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Profile and consumption risk assessment of trace elements in megamouth sharks (*Megachasma pelagios*) captured from the Pacific Ocean to the east of Taiwan☆

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ABSTRACT

Focusing on 27 rare filter-feeding megamouth sharks (*Megachasma pelagios*) captured as a by-catch of drift gillnet fishery in the Pacific Ocean to the east of Taiwan, this study analyzes the concentrations of 24 elements in their muscle, discusses the bioaccumulation of each element and the correlation between different elements, and assesses the potential health risks of consuming megamouth shark muscle. Among the 24 elements, mean concentrations of Ga, Ag, Li, Bi, Hg, Co, and Cd were relatively low ranging from 10^{-3} to 10^{-1} mg/kg, those of Pb, Ba, Mn, Ni, As, Cr, B, Sr, Cu, and Zn ranged from 10^{-1}–10^{1} mg/kg, and those of Fe, Ca, Al, K, Mg, Ti, and Na were relatively high ranging from 10^{1} to 10^{3} mg/kg. The toxic element content index was most significantly correlated with the concentration of Cu. Hence, this study recommends that the concentration of Cu could be used as an indicator of metal accumulation in megamouth shark muscle. The log bioconcentration factor (BCF) ranged from less than 0 to 7.85 in shark muscle. For elements with a concentration of less than 100 μg/L in seawater, the log BCF was inversely proportional to their concentration in seawater. According to the correlation analysis, the accumulation of elements in muscle of megamouth sharks is primarily affected by the concentrations of dissolved elements in seawater, except that the accumulation of Hg, As, Cu, Ti, Al, and Fe appears to be mainly affected by feeding behaviors. The assessment of the health risk of consuming megamouth shark muscle showed that its total hazard index was greater than 1. This suggests that the long-term or high-frequency consumption of megamouth shark muscle may cause health hazards due to the accumulation of trace elements, particularly those with a large contribution of health risk, including As, Hg, and Cu.

1. Introduction

Megamouth sharks (*Megachasma pelagios*) are a species of rare sharks. Since its discovery in 1976, only approximately 138 megamouth sharks had been captured or discovered worldwide till 2019 (www.sharkmans-world.eu). According to records, megamouth sharks are mainly distributed in the Indian Ocean, Pacific Ocean, and Atlantic Ocean, and are found most frequently (105 out of 138) in sea areas along the Kuroshio currents (e.g., sea areas near Taiwan, The Philippines, and Japan) (Liu et al., 2018; Watanabe and Papastamatiou, 2019). Because megamouth sharks are a species of extremely rare deep-water sharks, information regarding their biological characteristics, feeding, and migration behaviors is still insufficient or yet to be verified (Moura et al., 2015; Liu et al., 2018; Watanabe and Papastamatiou, 2019). However, available literature shows that megamouth sharks can be as long as 710 cm, can weigh

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up to 1,137 kg (Watanabe and Papastamatiou, 2019), and are the third largest filter-feeding sharks in existence, ranking only next to whale sharks and basking sharks. They prey on krill, copepods, and gelatinous zooplankton in a manner similar to that of humpback whales (Taylor et al., 1983; Berra and Hutchins, 1990; Yano et al., 1999; Sawamoto and Matsumoto, 2012; Moura et al., 2015). Similar to other filter-feeding sharks, they can migrate vertically, possibly because they follow vertically migrating zooplankton (i.e., deep scattering layer) to improve foraging efficiency (Watanabe and Papastamatiou, 2019). They swim at a depth of approximately 120 m–166 m during the day, stay at a depth of approximately 12 m–25 m at night, and move downward to a deeper layer at dawn (Nelson et al., 1997).

Globally, the largest number of megamouth sharks have been captured or discovered in Taiwan, accounting for approximately 75% of global captured or discovered sharks (57 out of 138). Most of the captured megamouth sharks are sold by fishermen to restaurant operators for human consumption (Liu et al., 2018). Although there have been very few studies on the concentration distribution and bioaccumulation of heavy metals in megamouth sharks, to the best of our knowledge, only one megamouth shark that was stranded on the southeast coast of Brazil was investigated to analyze the concentrations of trace elements in its muscle and liver (Moura et al., 2015). Intra-specific variation of toxicological profiles is high among sharks and one sample is not enough to describe a population. Moreover, the only study that has previously addressed this issue, analyzed an individual from another ocean basin, which greatly influences the results of trace element accumulation patterns. The mechanism underlying the bioaccumulation of trace elements in aquatic organisms is extremely complex; specifically, it is affected not only by biodynamics (e.g., uptake, assimilation, elimination, and individual physiology, including uptake rate and growth rate) but also by environmental conditions (Wang and Lu, 2017; Ju et al., 2017). In addition to biomagnification, the concentration of elements in aquatic organisms may be affected by the age, sex, weight, habitat, and mobility of aquatic species (Ju et al., 2020).

The bioaccumulation of metal elements in fishes is tissue-specific; hypermetabolic tissues (e.g., the kidney) accumulate a high concentration of metals, whereas muscle accumulate a relatively low concentration of metals (Ju et al., 2017). The accumulation of metals is low in muscular tissues because they are not the main target ligand of biotransformation and accumulation of metals (El-Moselhy et al., 2014) and/or are affected by growth dilution (Gbem et al., 2001; Farkas et al., 2003). Nevertheless, fish muscle is the main edible part for humans; hence, it is necessary to assess the health risk of consuming fish muscle. In general, the megamouth shark is not the main source of fishermen's income, and it has been caught accidently and sold to seafood restaurants for human consumption (Taiwan News, 2019). The consumption of megamouth shark by humans is rare and opportunistic, and there are no reports of long-term consistent consumption of megamouth shark as it is rare to find and catch. However, once the accidental catches of megamouth sharks occur, megamouth sharks are highly possible to be sought after by restaurants. Therefore, this study attempts to quantitatively assess the potential noncarcinogenic risk of eating megamouth shark in terms of the hazard quotient (HQ) and the total hazard index (THI) proposed by the USEPA (Gu et al., 2015; Abdel-Khalek et al., 2016; Zhang et al., 2016; Ju et al., 2017) to be a reference for future consideration responding to megamouth shark becomes more prevalent in the shark trade.

In this study, we collected muscular tissue samples of 27 rare filter-feeding megamouth sharks captured as a by-catch of drift gillnet fishery in the sea areas to the east of Taiwan and measured the concentration of 24 elements in the muscular tissues. Accordingly, this study discusses the bioaccumulation of different elements in the muscular tissues and the correlation between them. Finally, the potential health risk of eating megamouth shark muscle in terms of the HQ and THI are assessed. The results of this study are the most comprehensive toxicological profile for megamouth sharks in the Pacific Ocean, known to date as the most important area in terms of relative abundance for this species. More critical reference information can also be obtained from this study for future studies, such as the bioaccumulation of elements and the possible human health risk of eating megamouth shark muscle.

2. Materials and methods

2.1. Sample collection

This study collected the muscular tissue samples of 27 megamouth sharks captured as a by-catch of drift gillnet fishery in the sea area of Hua-lien to the east of Taiwan from 2013 to 2015. The muscular tissues of captured sharks were sampled at discharging quays for fishing ships and were stored in trash ice buckets. The weight of the 27 captured megamouth sharks ranged from 210 to 1,147 kg, and their full length ranged from 341 to 710 cm. The female:male proportion was 16:11; five megamouth sharks were adults (the female:male proportion was 4:1), and the remaining were sub-adults. The detailed information (e.g., capture date, sex, weight, and full length) of the 27 megamouth sharks was discussed in a previous study (Liu et al., 2018).

2.2. Sample preparation and determination of elements

The muscular tissues of megamouth sharks were digested according to the method reported by Ju et al. (2017), with slight modifications. Specifically, 0.10 g of fresh muscle tissues were placed in a polypropylene centrifuge tube for 30 min after the addition of ultrapure nitric acid (67%–70% HNO_3, Fisher Scientific, USA) and hydrogen peroxide (>35% H_2O_2, SHOWA, Japan). Then, the samples were fed to a graphite digestion and decomposition furnace (SETON, Taiwan) and heated at a temperature of 95 °C for reflux digestion until the digestive fluid became clear. After the clear digestive fluid cooled to room temperature (~25 °C), it was filtered using a glass filter paper with a pore diameter of 0.22 μm, and diluted to 10 mL using ultrapure water (Milli-Q Millipore 18.2 MΩ•cm). The concentration of metal elements in the digestive fluid was measured using inductively coupled plasma mass spectrometry (ICP-MS, iCAP RQ, Thermo Fisher Scientific, USA). ICP-MS analysis was conducted under the kinetic energy discrimination (KED) mode. Each sample was injected and diluted using an automatic sampler (Elemental Scientific, USA), and Yttrium (^{89}Y), Rhodium (^{103}Rh), and Platinum (^{195}Pt) were added as internal standards.

For quality control, calibration curves, procedure blank, check standards, duplicated analysis, and certified reference material (CRM) analysis were performed. The element standard solution used for the calibration curves was the 1,000 mg/L ICP multielement standard solution IV with As and Hg provided by Merck. The standard solution comprised 24 elements, namely, Ag, Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Ti, and Zn. The correlation coefficients of calibration curves (0.01–50 μg/L) of all tested elements were greater than 0.995; the measured values of program blanks were lower than the detection limit; the recovery rate of check standards ranged from 86.7% to 115.3%; the percentage of relative difference of repeated analysis was lower than 10%. The mean recovery rate of the fish protein CRM (DORM-4, National Research Council of Canada) ranged from 95.2% to 112.5% (Table S1). In addition, all used glassware was soaked overnight in a 5 M HNO_3 solution and then washed using ultrapure water. All used reagents were of analytical grade or higher and were diluted using ultrapure water (18.2 MΩ•cm). In this study, the element concentration of muscular tissue samples was measured in terms of the element mass per unit wet weight (mg/kg wet weight).

2.3. Data analyses

All data were subjected to statistical analysis to calculate minimum, maximum, and mean values, along with standard deviations. In addition, the allometric growth model was adopted to describe the relationship between the full length and weight of each megamouth shark (Kumar et al., 2018), that is, $W = aL^b$, where W is the whole body wet weight, L is the full length, a is the intercept constant, and b is the length–weight relationship index. The b value, which is determined using the allometric growth model, can be used to calculate the conditional factor ($K = W/L^b$) for evaluating the biological and physiological condition (Sharma et al., 2005). In addition, the toxic element content in the muscle of individual megamouth sharks was compared in terms of the toxic element content index (TECI). The TECI is calculated using the equation $\mathrm{TECI} = (C_{E1} \times C_{E2} \times C_{E3} \times \ldots C_{En})^{1/n}$ (Usero et al., 1997), where C_{En} denotes the concentration of element n in muscular tissues (mg/kg wet weight), including nine toxic elements (Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn). The bioconcentration factor (BCF) was also used to evaluate the element accumulation capability of megamouth sharks exposed to water. BCF is calculated using the equation $\mathrm{BCF} = C_E/C_w$, where C_E and C_w denote the concentrations of elements in the muscular tissues of megamouth sharks (mg/kg wet weight) and seawater (mg/L), respectively. Because the concentration of elements in seawater usually remains within a narrow range, the difference in the element concentration between inshore seawater and ocean seawater is usually less than one order of magnitude (Fang et al., 2006, 2014). The mean concentration of dissolved elements in seawater used in this study was adopted from Bruland and Lohan (2003). The Spearman's rank correlation coefficient (r) was used to evaluate the correlation between various parameters, including element content in muscular tissues, TECI, weight (W), full length (L), and conditional factor (K). When the p value was less than 0.05, the results were considered significant. All statistical analyses were conducted using SPSS software 20.0 (IBM, Armonk, NY, USA).

2.4. Human health risk assessments

The potential human health risk of consuming megamouth shark muscle was measured in terms of the HQ and THI. The HQ is a proportion of estimated daily intake (EDI, μg/kg/day) to chronic oral reference dose (RfD, μg/kg/day); HQ = EDI/RfD. The EDI was estimated according to the element concentration (C_E, μg/g wet weight), the daily consumption (DC_{fish}, g/day) of megamouth shark muscle, and the mean body weight (BW, kg) of Taiwanese adults; EDI = (CE × DCfish)/BW. According to the Taiwan National Food Consumption Database (TNFCD, 2017; http://tnfcds.cmu.edu.tw/), the mean body weight of male and female Taiwanese adults aged 19 to 65 is 69.3 ± 11.1 kg and 57.3 ± 9.8 kg, respectively, and their mean daily intake of saltwater fishes is 86.78 ± 97.99 g/day and 69.85 ± 87.39 g/day, respectively. RfD was determined according to the data of the Integrated Risk Information System (https://www.epa.gov/iris) developed by the USEPA in 2020 (USEPA, 2020), except the doses of Pb and Co, which were determined according to the studies reported by Orisakwe et al. (2017) and Finley et al. (2012). The THI is a summation of HQ of all metal elements. When the HQ or THI is less than 1.0, eating megamouth shark muscle does not pose a significant noncarcinogenic risk. The greater the HQ or THI value, the higher the possible health risk. When the THI is greater than 10, the noncarcinogenic risk is considered to be extremely high (Ahmed et al., 2019).

3. Results and discussion

3.1. Trace element concentrations in megamouth sharks

Among the 138 megamouth sharks reported in literature from 1977 to 2019, full length and body weight of only 39 sharks (including 27 in this study) have been reported (Watanabe and Papastamatiou, 2019). According to statistical data, four outliers of sharks were excluded, and the remaining 35 sharks were used to establish the relationship between full length (L) and body weight (W) as $W = 4.01\ L^{3.24}$ ($n = 35$, $r = 0.87$) (as shown in the supplement; Figure S1). According to the length–weight relationship index ($b = 3.24$), the condition factor (K) (as described in the supplement; Table S2) of female and male megamouth sharks was 4.1 ± 1.1 and 4.5 ± 0.9, respectively, without a significant difference ($p > 0.05$). The concentration distribution of 24 selected elements in the muscular tissues of 27 megamouth sharks is listed in Table 1 (as detailed in the supplement; Table S3). There was no significant difference in the mean concentration of each element in muscular tissues between female and male megamouth sharks ($p > 0.05$). The concentration range of each element in muscular tissues was different among all megamouth sharks, and varied by approximately one order of magnitude. The mean concentration of 24 elements in ascending order is shown in Fig. 1. The concentration of Ga, Ag, Li, Bi, Hg, Co, and Cd in the muscular tissues was low ranging from 10^{-3} to 10^{-1} mg/kg (Fig. 1a); the concentration of Pb, Ba, Mn, Ni, As, Cr, B, Sr, Cu, and Zn ranged from 10^{-1}–10^{1} mg/kg (Fig. 1b); and the concentration of Fe, Ca, Al, K, Mg, Ti, and Na was relatively high ranging from 10^{1} to 10^{3} mg/kg (Fig. 1c).

Table 2 compares the concentration of elements in the muscular tissues of megamouth sharks specified in this study, megamouth sharks stranded in Praia Grande, Arraial do Cabo, southern Brazil (Moura et al., 2015), filter-feeding whale sharks (*Rhincodon typus*) (Wang et al., 2015; Pancaldi et al., 2019), apex predator white sharks (*Carcharodon carcharias*) (Mull et al., 2012), and predatory benthic sharks (*Chiloscyllium plagiosum*) (Cornish et al., 2007). The mean concentration of most elements in the muscle of megamouth sharks specified in this study was different from that of the megamouth sharks stranded in Brazil by less than one order of magnitude, except the difference in the mean concentration of Pb, Ni, B, Cu, Al, and K reached one to two orders of magnitude. The concentration of B (1.63 ± 1.25 mg/kg) and K (133 ± 93.1 mg/kg) in the muscle of megamouth sharks specified in this study was relatively low compared with that (363 mg/kg and 1,103 mg/kg, respectively) in the muscle of megamouth sharks discovered in Brazil. However, it is difficult to provide an explanation for such a difference because limited data are available for reference. Compared with other giant filter-feeding sharks, the concentration of Hg (0.058 ± 0.075 mg/kg) and As (1.37 ± 0.95 mg/kg) in the muscular tissues of megamouth sharks specified in this study is higher than that (0.0004–0.0023 mg/kg and 0.16–0.17 mg/kg respectively) of the muscular tissues of whale sharks discovered in China; however, it is similar to that (0.029–0.045 mg/kg and 0.707–1.050 mg/kg) of the muscular tissues of whale sharks discovered in the Gulf of California, Mexico, that borders the Pacific Ocean. Compared with predatory sharks, Hg and As concentrations in muscular tissues of filter-feeding megamouth sharks and whale sharks were relatively low (Table 2). In general, Hg and As concentrations increase with the increase in trophic level, which is referred to as the biomagnification effect (Eisler, 2010; Bosch et al., 2012). Therefore, Hg and As concentrations in the muscle of megamouth sharks, which feed on plankton by filter feeding, may

Table 1
Concentration (mg/kg ww) of twenty four elements in muscular tissues of *Megachasma pelagios* captured from the Pacific Ocean to the east of Taiwan.

Element	Total (n = 27)[a]				Females (n = 16)				Males (n = 11)			
	Min.[b]	Max.[b]	Mean[c]	SD[b]	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Ga	ND[b]	0.021	0.008	0.006	ND	0.020	0.007	0.006	ND	0.021	0.009	0.006
Ag	ND	0.144	0.027	0.036	ND	0.144	0.033	0.044	ND	0.052	0.018	0.017
Li	ND	0.080	0.034	0.018	ND	0.080	0.035	0.020	ND	0.068	0.033	0.016
Bi	0.009	0.115	0.042	0.031	0.009	0.114	0.035	0.025	0.010	0.115	0.053	0.037
Hg	ND	0.327	0.058	0.075	ND	0.260	0.053	0.065	ND	0.327	0.065	0.090
Cd	ND	0.363	0.075	0.101	ND	0.363	0.086	0.119	0.015	0.260	0.059	0.072
Co	0.016	0.204	0.071	0.055	0.016	0.204	0.066	0.056	0.018	0.203	0.078	0.055
Pb	ND	0.80	0.25	0.23	0.04	0.77	0.25	0.24	ND	0.80	0.25	0.22
Ba	0.07	1.97	0.49	0.42	0.08	0.78	0.38	0.21	0.07	1.97	0.66	0.57
Mn	0.13	1.98	0.68	0.45	0.24	1.98	0.69	0.50	0.13	1.55	0.67	0.39
Ni	0.11	2.89	1.02	0.74	0.20	2.17	0.92	0.60	0.11	2.89	1.16	0.92
As	0.34	4.73	1.37	0.95	0.34	4.73	1.40	1.16	0.68	2.57	1.33	0.56
Cr	0.16	5.40	1.63	1.25	0.55	4.02	1.47	1.00	0.16	5.40	1.86	1.57
B	0.36	2.73	1.63	0.60	0.36	2.73	1.62	0.62	0.75	2.64	1.64	0.59
Sr	0.58	5.33	2.96	1.26	0.58	5.20	2.96	1.28	1.13	5.33	2.98	1.30
Cu	1.13	15.28	5.47	3.53	1.80	15.28	5.08	3.32	1.13	12.57	6.05	3.89
Zn	1.43	20.43	7.82	5.18	1.43	20.43	8.88	6.34	3.45	11.36	6.29	2.26
Fe	3.79	60.7	20.0	14.8	7.43	58.9	17.4	12.4	3.79	60.7	23.8	17.8
Ca	13.2	88.9	51.1	20.3	13.2	80.9	50.9	20.3	16.8	88.9	51.3	21.3
Al	6.88	187	58.8	46.8	11.7	148	51.4	39.7	6.88	187	69.7	55.9
K	31.9	403	133	93.1	31.9	274	130	77.8	49.4	403	137	116
Mg	54.5	441	153	84.5	68.5	441	156	90.1	54.5	344	149	79.8
Ti	126	1169	547	276	251	1072	525	236	126	1169	579	335
Na	299	2591	1104	590	400	1929	1025	439	299	2591	1218	768

[a] n: Number of samples.
[b] Min: Minimum, Max: Maximum, SD: standard deviation; ND: the measured value is less than the method detection limit (MDL).
[c] When the measured value is less than the MDL, the mean is calculated based on the MDL value.

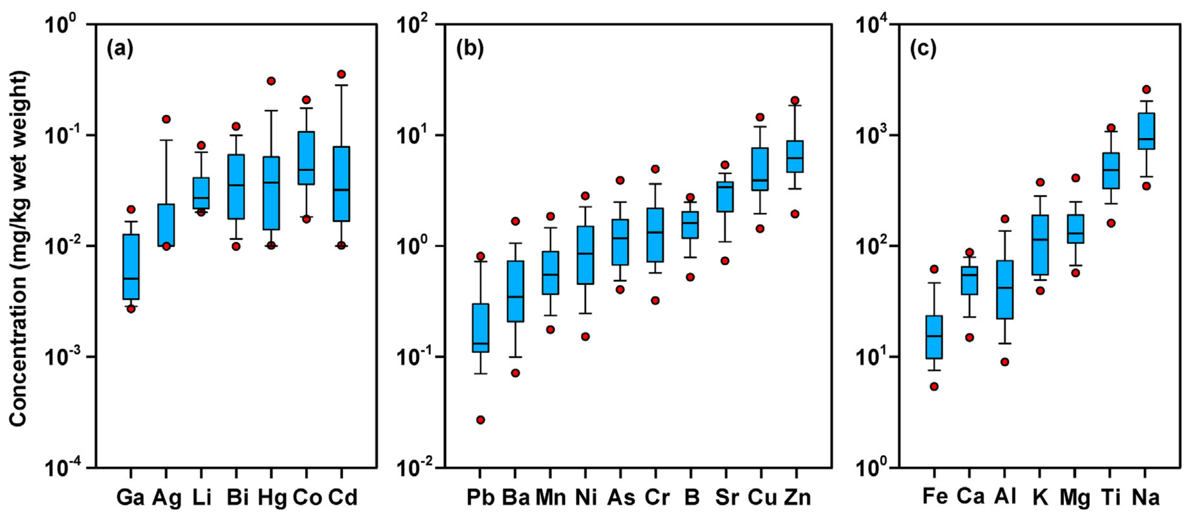

Fig. 1. Box-whisker plots shown the concentration distributions of elements in the muscular tissues of *Megachasma pelagios*. Line inside the box is the median, lower and upper limits of box respectively represent 25th and 75th percentiles; whiskers extend to the range between the 10th and 90th percentiles; the circles above and below respectively represent to the range between the 5th and 95th percentiles.

be relatively low. The concentration of Cd (0.075 ± 0.101 mg/kg) and Pb (0.25 ± 0.23 mg/kg) in the muscular tissues of megamouth sharks specified in this study is higher than that (0.05 mg/kg and 0.01 mg/kg, respectively) in the muscular tissues of megamouth sharks discovered in Brazil, as well as than that (0.010–0.033 mg/kg and <0.001–0.077 mg/kg, respectively) in the muscular tissues of predatory sharks, but similar to that (0.11–0.12 mg/kg and 0.30–0.33 mg/kg, respectively) in the muscular tissues of whale sharks discovered in China. The difference in the concentration of Cd (0.03 and 2.79 mg/kg) and Pb (0.016 and 13.70 mg/kg) in the muscular tissues of two whale sharks discovered in the Gulf of California, Mexico is significant as two and three orders of magnitudes, respectively. The concentration of Cu (5.47 ± 3.53 mg/kg) in the muscular tissues of megamouth sharks specified in this study is higher than that (0.23 mg/kg) in the muscular tissues of megamouth sharks discovered in Brazil, but similar to that (1.69–3.58 mg/kg) in the muscular tissues of whale sharks discovered in China and that (0.92–3.03 mg/kg) in the muscular tissues of whale sharks discovered in the Gulf of California, Mexico.

The concentration of trace elements accumulated in marine fishes varies greatly, and is mainly affected by the ambient media, body type, physiological metabolism, food sources, and feeding behaviors (Neff, 2002; Eisler, 2010). However, by the stable Sr–Ca ratio displayed in the muscle of megamouth sharks indicated that

Table 2
Comparison for elements concentrations (mg/kg ww) among different shark species.

Location	Taiwan	Brazil	China	China	Mexico	Mexico	California	Hong Kong
Species	*M. pelagios*	*M. pelagios*	*R. typus*	*R. typus*	*R. typus*	*R. typus*	*C. carcharias*	*C. plagiosum*
Feed behavior	Filter feeder	Filter feeder	Filter feeder	Filter feeder	Filter feeder	Filter feeder	Apex predator	Bottom-dwelling predator
Sample number	27	1	1	1	1	1	20	35
Total length (m)	3.41–7.10	5.39	NA	NA	9.44	5.48	1.73–3.30	0.29–0.76
Ag	0.027 ± 0.036	0.05					0.406	<0.001
Li	0.034 ± 0.018	0.02						
Bi	0.042 ± 0.031	0.03						
Hg	0.058 ± 0.075	0.0996	0.0004	0.0023	0.029	0.045	2.78	
Cd	0.075 ± 0.101	0.05	0.12	0.11	0.03	2.79	0.033	0.010
Co	0.071 ± 0.055	0.01					0.037	
Pb	0.25 ± 0.23	0.01	0.33	0.30	0.016	13.70	0.077	<0.001
Mn	0.68 ± 0.45	0.10					0.189	0.090
Ni	1.02 ± 0.74	0.09					0.18	<0.001
As	1.37 ± 0.95	5.54	0.16	0.17	1.050	0.707	2.58	
Cr	1.63 ± 1.25	0.67					1.09	0.210
B	1.63 ± 0.60	363						
Sr	2.96 ± 1.26	0.85					0.56	
Cu	5.47 ± 3.53	0.23	3.58	1.69	0.92	3.03	0.52	0.15
Zn	7.82 ± 5.18	18.4	11.12	7.43	8.49	26.2	3.19	7.42
Fe	20.0 ± 14.8	5.18					12.8	
Ca	51.1 ± 20.3	41.1					41.2	
Al	58.8 ± 46.8	4.6						
K	133 ± 93.1	1103						
Mg	153 ± 84.5	276						
Na	1104 ± 590	3153						
References	This study	Moura et al. (2015)	Wang et al. (2015)	Wang et al. (2015)	Pancaldi et al. (2019)	Pancaldi et al. (2019)	Mull et al. (2012)	Cornish et al. (2007)

Species: *Megachasma pelagios, Rhincodon typus, Carcharodon carcharias, Chiloscyllium plagiosum*.
ND: Not *available*.

they inhabit similar water masses over a long period (Liu et al., 2018; Watanabe and Papastamatiou, 2019). The shark species listed in Table 2 were mostly collected from the regions around the North Pacific except a megamouth shark discovered on the coast of Brazil connecting the Atlantic Ocean, therefore the differences of metal loads in their muscle might be not mainly caused by the differences of these metals in the water column that they inhabit. Turoczy et al. (2000) and Boldrocchi et al. (2019) also suggested that trophic level and associated food sources, and the species ecology seem to be the major factors determining the differences of metal loads between shark species. Compared with predatory sharks, the concentration of Cu in the muscular tissues of filter-feeding megamouth sharks (except those discovered in Brazil) and whale sharks is relatively higher. The concentration of Zn in the muscular tissues of filter-feeding megamouth sharks and whale sharks (7.43–26.2 mg/kg) is also higher than that in the muscular tissues of predatory sharks (3.19–7.42 mg/kg). Copper and Zn could be absorbed via the water column and the food sources, and they play an important role in enzymatic components related to fish metabolism and development (Al-Weher, 2008; Ong and Gan, 2017). Not only the concentration of Cu and Zn but also Pb and Cd in the muscular tissues of megamouth sharks is relatively higher than that in the muscular tissues of predatory sharks, which may be because plankton, the main food source of megamouth sharks, has a remarkable bioaccumulation effect on such elements (Martin and Knauer, 1973; Fang et al., 2014; Pancaldi et al., 2019). The higher Cd concentration has been contributed to the food source richer in crustaceans and cephalopods than in fish resulting that Cd load in the muscle of filter feeder higher than that of the predator in marine fish (Bustamante et al., 1998). Due to the trophic level, filter feeders as megamouth shark and *R. typus* are not expected to exhibit Hg and As at a similar concentration than carnivorous shark such as *C. carcharias*. Overall, the biomagnification effect of Hg and As is not the dominant mechanism for Hg and As bioaccumulation of megamouth sharks. The weak bioconcentration ability of megamouth sharks to As and Hg may make them have a relatively lower concentration of Hg and As in muscular tissues.

3.2. Elemental bioaccumulation in megamouth sharks

The TECI was used to describe the bioaccumulation level of toxic elements (including Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) in the muscular tissues of megamouth sharks. The larger the TECI value, the higher the content of toxic elements (Usero et al., 1997; Ju et al., 2017). The TECI of muscular tissues of 27 megamouth sharks specified in this study ranged from 0.16 to 0.92, with an average of 0.41 ± 0.20. The TECI did not significantly differ between female and male megamouth sharks ($p > 0.05$), and the mean TECI was 0.41 ± 0.20 for both female and male megamouth sharks. There was no significant correlation of TECI with the body weight, full length, and conditional factor (K) of megamouth sharks ($r = 0.04$–0.09, $p > 0.05$). Table 3 describes the correlation between the TECI and the concentration of nine toxic metal elements. The concentration of Ag ($r = 0.24$, $p > 0.05$) and Cd ($r = 0.16$, $p > 0.05$) had no significant correlation with the TECI ($p > 0.05$), whereas the concentration of the other seven metal elements showed a significantly positive correlation with the TECI ($r = 0.47$–0.83, $p < 0.05$). The concentration of Cu and TECI showed the most significant correlation ($r = 0.83$, $p < 0.01$), implying that Cu can be used as an indicator of accumulation of toxic elements in the muscular tissues of megamouth sharks.

When aquatic organisms absorb a substance at a rate higher than they can metabolize and eliminate, the bioaccumulation occurs. Hence, toxic substances with a long biological half-life (e.g.,

Table 3
Spearman's rho coefficient matrix among the elements in the muscular tissues of megamouth sharks.

Element	Ga	Bi	Co	Pb	Ba	Mn	Ni	Cr	Cu	Fe	Al	Ti	Na	As	B	Ca	Sr	Zn	K	Mg	Li	Hg	Cd
Bi	0.77[a]																						
Co	0.89[a]	0.81[a]																					
Pb	0.76[a]	0.68[a]	0.76[a]																				
Ba	0.58[a]	0.60[a]	0.63[a]	0.71[a]																			
Mn	0.66[a]	0.51[a]	0.57[a]	0.48[b]	0.54[a]																		
Ni	0.73[a]	0.79[a]	0.80[a]	0.66[a]	0.57[a]	0.42[b]																	
Cr	0.80[a]	0.75[a]	0.83[a]	0.79[a]	0.68[a]	0.57[a]	0.74[a]																
Cu	0.81[a]	0.64[a]	0.87[a]	0.67[a]	0.65[a]	0.60[a]	0.68[a]	0.72[a]															
Fe	0.68[a]	0.51[a]	0.54[a]	0.65[a]	0.47[b]	0.71[a]	0.59[a]	0.65[a]	0.46[b]														
Al	0.94[a]	0.82[a]	0.94[a]	0.80[a]	0.67[a]	0.62[a]	0.82[a]	0.85[a]	0.88[a]	0.59[a]													
Ti	0.76[a]	0.73[a]	0.65[a]	0.71[a]	0.76[a]	0.64[a]	0.65[a]	0.67[a]	0.73[a]	0.60[a]	0.76[a]												
Na	0.85[a]	0.81[a]	0.81[a]	0.72[a]	0.61[a]	0.56[a]	0.77[a]	0.81[a]	0.82[a]	0.54[a]	0.93[a]	0.81[a]											
As	0.68[a]	0.46[b]	0.62[a]	0.51[a]	0.23	0.61[a]	0.40[b]	0.55[a]	0.44[b]	0.56[a]	0.59[a]	0.33	0.44[b]										
B	0.64[a]	0.54[a]	0.63[a]	0.50[a]	0.36	0.42[b]	0.61[a]	0.58[a]	0.54[a]	0.35	0.71[a]	0.47[b]	0.65[a]	0.41[b]									
Ca	0.37	0.45[b]	0.24	0.44[b]	0.52[a]	0.41[b]	0.39[b]	0.35	0.38	0.44[b]	0.37	0.82[a]	0.53[a]	−0.05	0.19								
Sr	0.17	0.29	0.04	0.26	0.42[b]	0.27	0.25	0.23	0.18	0.34	0.17	0.65[a]	0.36	−0.17	0.06	0.95[a]							
Zn	0.41[b]	0.16	0.47[b]	0.31	0.13	0.28	0.27	0.28	0.49[a]	0.31	0.37	0.19	0.28	0.31	0.29	0.01	−0.10						
K	−0.30	−0.26	−0.35	−0.33	−0.37	−0.22	−0.18	−0.26	−0.37	−0.15	−0.35	−0.30	−0.17	−0.12	0.02	−0.05	0.11	0.07					
Mg	−0.09	0.04	−0.17	0.02	0.14	0.00	0.02	0.04	−0.09	0.10	−0.10	0.19	0.03	−0.43[b]	0.04	0.46[b]	0.57[a]	0.08	0.46[b]				
Li	0.37	0.32	0.29	0.20	0.13	0.21	0.31	0.27	0.22	0.18	0.40[b]	0.24	0.50[a]	0.11	0.59[a]	0.24	0.19	−0.04	0.31	0.12			
Hg	0.21	0.11	0.13	0.04	0.01	0.23	0.25	0.07	0.36	0.23	0.22	0.30	0.39[b]	−0.09	0.11	0.39[b]	0.32	0.30	0.07	0.20	0.14		
Cd	−0.17	−0.37	−0.04	−0.28	−0.29	−0.19	−0.12	−0.18	−0.06	−0.11	−0.20	−0.38	−0.36	−0.05	0.09	−0.34	−0.32	0.17	−0.04	−0.27	0.00	−0.01	
TECI	0.76[a]	0.56[a]	0.82[a]	0.63[a]	0.48[b]	0.59[a]	0.74[a]	0.69[a]	0.83[a]	0.65[a]	0.78[a]	0.60[a]	0.70[a]	0.50[a]	0.59[a]	0.34	0.19	0.66[a]	−0.11	0.02	0.24	0.47[b]	0.16

[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).

trace metals) can pose a high chronic health risk due to bioaccumulation even if their concentration in water is extremely low. The BCF can provide information about the bioconcentration of specific elements in the surrounding environment. The BCF of each element in the muscular tissue samples of megamouth sharks specified in this study varied by approximately one to two orders of magnitude (Fig. 2). The mean log BCF of various elements in descending order was as follows: Ti (7.85) > Bi (6.46) > Ai (6.04) > Fe (5.86) > Hg (5.46) > Pb (5.08) > Co (4.78) > Mn (4.62) > Cu (4.46) > Zn (4.38) > Ag (4.09) > Cr (3.89) > Ga (3.73) > Ni (3.34) > Cd (3.05) > As (2.90) > Ba (1.51) >Sr (−0.42) > B (−0.44) > K (−0.48) > Li (−0.72) > Mg (−0.93) >Ca (−0.91) > Na (−0.99). Interestingly, the concentrations of Na, Ca, Mg, Li, K, B, and Sr in seawater reached the μM level and these elements were not considered to be the essential elements of marine organisms, but their BCF was less than 1 (Fig. 2c) (Neff, 2002; Fang et al., 2014). The BCF of Ba was also relatively low (4.4–130; average: 33 ± 27), indicating that Ba may not be biologically accumulated in the muscular tissues of megamouth sharks (Arnot and Gobas, 2006). Due to low water solubility, Ba is not prone to accumulation in marine animals (Neff, 2002). In addition, previous studies have shown that the Ba content accumulated in the tissues of carnivorous fishes at a high trophic level or mammals is not higher than that accumulated in carnivorous fishes at a low trophic level, indicating that Ba is not biomagnified in the marine food web (Neff, 2002).

The mean log BCF of As and Cd in the muscular tissues of megamouth sharks was 2.09 and 3.05, respectively. Most marine animals have a limited ability to accumulate As from seawater. For example, the BCF of marine shrimps (*Lysmata seticaudata*) and mussels (*Mytilus galloprovincialis*) ranges from 2 to 10 (Neff, 2002). In general, the concentration of As in marine animals increases with the rise in trophic level (Bosch et al., 2012), but may also be affected by dietary intake and the ability to absorb, metabolize, and accumulate As in their bodies (Rahman et al., 2012a). The concentration of As in the muscular tissues of megamouth sharks specified in this study (1.37 ± 0.95 mg/kg) was lower than that in the muscular tissues of predatory sharks, specifically, *Sphyrna lewini* (12.2 ± 1.08 mg/kg) and *Rhizoprionodon acutus* (19.7 ± 5.7 mg/kg) (Boldrocchi et al., 2019). This may be because megamouth sharks are filter-feeding sharks, which mainly feed on krill, copepods, and gelatinous zooplankton, and are secondary consumers. The log BCF of Cd in the muscular tissues of megamouth sharks ranged from 2.17 to 3.73 and averaged 3.05. The concentration of Cd in fish tissues depends on its concentration in the surrounding water and food intake. However, the concentration of Cd in the muscular tissues of fishes is typically low, because Cd from water or food sources is prone to accumulate preferentially in organ tissues (e.g., livers, digestive glands, and kidneys) rather than muscular tissues (Neff, 2002).

The mean log BCF of Ni, Ga, Cr, and Ag ranged from 3.34 to 4.09. Ni and Cr are considered to be biological essential elements because they play an important role in a variety of enzymes and other cellular components; however, their excessive intake has adverse impact on human health (Demirezen and Uruc, 2006). Ni concentration in the muscular tissues of megamouth sharks ranged from 0.11 mg/kg to 2.89 mg/kg, which is similar to that of other sharks (<0.001–4.20 mg/kg) previously reported (Cornish et al., 2007; Eisler, 2010; Nicolaus et al., 2016; Boldrocchi et al., 2019). The mean concentration of Ni in seawater is 0.47 μg/L (Bruland and Lohan, 2003). According to the above analysis, the log BCF of Ni in shark muscle should range from 3 to 4. Because Cr is an essential micronutrient for organisms, marine organisms have developed mechanisms to accumulate Cr from water and food and regulate its species form, distribution, and concentration in tissues and body

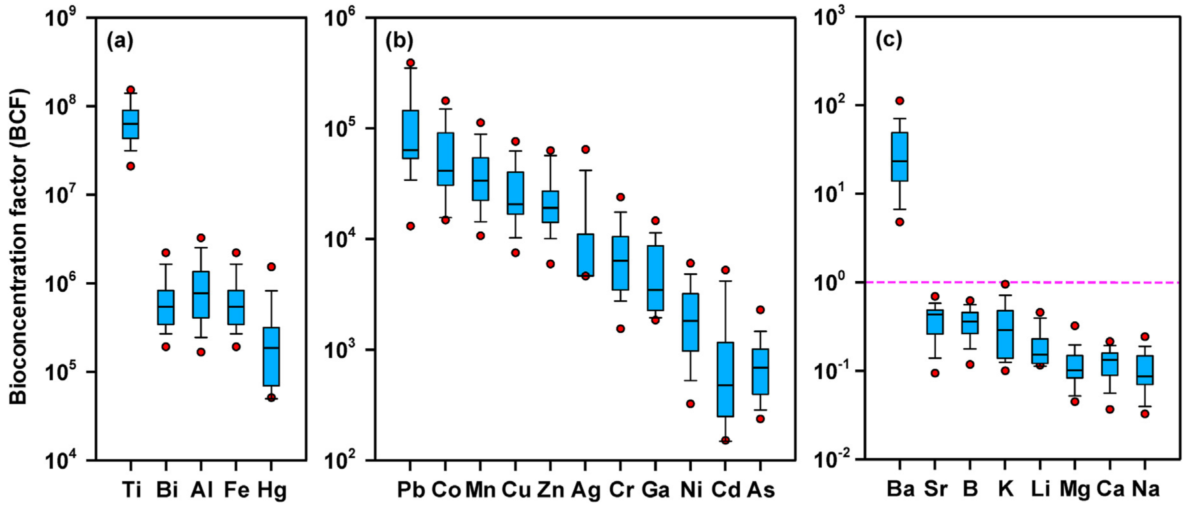

Fig. 2. Box-whisker plots shown the distributions of bioaccumulation factors of elements in the muscular tissues of *Megachasma pelagios*. Line inside the box is the median, lower and upper limits of box respectively represent 25th and 75th percentiles; whiskers extend to the range between the 10th and 90th percentiles; the circles above and below respectively represent to the range between the 5th and 95th percentiles.

fluids (Simkiss and Taylor, 1989). Cr in seawater mainly exists in the form of Cr (III) and Cr (VI). Cr (III) is prone to be adsorbed on suspended particles, and Cr (VI) is prefer to dissolve in seawater. However, Cr (VI) in seawater can be easily reduced to less toxic Cr (III) by marine organisms, dissolved organic matter, and ferrous ion (Fe (II)) (Nakayama et al., 198l, Eary and Rai, 1988). The mean concentration of Cr in seawater is 0.208 μg/L (Bruland and Lohan, 2003). In addition, there is no definite relationship between the concentration of Cr in marine organisms, trophic level, and body burden, indicating that Cr is not biomagnified in the marine food web. The concentration of Cr in the muscle of most fishes is lower than 5 mg/kg (Neff, 2002). Based on the observations of this study and previous literature data, the log BCF of Cr in fish muscle should range from 3 to 5.

Because Ag and Ga are biological nonessential elements, it is expected that megamouth sharks have a low uptake of these elements. However, the mean log BCF of Ag and Ga (4.09 and 3.73, respectively) in the muscular tissues of megamouth sharks was close to the that of essential elements Ni and Cr (Fig. 2b). The log BCF of Ag and Ga was relatively high, possibly because the concentration of Ag (2.16 ng/L) and Ga (1.46 ng/L) dissolved in seawater is far lower than that of essential elements dissolved in seawater (Fang et al., 2014), resulting in high BCF in the muscular tissues of megamouth sharks. Available data on the concentration of Ag and Ga in the muscular tissues of sharks are extremely rare. The mean concentration of Ag (0.027 ± 0.036 mg/kg) in the muscular tissues of megamouth sharks specified in this study is lower than that of the red muscle and white muscle (0.204 mg/kg and 0.406 mg/kg, respectively) of white sharks (*Carcharodon carcharias*) captured in the Southern California Bight (Mull et al., 2012). Previous studies have reported Ag concentrations in the muscular tissues of scalloped hammerhead sharks (*Sphyrna lewini*) and milk sharks (*Rhizoprionodon acutus*) captured in Djibouti (Gulf of Aden) of 0.002 ± 0.003 mg/kg and 0.02 ± 0.01 mg/kg, respectively (Boldrocchi et al., 2019). The mean concentration of Ga in the muscular tissues of megamouth sharks specified in this study was 0.008 ± 0.006 mg/kg. Although there is no literature data on the concentration of Ga in the muscular tissues of sharks, in zooplankton, the main food of megamouth sharks, Ga concentration ranges from 0.07 to 0.87 mg/kg (Fang et al., 2014).

Zn, Cu, Mn, and Co are biological essential elements, and their mean log BCF ranged from 4.38 to 4.78. Zn is ubiquitous in the tissues of marine organisms, which mainly absorb Zn from seawater and food. The concentration of Zn in the muscular tissues of megamouth sharks stranded in Praia Grande, Arraial do Cabo, southeastern Brazil was 18.37 mg/kg (Moura et al., 2015). The concentration of Zn in the muscular tissues of two whale sharks (*Rhincodon typus*) stranded in the Gulf of California, Mexico was 8.49 mg/kg and 26.2 mg/kg (Pancaldi et al., 2019). According to the concentration of Zn in the muscular tissues of the three filter-feeding sharks, the log BCF was 4.41, 4.75, and 4.90. The log BCF of Zn in the muscular tissues of 27 megamouth sharks specified in this study ranged from 3.64 to 4.80, with an average of 4.38. Accordingly, the log BCF of Zn in the muscular tissues of megamouth sharks should range from 4 to 5. Similar to Zn, Cu is also ubiquitous in the tissues of marine organisms. However, the concentration of Cu in the muscular and organ tissues of marine fishes is usually not high, and even the concentration of Cu in the muscular tissues of apex predatory fishes (e.g., tunas and diverse sharks) is less than 2 mg/kg (Neff, 2002). The mean concentration of Cu in the muscular tissues of megamouth sharks was 5.47 ± 3.53 mg/kg, and its log BCF ranged from 3.77 to 4.90, with an average of 4.46. The concentration of Cu is generally higher in the muscular tissues of sharks (e.g., *Rhizoprionodon acutus*) that feed on micronekton (Cornish et al., 2007). As aforementioned, this may explain the relatively high log BCF of Cu in the muscular tissues of megamouth sharks.

The concentration of Mn in the muscular tissues of fishes is rarely higher than 0.5 mg/kg (Eisler, 2010); in other words, the log BCF of Mn in the muscular tissues of fishes is usually lower than 4.48. The mean concentration of Mn in the muscular tissues of megamouth sharks specified in this study was 0.68 ± 0.45 mg/kg, and their mean log BCF was 4.62, indicating a relatively high accumulation of Mn in the muscular tissues of megamouth sharks. The content of Mn in fish tissues increases with increasing fish size (Burger et al., 2007). Petkevich (1967) concluded that the bony tissues of plankton-feeding fishes contain a higher concentration of Mn, Ni, Cr, V, Ti, Sn, Pb, and Sr than those of benthon (Eisler, 2010; El-Moselhy et al., 2014). In addition, some studies have shown that the muscular tissues of sardines contain higher concentrations of Mn, Zn, Fe, Cu, Pb, and Cd than those of other fish species (Chen and Chen, 2001; Abdallah, 2008; Alturiqi and Albedair, 2012).

Therefore, the relatively high accumulation of Mn in the muscular tissues of megamouth sharks may be related to the food they consume. The concentration of Co in the muscular tissues of megamouth sharks specified in this study ranged from 0.016 mg/kg to 0.204 mg/kg, which is similar to that in the muscular tissues of marine fishes previously reported (0.002 mg/kg to 0.36 mg/kg) (Eisler, 2010). The mean concentration of Co dissolved in seawater is 1.18 pg/L (Bruland and Lohan, 2003). According to the measured values in this study, the log BCF of Co ranged from 4 to 6.

Pb is a nonessential element for marine organisms. The content of Pb in marine organisms is accumulated from seawater (Szefer, 1991); moreover, Pb does not post biomagnification in the marine food chain (Dietz et al., 2000; Neff, 2002). However, the bioavailability or toxicity of dissolved Pb to marine organisms is not high (Amiard et al., 1985). The mean concentration of Pb dissolved in seawater is 2.07 μg/L (Bruland and Lohan, 2003). The log BCF of Pb in the muscular tissues of megamouth sharks specified in this study ranged from 3.91 to 5.59, with an average of 5.08. The high BCF of Pb in the muscular tissues of megamouth sharks may be because Pb in organisms enters biological cells through the Ca transportation mechanism and complexes with cytoplasmic calcium-binding proteins (Evans et al., 2003; Marchetti 2013). This may explain higher concentration of Pb in the bone, skin, muscular, and gill tissues of fishes than that in livers and other viscera (Eisler, 2010; Pancaldi et al., 2019).

The mean log BCF of Hg, Fe, Al, and Bi in the muscular tissues of megamouth sharks ranged from 5.46 to 6.46. The log BCF of Hg in the muscular tissues of megamouth sharks ranged from 4.70 to 6.21, with an average of 5.46. The Hg accumulated in marine fish includes organic Hg and inorganic Hg; organic Hg (especially methyl Hg) is far more toxic than inorganic Hg. However, most of the Hg in fish muscle exists in the organic form (usually accounting for 95%), mainly methyl Hg, because fishes absorb methyl Hg more efficiently than inorganic Hg from environmental media and foods and eliminate inorganic Hg faster than methyl Hg (Kehrig et al., 2001; Mason et al., 2006; Moura et al., 2015). In addition, as the accumulation of Hg is related to the age or size of fishes, the muscles of adult or large fishes usually contain a relatively high content of Hg (Adams and Onorato, 2005). The mean concentration of Hg in seawater is as low as 0.20 ng/L (Bruland and Lohan, 2003), and Hg is prone to accumulate in muscular tissues of marine fishes (Mason et al., 2006; Eisler, 2010); therefore, the BCF of Hg in fish muscle is relatively high.

Fe and Al are also considered to be essential elements for marine organisms, but field survey data on their content in fishes are not sufficient. The concentration of Al and Fe in the muscular tissues of megamouth sharks stranded in Praia Grande, Arraial do Cabo, southeastern Brazil was 4.60 mg/kg and 5.18 mg/kg, respectively (Moura et al., 2015). In addition, the concentration of Al and Fe in the muscular tissues of certain predatory sharks ranged from 0.71 to 4.60 mg/kg and from 2.03 to 37.7 mg/kg, respectively (Mull et al., 2012; Kim et al., 2019; Endo et al., 2008; Moura et al., 2015; Nicolaus et al., 2016). The mean concentration of Al and Fe in the muscular tissues of megamouth sharks specified in this study was 58.8 ± 46.8 mg/kg and 20.0 ± 14.8 mg/kg, respectively. According to the above literature review and the values measured in this study, the log BCF of Al and Fe in the muscular tissues of megamouth sharks ranged from 5 to 7. Field survey data on the concentration of Bi in the muscular tissues of sharks are rare, with only one study on the muscular tissues of megamouth sharks stranded in Brazil, which reported Bi concentration of 0.03 mg/kg (Moura et al., 2015). The mean concentration of Bi in the muscular tissues of megamouth sharks specified in this study was 0.042 ± 0.031 mg/kg, and its log BCF ranged from 5 to 7.

Among the 24 elements in this study, Ti in the muscular tissues of megamouth sharks had the highest BCF (Fig. 2a), and its log BCF ranged from 7.22 to 8.18, with an average of 7.85. Few marine organisms accumulate a high concentration of Ti, and its concentration in most marine organisms is lower than 100 mg/kg dry weight; however, some marine organisms (e.g., diatoms, dinoflagellates, sponges, and ascidians) have a high Ti concentration of as high as more than 1,000 mg/kg dry weight (Buettner and Valentine, 2012). Studies have shown that the concentration of Ti in the silica fractions of marine phytoplankton can be as high as 1,254 mg/kg dry weight (Martin and Knauer, 1973). The foods for filter-feeding megamouth sharks are not only zooplankton but also phytoplankton that contained relatively high Ti, which may result in the high bioaccumulation of Ti in their muscular tissues. However, environmental factors and metabolic effects may also be other important influencing factors; for example, the high accumulation of Ti is possibly related to the heavy use of nano-TiO_2 by humans and releasing to environments (Zhu et al., 2011).

To comprehensively understand the distribution of trace elements in megamouth sharks and seawater, we plotted log BCF of all analyzed elements in the muscular tissues of megamouth sharks and their mean dissolved concentration in seawater (Bruland and Lohan, 2003) (as shown in Fig. 3). For elements with a dissolved concentration of 100 μg/L or above in seawater, the log BCF was less than 0. For elements with a dissolved concentration of less than 100 μg/L in seawater, the BCF in the muscular tissues of megamouth sharks appeared to increase with the decline in the dissolved concentration in seawater. Similar results were also obtained in a study on the relationship between the log BCF of zooplankton and the concentration of dissolved elements in seawater (Fang et al., 2014). Interestingly, for elements other than Ti, Al, and Fe, the log BCF was significantly correlated with the dissolved concentration in seawater ($p < 0.01$), wherein the slope of the regression curve was −0.620 and the correlation coefficient was 0.849. According to the linear correlation, the BCF of Ti, Al, and Fe was higher than that of the other elements. As analyzed above, the relatively high BCF of Ti may be because megamouth sharks feed on phytoplankton. The relatively high BCF of Al and Fe may be related to the inorganic suspended solids in seawater. When megamouth sharks filter feed on plankton, they also uptake a large amount of inorganic suspended particles, which usually contain a high proportion of Al (7.70%–8.23%) and Fe (2.7%–8.56%) (Taylor, 1964).

3.3. Relationships between trace elements in muscle tissue

The accumulation of elements in fishes may be correlated with

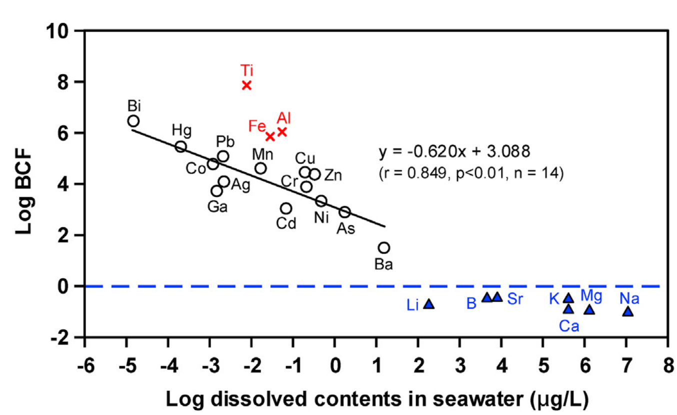

Fig. 3. Scatter plot for log bioconcentration factor (BCF) of varied element in the muscular tissues of *Megachasma pelagios* and the mean dissolved concentrations in seawater (Bruland and Lohan, 2003).

their body parameters (e.g., body weight and full length), and primarily depends on the change in the metabolic activities of organisms. The metabolic activity of fishes decreases as they grow, and young fishes have a higher metabolic rate and higher element accumulation (Canli and Atli, 2003; Bosch et al., 2016). The accumulated elements may also be correlated with each other. Specifically, they may be negatively correlated at the competitive binding sites of elements but positively correlated when elements interact with each other. In addition, elements with a common source or similar diffusion process may also be positively correlated (Rahman et al., 2012b; Bosch et al., 2016). In this study, no significant correlation ($p > 0.05$; the data on r are not available) was observed between the concentration of elements in the muscular tissues and body weight, full length, and conditional factor (K). Previous studies have shown that the correlation between the concentration of various elements in the muscular tissues of fishes and fish size mainly depends on the specific types of elements and fishes (Canli and Atli, 2003; Bosch et al., 2016). In addition, a constant dietary structure may also explain why there is no correlation between element concentration and fish size (Bosch et al., 2016). Megamouth sharks mainly filter feed on plankton, and their dietary structure is relatively constant, which may explain why there is no correlation between the concentration of elements in their muscular tissues and body parameters. To identify the primary reason, it is essential to further explore other factors, including environmental factors and specific metabolic effects of megamouth sharks.

Table 3 lists the Spearman's correlation coefficients between different elements in the muscular tissues of megamouth sharks. Sr, Zn, K, Mg, Li, Hg, and Cd are not significantly correlated with most of the other elements ($p > 0.05$). However, there is a strong significant correlation between Sr and Ca ($p < 0.01$, $r = 0.95$). The hard tissues of many marine organisms (e.g., coral bones, shells of crustacean plankton, and fish otoliths) mainly comprise calcium carbonate. For tissues that mainly comprise calcium carbonate, the Sr–Ca ratio is closely related to water temperature, salinity, and water mass, and is often used to reconstruct the external environmental characteristics experienced by organisms (DeLong et al., 2012). In the muscular tissues of megamouth sharks specified in this study, the slope of linear regression between Sr and Ca was 0.0265 mol/mol, and the correlation coefficient between them was 0.944. The Sr–Ca ratio in the muscular tissues of megamouth sharks was relatively constant; it is important to further explore whether this is because they filter feed crustacean plankton. In addition, a stable Sr–Ca ratio may imply that megamouth sharks inhabit similar water masses over a long period; this is consistent with the fact that megamouth sharks are most commonly found in sea areas along the Kuroshio Current (Liu et al., 2018; Watanabe and Papastamatiou, 2019).

In addition to the above elements, most other elements were significantly correlated to each other ($p < 0.05$ or $p < 0.01$) (Table 3). These correlated elements may come from common sources or share similar diffusion processes (Rahman et al., 2012b; Bosch et al., 2016). The accumulation of elements in the muscular tissues of megamouth sharks is mainly related to their dissolved concentration in seawater (as shown in Fig. 3). However, diet is another important influencing factor in the accumulation of elements. The analysis of gastric contents has shown that megamouth sharks mainly feed on krill, as well as on copepods and gelatinous zooplankton (Sawamoto and Matsumoto, 2012; Moura et al., 2015). The content of Cu in these preys is relatively high because mollusks and crustaceans (including krill) transport oxygen through hemocyanin (Fredrick and Ravichandran, 2012). In addition, these preys also comprise some essential trace elements (e.g., Zn, Fe, Mn, Ni, and Cr) and nonessential elements (e.g., Co, Pb, Ba, Al, and Ti) (Martin and Knauer, 1973; Palmer Locarnini and Presley, 1995; Spicer and Saborowski, 2010; Fang et al., 2014). Therefore, the correlated elements in the muscular tissues of megamouth sharks may also be affected by foods.

According to the correlation, concentration distribution, and BCF between different elements, element accumulation in the muscular tissues of megamouth sharks is primarily affected by the concentration of dissolved elements in seawater, and the accumulation of certain elements (e.g., Hg, As, Cu, Ti, Al, and Fe) appears to be primarily affected by feeding behaviors.

3.4. Estimation of human health risks by consumption

The megamouth shark is a rare shark species not targeted by fishermen. The probability of consuming megamouth shark by humans is low. However, we have seen an increase on species sightings and catches and the information this paper provides can be used as future reference if the megamouth shark became more prevalent in the trade. Table 4 lists the HQ and THI values estimated based on the mean concentration of various elements in the muscular tissues of megamouth sharks and the mean consumption of saltwater fishes by Taiwanese male and female adults. The content of inorganic As in fishes usually accounts for 0.5%–10% of total As; therefore, in this study, the HQ of As was estimated by converting 6% of the total As into the content of inorganic As (Rahman et al., 2012a; Copat et al., 2013; Boldrocchi et al., 2019). The HQ of all estimated elements was lower than 1; therefore, regardless of whether Taiwanese male and female adults eat megamouth shark muscle, there will be no chronic noncarcinogenic health hazard. However, cumulative health risk arises when people are exposed to mixed elements on eating megamouth shark muscle. In view of this, the THI can be determined by summating the HQ of all estimated 15 elements. The results of THI show that the THI of both male and female adults was greater than 1, and the health risk faced by male adults (THI = 1.110) was slightly higher than that by female adults (THI = 1.080). This implies that long-term consumption of megamouth sharks by adults may cause chronic noncarcinogenic health hazards. Obviously, it is necessary to consider the cumulative health risk caused by mixed elements. Even if a single element does not cause any health risk (HQ < 1), this

Table 4
Risk assessment of consuming megamouth shark for Taiwanese male and female adults.

Trace element	C_m (μg/g)	RfD[a] (μg/kg/day)	Hazard quotient (HQ)	
			Male	Female
Ag	0.027	5	0.007	0.007
Hg	0.058	0.3	0.242	0.236
Cd	0.075	1	0.094	0.092
Co	0.071	30	0.003	0.003
Pb	0.25	3.5	0.090	0.088
Ba	0.49	200	0.003	0.003
Mn	0.68	140	0.006	0.006
Ni	1.02	20	0.064	0.062
As	0.08[b]	0.3	0.343	0.334
Cr	1.63	3	0.001	0.001
B	1.63	200	0.010	0.010
Sr	2.96	600	0.006	0.006
Cu	5.47	40	0.171	0.167
Zn	7.82	300	0.033	0.032
Fe	20.0	700	0.036	0.035
Total hazard index (THI)			**1.110**	**1.080**

[a] RfD adopted from USEPA (2020), and Pb and Co from Orisakwe et al. (2017) and Finley et al. (2012).
[b] The value represents the As inorganic form that were calculated taking into account 6% of total As (Boldrocchi et al., 2019).

does not imply that the cumulative health risk of all elements will not cause adverse health hazards; moreover, the consumption of fish muscle involves a mixture of all analyzed elements (Boldrocchi et al., 2019). Among the estimated elements, the HQ of As, Hg, and Cu account for up to 30.9%, 21.8%, and 15.4% of the THI, respectively; therefore, the potential noncarcinogenic health effects may be lesions in the nervous system, reproductive system, blood vessels, kidneys, and livers (Ju et al., 2020; Luvonga et al., 2020). Because of the accumulation of As and Hg in fish muscle and their high toxicity to humans, they are often considered as noteworthy elements in health risk assessment (Ju et al., 2017; Adel et al., 2018; Boldrocchi et al., 2019). However, Cu is also a remarkable element in this study, even though it is an essential element for humans and is quickly excreted through urine and bile after absorption in the human body. Overall, long-term or high-frequency consumption of megamouth shark muscle is not recommended because the metal elements present in them may be harmful to human health.

4. Conclusions

This study is the first to present a profile of 24 trace elements present in the muscular tissues of rare filter-feeding megamouth sharks captured in the Pacific Ocean to the east of Taiwan. The concentrations of elements in the muscular tissues of megamouth sharks ranged from 0.008 mg/kg (Ga) to 1,104 mg/kg (Na), with a difference of approximately six orders of magnitude, and the concentration of each element ranged to an extent of approximately one order of magnitude. The TECI had the strongest correlation with the concentration of Cu in megamouth shark muscle. Therefore, the concentration of Cu in megamouth shark muscle can be used as an indicator of the accumulation of toxic elements in megamouth sharks. For each element in the muscular tissues of megamouth sharks, the log BCF ranged from less than 0 to 7.85. For elements with a dissolved concentration of more than 100 μg/L in seawater, the log BCF was less than 0; for elements with a dissolved concentration of less than 100 μg/L in seawater, the log BCF was inversely proportional to their concentration in seawater. The accumulation of elements in the muscular tissues of megamouth sharks is primarily affected by the concentration of dissolved elements in seawater, and the accumulation of certain elements (e.g., Hg, As, Cu, Ti, Al, and Fe) appears to be primarily affected by feeding behaviors. Long-term consumption of megamouth shark muscle is not recommended because metal elements present in them may be harmful to human health. In particular, the contributions of As, Hg, and Cu to human health risk are 30.9%, 21.8%, and 15.4% respectively.

CRediT authorship contribution statement

Yun-Ru Ju: Conceptualization, Data curation, Writing - original draft, Writing - review & editing. **Chih-Feng Chen:** Methodology, Writing - original draft, Data curation, Formal analysis, Visualization. **Chiu-Wen Chen:** Resources, Supervision, Project administration. **Ming-Huang Wang:** Data curation, Investigation, Validation. **Shoou-Jeng Joung:** Resources, Writing - review & editing. **Chi-Ju Yu:** Resources, Writing - review & editing. **Kwang-Ming Liu:** Resources, Writing - review & editing. **Wen-Pei Tsai:** Resources, Investigation. **Shang Yin Vanson Liu:** Resources, Writing - review & editing. **Cheng-Di Dong:** Resources, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.116161.

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