	F	2	1	Site
Site(Loc,Seas)	R	3,3;2,3	2	Residual
Loc x Seas			1	Residual
Residual				

2.4 The effect of sex: Metal levels in tail flesh of male and female *P. ornatus* with tail width of 40-60 millimetres, collected from all sites combined in October/November 1992. (table 32 refers) n=15 per sex.

Factor	R F	No Levels	degrees of freedom	MS denominator in F ratio
Sex	F	2	1	Residual
Residual			28	

2.5 The effect of handling: Metal levels in tail flesh of *P. ornatus* collected from Sth Orman Rfs in June 1992, collected according to 'clean' and 'industry' handling protocols. (tables 33 and 34 refer) n=5 per site per treatment.

Factor		No Levels	degrees of freedom	MS denominator in F ratio
Treatment	F	2	1	Site
Site(treat)	R	2	2	Residual
Residual			16	

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