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Sequestration of total and methyl mercury in different subcellular pools in marine caged fish

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ABSTRACT

Mercury contamination is an important issue in marine fish, and can cause toxicity to human by fish consumption. Many studies have measured the mercury concentrations in fish and estimated the threshold levels of its risk to human, but the mercury sequestration in different subcellular pools of fish is unclear. In this study, we investigated the concentration and distribution of total mercury (THg) and methylmercury (MeHg) in different subcellular fractions in the farmed red seabream, red drum, and black seabream from Fujian marine fish farms, China. We found that both THg and MeHg were dominantly bound with the cellular debris, followed by metallothionein-like protein > metal-rich granule > heat-denatured protein > organelles pools. In general, Hg bound with the metal-sensitive fraction was small, indicating that Hg may have little toxicity to the fish (muscle). For the first time we showed that MTLP fraction had the highest % of total Hg as MeHg (88–91%) among all the subcellular fractions. Furthermore, the mercury concentration and subcellular distribution in the black seabream were both dependent on the fish size. Subcellular study may shed light on the detoxification of marine fish to Hg exposure and the potential bioavailability to humans due to fish consumption.

1. Introduction

Mercury (Hg) is a non-essential metal and toxic substance, and may cause toxicity to human as a result of dietary exposure. Among the different Hg compounds, methylmercury (MeHg) is a neurotoxin and may cause myocardial infarction risk and coronary heart disease to human [1–3]. In fish, more than 90% of mercury accumulation is in MeHg form [1,4]. Recently, mercury contamination in marine farmed fish has drawn much attention [5–8]. Unlike the wild fish with complex food web dynamics, the fish farming is governed by economic value manipulation, and its food chain simply consists of fish feeds and farmed fish. Mercury levels can rise up in the fish as a result of trophic transfer, which is a dominant pathway for most of metals accumulation in fish [9–11]. Due to the concerns for Hg contamination, most previous studies determined the Hg concentrations in fish body, as well as the environmental or biological (e.g., age and size) factors impacting Hg accumulation [12,13].

Recently, differential fractionation approach has been used to identify the subcellular fates of metals in aquatic organisms. With this approach, metals can be fractionated to examine their distributions in several operationally defined subcellular fractions, including the metal-rich granules (MRG), cellular debris, organelles, heat denatured protein (HDP), and metallothionein-like protein (MTLP) [14]. The subcellular compartmentalization of various metals in aquatic animals has been investigated in many invertebrates such as polychaetes, molluscs, and crustaceans, and in fish [14–20]. Several laboratory studies have also attempted to understand the ecotoxicological relevance of such subcellular fractions, e.g., linking the metal subcellular distribution in prey and their assimilation by predators through trophic transfer [21–23]. In contrast to these earlier studies, the subcellular fates of metals in fish collected from the fields (natural populations) have been less well known [24–27]. This is especially true for Hg (including both inorganic and methylmercury), although limited studies in the laboratory had used radiotracers to explore their subcellular compartmentalization [28,29]. Bebianno et al. [30] investigated the total mercury and MTLPs levels in the black scabbard fish (*Aphanopus carbo*) from Portugal waters, in which the size dependent relationship was explored.

Examination of the intracellular distribution may give clue to the mercury speciation, sequestration and detoxification, as well as the bioavailability [31]. In a biological system (e.g., intracellular unit), Hg does not exist as free ion (unbound) or inorganic salts form. Instead, Hg^{2+} has a high affinity for thiol-complexes and always conjugates to one or more of glutathione, cysteine, homocysteine,

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Table 1
Total mercury, methymercury, and percentage of total Hg as methylmercury in marine cage fishes collected from Fujian farming sites. Different letters indicate significant difference between farming sites ($p < 0.05$). Mean ± SD.

Fish species	Cage site	Weight (g)	Length (cm)	THg (ng/g ww.)	MeHg (ng/g ww.)	% Total Hg as MeHg
Red seabream						
($n = 4$)	Dongshan	713 ± 28	33 ± 2	251 ± 31.8^{b}	223 ± 12.3^{c}	90.1 ± 13.2^{b}
	Xiamen	540 ± 20	30 ± 1	54.4 ± 3.4^{a}	30.8 ± 3.2^{a}	56.6 ± 4.7^{a}
	Xinghua	383 ± 9	27 ± 0.3	79.3 ± 12.1^{a}	68.2 ± 6.9^{b}	87.2 ± 13.5^{b}
	Fuqing	536 ± 26	31 ± 1	71.5 ± 8.0^{a}	62.7 ± 11.3^{b}	87.2 ± 7.1^{b}
	Luoyuan	749 ± 12	35 ± 1	65.9 ± 9.1^{a}	61.8 ± 6.7^{b}	94.1 ± 4.6^{b}
Red drum						
($n = 4$)	Dongshan	770 ± 84	42 ± 2	109 ± 7.4^{b}	90.5 ± 16.3^{b}	82.4 ± 10.2^{ns}
	Xiamen	669 ± 29	40 ± 0.4	35.9 ± 4.5^{a}	39.2 ± 10.1^{a}	88.3 ± 9.9^{ns}
	Xinghua	1075 ± 87	44 ± 2	62.9 ± 4.2^{a}	54.8 ± 6.8^{ab}	87.0 ± 7.1^{ns}
	Fuqing	729 ± 15	39 ± 2	59.3 ± 8.3^{a}	50.4 ± 5.4^{ab}	85.5 ± 8.7^{ns}
	Luoyuan	884 ± 44	42 ± 1	91.8 ± 23.5^{b}	62.6 ± 41.7^{ab}	65.7 ± 31.1^{ns}
Black seabream						
($n = 29$)	Xiamen	304 ± 125	24 ± 4	177 ± 57.4	171 ± 65.8	95.5 ± 15.5

N-acetylcysteine, metallothionein, or other S-containing moleculars [32]. Previously, it was suggested that MRG and MTLP can be combined as the biologically detoxified metals (BDM), while the metal soluble fractions (MTLP + HDP) and organelles can be combined as the trophically available metals (TAM) [14]. Metals in the HDP and organelles are considered as the metal-sensitive fraction (MSF) [15]. Such combination of different subcellular pools has ecotoxicological relevance for the study of subcellular distributions of metals in aquatic animals.

In the present study, we first investigated the mercury concentrations (total mercury and methylmercury) in three marine caged fishes (red seabream, red drum, and black seabream) collected from different farming sites in the rural areas of Fujian province, Southern China. The concentrations of Hg in the five subcellular binding pools, including MRG, cellular debris, organelles, HDP, and MTLP, were then determined. Mercury concentrations and subcellular distribution in two of these fish (red seabream and red drum) among different sites were compared. More importantly, we for the first time quantified the % of total Hg as MeHg in each of these subcellular fractions. Finally, we examined the influences of body size (weight and length) on mercury tissue concentration, subcellular distribution, as well as the % of total Hg as MeHg in these fractions in the black seabreams.

2. Materials and methods

2.1. Samples

Two species of marine fish, the red seabream (*Pagrus major*) and the red drum (*Sciaenops ocellatus*) of marketed sizes were collected from five fish cages along the Fujian coastline. The sampling stations from south to north were Dongshan, Xiamen Bay, Xinghua, Fuqing and Luoyuan cage farmings. The mean fish sizes were 584 ± 138 g in weight and 31 ± 3 cm in standard length for red seabream, and 825 ± 156 g in weight and 42 ± 2 cm in standard length for red drum. In addition, another economically marine fish of different sizes ($n = 29$) of black seabream (*Acanthopagrus schlegelii*) was purchased from the Xiamen farming site (159–660 g in weight and 19.0–33.2 cm standard length), to examine the relationship between fish body size and mercury concentration in different subcellular binding pools. At the sampling sites, the fish flesh muscles were dissected with stainless steel knife, washed with deionized water, and then placed in clean small polyethylene bottles kept in liquid nitrogen. The samples were subsequently transported back to the laboratory and stored at −80 °C prior to analysis.

2.2. Chemical analysis

2.2.1. Subcellular fractionation for mercury analysis

Subcellular metal fraction analysis was performed using the modified method of Wallace and Luoma [14]. This operationally defined method has been widely used to study the metal subcellular partitioning in fish in previous studies [29,33,34]. In the present study, we did not specifically optimize the experimental conditions for the new species of fish samples, but instead used the methods published previously [14,29,33]. One gram of fish muscle samples were added with cold 20 mM Tris–HCl buffer containing Tris (hydroxymethyl) aminomethane (50 mM, pH 7.4) at a weight ratio of 1:10. An anti-oxidant (2-mercaptoethanol, 5 mM) and a protease inhibitor (phenylmethanesulfonyl fluoride, 0.1 mM) were also added to the buffer. The tissues were homogenized (Ultra-Turrax T25 Basic, IKA, Staufen, Germany) for 5 min at 4 °C and then centrifuged at 1500 × *g* for 15 min, producing a pellet containing tissue fragments and other cellular debris, and a supernatant. The pellets were examined under the microscope, and it was found that the cells were completely broken. Similarly, Dang and Wang [29] found that more than 95% of the cells were completely broken with the same homogenization protocol. Pellets were added with 1 N NaOH (at a ratio of 1:1) and boiled in a water bath (100 °C) for 10–15 min and then centrifuged at 5000 × *g* for 10 min to separate the pellet (metal-rich granule, MRG) and second supernatant (cellular debris). The supernatants were collected and placed in vials, and then centrifuged at 100,000 × *g* and 4 °C for 1 h to separate the pellet (organelles) and protein supernatant. The protein supernatant was boiled at 80 °C for 10 min, and then placed in ice box for 1 h, after which it was centrifuged at 50,000 × *g* and 4 °C for 20 min to separate the two final products, including the heat-denatured protein (HDP) and the metallothionein-like proteins (MTLP) [14]. All the five fractions were freeze-dried and then subjected to total mercury and methylmercury measurements described below. A mass balance calculation showed an 85–107% recovery of Hg from all the subcellular fractions.

2.2.2. Total mercury analysis

All the glassware used for sample and standard preparation were soaked in 4 N HCl for at least 48 h, rinsed four times with Milli-Q water, and dried at 60 °C prior to use. Total mercury residue in fish muscle was determined by the method of EPA 7474 [35]. Around 20 mg of freeze-dried muscle tissues were placed in a 20 mL glass vial with Teflon lining cap. Two milliliters ultrapure HNO_3 (65%) was added to the vial which was then capped tightly.

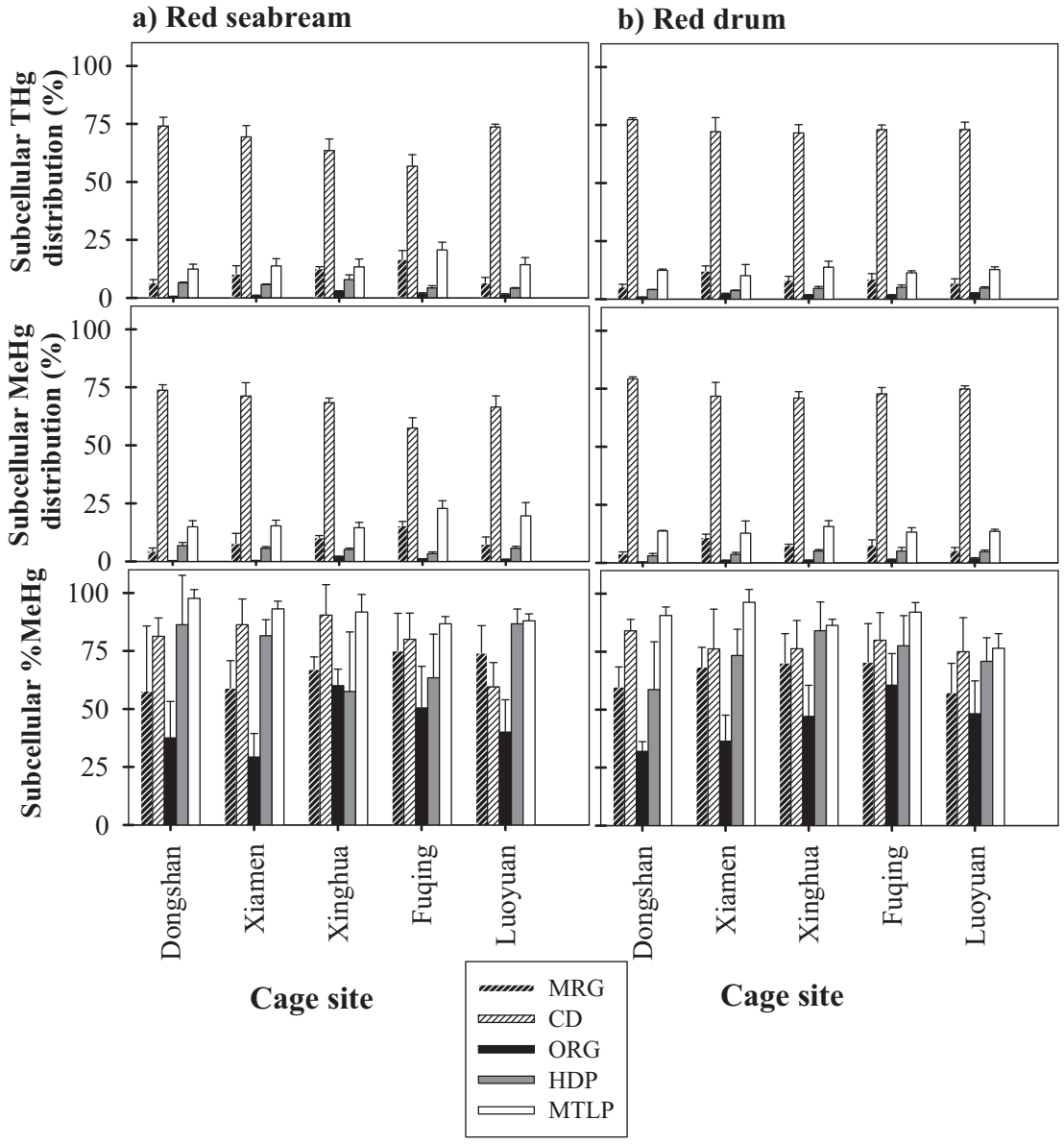

Fig. 1. Subcellular distributions of total mercury and methylmercury in red seabream (a) and red drum (b) from different cage sites. The % of total Hg as methylmercury in different subcellular fractions is also shown on the bottom. MRG: metal-rich granules; CD: cellular debris; ORG: organelles; HDP: heat-denatured proteins; MTLP: metallothionein-like proteins. Mean ± SD ($n = 4$).

Also, the separated subcellular fractions were dissolved in ultrapure HNO_3 and transferred into clean glass vials. All samples were heated at 60 °C in an oven for 16 h. After cooling, the digested sample was adjusted to 5 ml with Milli-Q water. An aliquot of digested samples was taken as appropriate and diluted to 10 mL. For complete oxidization, diluted sample was spiked with BrCl solution and was further digested overnight at room temperature for subsequent THg analysis. All digested samples were pre-reduced by addition of $NH_2OH{\cdot}HCl$. THg was analyzed using the single gold trap amalgamation technique by Cold Vapor Atomic Fluorescence Spectrometry (CVAFS, CETAC® Quick Trace M-8000). A standard reference material (SRM) mussel tissue (IAEA 142) was digested simultaneously and the recovery was 97–108% (a range of 90–110% recovery was considered acceptable [36]). Each batch running included 30 samples and blanks which were used to correct for background levels of Hg and to calculate the method detection limits (2–10 ng/g).

2.2.3. Methylmercury analysis

Matrix of methylmercury in fish tissue and subcellular fractions were determined by modified method from USEPA 1630 [37]. Freeze-dried muscle tissues (15–20 mg) and also the separated subcellular fractions were added with 3 mL of 25% (w/v) KOH methanol solution. The vial was capped tightly and was heated at 65 °C for 3–4 h. The digested samples (10–40 μL) were buffered with sodium acetate at pH 4.9, and ethylated by sodium tetraethylborate in a 40 mL borate glass bottle. The quantification of MeHg was carried out by an automated MeHg analyzer (methylmercury analysis by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry, MERX, Brooks Rand). Standard reference material of mussel tissue (IAEA 142) was concurrently digested and analyzed, with a recovery of 94–117%. The acceptable spiked recovery range was 80–120% [36]. Each batch of analysis included blanks, a SRM, and 30 samples. The method detection limit for MeHg analysis was 1–5 ng/g.

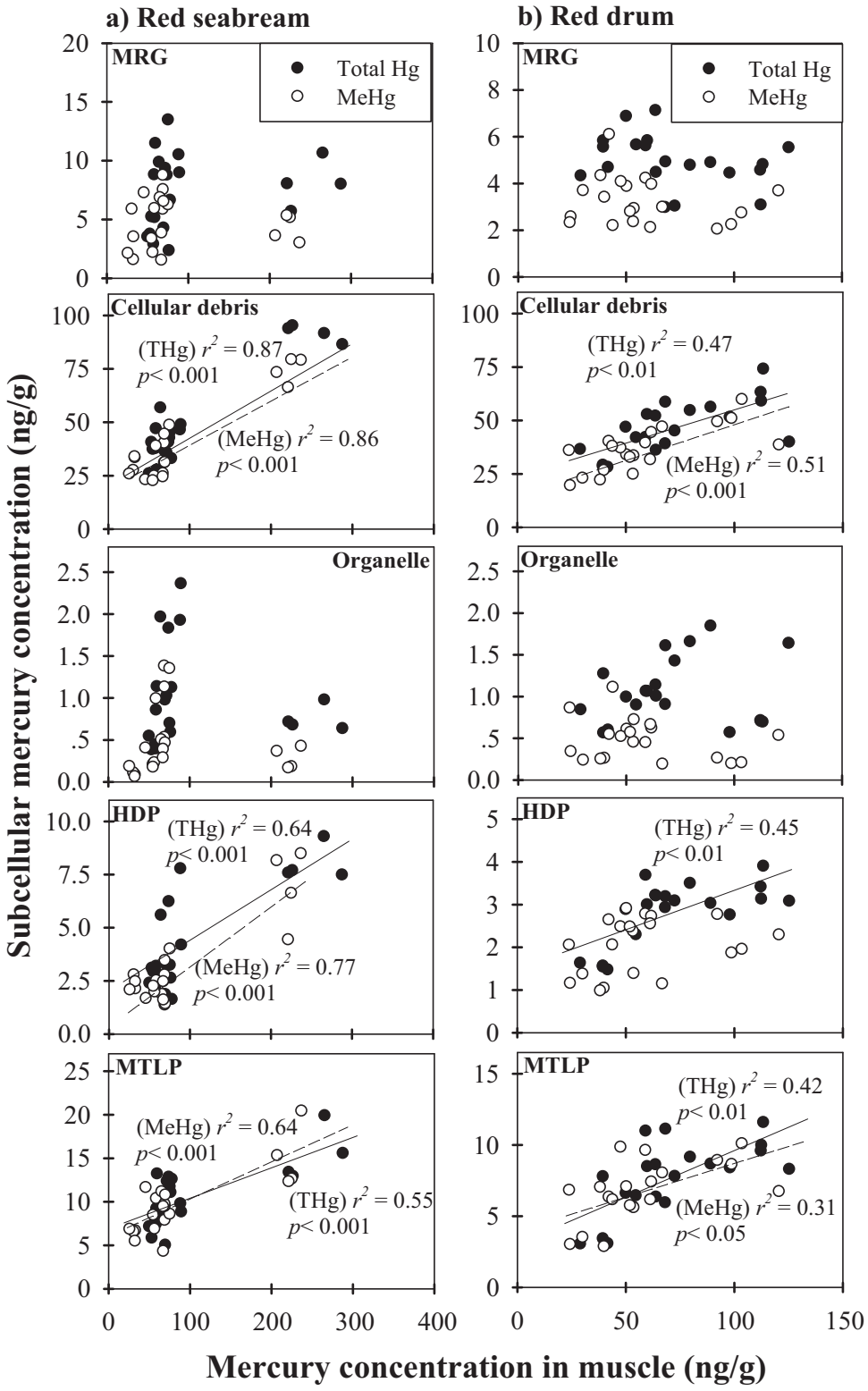

Fig. 2. Relationships between mercury concentration (THg and MeHg) in different subcellular fractions and total mercury concentration in muscles of red seabream (a) and red drum (b). Each dot represents one individual fish. HDP: heat-denatured proteins; MTLP: metallothionein-like proteins.

2.3. Statistical analysis

The % of subcellular mercury distribution and % of total Hg as MeHg were all arcsine transformed before statistical tests. The subcellular mercury (concentration and distribution) in each binding pool of two fish (red seabream and red drum) among different cage sites was tested for statistically significant difference by one-way ANOVA using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA). The relationships between fish body size (weight and length) and mercury concentration and subcellular mercury (concentration and distribution) in each binding pool of black seabream were tested using SPSS correlation analysis.

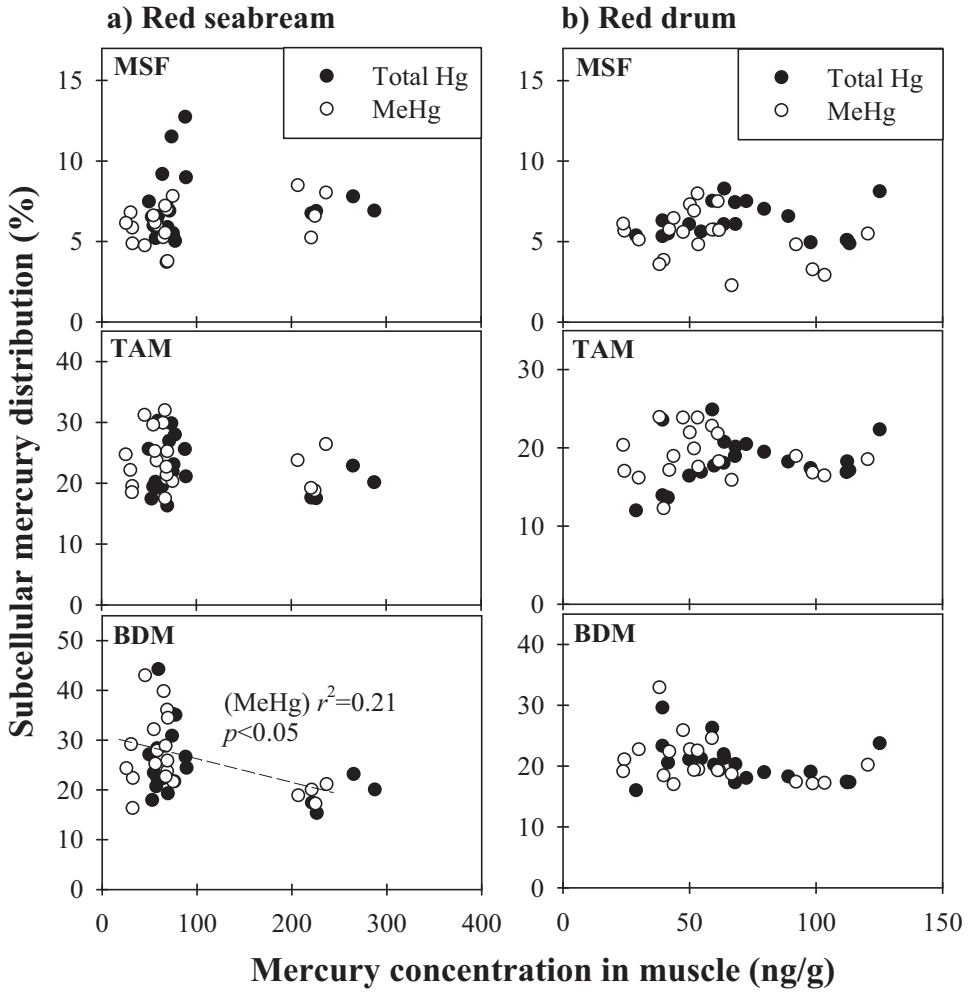

Fig. 3. Relationships between the mercury distribution in the metal-sensitive fraction (MSF), trophically available metal fraction (TAM), and biologically detoxified metal fraction (BDM) in fish muscle and the mercury concentration in red seabream (a) and red drum (b). Each dot represents one individual fish.

3. Results and discussion

3.1. Subcellular mercury distributions in fish from different caged sites

The total Hg and MeHg concentrations in the muscle tissues of two fish species are shown in Table 1. The Hg concentrations in red seabream dorsal muscles collected from Dongshan, Xiamen, Xinghua, Fuqing, and Luoyuan were 251, 54, 79, 72 and 66 ng/g THg, and 223, 31, 68, 63 and 62 ng/g MeHg, respectively (Table 1). In red drum, the Hg concentrations from Dongshan, Xiamen, Xinghua, Fuqing, and Luoyuan were 109, 36, 63, 59, and 92 ng/g THg, and 91, 39, 55, 50, 63 ng/g MeHg, respectively. All these values did not exceed the safety criterion of 0.30 μg/g ww established by USEPA. By comparison, Dongshan site had 3–5 times higher THg and 3–7 times higher MeHg concentration than the other stations, whereas the Xiamen site had the lowest Hg concentrations. This was primarily due to the different diets used in different sites. Fish from Dongshan site were generally fed with the viscera of other small fish, whereas fish from Xiamen were fed with artificial pellets [38]. The THg as MeHg was 90%, 57%, 87%, 87% and 94% in red seabream, and 82%, 88%, 87%, 86% and 66% in red drum from Dongshan, Xiamen, Xinghua, Fuqing, and Luoyuan sites, respectively.

The distribution of Hg in the five subcellular pools (MRG, cellular debris, organelles, HDP, and MTLP) is shown in Fig. 1. In the red seabreams, cellular debris was the dominant fraction binding with Hg (57–74% for THg and 57–73% for MeHg, respectively). The orders of THg and MeHg distribution followed cellular debris > MTLP > MRG > HDP > organelles. Approximately 12–21% of Hg was found in the MTLP fraction, and <2.7% was in the organelles. There was significant difference among the 5 caged sites for THg and MeHg distribution in all the subcellular fractions, except for MeHg distributions in organelles and HDP (Table 2). One possible explanation for such inter-site difference may have been due to their different feeding habits. The distributions in different subcellular fractions were similar for both THg and MeHg, mainly

Table 2
Statistical analysis of differences in subcellular distribution of THg, MeHg and % of total Hg as MeHg among cage sites by ANOVA test (*p < 0.05, **p < 0.01), after arcsine transformation of the percentage data. MRG: metal-rich granules; HDP: heat-denatured proteins; MTLP: metallothionein-like proteins.

	MRG	Cellular debris	Organelles	HDP	MTLP
Red seabream					
THg distribution (%)	**	*	**	**	*
MeHg distribution (%)	**	**			**
% of total Hg as MeHg					
Red drum					
THg distribution (%)	**		**		
MeHg distribution (%)	**				
% of total Hg as MeHg				*	

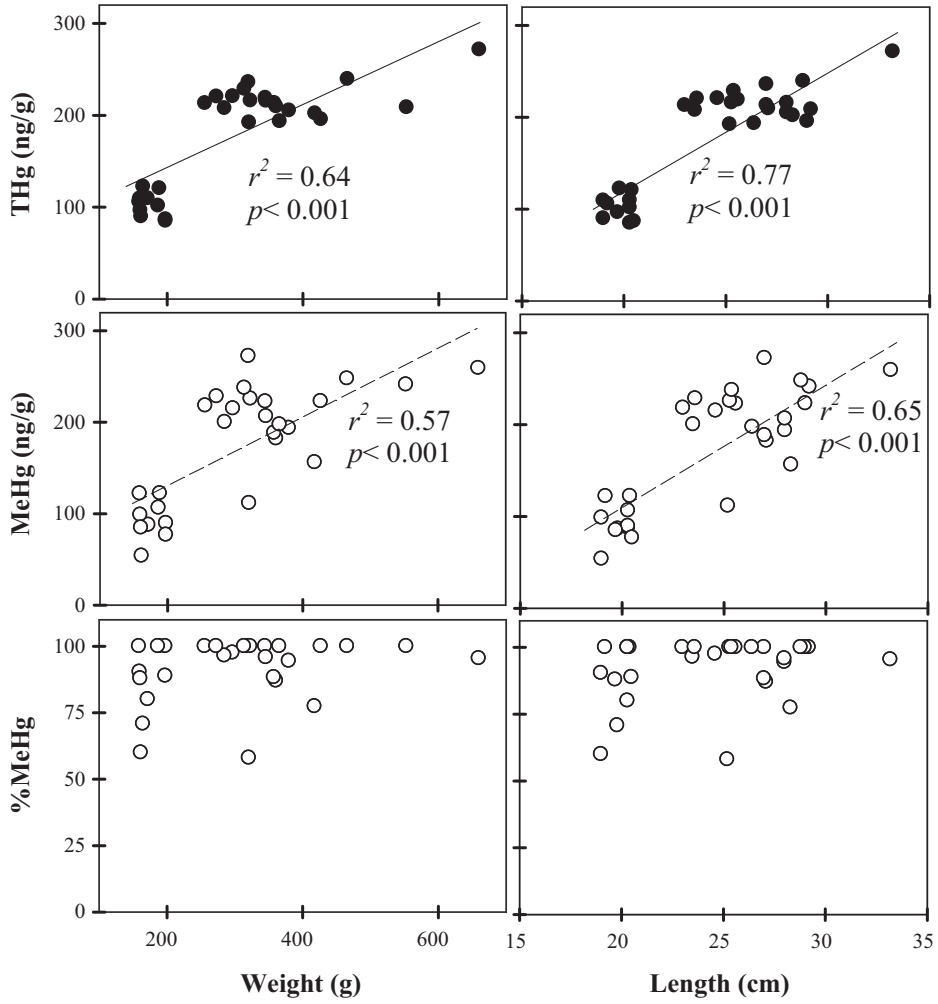

Fig. 4. Relationships between mercury concentrations (THg, MeHg, %MeHg) in black seabream muscle tissues and the body size (weight and length). Each dot represents one individual fish.

because >80% of total mercury in all the major fractions was in MeHg form.

In the red drum, the cellular debris also played an important role as the major binding pool of mercury, representing 71–77% of THg and 70–79% of MeHg. Approximately 10–13% of Hg was found in the MTLP fraction, and <2.5% was in the organelles. Among the different fish caged sites, significant difference was found for THg distribution in MRG and organelles, for MeHg distribution in MRG (Table 2). Mercury distributions in cellular debris, HDP, and MTLP showed no significant difference among cage sites.

A few earlier laboratory studies have examined the Hg distribution in the subcellular pools using radiolabeling methodology [28,29]. Bose et al. [28] injected the ^{203}Hg(II) and then quantified the subcellular ^{203}Hg fractions in livers of freshwater perch. They found that Hg in the cytosol (soluble fraction including HDP and MTLP) was a major pool for Hg binding. Dang and Wang [29] examined the subcellular pools of ^{203}Hg(II) and Me^{203}Hg in a juvenile grunt, *Terapon jarbua*, after pulse fed with radiolabeled prey. In their study, 47% of ^{203}Hg(II) was bound with HDP, and 47% of Me^{203}Hg was bound with MTLP. These laboratory results suggested that proteins pools (cytosolic fraction) were important in binding with both Hg(II) and MeHg, and were consistent with the general idea that mercury was sequestered by protein or larger peptides including glutathione, methylmercury–cysteine complex in the MTLP fraction in fish [1,29]. However, the field studies appeared to be different from these laboratory studies. Bebianno et al. [30] reported the total mercury and MTLP from black scabbardfish *A. carbo* collected from Portugal water, and found that the insoluble fraction (pellet) represented the major pool (89–94%) in the fish muscle tissue, while the heat-treated cytosol only amounting to 1–3% of total mercury distribution. By comparison, our study similarly showed that over 80% of MeHg was distributed in the insoluble fraction, especially in the cellular debris fraction. For other metals such as Cd, its subcellular distribution in the livers of yellow perch collected from eight natural lakes along a Cd, Cu, Ni and Zn concentration gradient was dominated by binding with MTLP [25]. It was difficult to compare our study with these previous studies [28,39] which only measured the cytosolic fractions (including MTLP and HDP) in the liver, kidney or gills. MeHg accumulation in muscle was probably mainly redistributed from other tissue (i.e., blood, gill, intestine and liver) [40]. Harris et al. [1] found that MeHg was sequestrated in thiols likely due to its high affinity for thiols group (cysteine), but they did not quantify the MeHg distribution in other cellular fractions. Our results were consisted with those of Barghigiani et al. [41] who found that over 92% of THg in fish muscle was sequestrated in the insoluble cellular fraction (including cellular debris, MRG and organelles). Fish muscle was hypothesized to be the inert storage site for Hg [42].

The subcellular distribution may have an important implication for the trophic transfer of Hg from the fish to humans. Recently, He and Wang [43] examined the factors affecting the bioaccessibility of methylmercury (MeHg) in nine species of marine fish from Hong

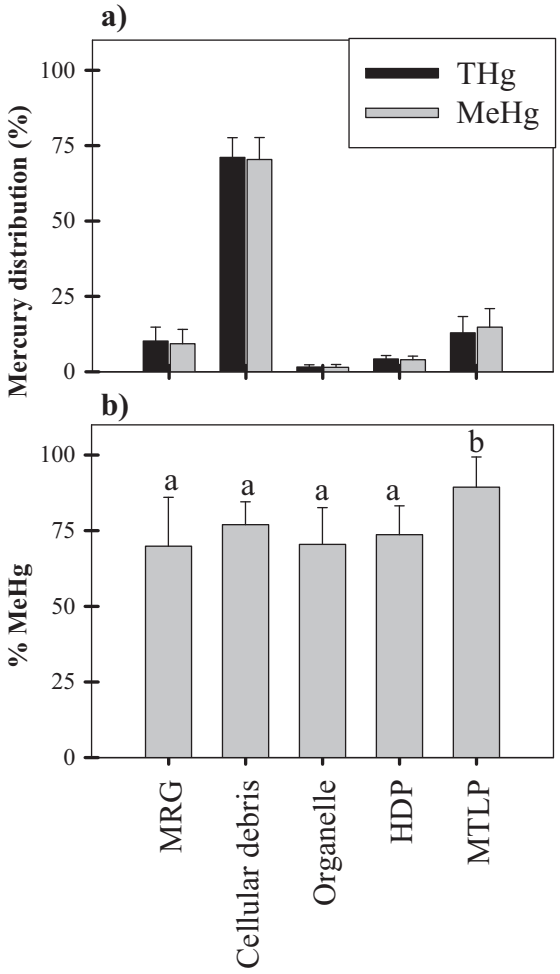

Fig. 5. (a) Subcellular distributions of total mercury and methylmercury in black seabream from Xiamen cage site; (b) percentage of total Hg as methymercury in each subcellular fraction. Mean ± SD (n = 29). Different letters indicate significant difference between two fractions.

Kong. In eight of the nine species studied, bioaccessibility was negatively correlated with the extent to which MeHg was partitioned into metal-rich granule fraction and the trophically available fraction, but was positively correlated with its partitioning into the cellular debris fraction. In their study, MeHg was also mainly partitioned in the cellular debris and in the MTLP fractions, but the range of variation was much greater than found in this study. For example, the % of MeHg in the cellular debris fraction was much higher (40–64%) for rabbitfish, grouper, sillago and golden thread than for mullet, horsehead, mackerel and yellow croaker (6–24%). It was difficult to conclude that the subcellular distribution was dependent on the feeding habits of the fish from their study. Cellular debris was mainly composed of tissue fragments and cell membranes. The much high distribution of Hg in the cellular debris fraction indicated that the potential bioaccessibility to human consumers may be high for these marine caged fish [43].

In previous study [44], the liver was the majority site for *in vivo* methylation in fish. Simon and Boudou [45] exposed the carp *Ctenophatyngodon idella* to Hg(II), and found that MeHg increased to 28% of the total muscle Hg, suggesting *in vivo* methylation. We also for the first time quantified the % of total Hg as MeHg in each binding pool of two fishes. The highest degree of %MeHg was found in the MTLP fraction (86–98% for red seabream, and 76–96% for red drum) (Fig. 1). Among the different fish caged sites, significant difference was only found HDP in red drum (Table 2). In contrast, the organelles fraction had the lowest % of total Hg as MeHg (30–60% for red seabream, and 32–61% for red drum). MTs had the capacity to bind both essential (e.g. Zn, Cu) and non-essential (e.g. Hg, Cd, Ag, As and Pb) metals since they were enriched with thiol groups of cysteine residues [46]. Cysteine is abundant in thiols in the cytosolic pool. Mercury binding as methylmercury–cysteine complex may thus form an important detoxification mechanism in fish muscle. Whether there was any *in vivo* methylation in the fish muscle tissue needs to be studied further.

Fig. 2 shows the relationship between mercury concentration in fish muscle and in each subcellular fraction. Significant relationships of THg/MeHg were found between their total concentrations and subcellular concentrations in cellular debris, and HDP and MTLP for both fish species. This was mainly due to the predominance of both THg and MeHg in cellular debris and the cytosol protein reservoir (HDP and MTLP). Earlier, Bebianno et al. [30] found no relationship between the MT levels and the Hg concentrations, or between the heat-treated cytosol fraction and the Hg concentration, in the black scabbardfish *A. carbo*, mainly because 90% of Hg was in the insoluble cellular debris of muscles. In contrast, no relationship was detected between the Hg concentration and the % Hg subcellular distribution, except for the negative correlation found for the MRG fraction in both species of fish ($p < 0.05$ for MeHg).

The metal-sensitive fraction (MSF) is defined as metals in HDP and organelles. It was rather surprising that both THg and MeHg in the MSF in the two fish species were very low (4–13% for red seabream and 2–8% for red drum), which strongly suggested that Hg may present little toxicity to the fish muscle due to its little partitioning in the MSF. There was no relationship between the Hg in the MSF and the Hg concentrations in the two fish species (Fig. 3). No relationship between the Hg in the TAM and the Hg concentrations in the two fish species was also found. Only MeHg showed a weak negative relationship between its distribution in BDM and its concentrations in the red seabreams (Fig. 3).

3.2. Influence of body size on mercury concentration and subcellular distribution

We also specifically examined the influence of body size on Hg subcellular fractionation. The black seabreams were collected from the same Xiamen farming site, with the body size range of 159–660 g in weight and 19.0–33.2 cm in length. The mean mercury body burdens were 177 ± 57 ng/g THg and 171 ± 66 ng/g MeHg, respectively. The average of %MeHg in black seabream was 95.5%, indicating that almost all mercury in black seabream were MeHg (Table 1).

In the present study, mercury concentrations in fish were positively related to body weight and standard length, however, the % of THg as MeHg was not dependent on the size of fish (Fig. 4). In general, mercury (and MeHg) concentrations in fish increased with age and body size (weight and length) [13,47–51], although exceptions also existed [6]. Qiu et al. [8] also confirmed that mercury bioaccumulation was related to body weight of snapper *Lutjanus malabaricus* and pompano *Trachinotus blochii* from fish farms in Southern China. In contrast, total mercury levels in farmed Atlantic salmon and Atlantic cod was not related to folk length [5]. It should be noted that fish farming has a very short food chain (i.e., from fish feeds to fish) [7,8]. Recently, Dang and Wang (unpublished) investigated the biokinetic factors leading to body size dependence of mercury concentration in the juvenile blackhead seabream *Acanthopagrus schlegeli*. They quantified the key size-dependent biokinetic parameters for both Hg(II) and MeHg, including the dissolved uptake rate constant, assimilation efficiency and the elimination rate constant. Among the

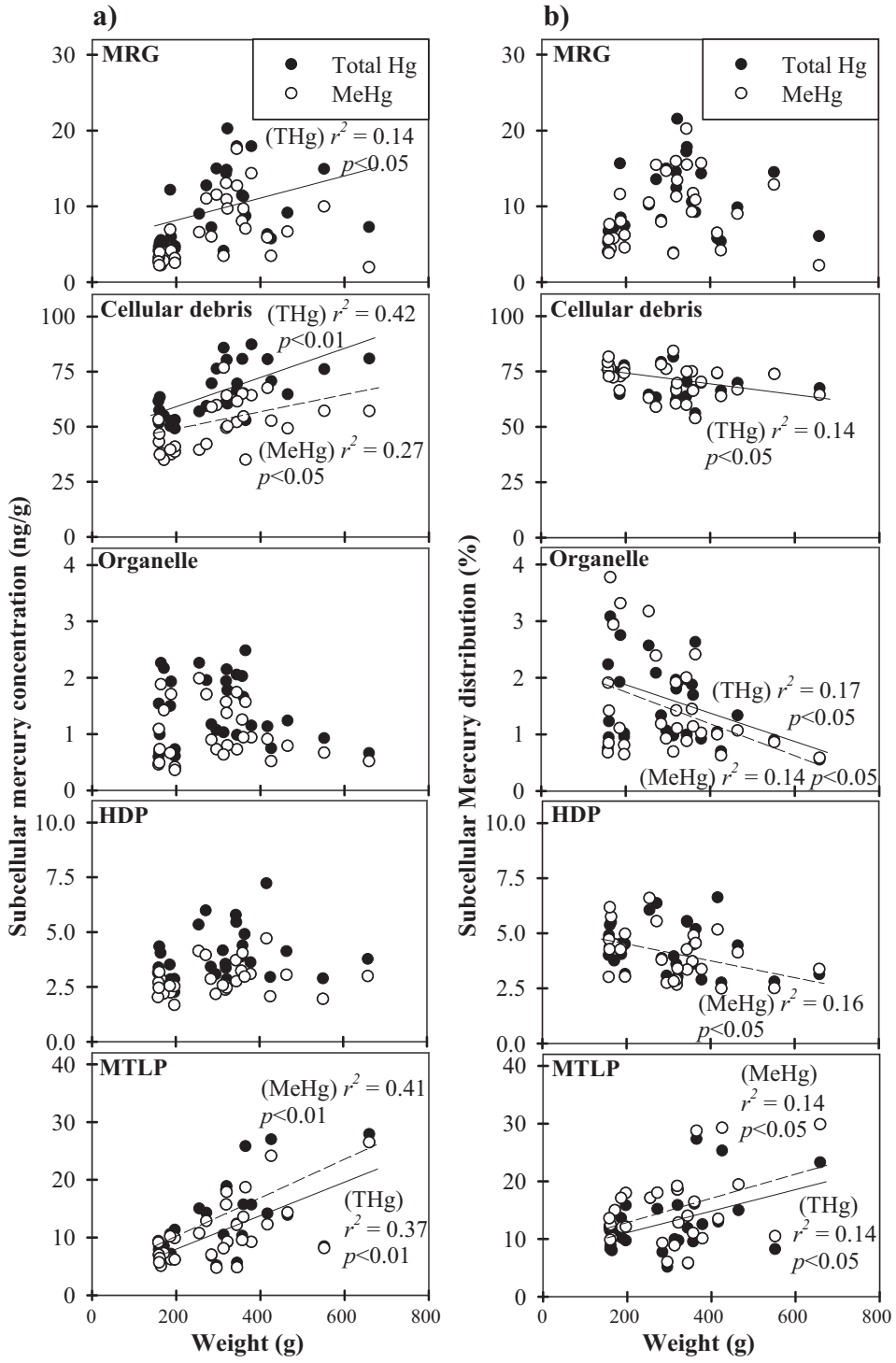

Fig. 6. Relationships between subcellular total mercury and MeHg (concentration and percentage of distribution) and body size (weight) of black seabream from Xiamen cage site. Each dot represents one individual fish.

examined kinetic parameters, both growth rate and Hg elimination differences explained most of the size-dependence of mercury concentration. Specifically, the slower growth and elimination of Hg in larger size of fish may account for the increasing concentrations of Hg in the fish. Consequently, one possibility to reduce the Hg body burden in the fish is to increase the growth of fish (e.g., growth dilution for the growing fish). Ward et al. [52] found that larger (fast growing) salmon had lower mercury levels than smaller (slow growing) fish, probably caused by the somatic growth dilution.

For mercury subcellular distribution in all sizes of black seabream, THg and MeHg were bound with different fractions in order of cellular debris > MTLP > MRG > HDP > organelles (Fig. 5a). Again, the cellular debris was the major fraction (71%) for both THg and MeHg, and the MTLP was the second important fraction (13–15%). Similarly, the MTLP fraction also contained the highest % of total Hg as MeHg (89%, Fig. 5b), and organelles and MRG had the lowest % of total Hg as MeHg (70%). These data were consistent with those found for the red seabreams and red drums, except that

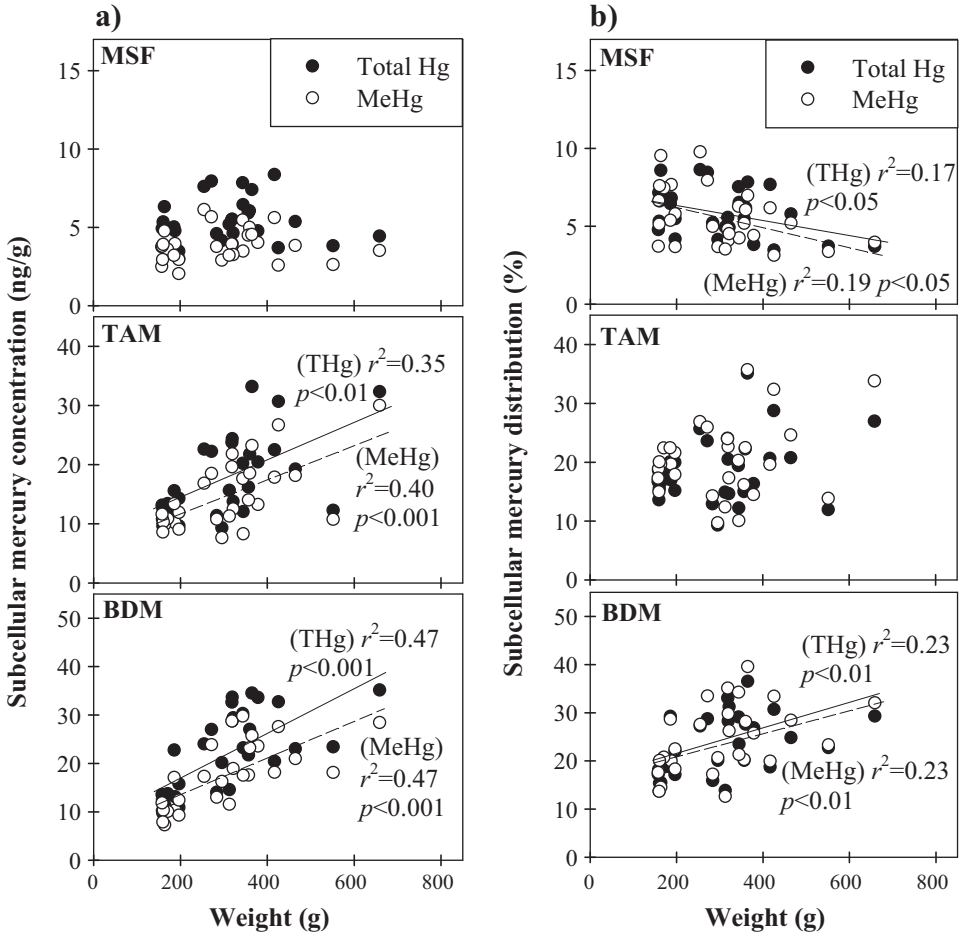

Fig. 7. Relationships between mercury distribution in the metal-sensitive fraction (MSF), trophically available metal fraction (TAM), and biologically detoxified metal fraction (BDM) in fish muscles and body size (weight) of black seabream from Xiamen cage site. Each dot represents one individual fish.

the black seabreams had a somewhat higher % of total Hg as MeHg in the organelles fraction.

Influence of body size on subcellular mercury fractionation was conducted by linear regression analysis (Fig. 6). Positive relationships were found for cellular debris and MTLP fractions for both THg and MeHg concentrations, whereas Hg in organelles and HDP was not related to the body weight of the fish. The % of THg and MeHg subcellular distribution in the MTLP fraction was also positively correlated with the fish weight, and negative correlations were found for cellular debris, organelles and HDP. Bebianno et al. [30] showed that the MT concentration in the muscle decreased with increasing size and tissue Hg concentrations of black scabbardfish, but the subcellular fraction of Hg was not related to the body size of fish since the majority of Hg was in the insoluble fraction. The increasing partitioning of Hg in the MTLP suggested that detoxification may increase in larger fish. Consistently, we found a positive relationship between the Hg partitioning in BDM (MRG + MTLP) and the fish weight (Fig. 7). A negative relationship between the Hg partitioning in MSF (HDP + organelles) and fish weight was also documented, indicating less toxicity in muscle of larger fish. Overall, these data suggested that muscle of larger fish may have a greater detoxification ability than the smaller fish.

4. Conclusions

In this study, we compared the mercury speciation in subcellular fraction (concentration and distribution) of caged fish from Fujian marine cage farming. Mercury (THg and MeHg) was accumulated mainly in the cellular debris, followed by MTLP > MRG > HDP > organelles. Interestingly, %MeHg was the highest in MTLP pool, which was considered as the biologically detoxified metal pool. Hg distribution in the metal-sensitive fraction was generally low for the marine fish, suggesting that Hg may present little toxicity to the fish. However, the predominance of Hg in the cellular debris fraction may facilitate its bioavailability to humans due to fish consumption. In addition, the body size of fish both affected the mercury tissue concentration and its distribution in different subcellular pools. Measurements of subcellular distribution of Hg in marine fish may provide important information for the Hg sequestration and detoxification, as well as potential bioaccessibility to human consumers.

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