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Trace Metal Concentrations in the Balmain Bug (*Ibacus peronii* Leach, 1815) from Southwest Victoria, Australia

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The tissues of crustaceans are frequently consumed by humans, even though they are known to accumulate metals within their tissues. A number of studies have determined metal concentrations in rock lobsters (Palinuridae) and scampi (Nephropidae), but not in shovel-nosed lobsters or slipper lobsters (Scyllaridae), despite there being more than seventy species in this taxonomic group (Jones & Morgan, 2002). There are two species of slipper lobsters found around the coast of Australia, *Thenus orientalis* Lund, 1793, found in Australia's warmer, northern waters, and *Ibacus peronii* Leach, 1815, which is restricted to cooler southern waters. *Ibacus peronii*, or the Balmain Bug, is part of the commercial catch of the Victorian fisheries, and legally caught and sold for human consumption. *Ibacus peronii* are relatively small organisms (total body length < 200 mm) found in coastal waters at 20 to 250 m depth, in locations where the sediments consist of fine sand or silt. This species is considered an important nocturnal benthic carnivore, which feed on crustaceans, polychaetes, molluscs and echinoderms (Jones & Morgan, 2002).

In this basket survey, we investigated the concentrations of a range of metals, including As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, and Zn, in the edible muscle of Balmain Bugs on sale to the general public in the towns of Warrnambool and Portland in south-west Victoria, Australia. South-west Victoria is often considered to be a relatively uncontaminated region due its low scale industrial base and the distance from major sources of metal pollution. Herein we report the results of our survey, and where relevant compare our results to the maximum values permitted by the Australian food standards and WHO/FAO Joint Expert Committee on Food Additives (JECFA) maximum tolerable intakes, and assess the human health implications for consumers of this decapod crustacean.

MATERIALS AND METHODS

Thirty specimens of *I. peronii* were purchased from a seafood retailer in Portland, who reported that the organisms had been caught off the coast of Portland (38`29°S, 142`44 °E) during July and August 2004. Similarly, a further thirty specimens were purchased from a retailer in Warrnambool in early summer 2005,

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having been caught off the coast of Warrnambool (38`19° S, 141`29° E) during December 2004 and January 2005. The animals were thawed and the carapace size and sex of each organism was determined before dissection. Sex was determined by examining the 3rd and 5th leg base of each animal for a sexual pore. Male Balmain Bugs have a sexual pore on the 5th leg, females have a sexual pore on the 3rd leg. Sex distribution was almost the same between sites (Portland, 20 male and 10 female animals; Warrnambool, 19 male and 11 female animals).

All dissection equipment, glassware, plasticware used during the processing and preparation of samples were soaked prior to use for at least 24 hours in a 5% solution of Extran 300, followed by three rinses with deionised water prior to use. The glassware and plasticware was soaked in a 10% nitric acid solution for 1 week. Polyethylene gloves were worn when handling all samples, equipment and reagents. The tail muscle of each animal was cut away from the exoskeleton, any excess shell removed, and the muscle weighed. From each tail, two sub-samples of approximately 2.5 g were excised, and accurately weighed (OHAUS analytical scale model GT410). Each sub-sample of muscle was placed in an individual, labelled polythene bag and frozen prior to further preparation and analysis.

The tail muscle was digested using the EPA Method 200.3 for Total Recoverable Elements in Biological Samples (Environmental Protection Agency, 1991), with minor modification. The EPA method recommends 5 g (wet weight) of sample. This sample weight was halved in the current study, because of the small size of the organisms. All reagent volumes used were adjusted accordingly. In short, the samples were digested in four batches in a temperature controlled digestion block (AIM500 digestion block, A.I. Scientific). Field and quality control/quality assurance samples (eg. spiked samples, blanks, certified reference material (CRM; Tort 1 lobster hepatopancreas, National Research Council of Canada (NRCC))) were haphazardly placed into separate glass tubes on the digestion block. Thereafter, concentrated nitric acid (5 mL) was added to each tube, and the stoppered tube left for 24 h at room temperature. A small number of anti-bumping granules were placed into each of the tubes, which were then heated to 40°C for 30 min, then allowed to cool to room temperature. Thereafter, nitric acid (4.5 mL) was added to each tube, the solutions heated to 90-95°C for 3 h, and then again allowed to cool to room temperature. Once the solution had cooled, hydrogen peroxide (2 mL) was added, and the solution was heated to boiling for 30 min with the stoppers off. The step requiring hydrochloric acid (as suggested by EPA) was not employed for the current study as it is mainly applied to extract methyl-mercury from biological tissues. Samples were cooled to room temperature, transferred to 50 mL volumetric flasks and made up to the mark with deionised water. The digests were filtered through 0.45 μm cellulose acetate disposable syringe filters (Sartorius CE Minisart RC15, Sartorius, Germany) into sterile centrifuge tubes for transport to Japan, and analysis by ICP-AES. Digests were analysed using an IRIS ICP-61E (Thermo Jarrell Ash, Japan). The following analytical wavelengths were monitored: Al, 396.1; As 189.0; B 208.9; Ba, 233.5; Be, 313.1; Ca, 317.9; Cd, 214.4; Co, 228.6; Cr, 205.5; Cu, 324.7; Fe, 239.5; Hg,

194.2; K, 766.4; La, 408.6; Mg, 279.0; Mn, 260.5; Mo, 202.0; Na, 588.9; Ni, 231.6; P, 213.0; Pb, 220.3; Sc, 361.3; Se, 196.0; Sr, 421.5; Ti, 336.1; V, 311.0; Y, 395.0; Zn, 206.5 nm, respectively. Instrument limits of determination (LOD) were: Be, Cd, Cu, Mn, Sc, Sr, 0.01 mg L^{-1}; Ba, Co, Fe, Ni, Ti, V, Zn, 0.02 mg L^{-1}; Ca, Y, 0.03 mg L^{-1}; Cr, La, Mo, 0.05 mg L^{-1}; Al, As, B, Hg, Mg, P, Pb, 0.1 mg L^{-1}; and K, Na, Se, 0.5 mg L^{-1}, respectively. Method detection limits, ie. the minimum concentration that could be quantified in 2.5 g raw muscle, were twenty times higher than these values. Deionised water with a resistivity of at least 18M Ω cm was prepared by passing singly distilled water through a Milli-Q water Purification System. Nitric acid (Univar grade, AnalaR grade), hydrochloric acid (AnalaR grade), hydrogen peroxide (AnalaR grade), ammonium dihydrogen phosphate (Univar grade) and Extran 300 detergent were obtained from BDH Chemicals.

Statistical analysis: Of the 28 elements measured in the digests, the concentrations of 16 were certified by the NRCC in TORT-1 CRM, namely As, Ca, Cd, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Se, Sn, Sr, Zn. Sn was not measured in this study. Although measured, the digestion method used is not appropriate for Se. CRM digest concentrations of Hg and Co were all <LOD. Hence, these elements were not included in statistical analysis. The concentrations of Al, B, Ba, Be, La, Sc, Ti, V, Y were not certified in TORT-1, and, with few exceptions, were all <LOD. Hence, these elements too were removed from statistical analysis and subsequent discussion. For statistical comparison, where the concentration of a small proportion of measurements for particular analyte was below determinable limits, that concentration was incorporated into the analysis as 0 mg kg^{-1}. Carapace length and organism weight data were normally distributed about a central arithmetic mean, and hence complied with prerequisutes for parametric tests. However, chemical residue data did not comply with prerequisites of homogeneity of variance and normality of data required for parametric tests, so the non parametric tests Mann-Whitney U test was applied to the residue data using SPSS version 12 (SPSS Inc, Chicago) to test for differences in analyte concentrations between sites, and gender within and between sites. To assess the similarity in metal residue concentrations between sites, Principal Components Analysis (PCA) ordination plots were obtained from a matrix of Pearson's correlation coefficients. Three analytes with fewer than ten observations >LOD for a site, namely Cd, Cr and Pb, were removed from the matrix prior to generation of the ordination plots.

RESULTS AND DISCUSSION

There was no difference in carapace length or body weight of animals caught off the two cities (Portland, 49.7 ± 3.8 mm and 46.3 ± 13.7 g *cf.* Warrnambool, 44.2± 3.7 mm and 42.9 ± 13.2 g). To check analytical accuracy and precision, analysis of a certified reference material (Tort 1 lobster hepatopancreas) was undertaken. For the most part, metal concentrations were found to be within 25% of expected values (77-114% recovery). The recovery of Ni (157% expected value) was deemed too high, and this element was removed from subsequent data analysis.

Table 1. Summary of metal concentrations (mg kg^{-1} wet weight) in the edible muscle meat of *I. peronii*, caught in south-west Victorian waters, 2004.

	Warrnambool			Portland		
Element	Mean *	CV (%)	Range	Mean *	CV (%)	Range
As	13.2	(25)	9.0 - 22	37.7	(36)	16.4 - 74.3
Ca	755	(40)	328 - 1398	430	(39)	239 - 1145
Cd	0.4	(81)	<LOD - 1.2	1.1	(286)	<LOD - 12.0
Cr	0.5	(200)	<LOD - 3.5	5.3	(148)	<LOD - 37.1
Cu	12.1	(41)	<LOD - 21.9	6.9	(59)	2.6 - 16.4
Fe	10	(71)	3.4 - 31.1	31	(129)	2 - 201
K	1271	(21)	785 - 1621	3506	(12)	2678 - 4476
Mg	518	(19)	395 - 786	630	(19)	500 - 1160
Mn	0.5	(47)	0.2 - 1.5	2.3	(179)	<LOD - 13.4
Na	3202	(11)	2485 - 3725	3626	(20)	2384 - 6134
Pb	1	(175)	<LOD - 4.9	1.1	(323)	<LOD - 14.7
Sr	11	(49)	4.0 - 25	7	(68)	2.2 - 19.4
Zn	35	(10)	28 - 43	25.7	(18)	17.5 - 37.0

* Means and ranges in this table include those samples for which a determination <LOD (as 0 mg kg^{-1}). Number of samples with measured concentrations >LOD, n = 30, except for: Warrnambool Cd, 23; Cr, 7; Cu, 29; Pb, 8; Portland Cd, 7; Cr, 21; Mn, 27; Pb, 3. CV, coefficient of variation in data.

In discussing elemental concentrations, data has not been corrected for recovery.

Some caution should be taken when assessing statistical variability in the biophysical or chemical data, since the samples were not collected from the field to a rigorous ecological protocol, but purchased from local retail outlets. No statistically significant differences in metal concentrations were observed between male and female animals at either site, with the exception of K, which was higher in Portland females than Portland males ($p < 0.05$), and Na, which was higher in Portland males ($p < 0.05$) (Table 1). Statistically significant differences were observed for all metals between sites, with the exception of Cd and Mn. For instance, As, Cr, Fe, K, Mg, Na and Pb are significantly higher in Portland samples than Warrnambool animals ($p < 0.05$), while Ca, Cu, Sr and Zn are higher in the Warrnambool animals ($p < 0.05$) (Table 1). PCA showed clustering by site, suggesting that even though the two towns are separated by only approximately 100 km, there were clear differences in metal concentrations in *I. peronii* at these two locations. Any, or all of a number of factors, may be the reason for the observed differences, including sex, size, diet, stage of moult cycle, location, season, ambient dissolved metal concentration, temperature, and salinity (Canli et al 1997; Páez-Osuna et al 1995).

Table 2. Average metal concentrations reported in lobsters in Australia, and worldwide (post-1990 publications only).

Species and location	Metal concentration (mg kg^{-1} wet weight)						Authors
	As	Cd	Cu	Fe	Mn	Zn	
Nephrops norvegicus,SCO*		0.35	5			12	Canli et al, 1993
Nephrops norvegicus, SWE					3.7		Eriksson, 2000
Thenus orientalis, KUW *	2						Bu-Olayan et al, 1998
Panulirus inflatus, MEX *		0.06	11	8	0.3	25	Páez-Osuna et al, 1995
Panulirus marginatus, USA	116	6.0	110			129	Miao et al, 2001
Panulirus cygnus, WA		0.02					Francesconi et al, 1994
Jasus edwardsii, VIC	54	0.02	9			19	Fabris et al, 2005
Ibacus peronii,VIC	13	0.5	13	10	0.5	35	This study (W)
	38	4.6	7	31	2.6	18	This study (P)

* Approximate wet weight concentration calculated from authors' manuscript information. KUW, Kuwait; MEX, Mexico; SCO, Scotland; SWE, Sweden; USA, United States of America; WA, Western Australia, VIC, Victoria.

The concentrations of metals determined in this study are within in the range reported in the few recent studies on metals in the edible tissues of other lobster species, either in Australia or internationally (Table 2). Food Standards Australia and New Zealand (FSANZ) has the responsibility of developing food standards and other food regulatory measures in Australia (Food Standards Australia and New Zealand, 1998). In order to regulate metals in food, FSANZ has established maximum levels (ML) for several metals in crustaceans (inorganic-As, Cu, Hg, Zn), and molluscs (Cd, Pb). Mercury concentrations must have been less than 2 mg kg^{-1} (our method detection limit) in these animals, since Hg concentrations in the digests were <LOD (note: this still leaves the possibility that Hg concentrations are higher than the ML (0.03 mg kg^{-1})). Copper and Cd levels were similar to their MLs, and Zn was much lower than the ML. The concentration of As exceeded the ML by more than tenfold (Table 3). However, exceeding food standards does not necessarily mean that the food is unfit for human consumption, since the maximum levels are set conservatively and assume worst case scenarios, eg. a food item is a major part of the total diet, and, for As, that all the As is in the most toxic form, inorganic-As. Moreover, nearly all the As in marine organisms is found as arsenobetaine and other nontoxic organic forms of arsenic. These organoarsenic compounds are readily excreted by mammals, and are not considered toxic to human consumers of fisheries products (Neff, 1997). A more meaningful exercise is to compare measured concentrations with the provisional maximum tolerable intakes set by JECFA (Ministry of Agriculture, Fisheries and

Table 3. Estimated daily intake of metals on consumption of 200 g week^{-1} *I. peronii* , and comparison with food standards.

Element	Standards			This study	
	JECFA [a]	FSANZ RDI	FSANZ ML	Concentration [b]	Intake [a]
	(mg day^{-1})	(mg day^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg day^{-1})
As	0.12 [c]		2 [d]	25.5	0.7
Ca		800		592	17
Cd	0.06		2 [e]	2.6	0.1
Cr		0.2		4.8	0.1
Cu	30 [f]	3	10	9.8	0.3
Fe		12		20.5	0.59
Mg		320		574	16
Mn		5		1.6	<0.1
Pb	3.6		2 [e]	7.3	0.2
Zn	1000	12	150	30	1

JECFA, WHO/FAO Joint Expert Committee on Food Additives; FSANZ, Food Standards Australia and New Zealand; RDI, Recommended Daily Intake; ML, Maximum Level; a, calculated from a weekly intake of 200 g lobster; b, pooled data; c, calculated from JECFA provisional tolerable weekly intakes (PWTI); d, maximum level allowed in crustacea; e, maximum level allowed in molluscs; f, calculated from JECFA provisional maximum tolerable daily intake (PMTDI).

Food, 1998). The daily intake of these metals via consumption of *I. peronii* is below the JECFA maximum tolerable intakes for all elements but As and Cd, when using the standard assumption that a 200 g portion is consumed each week (~29 g day-1) by a 60 kg adult (Table 3). Consumption of seventeen Balmain Bugs per week on a regular basis would be required to exceed the intake limits for As and Cd, and risk toxic effects. This is considered unlikely for all but very heavy consumers of seafood. In short, no adverse health affects are anticipated from moderate consumption of these animals, although a more detailed ecological survey should be undertaken to identify contamination hotspots from which these crustaceans should not be taken by commercial or recreational fishermen.

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