Journal of Hazardous Materials 203–204 (2012) 13–21

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

Mercury and stable isotope signatures in caged marine fish and fish feeds

Sarayut Onsanit, Min Chen, Caihuan Ke, Wen-Xiong Wang*

State Key Laboratory for Marine Environmental Science, College of Oceanography and Environmental Science, Xiamen University, Xiamen 361005, China

ARTICLE INFO

Article history:
Received 23 July 2011
Received in revised form 16 October 2011
Accepted 6 November 2011
Available online 26 November 2011

Keywords:
Caged fish
Total mercury
Methylmercury
Biomagnification
Stable isotope
Risk assessment

ABSTRACT

Total mercury (THg) and methylmercury (MeHg) concentrations were determined in four species of marine caged carnivorous fish, one species of herbivorous fish and three types of fish feeds (dried pellet feed, forage fish and fish viscera), collected from five cage sites in the rural areas along Fujian coastline, China. For the carnivorous fish, the concentrations of THg and MeHg ranged from 0.03 to 0.31 μg/g and from 0.02 to 0.30 μg/g on wet weight basis, respectively. The concentrations were lower for the herbivorous fish with both within the range of 0.01–0.03 μg/g. Out of the three tested fish feeds, tuna viscera contained the highest level of mercury (0.20 μg/g THg and 0.13 μg/g MeHg), with pellet feed containing the lowest level (0.05 μg/g THg and 0.01 μg/g MeHg). The calculated trophic transfer factor of MeHg was the highest (12–64) for fish fed on pellet feeds, and was the lowest for fish fed on tuna viscera. A significant relationship was found between Hg concentrations in caged fish and in fish feeds, thus Hg was primarily accumulated from the diet. Furthermore, the stable isotope $\delta^{15}N$ was positively correlated with the Hg concentration in two caged sites, indicating that $\delta^{15}N$ may be a suitable tool for tracking mercury in caged fish. We conclude that fish farming may be a good way of reducing the human exposure to Hg because mercury levels can be carefully controlled in such farming systems.

1. Introduction

Over the past decades, marine fish farming along the coastline of China has been experiencing a dramatic growth due to both domestic and foreign demands. The southern Fujian province has witnessed an unprecedented growth in its marine caged fish industry, with the farmed fish generally including species such as the red seabream (*Pagrus major*), the black seabream (*Acanthopagrus schlegelii*), the Japanese seabass (*Lateolabrax japonicus*), the red drum (*Sciaenops ocellatus*), the yellow croaker (*Larimichthys croceus*) and the grouper (*Epinephelus* spp.). Farming of these fish species requires low investment and easy routine farming management. As a result of fish farming, the fish feed industry has also grown dramatically in the region. Almost all the caged marine fish in this area are carnivorous fish that can grow rapidly with high commercial values.

In practice, the farmers generally feed the farmed fish with two different types of feed. One type is the forage fish or trash fish (any small fish that has little value as a food fish) such as the anchovy or clupeids. Moreover, tuna viscera and squid viscera as the byproducts from seafood processing are also used as feed for caged fish [1,2], which can increase the palatability of feeding for these carnivorous fish [3,4]. Another type is the dried artificial feed pellet produced by animal feed factories. The diet composition (fresh fish and artificial feed) of caged fish varies due to the instability of the raw material supply. Recently, Hardy and Lee [5] concluded that the challenges for the aquaculture industry in the 21st century include not only the large quantity needed to meet the increasing demand but also maintaining the seafood quality to prevent mercury contamination.

Due to the biomagnification of mercury (mainly MeHg) in marine fish, there is now a considerable concern regarding the safety of fish consumption. Mercury can build up in certain edible freshwater and marine fish as a result of trophic transfer, and food has been implied as the dominant pathway for mercury uptake in fish [6,7]. In fish, the dominant form of accumulated mercury is MeHg, and the highest concentration of mercury is commonly found in fish occupying the top trophic levels, as well as in larger and older fish [8]. Bioaccumulation of mercury in fish through dietary exposure and trophic transfer are the dominant processes defining the exposure to the fish itself and to human health from fish consumption. Previous studies have reported mercury contamination in Fujian coastal areas [8–10]. There have been some reports of total mercury (THg, ranging from 0.003 to 1.34 μg/g) in economic marine fish from estuary and coastal waters in China [10–12].

* Corresponding author at: Division of Life Science, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong.
Tel.: +852 2358 7346; fax: +852 2358 1559.
E-mail address: wwang@ust.hk (W.-X. Wang).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.11.021

The consumption of marine fish is recommended because they are good nutritional sources of omega-3 fatty acids associated with health benefits. MeHg is a form of mercury that is easily absorbed through the gastrointestinal tract with an efficiency of 90–95% [13]. Thus, although the consumption of marine fish is beneficial, it can also present a risk to humans [14–17]. Many countries are concerned with the health risk of mercury in edible fish. Human health issues from MeHg contamination in fish have been addressed by the World Health Organization (WHO), the UN Food and Agriculture Organization (FAO), the US Environmental Protection Agency (EPA), the US Food and Drug Administration (FDA), and other organizations in several countries [18–20]. These agencies have issued threshold guidance for fish consumers to limit their MeHg exposure from fish consumption. However, there has not been any report of mercury concentration in marine caged fish from Fujian, which now probably hosts one of the largest fish farms in China [21].

In this study, we specifically quantified the THg and MeHg in marine caged fish collected from five marine fish cage sites in Fujian province. Mercury concentrations in five species from different rural cage sites (from the south to the north of Fujian's coastal waters) were compared. Moreover, the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were used as a tool to track the trophic position of the caged fish. Human risk assessments of mercury for average Chinese people consuming cage-reared fish were performed. We focused our study on the caged fish farm mainly because of its very unique system in fish feed practice, thus the biomagnification of Hg may be very different from what is naturally observed in wild fish populations. We also examined the difference in Hg bioaccumulation in caged fish fed on different fish feeds.

2. Materials and methods

2.1. Fish samples

In June 2009, five marine fish species, including the red seabream (*P. major*), the red drum (*S. ocellatus*), the black seabream (*A. schlegelii*), the Japanese seabass (*L. japonicus*), and the rabbitfish (*Siganus fuscessens*), were collected from five fish cages along the Fujian coastline. Fish feeds including the dried pellet feed and fresh feeds (forage fish and fish viscera) were also sampled to monitor the mercury sources in the caged fish culture. The farming in Fujian marine caged fish generally used these fish diets. The forage fish included a variety of small fish such as anchovy and clupeids, while the commercial dried pellets were prepared from fish meal, bean meal, corn or wheat gluten, squid viscera meal, fish oil, and scarp of seed Agro byproducts. The sampling stations from south to north were Dongshan (23°44.539′N, 117°31.081′E), Xiamen Bay (24°21.353′N, 118°04.342′E), Xinghua (25°18.335′N, 119°14.303′E), Fuqing (25°41.169′N, 119°35.167′E), and Luoyuan (26°21.615′N, 119°43.163′E) (Fig. 1). Ten market-sized fish for each species were sampled from each cage station (approximately 500 g for red seabream, 800 g for red drum, 1000–1200 g for seabass, 400 g for black seabream and 100 g for rabbit fish, Table 1). *L. japonicus* and *A. schlegelii* were not available at the Dongshan cage site. *S. fuscessens* was available only at the Dongshan and Xiamen Bay cage sites. Only the Xiamen Bay site was sampled for all five fish species. No gender difference was considered in this study. Fish of similar size were collected to minimize any potential influence of fish size on mercury concentration. Dorsal fish muscle tissues (white muscle) were dissected with clean stainless steel knife and washed with deionized water and then placed in clean ziplock plastic bags

Fig. 1. Sampling sites of caged fish along the coastline of Fujian province.

Table 1
The weight, length, THg, MeHg, percentage of total Hg as MeHg, and $\delta^{13}C$ and $\delta^{15}N$ values in caged fish from Fujian waters. For the statistical analysis of mercury and stable isotope, different letters indicate significant differences between sampling sites ($p < 0.01$). Data are mean ± SD.

Fish species	Weight (kg)	Length (cm)	THg (μg/g ww)	THg (range)	MeHg (μg/g ww)	MeHg (range)	%MeHg	% Moisture	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Red seabream										
Dongshan ($n = 10$)	0.68 ± 0.07	31.9 ± 2.5	0.25 ± 0.03^{b}	0.21–0.31	0.23 ± 0.05^{b}	0.16–0.30	92.8 ± 22.1	71.2 ± 1.5	−16.8 ± 0.4^{b}	13.3 ± 0.3^{b}
Xiamen ($n = 10$)	0.50 ± 0.07	29.7 ± 1.5	0.06 ± 0.01^{a}	0.05–0.07	0.05 ± 0.02^{a}	0.03–0.09	85.5 ± 31.0	76.4 ± 1.3	−15.5 ± 0.2^{d}	13.8 ± 0.1^{bc}
Xinghua ($n = 10$)	0.35 ± 0.07	26.2 ± 1.4	0.08 ± 0.01^{a}	0.06–0.09	0.07 ± 0.01^{a}	0.06–0.08	87.7 ± 9.3	74.0 ± 1.0	−17.7 ± 0.1^{a}	11.9 ± 0.3^{a}
Fuqing ($n = 10$)	0.53 ± 0.10	30.9 ± 1.3	0.07 ± 0.01^{a}	0.06–0.08	0.06 ± 0.01^{a}	0.05–0.07	86.5 ± 5.2	75.6 ± 1.4	−15.7 ± 0.3^{cd}	13.8 ± 0.5^{bc}
Luoyuan ($n = 10$)	0.76 ± 0.06	34.5 ± 1.2	0.06 ± 0.01^{a}	0.05–0.08	0.06 ± 0.01^{a}	0.04–0.08	89.0 ± 12.5	76.0 ± 0.5	−16.1 ± 0.5^{c}	14.1 ± 0.2^{c}
Black seabream										
Xiamen ($n = 10$)	0.36 ± 0.04	26.7 ± 1.4	0.21 ± 0.01^{c}	0.19–0.27	0.20 ± 0.05^{c}	0.11–0.27	95.8 ± 17.6	77.3 ± 1.1	−16.8 ± 0.6^{ns}	14.3 ± 0.4^{ab}
Xinghua ($n = 10$)	0.21 ± 0.04	21.7 ± 1.1	0.16 ± 0.01^{b}	0.12–0.21	0.15 ± 0.02^{b}	0.12–0.17	91.1 ± 6.9	77.4 ± 0.4	−16.0 ± 0.6^{ns}	14.6 ± 0.3^{b}
Fuqing ($n = 10$)	0.27 ± 0.05	23.2 ± 1.5	0.18 ± 0.03^{b}	0.13–0.21	0.16 ± 0.01^{b}	0.13–0.18	91.6 ± 9.9	74.7 ± 0.8	−15.5 ± 0.3^{ns}	13.7 ± 0.5^{a}
Luoyuan ($n = 10$)	0.58 ± 0.08	29.8 ± 1.1	0.10 ± 0.01^{a}	0.09–0.12	0.09 ± 0.03^{a}	0.03–0.13	85.9 ± 21.7	74.9 ± 0.5	−15.9 ± 0.3^{ns}	13.7 ± 0.6^{a}
Seabass										
Xiamen ($n = 10$)	0.55 ± 0.11	38.7 ± 2.7	0.10 ± 0.02^{b}	0.08–0.13	0.09 ± 0.03^{b}	0.05–0.17	93.2 ± 20.8	81.4 ± 1.4	−16.9 ± 0.4^{a}	14.7 ± 0.3
Xinghua ($n = 10$)	1.46 ± 0.16	43.2 ± 3.8	0.09 ± 0.03^{b}	0.06–0.13	0.08 ± 0.02^{b}	0.06–0.10	90.8 ± 9.9	77.4 ± 1.6	−16.7 ± 0.2^{ab}	14.6 ± 0.3
Fuqing ($n = 10$)	2.21 ± 0.36	45.0 ± 3.7	0.09 ± 0.00^{ab}	0.08–0.09	0.07 ± 0.01^{ab}	0.05–0.08	80.7 ± 8.2	74.7 ± 0.8	−16.1 ± 0.7^{b}	14.5 ± 0.4
Luoyuan ($n = 10$)	1.01 ± 0.15	45.7 ± 2.3	0.07 ± 0.01^{a}	0.06–0.08	0.05 ± 0.01^{a}	0.04–0.07	79.7 ± 16.5	75.1 ± 0.8	−16.0 ± 4.3^{b}	14.5 ± 0.3
Red drum										
Dongshan ($n = 6$)	0.76 ± 0.23	41.7 ± 4.5	0.11 ± 0.01^{d}	0.10–0.13	0.09 ± 0.02^{c}	0.07–0.12	82.9 ± 8.6^{ab}	77.5 ± 0.7	−15.1 ± 0.2^{b}	14.8 ± 0.1^{b}
Xiamen ($n = 10$)	0.58 ± 0.13	37.6 ± 2.7	0.04 ± 0.004^{a}	0.03–0.04	0.04 ± 0.01^{a}	0.02–0.05	98.2 ± 20.7^{b}	78.1 ± 1.0	−17.7 ± 0.3^{a}	14.2 ± 0.2^{a}
Xinghua ($n = 10$)	1.04 ± 0.24	44.6 ± 3.5	0.06 ± 0.01^{b}	0.05–0.07	0.05 ± 0.01^{ab}	0.04–0.06	89.5 ± 5.6^{ab}	76.3 ± 0.7	−15.3 ± 0.4^{b}	14.1 ± 0.2^{a}
Fuqing ($n = 10$)	0.76 ± 0.09	39.6 ± 1.9	0.06 ± 0.01^{b}	0.05–0.07	0.05 ± 0.004^{ab}	0.04–0.06	84.5 ± 7.0^{ab}	76.2 ± 0.9	−15.5 ± 0.5^{b}	13.9 ± 0.2^{a}
Luoyuan ($n = 10$)	0.85 ± 0.12	41.7 ± 1.7	0.09 ± 0.02^{c}	0.07–0.13	0.06 ± 0.03^{b}	0.02–0.12	72.4 ± 24.2^{a}	69.7 ± 3.1	−15.2 ± 0.5^{b}	14.7 ± 0.4^{b}
Rabbitfish										
Dongshan ($n = 10$)	0.08 ± 0.00	16.7 ± 0.5	0.02 ± 0.004^{b}	0.01–0.03	0.01 ± 0.002^{a}	0.007–0.01	58.6 ± 23.2^{a}	69.1 ± 0.7	−20.9 ± 0.4^{a}	9.3 ± 0.5^{a}
Xiamen ($n = 10$)	0.10 ± 0.03	17.7 ± 1.5	0.02 ± 0.002^{a}	0.01–0.02	0.02 ± 0.004^{b}	0.01–0.03	88.0 ± 10.7^{b}	77.7 ± 2.5	−17.7 ± 0.4^{b}	11.7 ± 0.6^{b}

and kept in ice box before being transported back to the laboratory. Stainless steel knife was washed three times with deionized water between samples to avoid cross contamination. The fish were kept at −80 °C and all chemical analysis was completed within 3 months after the sampling. All the sample preparation and mercury analysis were conducted in the Hong Kong University of Science and Technology, while the stable isotopes were analyzed in Xiamen University.

2.2. Chemical analysis

2.2.1. THg analysis

All the glasswares used for sampling and standard preparation had been soaked in 4 N HCl for several days, then rinsed four times with deionized distilled water and dried at 80 °C for 24 h prior to use. Total Hg concentrations in the fish and fish diet were determined by the method of EPA 7474 [22]. Fish muscle samples were freeze-dried for 48 h and the average moisture of fish muscles was calculated (Table 1). 20 mg of freeze-dried fish sample and fish diets powder were added into the digesting bottles containing 1 ml of ultrapure HNO_3 (65%) at room temperature for 2 h. The samples were then digested at 80 °C in a heating block for 6 h until the sample became clear. After cooling, the volume of sample was adjusted to 5 ml by adding deionized distilled water. A 1-ml diluted sample was removed and added with 0.5 ml of concentrated HCl and 0.4 ml of bromide/bromate solution (containing 1.39 g of potassium bromate and 5.95 g of potassium bromide in 500 ml) to adjust the volume to 10 ml. A series of standard solutions (from standard stock, 0.1354 g of mercury chloride in 75 ml of deionized water) and sample solutions were analyzed with a THg analyzer (CETAC® Quick trace M-8000 Cold Vapor Atomic Fluorescence mercury analyzer). A standard reference material (mussel tissue, IAEA 142) was used simultaneously for tissue digestion. The spike recoveries of THg from the standard tissues were 97–108%, which were within the acceptable range (90–110%) [23]. All samples were run in batches (30 samples), including two blanks and three duplicates of standard reference sample which were used to correct for background Hg levels and to calculate the method detection limits (2–10 ng/g). The concentrations of THg in fish tissues were expressed as μg/g wet weight or μg/g dry weight, with a wet weight/dry weight ratio of 4.0 for the fish muscle tissues. The concentrations of THg in dried fish feeds were expressed on dry weight basis.

2.2.2. MeHg analysis

Protocols for MeHg analysis in fish tissues and fish diets were modified from USEPA method 1630 [24]. Dried samples (15–20 mg) were placed in 8-mL transparent vials, and added with 1.5 mL of 25% (w/v) KOH methanol solution. The mixed solution was shaken gently and then digested at 85 °C for 3–4 h. The solution was then placed under room temperature for several hours and the final volume was adjusted to 3 mL with methanol prior to analysis. After adding 0.5 mL of citrate buffer, the digested samples were added with 50 μL of sodium tetraethylborate and the volatile MeHg was analyzed with a methylmercury analyzer (methylmercury analysis by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry). Again, the mussel standard reference material (IAEA 142) was digested concurrently, and the MeHg recovery for the standard materials was found to be 94–117%, which was within the acceptable range (80–120%) [23]. Each batch of MeHg sample running included two blanks, three duplicates of standard materials, and 30 samples, with method detection limit for MeHg analysis of 1–5 ng/g. Finally, MeHg was expressed as μg/g wet weight for fish tissues and dry weight for dried fish diets.

2.2.3. Stable isotope analysis

Homogenized samples (fish muscles and fish diets) were dried at 60 °C for 24 h and ground into powder with a mortar and pestle. In this study, lipid fraction in the samples was removed by solvent extraction (the ratio of chloroform to methanol was 2:1), and the residues were centrifuged using a microcentrifuge tube at 6000 rpm and dried at room temperature and later at 60 °C for 24 h. Removing the lipids can eliminate the bias of $\delta^{13}C$ measurements but the solvent extraction can also alter the $\delta^{15}N$ values. However, previous study showed that such removal can only increase the $\delta^{15}N$ value by approximately 0.8‰ for the fish muscle tissues [25]. Thus, we did not correct the $\delta^{15}N$ values due to lipid removal in this study. The powder sample (1 mg) was packed into 4 × 6 mm tin capsules for stable isotope analysis. The stable isotope ratios of N ($\delta^{15}N$) and C ($\delta^{13}C$) were measured with an isotope ratio mass spectrometer (IRMS, Thermo Finnigan®, Bremen, Germany). The $\delta^{13}C$ and $\delta^{15}N$ values were expressed as the deviation from the standards in parts per thousand (‰) according to the following equation:

$$\delta X \ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] \times 1000$$

where $X = {}^{13}C$ or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Atmosphere nitrogen and the Pee Dee belemnite (PDB) were used as the isotope standards for N and C, respectively, in the calculation. In-house standards [oxalic acids (IAEA-C8, $\delta^{13}C = -18.3 \pm 0.2‰$) and KNO_3 (IAEA-N3, $\delta^{15}N = 4.7 \pm 0.2‰$)] were used in the actual measurements. The standards were used after every six sample measurements. Analytical precisions were less than 0.2‰ for C and N isotopic ratios [26–28].

2.3. Data statistical analysis

The total Hg, MeHg, percentage of MeHg, and $\delta^{13}C$ and $\delta^{15}N$ isotopes in the tissue of five species of marine caged fish and among the different caged sites were tested for any significant difference by one-way ANOVA and post hoc Tukey tests using the SPSS package (version 16.0, SPSS Inc., Chicago, IL, USA). Trophic transfer factor (TTF) was calculated as the ratio of MeHg concentrations in caged fish and in its diets. The TTFs of MeHg among the different caged sites were also tested by one-way ANOVA and post hoc Tukey test. Simple linear regression analysis was performed for mercury in fish tissues and in fish feeds, and for logarithm of mercury (THg and MeHg) residues and natural stable isotope ($\delta^{15}N$) to test the site-specific trophic transfer in edible fish.

2.4. Human risk assessment analysis

The human risk assessment for Chinese people was conducted using the reference daily dosage (RfD, 0.1 μg/kg bw/day) previously established by the USEPA [29]. The estimated daily intake (EDI, in μg/kg bw/day) can be calculated from the following equation [2]:

$$EDI = C_{fish} \times \left[\frac{dc_{fish}}{bw} \right]$$

where C_{fish} = average Hg concentration in fish muscle (μg/g wet weight), dc_{fish} = daily fish consumption (g/day) per capita for Chinese people (3 g/person/day as recorded by the FAO) [30], and bw = the average body weight (kg) of the target population. The average Chinese body weight based on 158,666 Chinese from all provinces was 58.1 kg [31]. The hazard quotient (HQ) is a ratio of the exposure estimate to an effect concentration of residues considered to represent a safe environmental dose, and can be calculated by the following equation:

$$HQ = \frac{EDIs}{RfDs}$$

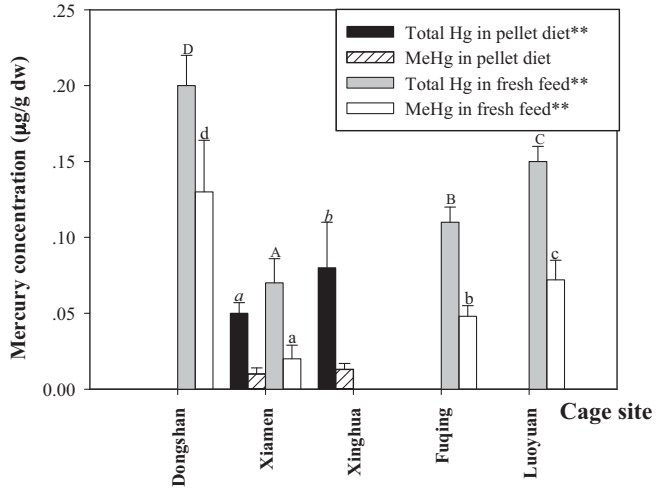

Fig. 2. The total mercury and methylmercury residues (μg/g dry weight) in fish feed from each cage site. Data are mean ± SD ($n = 4$), different letter show significant difference among sites ($p < 0.01$).

There would be no obvious risk if the HQ is less than 1 [2].

3. Results and discussion

3.1. Mercury in fish diets

Fish feed is the main source of mercury for the caged fish. Three types of caged fish feeds were collected, including fresh feeds (tuna viscera from the Dongshan cage site and forage fish from the Xiamen Bay, Fuqing and Luoyuan cage sites) and dried pellet feeds (from the Xiamen and Xinghua cage sites). In Xiamen Bay cage site, the farmed fish were raised on both forage fish and dried pellet feeds. The amount of feeds varied by fish species and by season.

THg and MeHg concentrations in fish feeds were analyzed based on dry weight (Fig. 2). The highest THg and the highest MeHg found in feeds were 0.20 ± 0.02 and 0.13 ± 0.03 μg/g, respectively, for fresh feed (tuna viscera) from Dongshan. The forage fish (the anchovy and clupeids) from Luoyuan contained the second highest concentration of THg and MeHg, where were 0.15 ± 0.01 and 0.07 ± 0.01 μg/g respectively. For the dried pellet feeds, the concentration of THg was higher in the batch from Xinghua than that from Xiamen, but the MeHg concentrations were comparable between these two cages. Our results are similar to those of Liang et al. [31] who found that mercury levels in forage fish were higher than those in feed pellet.

Dried feeds are mainly composed of raw plant materials (soybean meal, corn gluten meal and wheat), fish meal, fish oil and rendered marine animal products (e.g., squid viscera meal). Fish meal and fish oil are typically produced from short-lived small pelagic fish, such as anchovies, sardines, capelin and menhaden, none of which were likely to contain high levels of mercury contamination. In our study, the percentage of MeHg was 65% for the fresh tuna viscera from Dongshan, and 48%, 44% and 23% for the forage fish from Luoyuan, Fuqing and Xiamen, respectively. In previous studies, total mercury and methylmercury in tuna fish were reported to be high in the liver (0.27–3.5 μg/g for THg, and 1.1 ± 0.3 μg/g for MeHg, which was 32% of that for THg) [32–35]. In contrast, MeHg represented 67–91% of THg in tuna muscle [28,36]. It should be pointed out that the level of MeHg in tuna livers generally exceeded the threshold level for feed for caged marine fish. Hardy and Lee [5] noted that fish feed composed of the byproducts of large tuna, marlin, or swordfish may be the main source of Hg contamination in farmed fish.

By comparison, the percentage of MeHg in artificial feed (pellets) was only 21.6% from Xiamen and 17.7% from Xinghua, much lower than those in tuna viscera and in forage fish. Statistical analysis of variances for THg and MeHg in feed showed a significant difference ($p < 0.01$) among cage sites and feed types (Fig. 2). In fact, THg in pellet fish feed has previously been found to be very low (0.02–0.09 μg/g on a dry weight basis) [31,37,38], similar to our present measurements.

The raw materials used in commercial fish feeds are subject to quality control and must meet the food safety standards in terms of nutritional composition and low levels of toxic residues. Thus, it is possible to control the nutritional composition and the metal residues in caged fish farming, whereas wild fish are exposed to mercury via their prey [5]. Fish farmers generally feed the fish with pellets and fresh feed. In order to reduce the exposure of farmed marine fish to contaminants, farming practices and the level of contamination in fish feeds should be closely monitored, and feed production should focus on the quality of raw materials.

3.2. Mercury residue in caged fish

The THg and MeHg tissue concentrations in the five caged fish on a wet weight basis are shown in Table 1. The red seabreams were available from all cage sites. The mercury concentrations were 0.05–0.31 μg/g for THg and 0.03–0.30 μg/g for MeHg. The highest THg and MeHg concentrations of 0.25 μg/g and 0.23 μg/g, respectively, were from Dongshan where the fish was raised only on fresh tuna viscera. An average of 86–93% of THg appeared in the form of MeHg. Again, the highest %MeHg was found in Dongshan, but there was no significant difference among sites. Only about 2% of farmed red seabream exceeded the safety criterion of 0.30 μg/g ww established by USEPA [29].

The levels of mercury in caged black seabream were 0.08–0.27 μg/g (THg) and 0.03–0.27 μg/g (MeHg), with an order of Luoyuan < Xinghua = Fuqing < Xiamen Bay. The percentage of THg in the form of MeHg was 86–96%. Almost all black seabreams were under the safety criterion established by USEPA or the general requirements for biosafety (0.5 μg/g of MeHg in herbivorous fish and aquatic products, and 1.0 μg/g in carnivorous fish) established by the Chinese government [18]. These concentrations are in the lower ends of the range measured for wild seabreams, e.g., 0.10–0.92 μg/g (THg) [39–41]), and 0.13–0.79 μg/g (MeHg) [40,42], with 88% of THg in the form of MeHg [40].

The THg, MeHg and %MeHg in the Japanese seabass were 0.06–0.13 μg/g, 0.04–0.17 μg/g, and 80–93%, respectively. There was significant difference among cage sites for THg and MeHg ($p < 0.01$), but not for %MeHg. The mean mercury concentrations were Luoyuan < Fuqing = Xinghua = Xiamen Bay. These concentrations are below the safety thresholds.

Like seabass, the mercury residues in the red drum muscles were low (0.03–0.13 μg/g for THg, 0.02–0.12 μg/g for MeHg, and 72–98% THg appearing as MeHg). The mercury residues were Xiamen Bay < Fuqing = Xinghua < Luoyuan < Dongshan. Red drums collected from the Dongshan cage site where tuna viscera were used as the feed had the highest Hg concentrations. By comparison, the mercury concentrations in wild red drum measured in Texas and Florida, USA, were within the range of 0.02–3.6 μg/g (THg), with the highest concentration exceeding the EPA's safety criterion by 12 times [43,44].

Rabbitfish, the only herbivorous fish species in this study, were available from two cage sites (Dongshan and Xiamen Bay). In Dongshan, this fish was farmed in the same cage as carnivorous fish and fed the small tuna viscera scraps and grazed the small seaweeds that had grown on the cage net. In contrast, they fed on dried pellet feed prepared from crops in the Xiamen cage. The mean mercury residues in rabbitfish were 0.01–0.03 μg/g for THg, 0.007–0.03 μg/g

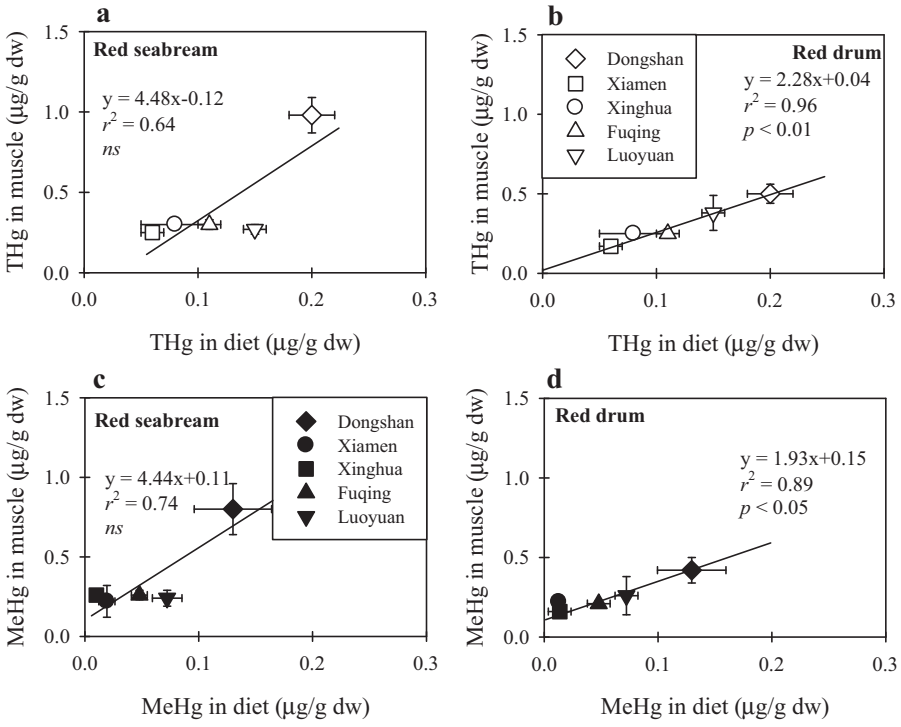

Fig. 3. Correlation between the total mercury and methylmercury concentrations in fish feeds and in caged fish muscles for red seabream (a, b) and red drum (c, d). All concentrations are expressed as μg/g dry weight. Data are mean ± SD (n = 4).

for MeHg, with 59–88% of THg appearing as MeHg. There was a significant difference between the two cage sites (p < 0.01). In previous studies, the Hg concentrations in rabbitfish from Hong Kong, Indonesia, Seychelles and Guam were 0.007–0.05 μg/g (THg), 0.007–0.012 μg/g (MeHg), and 64–69% of THg as MeHg [45–47]. The %MeHg displayed a wide range of variation, due possibly to differences in the rearing practices of caged fish.

Overall, the mercury levels in the farmed fish measured in this study were lower than those measured in the same species of wild fish [26,34,35,48,49]. Such low Hg levels may have primarily been due to the low concentrations of Hg in the fish feeds (except for the tuna viscera). Previous studies have also suggested that the reduced mercury contamination in farmed tuna was due to the growth dilution and the low levels of mercury in fish feed [50].

3.3. Relationships between mercury concentrations in feed and in fish

Fish accumulates mercury mainly through its food. In the present study, the correlation of mercury levels in feed and fish tissue was investigated for red seabream and red drum because both fish species were available in all cage sites (Fig. 3). Both species displayed significant correlations between mercury (THg and MeHg) concentrations in fish and in diet. These results strongly suggest that Hg levels in the fish were highly dependent on those in the fish feeds, and thus trophic transfer contributed predominantly to Hg accumulation in the fish. Magalhães et al. [40] studied the total and MeHg bioaccumulation in eight marine fish species, and similarly found positive correlation between mercury levels in diet and fish muscle. Based on such significant relationships, an important consideration in minimizing the Hg levels in farmed fish is to reduce the Hg levels in the fish feeds.

The biomagnification is defined as the increase in concentration between trophic levels, resulting in a trophic transfer factor (TTF) > 1. A TTF < 1 implies a biodilution of metals in the food chain transfer [27]. Fig. 5 shows the calculated TTF of Hg in the caged fish.

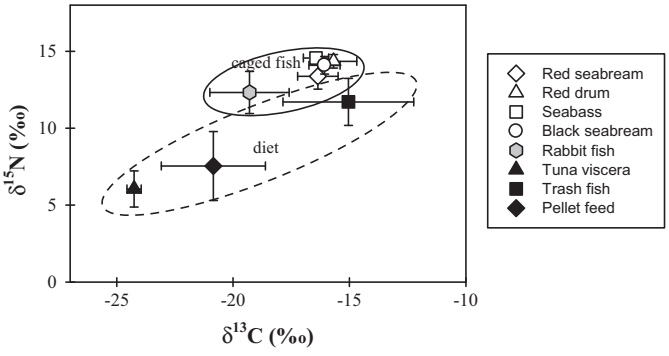

Fig. 4. The average and standard deviation of stable isotope (δ^{13}C and δ^{15}N) values (‰) in fish feed and caged fishes from each site in Fujian marine cage culture.

The highest TTF of MeHg was 20 for red seabream and 17 for red drum from Xinghua, 64 for black seabream, 30 for seabass and 8 for rabbitfish from Xiamen Bay. The TTF was significantly higher (p < 0.01) for caged fish from Xiamen Bay (both fresh and artificial diets fed fish) and Xinghua where dried pellets were used as feed than for caged fish from other sites where fresh feed was used (Dongshan, Fuqing and Luoyuan). Biodilution was only found for rabbitfish in Dongshan which was the only site where MeHg levels in fish were lower than those in feed.

3.4. Natural isotope compositions of feeds and caged fish

The isotope signatures of feeds collected from the Fujian cage sites were quite variable. The δ^{13}C and δ^{15}N values of feeds were −24.5 to −11.2‰ and 3.6 to 13.4‰, with mean values of −24.3‰ and 6.1‰ for tuna viscera, −20.8‰ and 7.9‰ for dried pellet feed, and -15.0‰ and 11.7‰ for forage fish, respectively. The δ^{13}C and δ^{15}N values of carnivorous caged fish muscle (red seabream, red drum, seabass and black seabream) were −17.7 to −15.1‰ and

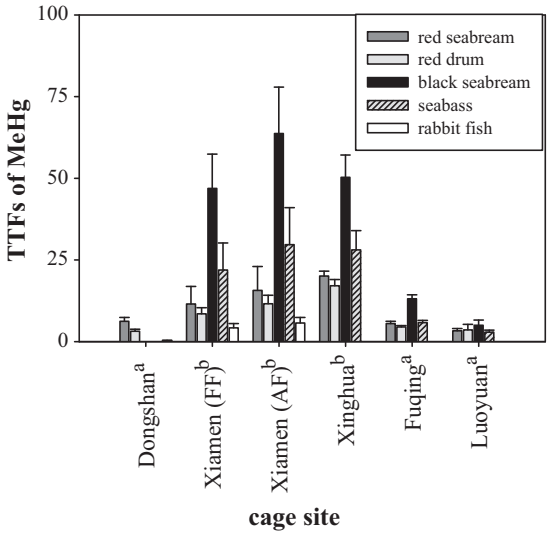

Fig. 5. Trophic transfer factor (TTF) of MeHg (dry weight basis) in caged fish in Fujian cage sites. The different superscript letters on cage sites show significant differences ($p < 0.01$).

11.9 to 14.8‰, and were −20.9 to −17.7‰ and 9.3 to 11.7‰ for rabbitfish, respectively (Fig. 4). The isotope values of tuna viscera and dried pellet feeds were different from those in caged fish, while they were similar between the forage fish and caged fish. The forage fish included anchovy, sardine and clupeids, and the ingredients of dried pellet feeds consisted mainly of plant products and fish meal and fish oil.

The $\delta^{13}C$ and $\delta^{15}N$ values in this study were similar to those of commercial feed produced in Australia [51] and dried pellets used in Japanese fish farms [52]. The $\delta^{15}N$ isotopic value generally increased in carnivorous fish and has been employed to determine metal biomagnification [26,28]. The $\delta^{13}C$ and $\delta^{15}N$ ratios for the rabbitfish were clearly different from those determined for the carnivorous fish, because this detritivorous fish also grazed the small seaweeds that had grown on the cage net.

Fig. 6 shows the regression between mercury concentration and $\delta^{15}N$ in marine fish from different sites. There was significantly positive linear relationship for THg or MeHg and $\delta^{15}N$ from Xiamen and Xinghua sites, whereas fish from other caged sites (Dongshan, Fuqing, Luoyuan) did not show significant relationship with the $\delta^{15}N$ values. The slopes of the linear regression for THg (0.02) were smaller than those found by Al-Reasi et al. [28] for the tropical marine food web in the Gulf of Oman. Moreover, the slopes for MeHg in caged fish were significantly greater in Xiamen ($p < 0.001$) and Xinghua ($p < 0.001$), indicating a stronger biomagnification potential in these two sites. However, these slopes were generally smaller than those documented in natural marine food webs [28]. Unlike wild fish, fish reared in caged culture systems had no choice but to eat whatever the farmers gave them. The positive correlation between MeHg and $\delta^{15}N$ may be used to trace the dominant diet of caged fish, but such correlations may also be dependent on the seasons and the economic values of raw materials, as well as the caged sites. The $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios are generally used to track the importance of a specific carbon source to a consumer and the trophic levels, respectively, and have been applied to investigate the Hg transfer in aquatic food webs [53–55]. For short food chain, as in the case of caged fish, the $\delta^{15}N$ is more suitable than $\delta^{13}C$ to clarify the trophic transfer. Previous studies on aquatic food webs showed that $\delta^{15}N$ values increased by 3–4‰ per trophic level [53,56–58].

3.5. Assessing the risk of caged marine fish consumption for Chinese people

Mercury residue in fish is a major safety concern for countries where large amounts of marine fish are consumed. In this study, the mean mercury concentrations in fish muscles were used to assess the risk of consuming caged marine fish for Chinese people. An average adult Chinese is 58.1 kg in weight [59]. The average daily consumption rate of marine fish was 3 g/person/day in China [29]. The EDIs of MeHg are shown in Table 2. These calculations show that the EDIs of five caged fish species were all lower than 0.1 μg/kg bw/day of the RfD guideline for MeHg established by the US EPA.

The use of RfDs in establishing safe exposures to hazardous substance is well accepted in toxicology [29]. In addition, the Agency for Toxic Substance and Disease Registry (ATSDR) evaluated the same literature used by EPA and estimated a minimum risk level (MRL) of 0.3 μg/kg bw/day for MeHg. In 2003, WHO recommended that the provisional tolerable weekly intake of MeHg be 1.6 μg/kg bw/week. This recommendation was made with the pregnant woman and developing fetus in mind [60]. According to these guidelines, MeHg concentrations in caged fish from Fujian waters did not pose an obvious risk for the average Chinese. Nevertheless, people living on the east coast of China are likely to consume more marine fish than people living further from the coast. In Hong Kong, for example, the estimated daily consumption of marine fish for an individual is 25 g/day [2]. Liang et al. [31] calculated a weekly MeHg intake of approximately 0.37 μg/kg from the consumption of cultured fish in Hong Kong. Dickman and Leung [45] noted that the

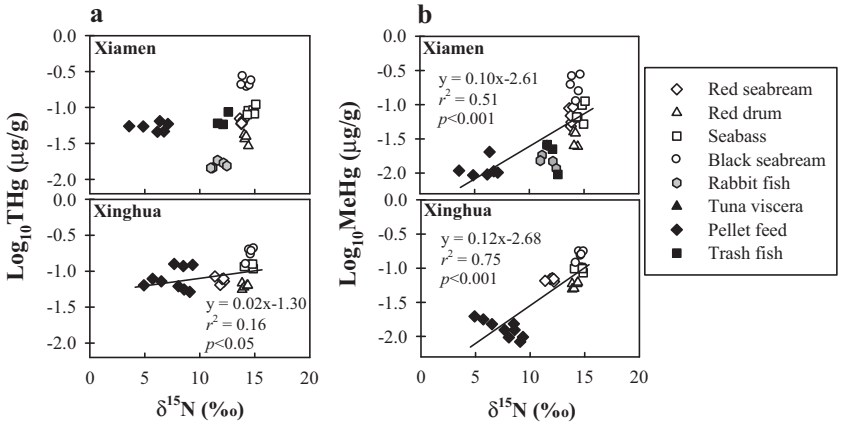

Fig. 6. Relationship between the $\delta^{15}N$ (‰) and the $\log_{10}$ total mercury (a) and $\log_{10}$MeHg (b) concentrations in diet (dry weight basis) and caged fish in Xiamen and Xinghua cage sites.

Table 2
Daily intake of MeHg through marine fish consumption by people in China; EDIs, estimated daily intakes; RfDs, reference doses of MeHg as established by the US EPA; Hazard quotient = EDIs/RfDs. If the ratio is <1, there is no obvious risk.

Fish species	Average concentration (μg/g ww)	EDIs (μg/kg bw/day)	RfDs (μg/kg bw/day)	Hazard quotient
Red seabream (*P. major*)	0.094	0.0049	0.1	0.05
Red drum (*S. ocellatus*)	0.057	0.0029	0.1	0.03
Black seabream (*A. schlegelii*)	0.141	0.0073	0.1	0.07
Seabass (*L. japonicus*)	0.070	0.0036	0.1	0.04
Rabbitfish (*S. fuscessens*)	0.013	0.0007	0.1	0.01

dietary intake of mercury for people in Hong Kong who consumed fish and shellfish four or more time a week might be as high as 1.44 μg/kg/week, which poses a risk to any woman of childbearing age [60]. In this study, the calculated EDIs for marine fish consumption were well below the recommended oral RfDs and MRLs.

In conclusion, this study has found that mercury levels in the five caged marine fish were mostly lower than the safety criterion. Three types of fish feeds including tuna viscera, forage fish and pellet feed were found to be the sources of mercury accumulation in the fish. The MeHg TTF was the highest in carnivorous caged fish raised on pellet feeds. There was a positive correlation between THg and MeHg in feeds and in fish. Indeed, cage-reared marine fish are part of a very short food chain since they are fed directly by farmers.

Acknowledgements

We thank the anonymous reviewers for their helpful comments on this work. Sarayut Onsanit thanks the China Scholarship Council and Thailand's International Development Cooperation Agency for financial support. This study was supported by program for Changjiang Scholars and Innovative Research Team in university (PCSIRT, IRT0941).

References

[1] Y. Guo, H.Y. Yu, B.Z. Zhang, E.Y. Zeng, Persistent halogenated hydrocarbons in fish feeds manufactured in South China, J. Agric. Food Chem. 57 (2009) (2009) 3674–3680.
[2] S. Onsanit, C.H. Ke, X.H. Wang, K.J. Wang, W.-X. Wang, Trace elements in two marine fish cultured in fish cages in Fujian province, China, Environ. Pollut. 158 (2010) 1334–1342.
[3] K. Mai, H. Li, Q. Ai, Q. Duan, W. Xu, C. Zhang, L. Zhang, B. Tan, Z. Liufu, Effects of dietary squid viscera meal on growth and cadmium accumulation in tissue of Japanese seabass, *Lateolabrax japonicus* (Cuvier 1828), Aquaculture Res. 37 (2006) 1063–1069.
[4] F. Dang, W-X. Wang, Assessment of tissue-specific accumulation and effects of cadmium in a marine fish fed contaminated commercially produced diet, Aquat.Toxicol. 95 (2009) 248–255.
[5] R.W. Hardy, C.-S. Lee, Aquaculture feed and seafood quality, Bull. Fish. Res. Agency 31 (2010) 43–50.
[6] B.D. Hall, R.A. Bodaly, R.J.P. Fudge, J.W.M. Rudd, D.M. Rosenberg, Food as the dominant pathway of methylmercury uptake by fish, Water Air Soil Pollut. 100 (1997) 13–24.
[7] W.-X. Wang, P.S. Rainbow, Comparative approach to understand metal accumulation in aquatic animals, Comp. Physiol. Biochem. 148C (2008) 315–323.
[8] L. Zhang, M.H. Wong, Environmental mercury contamination in China: sources and impacts, Environ. Int. 33 (2007) 108–121.
[9] Z.H. Ding, J.L. Liu, L.Q. Li, H.N. Lin, H. Wu, Z.Z. Hu, Distribution and speciation of mercury in surficial sediments from main mangrove wetlands in China, Mar. Pollut. Bull. 58 (2009) 1319–1325.
[10] M. Yatawara, S.H. Qi, O.J. Owago, Y. Zhang, D. Yang, J.P. Zhang, J.E. Burnet, Organochlorine pesticide and heavy metal residues in some edible biota collected from Quanzhou Bay and Xinghua Bay, Southeast China, J. Environ. Sci. China 22 (2010) 314–320.
[11] J. Fang, K.H. Wang, J.L. Tang, Y.M. Wang, S.J. Ren, H.Y. Wu, J. Wang, Copper, lead, zinc, cadmium, mercury, and arsenic in marine products of commerce from Zhejiang coastal area, China, May 1998, Bull. Environ. Contam. Toxicol. 73 (2004) 583–590.
[12] J.L. Gan, X.P. Jia, Hygienic quality for heavy metals of economic fish in coastal water of China, Mar. Sci. Bull. 15 (1997) 88–93 (in Chinese).
[13] M.I. Castro-Gonzalez, M. Mendez-Armenta., Heavy metals: implications associated to fish consumption, Environ. Toxicol. Phamacol. 26 (2008) 263–271.
[14] K.R. Mahaffey, Fish and shellfish as dietary sources of methylmercury and the omega-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits, Environ. Res. 95 (2004) 414–428.
[15] G.J. Myers, P.W. Davidson, J.J. Strain, Nutrient and methyl mercury exposure from consuming fish, J. Nutr. 137 (2007) 2805–2808.
[16] K.L. Smith, J.L. Guentzel, Mercury concentration and omega-3 fatty acid in fish and shimp preferential consumption for maximum health benefits, Mar. Pollut. Bull. 60 (2010) 1615–1618.
[17] J.K. Virtanen, D. Mozaffarian, S.E. Chiuve, E.B. Rimm, Fish consumption and risk of major chronic disease in men, Am. J. Clin. Nutr. 88 (2008) 1618–1625.
[18] G.B. Wiersma, Maximum levels of contaminants in foods, General requirements for Biosafety (GB 2762–2005), National standards for the People's Republic of China, 2005, 7 pp. (in Chinese).
[19] Food Standards Australia New Zealand, Mercury in fish, Factsheet, 2004. http://www.foodstandards.gov.au/_srcfiles/FS_Mercury_in_fish_final.pdf.
[20] Japanese Ministry of Health, Labour and Welfare, Advice for pregnant woman on fish consumption concerning mercury contamination, 2003. http://www.mhlw.go.jp/english/wp/other/councils/mercury/index.html.
[21] J.X. Chen, H. Xu, Z.X. Chen, Y.T. Wang Marine fish cage culture in China. Network of Aquaculture Centers in Asia-Pacific (NACA), 2006, pp. 1–22. http://library.enaca.org/NACA-Publications/MaricultureWorkshop/SpecialReview_MarineFishCageFarminginChina.pdf.
[22] USEPA, U.S. Environmental Protection Agency, Method 7474 Mercury in sediment and tissue samples by atomic fluorescence spectrometry, 2007.
[23] M.L. Olson, J.F. DeWild, Techniques for the collection and species-specific analysis of low level of mercury in water, sediment, and biota. U.S. Geological Survey, Water Resources Investigations Report 99-4018B (1999) 191–202.
[24] USEPA, U.S. Environmental Protection Agency, Method 1630 Methylmercury in Water by distillation, Aqueous Ethylation, Purge and Trap, and CVAFS, 2001.
[25] C.J. Sweeting, N.V.C. Polunin, S. Jennings, Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues, Rapid Commun. Mass Spectrom. 20 (2006) 595–601.
[26] S. Vizzini, C. Tramati, A. Mazzola, Comparison of stable isotope composition and inorganic and organic contaminant levels in wild and farmed bluefin tuna, *Thunnus thynnus*, in the Mediterranean Sea, Chemosphere 78 (2010) 1236–1243.
[27] L.M. Campbell, R.J. Norstrom, K.A. Hobson, D.C.G. Muir, S. Backus, A.T. Fisk, Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay), Sci. Total Environ. 351 (2005) 247–263.
[28] H.A. Al-Reasi, F.A. Ababneh, D.R. Lean, Evaluating mercury biomagnification in fish from a tropical marine environment using stable isotopes (^{13}C and ^{15}N), Environ. Toxicol. Chem. 26 (2007) 1572–1581.
[29] EPA, US Environmental Protection Agency, Integrated Risk Information System: Methylmercury (MeHg), 2001. http://www.epa.gov/iris/subst/0073.htm.
[30] FAO, Food security statistics: food consumption. Statistics Division, Food and Agricultural Organization of the United Nations, 2008. http://www.fao.org/es/ESS/faostat/foodsecurity/index_en.htm.
[31] P. Liang, D.-D. Shao, S.-C. Wu, J.-B. Shi, X.-L. Sun, F.-Y. Wu, S.C.L. Lo, W.-X. Wang, M.H. Wong, The influence of mariculture on mercury distribution in sediments and fish around Hong Kong and adjacent mainland China waters, Chemosphere 82 (2011) 1038–1043.
[32] P. Licata, D. Trombetta, M. Cristani, C. Naccari, D. Martino, M. Calo, F. Naccari, Heavy metals in liver and muscle of bluefin tuna (*Thunnus thynnus*) caught in the straits of Messina (Sicily, Italy), Environ. Moni. Assess. 107 (2005) 239–248.
[33] M.M. Storelli, R.G. Stuffler, G.O. Marcotrigiano, Total and methylmercury residues in tuna-fish from the Mediterranean Sea, Food Additives Contam. 19 (2002) 715–720.
[34] Y. Yamashita, Y. Omura, E. Okazaki, Total mercury and methylmercury levels in commercially important fishes in Japan, Fish. Sci. 71 (2005) 1029–1035.
[35] E. Srebocan, J. Pompe-Gotal, A. Prevendar-Crnic, E. Ofner, Mercury concentrations in captive Atlantic bluefin tuna (*Thunnus thynnus*) farmed in the Adriatic Sea, Vet. Med. 52 (2007) 175–177.
[36] M.M. Storelli, R. Giacominelli-Stuffler, A. Storelli, G.O. Marcotrigiano, Accumulation of mercury, cadmium, lead and arsenic in swordfish and bluefin tuna from the Mediterranean Sea: A comparative study, Mar. Pollut. Bull. 50 (2005) 1004–1007.
[37] M.H. Choi, J.J. Cech, Unexpectedly high mercury level in pelleted commercial fish feed, Environ. Toxicol. Chem. 17 (1998) 1979–1981.
[38] M.D.L. Easton, D. Luszniak, E. Von der Geest, Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed, Chemosphere 46 (2002) 1053–1074.

[39] T. Agusa, T. Kunito, A. Sudaryanto, I. Monirith, S. Kan-Atireklap, H. Iwata, A. Ismail, J. Sanguansin, M. Muchtar, T.S. Tana, S. Tanabe, Exposure assessment for trace elements from consumption of marine fish in Southeast Asia, Environ. Pollut. 145 (2007) 766–777.
[40] M.C. Magalhães, V. Costa, G.M. Menezes, M.R. Pinho, R.S. Santos, L.R. Monteiro, Intra- and inter-specific variability in total and methylmercury bioaccumulation by eight marine fish species from the Azores, Mar. Pollut. Bull. 54 (2007) 1654–1662.
[41] E. Groth, Ranking the contributions of commercial fish and shellfish varieties to mercury exposure in the United States: implications for risk communication, Environ. Res. 110 (2010) 226–236.
[42] Y. Zhang, S. Nakai, S. Masunaga, An exposure assessment of methyl mercury via fish consumption for the Japanese population, Risk Anal. 29 (2009) 1281–1290.
[43] D.R. Sager, Long-term variation in mercury concentrations in estuarine organisms with changes in releases into Lavaca Bay, Texas, Mar. Pollut. Bull. 44 (2002) 807–815.
[44] D.H. Adams, G.V. Onorato, Mercury concentrations in red drum, Sciaenops ocellatus, from estuarine and offshore waters of Florida, Mar. Pollut. Bull. 50 (2005) 291–300.
[45] M.D. Dickman, K.M.C. Leung, Mercury and organochlorine exposure from fish consumption in Hong Kong, Chemosphere 37 (1998) 991–1015.
[46] G.R.W. Denton, L.P. Concepcion, H.R. Wood, R.J. Morrison, Trace metals in marine organism from four harbours in Guam, Mar. Pollut. Bull. 52 (2006) 1784–1804.
[47] Centre for Food Safety, Mercury in Fish and Food Safety, Risk Assessment Studies Report No. 31. Food and Environmental Hygiene Department, The Government of the Hong Kong Special Administrative Region, 2008.
[48] B.C. Kelly, M.G. Ikonomou, D.A. Higgs, J. Oakes, C. Dubetz, Mercury and other trace elements in farmed and wild salmon from British Columbia, Canada, Environ. Toxicol. Chem. 27 (2008) 1361–1370.
[49] Y.-W. Qiu, D. Lin, J.-Q. Liu, E.Y. Zeng, Bioaccumulation of trace metals in farmed fish from South China and potential risk assessment, Ecotoxicol. Environ. Safety 74 (2010) 284–293.
[50] S. Balshaw, J.W. Edwards, K.E. Ross, D. Ellis, D.J. Padula, B.J. Daughtry, Empirical models to identify mechanisms driving reductions in tissue mercury concentration during culture of farmed southern bluefin tuna *Thunnus maccoyii*, Mar. Pollut. Bull. 56 (2008) 2009–2017.
[51] G.M. Turchini, G.P. Quinn, P.L. Jones, G. Palmeri, G. Gooley, Traceability and discrimination among differently farmed fish: a case study on Australian murrey cod, J. Agric. Food Chem. 57 (2009) 274–281.
[52] H. Yokoyama, K. Abo, Y. Ishihi, Quantifying aquaculture-derived organic matter in the sediment in and around a coastal fish farm using stable carbon and nitrogen isotope ratios, Aquaculture 254 (2006) 411–425.
[53] M.S. Gustin, L. Saito, M. Peacock, Antropogenic impacts on mercury concentrations and nitrogen and carbon isotope ratios in fish muscle tissue of the Truckee River water shed, Nevada, USA, Sci. Total Environ. 347 (2005) 282–294.
[54] G. Cabana, J.B. Rasmussen, Modeling good chain structure and contaminant bioaccumulation using stable nitrogen isotopes, Nature 372 (1994) 255–257.
[55] L. Atwell, K.A. Hobson, H.E. Welch, Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis, Can. J. Fish. Aquat. Sci. 55 (1998) 1114–1121.
[56] B.J. Peterson, B. Fry, Stable isotopes in ecosystem studies, Ann. Rev. Ecol. Syst. 18 (1987) 293–320.
[57] G.W. Kling, B. Fry, W.J. O'Brien, Stable isotopes and planktonic trophic structure in arctic lakes, Ecology 73 (1992) 561–566.
[58] M.S. Cheung, W.-X. Wang, Analyzing biomagnifications of metals in different marine food webs using nitrogen isotopes, Mar. Pollut. Bull. 56 (2008) 2082–2105.
[59] D.F. Gu, J. He, X.F. Duan, K. Reynolds, X.G. Wu, J. Chen, G.Y. Huang, C.H. Chen, P.K. Whelton, Body weight and mortality among man and woman in China, J. Am. Med. Assoc. 295 (2006) 776–783.
[60] JECFA, Summary and Conclusions of the 61st Meeting of the Joint FAO/WHO Expert Committee on Food Additives, JECFA/61/Sc, Rome, Italy, 10–19 June 2003, pp. 1–22.