Marine Pollution Bulletin 94 (2015) 251–259

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

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 japanica
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 japanica* 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. japanica* 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.

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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http://dx.doi.org/10.1016/j.marpolbul.2015.02.005
0025-326X/

(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 HNO_3 (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