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Heavy metal concentrations in wild and cultured Blacklip Abalone (*Haliotis rubra* Leach) from southern Australian waters

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Abstract

The concentrations of 12 trace metals were assessed in wild and cultured specimens of blacklip abalone, *Haliotis rubra,* from each of two sites, Geelong and Port Fairy, in Victoria, Australia. Cadmium, copper, iron and zinc were quantified in the foot muscle of specimens from all four populations but the concentrations of aluminium, arsenic, beryllium, chromium, lead, manganese, nickel and vanadium were below the detection limits of the instrumental techniques employed. When similar sized specimens from each population were compared, the concentrations of each of the quantifiable metals varied according to location. The Geelong wild population had the highest or equal highest concentrations of each metal. Metal concentrations in the wild populations were usually greater than or equal to the concentrations in the corresponding cultured population. The concentrations of the regulated essential elements, copper and zinc, decreased with an increase in abalone length whereas the concentrations of iron, manganese and cadmium were independent of length. Metal concentrations in *H. rubra* from all sites complied with the Australian Food Code and other standards of food safety.

Keywords: Abalone; Metals; Aquaculture; Food safety

1. Introduction

The concentrations of trace metals in marine gastropods have been determined in a variety of contexts, including food safety (e.g. MAFF, 1998; Fabris, 2000), bioindication of pollutants (e.g. Watling & Watling, 1983), and basal metabolic requirements (White & Rainbow, 1985). Within a given species, the concentrations of trace metals may depend on factors such as location and season and, within an individual organism the concentrations may vary from tissue to tissue and with age. Tissue concentrations of dietary concern have often been reported, typically in the viscera of larger organisms.

Haliotis rubra (Leach) is the third most valuable fishery species in Australia, generating an annual catch worth approximately $100 million (Edgar, 1997), of which most is exported. The Australian harvest of *H. rubra* represents about 40% of the total world catch of abalone, making *H. rubra* the most important of the world's commercial abalone species (McShane, 1999, chap. 8).

Since the 1960s, *H. rubra* fisheries in Australia have been regulated by a variety of measures, such as commercial and recreational licensing, minimum size limits, total allowable catches and seasonal and area closures. These measures were introduced to prolong and sustain the abalone resource in Australia after the collapse of abalone fisheries in North America and other areas. To meet the commercial demand for abalone, several abalone farms have been established, particularly along the southern coast of Australia, and these produce a growing proportion of the total abalone harvest.

Trace metal concentrations in the foot muscle (the commonly consumed portion) of wild *H. rubra* and other abalone species have previously been reported

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(Anderlini, 1974; Fabris 2000; Hyne, Smith & Ellender, 1992; Ikuta, 1985; Kamimura, 1980; Maher, 1986; Walker, 1982; Young, Moore, Jan, & Eganhouse, 1981). The concentrations present in the foot muscle are generally low but are commonly found to be site-specific. To the best of our knowledge the concentrations in cultured abalone have not previously been investigated in detail. As the diet of cultured abalone is generally quite different from that of wild abalone, and cultured abalone are exposed to more manufactured materials than wild abalone, there may be significant differences in trace metal concentrations in edible tissues. These concentrations should be assessed in order to determine the dietary suitability of cultured *H. rubra*.

In this study, we examined the concentrations of 12 trace metals in the foot muscle of both wild and cultured *H. rubra* from Victoria, Australia. Specimens were obtained from Port Fairy, on the coast of western Victoria, and from Geelong, on the coast of Corio Bay (Fig. 1). The Corio Bay site shows elevated levels of several metals, particularly cadmium, lead and zinc, in both water and sediments (Fabris, Monahan, & Batley, 1999). By contrast, the Port Fairy site is hundreds of kilometres from the nearest major city (Melbourne) and there is no known source of heavy metal contamination of this area. Both wild and cultured populations of *H. rubra* are available at each of these locations, the cultured population at each location growing in water sourced from the vicinity of the corresponding wild population. Comparison of wild and cultured populations can therefore be undertaken at each location. The concentrations measured are interpreted in terms of dietary suitability, and the underlying factors influencing trace metal concentrations in these populations of abalone are investigated.

Fig. 1. Locations of the abalone sources.

2. Materials and methods

2.1. Reagents

Deionised water with a resistivity of at least 18 MΩ cm^{-1} was prepared by passing singly distilled water through a Milli-Q water purification system. Concentrated nitric acid (both AnalaR and Aristar grades), ammonium dihydrogen phosphate (AnalaR grade) and Extran 300 detergent were obtained from BDH Chemicals. Cadmium and lead atomic spectroscopy standard solutions, containing the metal at 1.00 g/l, were obtained from AJAX Chemicals. A multielement atomic spectroscopy standard solution for ICP–AES was obtained from Perkin-Elmer (Solution N9300211).

All dissection equipment, glassware and plasticware were soaked for at least 24 h prior to use in a 5% solution of Extran 300, followed by three rinses with deionised water. Glassware and plasticware were further cleaned by soaking in a 1 M nitric acid solution (prepared from the AnalaR grade reagent) for at least 2 days, and then rinsing at least three times with deionised water.

2.2. Sample collection and processing

Specimens of *H. rubra* of a range of sizes were collected from each of the four populations. Wild specimens greater than 12 cm in length from Port Fairy and greater than 10 cm from Geelong are of considerable interest, as these are the size classes legally harvestable by amateur and professional fishermen. In order to investigate relationships between size and metal concentrations approximately, 20 oversize and 20 undersize wild specimens were collected from each site. Cultured specimens are harvested before reaching the legal size of wild caught animals, so approximately 20 cultured specimens were collected from each of the aquaculture sites.

Wild abalone were collected by hand, while cultured abalone were provided by Southern Ocean Mariculture Pty Ltd. of Port Fairy and Bay City Sea Farming Pty Ltd. of Geelong. In the field, the samples were placed on ice in individual polythene bags, and, on return to the laboratory, were frozen until further processing.

2.3. Sample preparation

After defrosting, the abalone were weighed and the maximum shell length measured. Abalone were dissected on a polythene board. A small block of muscle tissue was removed from the foot using one scalpel, and a second scalpel was used to trim all around this block to ensure that extraneous matter on the skin did not contaminate the sample.

Tissue samples were digested using the closed vessel technique described by the Association of Official

Analytical Chemists (1990). Approximately 0.8 g of wet tissue was accurately weighed into a 70 ml teflon-lined digestion bomb, and 5 ml of concentrated nitric acid (Aristar grade, BDH Chemicals) was added. The vessel was sealed, placed in an oven at 150 °C for 2 h, and allowed to cool in a fume hood. The digest was transferred to a 25 ml volumetric flask and diluted to volume. Reagent blanks and 0.3 g samples of Standard Reference Material (SRM) 1566a Oyster Tissue from the National Institute of Standards and Technology were also processed using this digestion technique.

2.4. Analysis

Digests were analysed for cadmium and lead using a Hitachi 7000 Polarised Zeeman atomic absorption spectrophotometer (graphite furnace method). The wavelengths monitored were 228.8 nm for Cd and 283.3 nm for Pb. The purified ammonium dihydrogen phosphate matrix modifier and modified temperature programme described by Van Loon (1985) were utilised. Aluminium, arsenic, beryllium, chromium, copper, iron, manganese, nickel, vanadium and zinc were determined using either a Perkin-Elmer Optima 3000 inductively coupled plasma atomic emission spectrometer or a Varian Liberty 200 inductively coupled plasma atomic emission spectrometer, depending on availability. The wavelengths monitored were 396.152 nm for Al, 193.697 nm for As, 234.862 nm for Be, 205.551 nm for Cr, 324.756 nm for Cu, 259.941 nm for Fe, 294.922 nm for Mn, 231.603 nm for Ni, 214.361 nm for V and 213.856 nm for Zn. Several digests were analysed using both ICP–AES instruments, and no significant difference was found between the results.

Table 1 summarises the performance of the analytical methods. Limits of quantification are based on mean + 10 standard deviations for the digested blanks. (APHA, 1995). Quantitative recoveries of Cd, Cu, Fe, Mn and Zn from the SRM were obtained. Other metals were either not certified in the SRM or were present at concentrations less than the detection limits of the techniques employed. The performance of the analytical methods for these metals was checked by digesting standard additions of set point standards (Trace Metals, Analytical Products Group). Quantitative recoveries were obtained for all metals.

Table 1
Summary of analytical parameters for metals under investigation

Element	Detection limit (μg/g)	Recovery ± standard deviation (%)
Al	1	111 ± 2[b]
As	1	101 ± 5[b]
Be	0.1	100 ± 3[b]
Cd	0.02	99 ± 5[a]
Cr	2	101 ± 3[b]
Cu	0.5	103 ± 2[a]
Fe	4	101 ± 3[a]
Mn	4	97 ± 2[a]
Ni	0.8	105 ± 3[b]
Pb	0.3	92 ± 5[b]
V	0.2	98 ± 3[b]
Zn	0.4	97 ± 5[a]

[a] From SRM 1566a Oyster Tissue.
[b] From digested APG Standard.

2.5. Statistical analysis

SPSS Version 10 was used to investigate differences between populations and to examine correlations between metal concentrations.

The techniques of multivariate analysis of variance (MANOVA) were not applicable for many of the populations to be compared, as the data regularly violated the requirements of univariate normality, multivariate normality or homogeneity of variance, as assessed using the Shapiro–Wilks test, Box's test, and Levene's test, respectively. Preliminary transformation of the data, using functions such as $\ln x$ or $\sqrt{x}$ was not able to satisfactorily correct the violations. Univariate analysis of variance (ANOVA), which has been found to be robust to violations of the assumption of normality, was therefore used to test for differences between populations. Post-hoc testing was performed using the Games–Howell test, which does not require homogeneity of variance.

Principal components analysis (PCA) was used to investigate correlations between the metal concentrations and to further examine the different populations. Varimax rotation was utilised to aid interpretation of the factors extracted.

3. Results and discussion

Cd, Cu, Fe and Zn were present at quantifiable concentrations in the foot muscle of all populations of *H. rubra* (Table 2). The concentrations of the other metals were below the limits of quantification described earlier. Mean concentrations in the cultured populations are

Table 2
Concentrations of metals found in different populations of *H. rubra* (± standard deviation)

pop[n]	*n*	Metal concentration (μg/g wet wt.)			
		Cd	Cu	Fe	Zn
Geelong wild (small)	24	0.11 ± 0.05	1.7 ± 0.8	31 ± 12	11 ± 2
Geelong wild (large)	20	0.07 ± 0.08	2.4 ± 1.5	27 ± 19	10 ± 2
Geelong cultured	20	0.04 ± 0.03	1.0 ± 0.5	13 ± 9	11 ± 2
Port Fairy wild (small)	25	0.16 ± 0.23	0.4 ± 0.3	10 ± 10	8 ± 2
Port Fairy wild (large)	19	0.10 ± 0.09	0.4 ± 0.4	8 ± 4	9 ± 3
Port Fairy cultured	21	0.04 ± 0.02	2.0 ± 0.8	7 ± 9	13 ± 4

similar to those in the wild populations and are similar to those in other wild populations of abalone. The concentrations of zinc in particular are very similar to other reported measurements, an observation which is consistent with this metal being strongly regulated. White and Rainbow (1985) have calculated the theoretical metabolic requirement for zinc in molluscs to be about 9 μg/g (wet wt. basis) which is very similar to all reported results for zinc. White and Rainbow (1985) also calculated the theoretical metabolic requirement for copper to be about 6.5 μg/g, which is greater than most reported measurements (as has also been found in other organisms). Low concentrations of copper in the environment may limit growth of abalone.

As is often found in studies of trace metals, there was considerable variability in the trace metal concentrations within each population. The factors that may affect trace metal concentrations within *H. rubra* include age, size, sex, short term diet and long-term diet. These factors cannot all be investigated using the data obtained in this study, but the populations can be compared, and relationships among the metal concentrations and variations with size can be investigated.

Within a particular population, several metals exhibit weak but highly significant bivariate correlations with length (Table 3). For this reason, initial comparisons between populations were made on the basis of abalone of a restricted size class (<10 cm in length) in which suitable numbers of specimens were available from each population.

Using one-way analysis of variance (ANOVA), significant differences between populations were detected for each metal. Table 4 shows the order of the populations for each metal, as detected using post-hoc testing. The Geelong-wild population had the highest or equal highest concentrations of each metal. Usually the metal concentrations in the wild populations were greater than or equal to the concentrations in the corresponding cultured population. As the wild and cultured populations at each site shared a common water source, it is proposed that the observed differences between wild and cultured animals is due to differences in substrate or food.

PCA (using concentrations of metals and length as variables) produces two factors with initial Eigen values greater than 1. Table 5 shows the factor loadings for the rotated solution. Zn, Cu and length load heavily on the first factor, while Cd and Fe load heavily on the second factor. A factor scores plot (Fig. 2) suggests significant separation between habitat type (i.e. wild vs. cultured), particularly on the basis of factor 2, but considerable overlap between sites (i.e. Geelong vs. Port Fairy). Factor 1 appears to represent essential elements whose concentrations are regulated at a level that decreases with length. Factor 2 represents those metals whose concentrations are either unregulated or regulated at a level that does not depend on length.

In terms of food safety, it is the concentrations in the edible size classes that are of particular interest. ANOVA and post-hoc testing were therefore conducted, using the data obtained for the edible size classes from each site. A more disordered ranking of concentrations is now obtained (Table 4), due to the comparisons of different size classes across the various populations, and it is now difficult to discern general patterns. It cannot be concluded that animals from one particular source were significantly 'cleaner' in all respects.

Food safety authorities around the world have set standards for the presence of metals in food. The Australian

Table 3
Significant correlations between metal concentrations and length of *H. rubra*

Site	Metal	Correlation coefficient	Significance
Geelong cultured ($n=20$)	Cu	−0.713	<0.001
	Fe	−0.874	<0.001
	Zn	−0.692	0.001
Port Fairy wild ($n=44$)	Cd	−0.620	<0.001
	Fe	−0.640	<0.001
	Zn	−0.378	0.011
Port Fairy cultured ($n=21$)	Zn	−0.438	0.047

Table 4
Ranking of populations by metal concentration for *H. rubra*

Metal	Concentration order[a] (highest to lowest)	
	Animals <10 cm in length	Harvestable animals[b]
Cd	GW = PW > GC = PC	PW > GW = GC = PC
Cu	GW = PC > GC > PW	GW = PC > GC > PW
Fe	GW > GC = PW = PC	GW > GC > PC = PW
Zn	GW = GC = PW = PC	GC = PC > GW = PW

[a] GW = Geelong wild, PW = Port Fairy wild, GC = Geelong cultured, PC = Port Fairy cultured.
[b] i.e. excluding undersize wild specimens.

Table 5
Factor loadings for PCA analysis of metal concentrations and length in *H. rubra*

Variable	Component 1	Component 2
Cd	−0.109	0.851
Cu	0.708	0.025
Fe	0.192	0.815
Zn	0.886	0.050
Length	−0.644	−0.026

Table 6
Metal intake from consumption of 200 g of *H. rubra* per week, and comparison with the PMTI and RNI

	Metal intake (mg/day)						
	Al	As	Cd	Cu	Fe	Pb	Zn
H. rubra	<0.03	<0.03	0.005	0.07	0.9	<0.009	0.4
PMTI	60	0.13	0.06	30	48	0.21	120
RNI	–	–	–	1.2	~10[a]	–	~10[a]

[a] Exact value depends on sex and/or age.

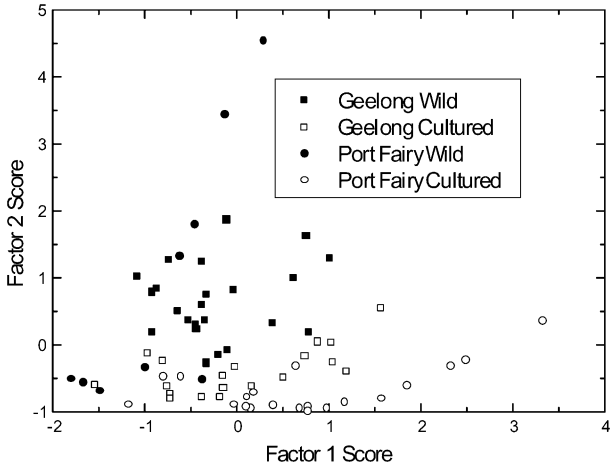

Fig. 2. Factor Scores plot for abalone <10 cm in length.

Food Safety Code specifies that, for this type of food, the concentrations of Cd and Pb should be less than 2 μg/g wet weight, and the concentration of As should be less than 1 μg/g (Australia New Zealand Food Authority, 1998). *H. rubra,* from each of the populations investigated here, comply with these limits. However, this type of food safety criterion does not consider the quantity of the food, and therefore the total amount of metal, consumed. The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organisation of the United Nations and the World Health Organisation has set a provisional maximum tolerable intake (PMTI) for certain metals. Table 6 compares the PMTI with the intake of metal from *H. rubra* if it is assumed that a 60 kg adult consumes one 200 g portion per week (29 g/day). To provide a conservative comparison, the *H. rubra* population having the highest concentration of metal was used in the calculation for each metal. The concentrations of all metals for which a comparison can be made are at least a factor of 10 below the PMTI (Table 6). On this basis, the abalone from each of the populations is considered an acceptable food item, although other items in the overall diet that contribute metal would also need to be considered.

Despite being toxic when ingested at excessive concentrations, copper, iron and zinc are also essential nutrients which must be present in the diet at some minimum level for the maintenance of human health. The Committee on Medical Aspects of Food Policy (COMA) has recommended reference nutrient intakes (RNIs) for essential elements (Department of Health, 1991). The quantities of copper, iron and zinc in the *H. rubra* populations examined are well below the respective RNIs (Table 6) when this food is consumed at the rate described above, indicating that this food is unlikely to be a significant source of these essential elements.

4. Conclusion

The concentrations of trace metals in the foot muscle of wild *H. rubra* were found to be low, but for some metals were site-dependent. Importantly, trace metal concentrations in cultured *H. rubra* were found to be generally lower than the corresponding wild population, which is attributed to the presumably lower concentrations of metals in the substrate or food. Concentrations of copper and zinc were generally found to be regulated according to size, but cadmium and iron concentrations were not found to depend on size. The foot muscle of *H. rubra* in each of the populations examined was an acceptable food item with respect to the concentrations of aluminium, arsenic, cadmium, copper, iron, lead and zinc.

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