



Levels of arsenic, cadmium, lead and mercury in the branchial plate and muscle tissue of mobulid rays



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ARTICLE INFO

Article history:

Available online 16 March 2015

Keywords:

Mobula japonica

Manta alfredi

As

Cd

Pb

Hg

ABSTRACT

Mobulid rays are targeted in fisheries for their branchial plates, for use in Chinese medicine. Branchial plate and muscle tissue from *Mobula japonica* were collected from fish markets in Sri Lanka, and muscle tissue biopsies from *Manta alfredi* in Australia. These were analysed for arsenic, cadmium, lead and mercury and compared to maximum levels (MLs) set by Food Standards Australia and New Zealand (FSANZ), European Commission (EC) and Codex Alimentarius Commission. The estimated intake for a vulnerable human age group was compared to minimal risk levels set by the Agency for Toxic Substances and Disease Registry. The mean inorganic arsenic concentration in *M. japonica* muscle was equivalent to the FSANZ ML while cadmium exceeded the EC ML. The mean concentration of lead in *M. alfredi* muscle tissue exceeded EC and Codex MLs. There were significant positive linear correlations between branchial plate and muscle tissue concentrations for arsenic, cadmium and lead.

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1. Introduction

Marine organisms, differentially exposed to metals, will accumulate the metals in their tissues to varying degrees as a result of differences in exposure routes and effects related to environmental chemistry (Adams et al., 2011). The primary routes of uptake by fish are via the gills and gut (McGeer et al., 2011; McIntyre and Linton, 2011). In response to public concern over exposure to metals through consumption of seafood, which caused incidents such as the outbreak of Minamata disease in Japan (Harada, 1995), international agencies (FAO/WHO and EU) have established limits for metals in various types of seafood (CODEX, 2012; Commission of the European Communities, 2006).

Mobulid rays (Family Myliobatidae) belong to two distinctive genera, *Manta* and *Mobula*, consisting of eleven planktivorous filter-feeding elasmobranchs that inhabit tropical, subtropical and

temperate seas worldwide (Eschmeyer and Fong, 2014; Couturier et al., 2012). These mobulid species are under threat from anthropogenic activities such as targeted fishing, fisheries bycatch, boat strikes and marine litter (Ibid). Targeted fishing is a major threat that occurs in many regions around the world including The Democratic Socialist Republic of Sri Lanka (RSL) and the Republic of Indonesia (Fernando and Stevens, 2011; Dewar, 2002). In Lamakera, Indonesia, the number of mobulid rays caught increased from historical levels of 200–300 individuals per season to ~1500 in 2002 (Ibid). This increase is attributed to the growing demand from the Chinese medicine market for dried mobulid branchial plates (Fernando and Stevens, 2011). At present, all mobulid species are classed as either Data Deficient, Near Threatened, Vulnerable or Endangered on the IUCN Red List of Threatened Species (International Union for Conservation of Nature, 2014), which makes the substantial market for branchial plates a concern for the long-term survival of the species.

Sharks, rays, and skates are capable of accumulating non-essential elements such as mercury (Hg) and lead (Pb) in their tissues

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(Lopez et al., 2013). While several studies have examined metal accumulation in some elasmobranchs (De Boeck et al., 2010; Marcovecchio et al., 1991; Storelli and Marcotrigiano, 2004), only two have reported metal concentrations from the genus *Manta* (Essumang, 2009, 2010). These two investigations reported elevated arsenic (As), cadmium (Cd), mercury (Hg) and platinum (Pt) concentrations in edible tissues and concluded that they pose a potential risk for people who consume *Manta birostris* meat on a daily basis. Unfortunately, analyses of the highly sought after branchial plates were not included in their assessment.

According to Jarup (2003), As, Cd, Pb and Hg are the main non-essential elements that contribute to human health risks via the consumption of food. Pb, Cd and Hg are not required by the human body and toxic effects have been recorded at extremely low concentrations (Goyer, 1995).

The aim of the present study was to determine the concentrations of As, Cd, Pb and Hg in the branchial plate and muscle tissue of *Manta alfredi* and *Mobula japanica* and identify whether they may be of potential concern to public health when consumed, based on established MLs. In addition, we investigated the potential for predicting branchial plate concentrations from biopsied muscle tissue, which are less invasive and simpler to obtain from wild animals than branchial plate samples.

2. Materials and methods

2.1. Sample collection

Tissue samples from dead specimens of *M. japanica* were collected at Republic of Sri Lanka (RSL) markets located in Negombo (7°12'N 79°50'E) and Mirissa (5°56'N 80°28'E). These specimens were caught 100–500 nautical miles offshore. The specimens may have been dead for ~1 week but carcasses were evaluated to be in good condition (condition code D2; adapted from Haines et al., 1999), with no significant decomposition of tissue as they were stored on ice. Disc width (wing-tip to wing-tip), disc length (from anterior margin of head, excluding cephalic lobes, to posterior margin of pectoral fin), gender and maturity of specimens are presented in Table 1. Maturity of male rays was determined by the size and extent of calcification of claspers (White et al., 2006). Evaluation of the maturity of female rays requires an assessment of internal organs (Ibid), which was not possible for these specimens.

A total of 15 muscle and 14 branchial plate tissue samples were collected from 15 *M. japanica*, following the method from Fernando (2012). Each animal was identified using the codes RSL 1–RSL 15. Specimens were cut in half along the midline and 20–30 g of muscle tissue taken from the region just posterior to the gills on the ventral side of the body. Paired branchial plate samples were taken from the ventral side antero-dorsal to the muscle sample site.

In addition, 12 muscle samples (0.02–0.3 g) were collected from *M. alfredi* by biopsy of free-ranging animals in February and June 2013 at Lady Elliot Island (LEI) reef, Australia, (24°07'S 152°42'E) following the method from Couturier et al. (2013). The corresponding samples were identified as LEI 1–LEI 12 and the data are presented in Table 1. *Manta* ray size and maturity could not be reliably assessed in these free-ranging animals.

2.2. Metal analysis

Upon collection at the fish markets, the 29 RSL samples were dehydrated within 2 h of collection, or frozen at –20 °C prior to dehydration. Dehydration was performed with a conventional kitchen food dehydrator for 5–10 h (depending on the size of the sample and the relative humidity) at 60 °C. Prior to digestion and

analysis, the 29 dehydrated *M. japanica* samples were freeze-dried (Christ, Alpha 2–4D) for 18 h to ensure uniform dehydration.

After collection at LEI, the fresh muscle samples were stored individually at –18 °C before being transported on ice off the island. These samples were not freeze-dried as their individual masses were too low. Tissue metal analyses were carried out at the Queensland Department of Health, Forensic and Scientific Services, following methods modified from Tinggi et al. (2004) as described below.

4 mL of 67–69% w/w HNO₃ (Australian Chemical Reagents, Australia) was added to ~0.1 mg of each sample. These were allowed to stand in a fume cupboard overnight at room temperature for an initial digestion phase, after which they were microwave digested (CEM MarsXpress, Mathews, NC) using a three-stage program (85 °C at 400 W for 14 min, followed by 110 °C at 800 W for 20 min and 160 °C at 1600 W for 10 min). The cooled solutions were washed into separate polypropylene tubes with Milli-Q water and made up to 20 mL.

Inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7700 Series was used to determine total concentrations of As, Cd, Pb and Hg in the sample digest. The instrument was calibrated using a serially diluted multi-element solution; from 0.1 to 200 µg/L for As, Pb, and Cd, and from 0.1 to 10 µg/L for Hg (High Purity Standards, HPS-Q19283). An internal standard solution, composed of iridium, scandium and rhodium, was added to each sample via the CETAC ASX-520 AutoSampler online injection system. To minimize polyatomic interferences, a collision cell using helium gas was used during data acquisition for each target element.

Because RSL samples were dried in the field for their preservation, wet mass was not obtained. The average water content of muscle and branchial plate tissue (70% and 80% respectively) was used for conversion of dry weight concentrations to wet weight concentrations. These averages were based on the muscle mass before and after dehydration of further RSL samples, and are consistent with reported tissue water content from other studies (Food Standards Australia and New Zealand (FSANZ), 2013a; The University of Western Australia (UWA), 2009).

2.3. Quality assurance/quality control

Four method blanks (one per ten samples) consisting of 16 mL of Milli-Q water were prepared identically to the samples. Four individual sets of certified reference material (CRM: dogfish liver, NSERC, Canada) were analysed with each batch of samples to monitor for instrument accuracy and method extraction efficiency. Average recoveries for Cd and Hg in the CRM were 112% and 93% respectively. The average recovery for As was 114%. All As results were re-sloped down by 10% because results from both the digested CRM and two independent QC solutions made from single element stocks indicated that the slope of the calibration curve resulted in a 10% overestimation of As concentrations. Initial average recovery of Pb was 67% but improved to 93% after sample dilution (factor 2.16 for the samples and 2.42 for DORM) and re-injection. The limit of detection (LOD) for each element was defined as three times the standard deviation of the average result for four blank replicates.

2.4. Statistical analysis

To test for significant differences in the suite of metal concentrations simultaneously in muscle tissue between location/species (*M. japanica* from RSL and *M. alfredi* from LEI), we conducted a MANOVA using a Pillai test. Only if there was a significant difference in metal concentration between locations/species in the MANOVA, did we then perform individual ANOVAs for each metal. This two-step

Table 1Tissue concentrations of total As, Cd, Pb and Hg (mg/kg wet weight) in *Mobula japonica* and *Manta alfredi* tissue.

Mobulid ray ID	Location	Species	Gender	Maturity (Yes/No)	Disc width (cm)	Body length (cm)	Branchial plate metal concentrations				Muscle tissue metal concentrations				Limit of detection (all metals)
							As	Cd	Pb	Hg	As	Cd	Pb	Hg	
RSL 1	RSL(Negombo)	<i>Mobula japonica</i>	M	Y	231	105	10	0.074	0.074	0.054	15	0.22	0.23	0.27	0.004 mg/kg dry weight
RSL 2	RSL(Negombo)	<i>Mobula japonica</i>	M	Y	222	105	9.8	0.056	0.046	0.013	12	0.054	0.057	0.25	0.004 mg/kg dry weight
RSL 3	RSL(Negombo)	<i>Mobula japonica</i>	M	Y	224	102	6.4	0.038	0.062	0.11	10	0.075	0.27	0.26	0.004 mg/kg dry weight
RSL 4	RSL(Negombo)	<i>Mobula japonica</i>	M	Y	209	101	6.2	0.026	0.026	0.018	20	0.036	0.14	0.15	0.004 mg/kg dry weight
RSL 5	RSL(Mirissa)	<i>Mobula japonica</i>	M	Y	218	99	3.4	0.026	0.052	0.011	15	0.042	0.090	0.18	0.004 mg/kg dry weight
RSL 6	RSL(Mirissa)	<i>Mobula japonica</i>	M	Y	196	96	2.8	0.042	0.050	0.072	16	0.060	0.054	0.19	0.004 mg/kg dry weight
RSL 7	RSL(Mirissa)	<i>Mobula japonica</i>	F	Unknown	238	121	7.4	0.052	0.020	0.14	16	0.18	0.11	0.42	0.004 mg/kg dry weight
RSL 8	RSL(Mirissa)	<i>Mobula japonica</i>	F	Unknown	222	115	6.8	0.048	0.16	0.076	15	0.096	0.42	0.22	0.004 mg/kg dry weight
RSL 9	RSL(Mirissa)	<i>Mobula japonica</i>	M	Y	230	105	11	0.14	0.080	0.032	39	0.033	0.026	0.15	0.004 mg/kg dry weight
RSL 10	RSL(Mirissa)	<i>Mobula japonica</i>	F	Unknown	230	108	–	–	–	–	11	0.18	0.13	0.16	0.004 mg/kg dry weight
RSL 11	RSL(Mirissa)	<i>Mobula japonica</i>	F	Unknown	222	113	4.4	0.11	0.066	0.024	15	0.12	0.030	0.19	0.004 mg/kg dry weight
RSL 12	RSL(Mirissa)	<i>Mobula japonica</i>	M	Y	206	106	14	0.026	0.11	0.020	66	0.057	0.45	0.24	0.004 mg/kg dry weight
RSL 13	RSL(Mirissa)	<i>Mobula japonica</i>	M	N	202	93	4.0	0.034	0.14	0.0074	15	0.039	0.30	0.051	0.004 mg/kg dry weight
RSL 14	RSL(Mirissa)	<i>Mobula japonica</i>	F	Unknown	218	101	2.8	0.13	0.10	0.010	13	0.072	0.24	0.11	0.004 mg/kg dry weight
RSL 15	RSL(Negombo)	<i>Mobula japonica</i>	M	N	160	70	13	0.013	0.16	0.010	24	0.0081	0.075	0.039	0.004 mg/kg dry weight
LEI 1	LEI	<i>Manta alfredi</i>	F	Unknown							0.16	0.020	0.16	<0.004	0.004 mg/kg wet weight
LEI 2	LEI	<i>Manta alfredi</i>	F	Unknown							0.14	0.027	1.1	<0.004	0.004 mg/kg wet weight
LEI 3	LEI	<i>Manta alfredi</i>	F	Unknown							0.32	0.019	0.36	<0.004	0.004 mg/kg wet weight
LEI 4	LEI	<i>Manta alfredi</i>	F	Unknown							1.1	0.0080	0.42	0.004	0.004 mg/kg wet weight
LEI 5	LEI	<i>Manta alfredi</i>	M	Unknown							0.081	0.071	0.22	0.045	0.004 mg/kg wet weight
LEI 6	LEI	<i>Manta alfredi</i>	F	Unknown							1.7	0.12	0.58	0.010	0.004 mg/kg wet weight
LEI 7	LEI	<i>Manta alfredi</i>	M	Unknown							0.55	0.030	0.37	0.0080	0.004 mg/kg wet weight
LEI 8	LEI	<i>Manta alfredi</i>	F	Unknown							0.14	0.019	0.68	0.0070	0.004 mg/kg wet weight
LEI 9	LEI	<i>Manta alfredi</i>	M	Unknown							0.12	0.010	0.41	<0.004	0.004 mg/kg wet weight
LEI 10	LEI	<i>Manta alfredi</i>	F	Unknown							0.47	0.051	0.37	0.010	0.004 mg/kg wet weight
LEI 11	LEI	<i>Manta alfredi</i>	F	Unknown							0.19	0.011	0.20	<0.004	0.004 mg/kg wet weight
LEI 12	LEI	<i>Manta alfredi</i>	F	Unknown							1.4	0.030	0.24	0.010	0.004 mg/kg wet weight
Mean ± SD	RSL	<i>Mobula japonica</i>			215 ± 19	103 ± 12	7.2 ± 3.5	0.058 ± 0.039	0.082 ± 0.044	0.042 ± 0.040	20 ± 15	0.084 ± 0.062	0.18 ± 0.14	0.19 ± 0.094	
Mean ± SD	LEI	<i>Manta alfredi</i>									0.53 ± 0.56	0.035 ± 0.032	0.43 ± 0.26	0.0091 ± 0.012	

< Denotes that Hg concentrations was below the limit of detection and a proxy value of 0.003 was used to calculate the mean tissue concentration.

The average water content of muscle and branchial plate tissue (70% and 80% respectively) (FSANZ, 2013a; UWA, 2009) was used for conversion of dry weight concentrations to wet weight concentrations.

All but one of the branchial plate and muscle tissue samples were paired (i.e. from the same animal).

Disc width and body length were rounded off to the nearest cm.

Values for metal concentrations were corrected to 2 s.f. as analysis methods were only accurate to 2 s.f.

procedure protected against automatically conducting multiple statistical tests and compounding the experiment-wise type I error rate in our analyses. To test for significant differences in metal concentrations between paired branchial plate and muscle tissue, we performed a paired MANOVA by differencing the concentrations of each metal in *M. japonica* samples, and tested if the intercept was different from zero using a Pillai test. If the difference was significant, we then performed individual paired ANOVAs on each metal. The relationship between muscle and branchial plate tissue was further represented through linear regression.

Model residuals were visually assessed for normality and homogeneity of variance and an untransformed response with a normal error structure was found to be appropriate. All data were analysed in R version 3.0.2.

2.5. Comparison to maximum levels in food standards and minimal risk levels (MRLs)

Average metal concentrations for each tissue type were compared with the maximum allowable levels (MLs) recommended for fish consumption per the FSANZ, European Commission (EC) and the Codex Alimentarius Commission (WHO/FAO). (CODEX, 2012; Commission of the European Communities, 2006; FSANZ, 2013). These comparisons were made to provide a frame of reference for the reported metal concentrations from these tissues.

Given the use of mobilised ray branchial plates in Chinese medicine as a cure for chicken pox, the highest non-outlier concentration of each metal from the samples of branchial plates was used to estimate the intake of each metal for a vulnerable age group. This was compared to the minimal risk levels (MRLs) set by the Agency

for Toxic Substances and Disease Registry (ATSDR) for an acute duration (1–14 days) of oral exposure.

3. Results

Table 1 presents the results of metal analyses of branchial plate and muscle tissue from 15 specimens of *M. japonica* from Sri Lankan markets (RSL) and muscle tissue samples from 12 specimens of *M. alfredi* from Lady Elliot Island reef (LEI). The samples were compared with each other, with Food Standards (Fig. 2) and with MRLs set by the ATSDR.

3.1. Comparison between samples

Significant differences were seen between the two muscle sample sets (MANOVA, Pillai = 0.768, $F = 18.2$, $df_1 = 4$, $df_2 = 22$, $p < 0.001$), with mean As, Cd and Hg significantly higher in the RSL samples and Pb higher in those from LEI (ANOVA for As, Cd, Hg and Pb respectively were: $F = 21.7$, $df = 1$, $p < 0.001$; $F = 6.31$, $df = 1$, $p = 0.018$; $F = 44.1$, $df = 1$, $p < 0.001$; $F = 10.3$, $df = 1$, $p = 0.004$). Metal concentrations in matched muscle and branchial tissue from the RSL samples differed significantly (Paired MANOVA, Pillai = 0.87, $F = 16.9$, $df_1 = 4$, $df_2 = 10$, $p < 0.001$), with mean As, Pb, and Hg much higher in the muscle (ANOVA for As, Pb and Hg respectively were: $F = 16.0$, $df = 1$, $p < 0.001$; $F = 8.50$, $df = 1$, $p = 0.012$, $F = 63.2$, $df = 1$, $p < 0.001$), while there was no significant difference between Cd in the two ($F = 1.32$, $df = 1$, $p = 0.271$). A significant linear correlation between metal concentrations of the two tissue types can be seen in Fig. 1.

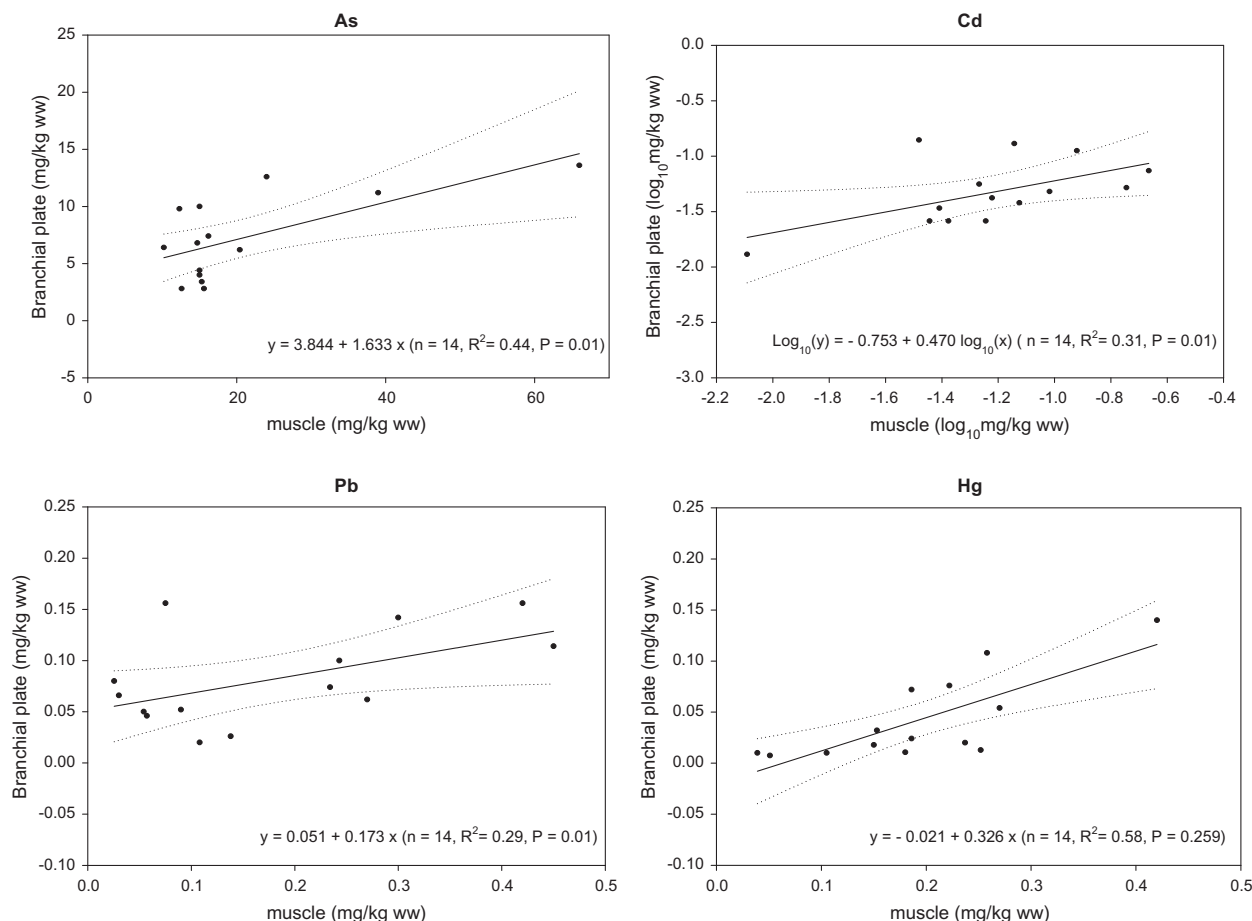


Fig. 1. Correlation between metal concentrations in muscle tissue and branchial plates.

3.2. Comparison to maximum levels in food standards

Comparison of metal concentrations in each tissue type with the MLs set by FSANZ, EC and Codex Alimentarius are represented in Fig. 2.

3.2.1. Arsenic

Among the three organisations, only FSANZ has established an ML for arsenic in fishes, which is based on its inorganic form. In the edible portion of marine fishes, ~10% of arsenic is generally present in inorganic forms (Rahman et al., 2012). Under the assumption that this is also valid for rays, the concentration of inorganic arsenic was estimated for each tissue sample, as only total arsenic was measured. Mean inorganic As concentrations in LEI and RSL branchial plate tissues were below the FSANZ ML, whereas the mean RSL muscle concentration (2 mg/kg wet weight) was equivalent.

3.2.2. Cadmium

Among the three organisations, only EC has established an ML for Cd in fishes. Mean Cd concentrations in RSL branchial plate and muscle samples were above the ML set by EC. Although the mean level of Cd in LEI muscle tissue did not exceed this limit, 25% of the LEI muscle samples (ranging from 0.05 to 0.12 mg/kg wet weight) exceeded this ML.

Table 2

Calculated intake of metals via consumption of branchial plates for a vulnerable age group.

Metal	Inorganic As	Cd	Pb	Hg
Amount in one serving of branchial plate	57.0 µg	5.9 µg	7.0 µg	0.80 µg
Calculated intake per day of a 5-year old Chinese girl weighing 15.9 kg (Marshall, 1981)	3.6 µg/kg/day	0.37 µg/kg/day	0.44 µg/kg/day	0.050 µg/kg/day
ATSDR oral exposure MRL for acute duration exposure	5 µg/kg/day	None available due to insufficient data	None available due to insufficient data	None available due to insufficient data

3.2.3. Lead

MLs for Pb set by EC and Codex are 0.3 mg/kg wet weight and 0.5 mg/kg wet weight for FSANZ. The RSL branchial plate and muscle samples had mean levels of Pb which were below both MLs. However, 13% of the RSL muscle samples had Pb concentrations that were >0.3 mg/kg wet weight. As for the muscle samples from LEI, the mean Pb concentration was above the EC and Codex limit. Although the mean level of Pb for LEI muscle samples did not exceed the FSANZ limit, 25% of the samples were above it.

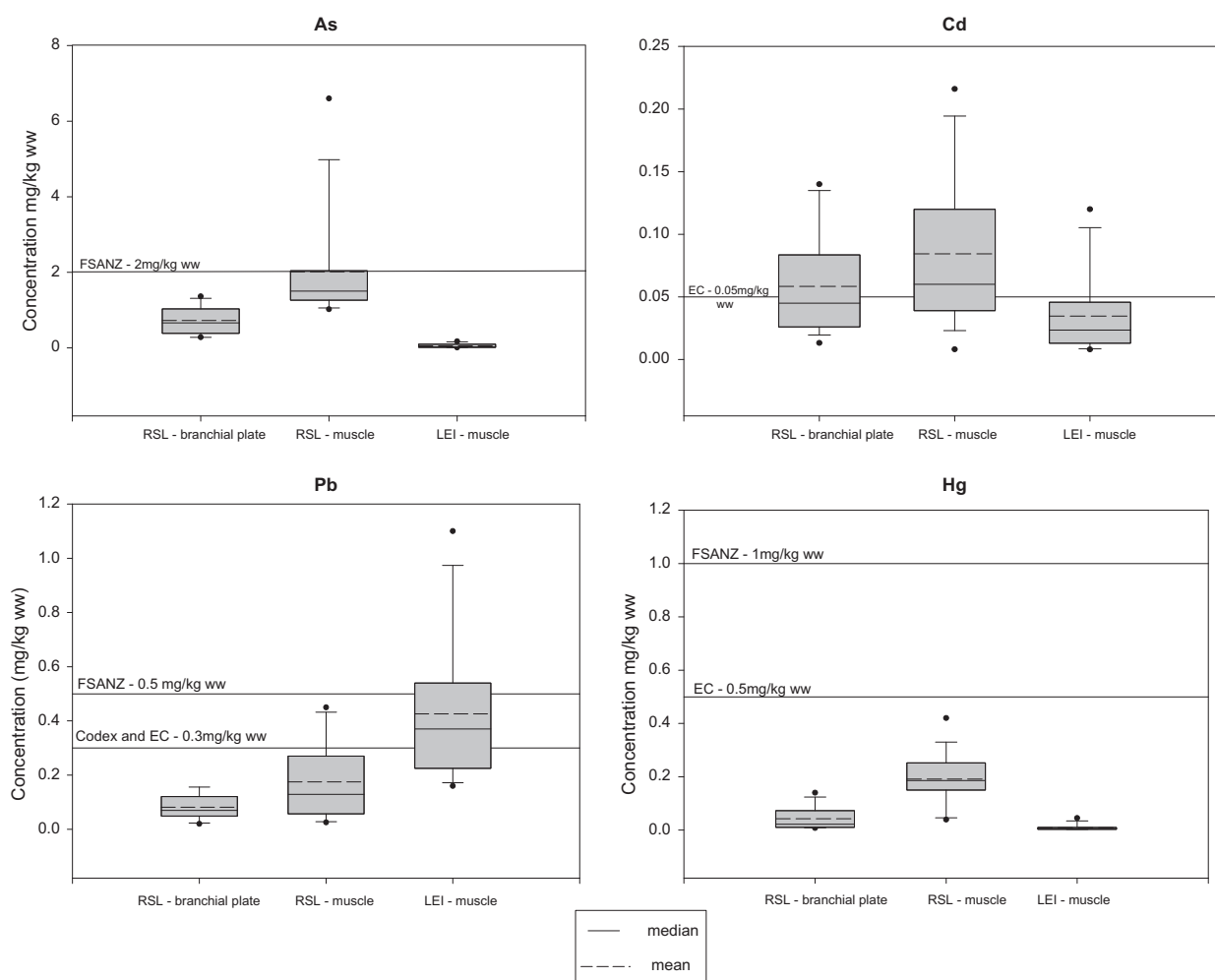


Fig. 2. Comparison of metal concentrations in samples to food standards.

3.2.4. Mercury

MLs for total mercury in fish set by FSANZ and EC are 1 mg/kg and 0.5 mg/kg wet weight respectively. All tissue samples had concentrations below these MLs.

Codex provided a guideline level (GL) for methyl mercury, defined as “the ML of a substance in a food or feed commodity which is recommended by the Codex Alimentarius Commission to be acceptable for commodities moving in international trade”, of 0.5 mg/kg wet weight instead of a ML for total Hg. Based on the assumption that methyl mercury is ~83% of total mercury (Kannan et al., 1998), the concentration of methyl mercury in our samples was calculated and compared with the Codex GL. Methyl mercury concentrations in all tissue samples had concentrations below the Codex GL of 0.5 mg/kg wet weight.

3.3. Comparison to ATSDR minimal risk levels

In Chinese medicine, the medicinal dose rate of mobulid ray branchial plates is 4.5–9 g (dried) once a month to stay healthy, and one dose daily for measles and chicken pox until the virus subsides (Paul Hilton, pers. comm., March 2014). In the case of chicken pox, the illness usually lasts from 5 to 10 days (Centers for Disease Control and Prevention, 2011), with the age group most likely to contract chickenpox being 3–8 year olds (Ma and Fontaine, 2006). Using this information and the highest non-outlier concentration of As out of all the RSL branchial plate samples, we calculated that there can be up to 57 µg of inorganic As in a single serving of branchial plates (assuming inorganic As is ~10% of total As (Rahman et al., 2012)). The calculations we made for a 5-year old Chinese girl of 15.9 kg body mass (Marshall, 1981) shows that her intake in a single serving could be 3.6 µg As/kg/day of inorganic As.

Using the same principles as above, a single serving of branchial plates could have 5.9 µg of Cd, 7.0 µg of Pb and 0.80 µg of Hg. This equates to intake of 0.37 µg/kg/day of Cd, 0.44 µg/kg/day of Pb and 0.050 µg/kg/day of Hg respectively. These calculations are tabulated in Table 2.

4. Discussion

Overall, our findings show that consumption of *M. japonica* branchial plates and muscle tissue from RSL and *M. alfredi* muscle tissue from LEI need further investigation to determine if they pose a risk to human health when consumed, especially to children.

4.1. Risks related to human consumption

4.1.1. Arsenic

Concentrations of inorganic As in all RSL branchial plate samples were lower than the ML set by FSANZ (Fig. 2), and the calculated intake of inorganic As by a vulnerable age group is lower than the oral exposure MRL set by the ATSDR (Table 2). However, the concentration of As contained varies with each individual sample. Furthermore, ingested As is readily absorbed (between 30% and 95%) by humans, and children are more susceptible than adults to toxicity due to the lack of hepatic detoxification enzymes (Fowler et al., 2011; ATSDR, 2007a). Anti-asthma preparations of 25–107,000 µg/g As prescribed in Chinese medicine have reportedly resulted in acute poisoning in children and adults (Ibid). A health risk assessment carried out by Essumang (2009) found that consuming As-containing *M. birostris* meat in Ghana poses some cancer risk. There is also potential for adverse, non-cancerous health effects for humans consuming As-contaminated meat and liver (Ibid). Therefore, although our results were lower than the standards set, further investigation is needed to evaluate the potential

health risk from consuming mobulid ray branchial plates containing inorganic As.

4.1.2. Cadmium

Essumang (2009) found that there is potential for adverse, non-cancerous health effects for humans consuming Cd-contaminated liver from manta rays. Although Cd levels in liver were not assessed in this study, some Cd concentrations in branchial tissues from RSL mobulid rays were over the ML set by EC, and a young child could have an intake of 0.37 µg/kg/day (Table 2) from a serving of mobulid branchial plates.

Horiguchi et al. (2004) found that higher rates of absorption and lower rates of excretion of Cd in humans were negatively correlated with age. Similarly, dietary toxicity experiments found that immature female rats had much lower calculated LD50 values (ATSDR, 2012). Therefore, younger age groups may be at higher risk of Cd toxicity. Consequences of Cd exposure to humans include renal disease and skeletal damage, with higher risk of osteoporosis and fractures (Hellström et al., 2001; Engstrom et al., 2011). In addition, Cd is classed as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC, 1993).

Although the ATSDR does not have an acute duration MRL for oral exposure to Cd due to insufficient data (ATSDR, 2012), given that these tissues are used in Chinese medicine to treat fevers and chicken-pox in children (Zhongguo ben cao tu lu, 1988), a detailed risk assessment is required to determine the potential health risk from Cd via consumption of mobulid branchial plates, particularly for young girls.

4.1.3. Lead

We found that the mean Pb concentration from LEI muscle samples as well as the concentration in several of the RSL muscle samples exceeded the limit of 0.3 mg/kg wet weight proposed by Codex and EC. Similarly, Lopez et al. (2013) found that the mean Pb concentration in muscle, liver and stomach samples taken from blue sharks (*Prionace glauca*), and in muscle and liver samples from mako short fin sharks (*Isurus oxyrinchus*) exceeded this limit. Lopez et al. (2013) concluded that their findings indicated a risk for human health from consumption of shark tissues by humans.

Lead toxicity has several negative effects on humans. Exposure to Pb in pre- and postnatal development periods cause delayed or reduced neurological and sexual development (ATSDR, 2007b). Short-term Pb exposure can cause gastrointestinal distress, anaemia, encephalopathy, and death (CODEX, 2004). Children are more susceptible than adults to effects of Pb toxicity (Jarup, 2003). Some of the health effects of Pb may occur at blood lead levels so low as to be essentially without a threshold (United States Environmental Protection Agency, 1988a). The IARC has classified inorganic Pb compounds as probably carcinogenic to humans (Group 2A), and determined organic Pb compounds to be non-classifiable as to their carcinogenicity to humans (IARC, 2006).

We calculated that a child of a certain age group (Table 2) may have an intake of 0.44 µg/kg/day of Pb when consuming mobulid branchial plates. Although the ATSDR does not have an acute duration MRL for oral exposure Pb due to insufficient data (ATSDR, 2007b), because of the potentially severe health effects, the concentrations reported here from mobulid ray tissues warrants further investigation into potential human health risks.

4.1.4. Mercury

Our comparison to the food standards had similar findings to Lopez et al., 2013: Hg concentrations in stomach, muscle and liver tissue samples from *P. glauca* and *I. oxyrinchus* did not exceed the limit proposed by Codex. However, it was mentioned that Hg levels may have been masked by another metal hence there could have been more Hg in the samples than what was detected (Ibid).

According to the study done by [Essumang \(2009\)](#), there is no potential health risk from Hg for human consumers of *M. birostris* meat in Ghana.

Concentrations of total Hg reported from fish tissues are expected to be mostly composed of methyl mercury ([Commission of the European Communities, 2006](#)). Since methyl mercury is capable of bioaccumulating in fish, larger long-lived species, such as sharks and swordfish, tend to be the focus for dietary restrictions ([FSANZ, 2013](#)).

The effects of oral exposure to methyl mercury can be severe. A human epidemiological study found that daily oral intake of methyl mercury concentrations as low as 0.86 mg/kg/day (from fish) in expectant mothers corresponded with neurophysiological disorders in their children by the age of 7 ([USEPA, 1988b](#)).

The intake of Hg by a child consuming mobulid branchial plates as a cure for chicken pox could be 0.050 µg/kg/day ([Table 2](#)). As with Cd and Pb, the ATSDR does not have an acute duration MRL for oral exposure to Hg ([ATSDR, 1999](#)). Although our reported concentration of total Hg and methyl mercury in mobulid rays does not generally exceed regulatory guidelines, due to the severity of the potential adverse health effects, a review of the use of mobulid tissues warrants further investigation into the potential human health risks.

4.2. Comparing paired branchial plate and muscle concentrations from the same ray

We found that for As, Pb and Hg, there was significantly more of each heavy metal accumulated in the muscle than branchial plates, while Cd had statistically similar levels in both tissues. Our findings for As were similar to [Allen et al. \(2004\)](#)'s who found that muscle levels of As exceeded gill levels in snakehead (*Channa punctatus*). However, concentrations of As, Cd and Pb were found to be higher in the gills as compared to muscle tissue in zebrafish (*Danio rerio*) and spotted dogfish (*Scyliorhinus canicula*) ([Hamdi et al., 2009](#); [De Boeck et al., 2010](#)). The higher accumulation of metals in some tissues over others could be because ionoregulatory organs have high accumulation rates ([De Boeck et al., 2010](#)) and the interaction between metals and cellular constituents, for As in particular ([Allen et al., 2004](#)).

There were significant positive correlations between branchial plate and muscle tissue concentrations for As, Cd and Pb in *M. japonica* tissue ([Fig. 1](#)). Therefore, it may be possible to estimate the concentrations of certain metals in branchial plates using muscle samples, which are less invasive to obtain. However, as the number of samples in this study was relatively low and the results showed some variability, these equations should be used with some caution until more samples can be collected and analysed.

4.3. Differences in metal concentration from two different species in separate locations

When we looked at the difference in metal concentrations in muscle tissue between *M. japonica* from RSL and *M. alfredi* from LEI, we found that As, Cd and Hg were significantly higher in samples from RSL than LEI. However, there were two different species being investigated at separate locations. Therefore, these differences may be due to species differences rather than differences in locality.

These differences between species could be due to differences in diet or inherent factors. [Marcovecchio et al. \(1991\)](#) found that the most important factor among 3 shark species analysed for tissue metal concentrations was variation in their feed, rather than location. [Storelli and Marcotrigiano \(2004\)](#) stated that different levels of As accumulation may differ between species of sharks

due to species-specific accumulation, or intrinsic factors such as uptake rate.

Besides natural sources, domestic waste, coal-burning power plants, metal smelters and sewage are sources of heavy metals in the marine environment ([Nriagu and Pacyna, 1988](#)). A possible reason for the relatively higher levels of As, Cd and Hg in RSL samples is the lack of proper waste management in Sri Lanka ([Pernetta, 1993](#)).

It is possible that the significantly higher Pb levels found in muscle samples from LEI than those from RSL could be linked to the large-scale mining activities in North-west Queensland, inland from the Great Barrier Reef ([Australian atlas of minerals resources, 2012](#)). According to [Mager \(2011\)](#), Pb mining is a major cause of concern for environmental contamination. Pb mining is a major industry in Australia ([Australian atlas of minerals resources, 2012](#)), with Australia being the second largest producer of Pb worldwide in 2008 ([Mager, 2011](#)).

Other marine organisms in the Great Barrier Reef have been found to be similarly affected by high Pb loads. For example, in the Townsville harbour, adjacent to the central Great Barrier Reef, concentrations of Pb in unfiltered seawater were found by [Esslemont \(2000\)](#) to exceed the recommended limits set by Australian and New Zealand Environmental and Conservation Council (ANZECC). The coral *Goniastrea aspera* sourced from the Townsville harbour also had high levels of Pb ([Esslemont, 2000](#)). These elevated levels were due to suspended, metal-bearing fine sediment present in the unfiltered seawater (*Ibid*). [Reichelt and Jones \(1994\)](#), who studied trace metals in Cleveland Bay, close to Townsville, similarly found that the range of metals (including Pb) present were mainly from detrital particles originating from re-suspended sediments or river discharge. In addition, concentrations of Pb in the livers of dugong (*Dugong dugon*) from the Southern Great Barrier Reef have increased from 20 years ago ([Haynes et al., 2005](#)).

In conclusion, mean As in *M. japonica* muscle tissue, mean Cd concentration in *M. japonica* muscle tissue and branchial plates, and concentrations of Cd and Pb in some individual samples of *M. alfredi* muscle were above the set MLs for food safety, with Hg concentrations in all tissues below the set MLs. The calculated intake of inorganic As for a vulnerable age group consuming branchial plates is below the acute duration MRL for oral exposure set by the ATSDR. There are no acute duration MRLs set by the ATSDR for oral exposure to Cd, Pb or Hg. Given that some metal concentrations exceeded set MLs and that children are especially vulnerable to the toxic effects of some metals, further investigation and an assessment of human health risk from consumption of mobulid branchial plates should be carried out.

Acknowledgements

We thank the staff at Queensland Department of Health, Forensic and Scientific Services for assistance with laboratory techniques and equipment. We thank Lydie Couturier, Fabrice Jaine, Katherine Burgess, Peran Bray and Julie Vercelloni for their assistance with this study. This study was supported by the Goodman Foundation, ARC Linkage Grant LP110100712, Earthwatch Institute Australia and Sibelco Pty Ltd. Field work was supported by Lady Elliot Island Eco Resort and was conducted under Fisheries Permit (165491), Great Barrier Reef Marine Park Permit (G09/2985.1) and Ethics approval (SBMS/071/08/SEAWORLD).

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