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Trace Metal Concentrations in Wild and Cultured Australian Short-Finned Eel (*Anguilla australis* Richardson)

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Eel is a very popular food in Europe and Asia, particularly in Japan. Fisheries Victoria (2003) reported that about 125-450 tonnes of eel, worth A$1.4-4.7 million per annum, are produced by the Australian eel fishery, with the Australian short-finned eel (*Anguilla australis* Richardson) accounting for 95% of total production. Although commercial sized, truly wild eels are harvested from rivers and lakes, the eel aquaculture industry generally relies on the capture of elver and sub-adult eels from natural water bodies, as their life cycle has not been fully closed (De Silva et al, 2001). Elvers and sub-adult eel are kept in either recirculation aquaculture systems (RAS) or earthen pond systems, where they are grown to commercial size and harvested. Earthen pond culture can be extensive or semi-intensive. Extensive culture refers to systems that use naturally occurring foods with no additional feed supplied, eg eels are translocated to natural, closed water systems such as lakes, swamps, where they are left to grow naturally. Semi-intensive culture systems use artificial water bodies, eg dams, in which the eels utilise naturally occurring food in conjunction with supplementary low protein feed. Earthen pond systems have low stocking densities and are low maintenance in comparison to RAS culture.

A number of studies have been conducted on trace metal concentrations in wild populations of eel, particularly in Europe on *Anguilla anguilla* (Bordajandi et al., 2003; Usero et al., 2003). However, to date, only Hg has been investigated in *A. australis* (Fabris and Theodoropoulos, 1999), and then only on the wild eel fishery, not cultured eel. In this 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 cultured and wild *A. australis* obtained from suppliers in rural Victoria, Queensland and Tasmania, Australia. Herein, we report the results of our survey, comparing and contrasting metal concentrations in wild, earthen-pond cultured and RAS-cultured eels, 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.

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MATERIALS AND METHODS

Eels were obtained from 7 aquaculture facilities around Australia, namely, from three RAS facilities growing out elvers to commercial size (Deakin University, Euroa, Tasmania), two RAS facilities growing out sub-adults (Warrnambool Trout Farm, Euroa restock), and one earthen pond facilities growing out sub-adults (Queensland). Eels were also obtained from two rivers (Curdies River, Merri River), and five lakes or dams (Lake Woolongoom, King Island, Skipton Private Dams 1 and 2, Trevallyn Dam). The number of sites available for testing in this study was limited, as both eel culture and the wild fishery have been affected by drought in recent years (Fisheries Victoria Management, 2003). In total, 128 eels were purchased, with 10 eels (replicates) obtained from all but one location (King Island, 8). Eels were typically at the optimum commercial weight of 700-800 g, with some exceptions.

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. From each eel, four sections approximately 2 cm thick were randomly excised from the length of the body, and accurately weighed (OHAUS analytical scale model GT410). The muscle sections were placed in an individual, labelled polythene bag and frozen prior to further preparation and analysis.

The eel muscle was digested using the EPA Method 200.3 for determination of metals and inorganic chemicals in environmental samples (Environmental Protection Agency, 1996), with minor modification. The EPA method recommends 5 g (wet weight) of sample, but in this study the sample weight was halved, because of the small size of digestion tubes. All reagent volumes used were adjusted accordingly. In short, the samples (2.5 g muscle blended from the four sections excised from each eel) 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; DORM 1, DORM 2, TORT 2, 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 ICAP (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 20 were certified by the NRCC in the three CRMs used in this study, namely Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, V, Zn. Silver was not measured in this study. Although measured, the digestion method used is not appropriate for Se. CRM digest concentrations of Co, Hg, Mo and Pb were all <LOD. Hence, these elements were not included in statistical analysis. The concentrations of B, Ba, Be, Ca, La, Sc, Ti, Y were not certified, and these elements too were removed from statistical analysis and subsequent discussion. For statistical comparison, the data did not comply with prerequisites of homogeneity of variance and normality of data required for parametric tests, so the non parametric Kruskal-Wallis H test was applied to the residue data using SPSS version 12 (SPSS Inc, Chicago) to test for differences in analyte concentrations between sites, with the Mann-Whitney U test used for post-hoc assessment. To assess the similarity in metal residue concentrations between sites, Principal Components Analysis (PCA) was used..

RESULTS AND DISCUSSION

For the most part, CRM metal concentrations were found to be within 25% of expected values (data not shown). However, the recoveries of Cr and Ni were outside this range, and these elements were removed from subsequent data

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

Because eel spend much of their time in contact with sediment (Bordajandi et al 2003), are long lived, and stay in the same areas for a long time, sometimes for up to 20 years, they may accumulate elevated concentrations of metals. Cultured organisms, however, are exposed to different environments and foods than wild species, to a greater or lessor extent. For instance, captured elvers are weaned from oocytes and different types of fish roe onto pelleted feed (De Silva et al, 2001), and sub-adult eels have spent spend a larger portion of their life in the wild than elvers prior to capture, and consumed a wider array of foods for longer, prior to weaning onto pelleted food. These factors may influence metal concentrations in elver and sub-adult eel cultured to commercial size, relative to truly wild eels. Although some caution should be taken when assessing statistical variability in the biophysical or chemical data obtained in this study, since the samples were not collected from the field to a rigorous ecological protocol, but purchased from commercial suppliers, PCA did not show clustering by site. In short, there was no difference in overall metal concentrations between wild and cultured eels. However, Na and Zn concentrations were statistically significantly higher in cultured compared to restock, and in restock compared to wild ($p<0.05$); K and P concentrations were statistically significantly higher in wild compared to restock, and in restock compared to cultured ($p<0.05$); Mg concentrations were statistically significantly higher in restock compared to cultured, and in wild compared to cultured ($p<0.05$) and Pb concentrations were statistically significantly higher in wild compared to cultured eels ($p<0.05$). No other statistically significant differences were observed. Metal concentrations did not depend on size (length and weight), eliminating these variables as being the underlying cause of the statistically significant differences observed.

The concentrations of metals determined in *Anguilla australia* in this study (Table 1) are, for the most part, lower than those reported in recent studies on metals in other eel species. For instance, Bordajandi et al (2003) reported As, Cd, Cu, Pb and Zn concentrations in of 0.228, 0.005, 0.977, 0.102, and 16.95 mg kg^{-1} (wet weight), respectively, in European eel (*Anguilla anguilla*) in Spain. Similar levels were reported by Perez Cid et al (2001) in Portugal, Usero et al (2003) in Spain, and Farkas et al (2000) in Hungary, although in the latter case much higher levels of Zn were reported (97-104 mg kg^{-1}).

Food Standards Australia and New Zealand (FSANZ) has the responsibility of regulate contaminants, including metals, in food. FSANZ has established maximum levels (ML) for several metals in fish (inorganic-As, Cu, Hg, and Pb; Table 2). Generally expected levels (GELs) have been established to complement MLs, and although not legally enforceable, they do provide a benchmark against which to measure contaminant levels in organisms (FSANZ, 2005).

Table 1. Summary of metal concentrations (mg kg^{-1} wet weight) in *Anguilla australis*, from south-eastern Australia, 2004.

Source	System	Length (cm)	Weight (g)	As	Ca	Cu	Fe	K
Deakin	C, RAS, E	56.8 (4)	421 (17)	0.7 (151)	192 (36)	0.2 (112)	6.5 (45)	2810 (38)
Euroa (elvers)	C, RAS, E	61.1 (8)	790 (14)	0.4 (216)	144 (79)	0.2 (60)	4.6 (24)	2450 (13)
Tasmania	C, RAS, E	67.1 (6)	742 (4)	<LOD	118 (30)	0.3 (48)	5.4 (32)	2710 (9)
Warrnambool Trout Farm	C, RAS, S	63.4 (9)	788 (19)	<LOD	103 (45)	0.3 (42)	6.2 (17)	2640 (5)
Euroa (restock)	C, RAS, S	71.9 (7)	816 (23)	<LOD	259 (39)	0.1 (300)	3.3 (22)	3540 (22)
Queensland	C, Et, S	78.6 (7)	1023 (14)	<LOD	125 (50)	0.1 (106)	5.0 (29)	2660 (6)
Warrnambool Trout Farm	C. Et, S	63.4 (9)	788 (19)	<LOD	103 (45)	0.3 (42)	6.2 (17)	2640 (5)
Curdies	W, R	65.5 (5)	593 (10)	<LOD	187 (46)	<LOD	3.5 (22)	3580 (7)
Merri River	W, R	75.0 (9)	778 (16)	<LOD	248 (68)	0.2 (117)	5.3 (35)	2760 (8)
Lake Woolongoom	W, R	71.4 (8)	800 (15)	<LOD	163 (39)	0.1 (213)	5.4 (53)	3090 (5)
King Island	W, L	74.7 (3)	1068 (8)	<LOD	160 (53)	0.2 (82)	6.2 (45)	2660 (60)
Skipton Private Dam #2	W, L	67.6 (3)	719 (3)	<LOD	129 (32)	0.0 (211)	3.9 (30)	3070 (7)
Skipton Private Dam #1	W, L	72.5 (5)	770 (13)	0.4 (211)	163 (37)	0.1 (131)	6.7 (35)	3100 (13)
Trevallyn Dam	W, L	70.1 (3)	733 (6)	<LOD	147 (62)	0.2 (43)	4.8 (26)	2790 (6)

Source	Mg	Mn	Na	P	Pb	Sr	Zn
Deakin	189 (32)	0.2 (62)	490 (31)	2410 (33)	0.7 (198)	0.2 (79)	18.4 (37)
Euroa (elvers)	182 (11)	0.1 (165)	529 (15)	2080 (8)	2.1 (46)	0.1 (236)	18.4 (12)
Tasmania	177 (7)	0.2 (95)	424 (9)	2180 (6)	<LOD	0.0 (300)	15.8 (12)
Warrnambool Trout Farm	189 (5)	0.2 (81)	465 (7)	2240 (4)	<LOD	0.1 (218)	18.2 (8)
Euroa (restock)	243 (18)	0.2 (101)	555 (41)	2540 (34)	<LOD	0.8 (69)	14.7 (43)
Queensland	200 (8)	0.1 (248)	448 (15)	2380 (9)	<LOD	0.1 (136)	16.0 (16)
Curdies	201 (7)	0.2 (82)	367 (8)	2360 (5)	<LOD	0.1 (128)	10.7 (13)
Merri River	169 (7)	0.3 (113)	392 (15)	2450 (4)	<LOD	0.6 (80)	14.4 (11)
Lake Woolongoom	199 (7)	0.2 (75)	494 (11)	2320 (5)	<LOD	0.2 (91)	14.1 (14)
King Island	185 (45)	0.3 (54)	469 (52)	2260 (50)	<LOD	0.6 (70)	15.5 (53)
Skipton Private Dam #2	203 (6)	0.2 (109)	367 (28)	2210 (4)	0.3 (316)	0.1 (163)	13.9 (15)
Skipton Private Dam #1	208 (14)	0.1 (130)	451 (19)	2450 (14)	1.2 (129)	0.3 (64)	15.6 (21)
Trevallyn Dam	169 (5)	0.2 (79)	483 (9)	2300 (4)	2.1 (75)	0.1 (198)	13.4 (17)

Figures in parenthesis represent coefficient of variation in data (%); C, culture; RAS, recirculating aquaculture system, E, elvers; S, sub-adults; Et, earthen, W, wild,; R, river; L, lake.

Table 2. Estimated daily intake of metals on consumption of 200 g week^{-1} *A. australis* , and comparison with food standards.

Element	Standards			This study	
	JECFA [a]	FSANZ RDI	FSANZ ML [GEL]	Concentration [b]	Intake [a]
	(mg day^{-1})	(mg day^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg day^{-1})
As	0.12 [c]		2	0.12	0.003
Ca		800		164	4.7
Cu	30 [d]	3	[5]	0.15	0.004
Fe		12		5.1	0.15
Mg		320		193	5.5
Mn		5		0.18	0.005
Pb	3.6		0.5	0.5	0.014
Zn	1000	12	[130]	15	0.4

JECFA, WHO/FAO Joint Expert Committee on Food Additives; FSANZ, Food Standards Australia and New Zealand; RDI, Recommended Daily Intake; ML [GEL], Maximum Level [Generally Expected Level] in fish; a, calculated from a weekly intake of 200 g eel; b, pooled data; c, calculated from JECFA provisional tolerable weekly intakes (PWTI); calculated from JECFA provisional maximum tolerable daily intake (PMTDI).

Arsenic concentrations in *A. australis* were well below the ML (Table 2). Lead concentrations were similar to the ML. Mercury concentrations must have been less than 2 mg kg^{-1} (our method detection limits) in these animals, since the concentrations of these elements in the digests were <LOD. (Note: this still leaves the possibility that Hg concentrations are higher than both the ML and GEL (0.5 mg kg^{-1}). Copper and Zn concentrations in *A. australis* were well below their respective GELs. The daily intake of metals via consumption of *A. australis* is also below the JECFA provisional maximum tolerable intakes (Ministry of Agriculture, Fisheries and Food, 1998), when using the standard assumption that a 200 g portion is consumed each week (~29 g day^{-1}) by a 60 kg adult (Table 2). Consumption of at least 1.5 kilograms of eel per week on a regular basis would be required to exceed the intake limits for Pb, at least 8 kilograms per week to exceed intake limits for As, and substantially greater quantities to approach the limits for Cu or Zn. This is considered unlikely for all but very heavy consumers of eel. In short, Australian short-finned eel, whether of wild or cultured origin, appear uncontaminated by the metals examined in this study, and no adverse health affects are anticipated from moderate consumption of these fish, whatever the source.

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