Environmental Pollution 243 (2018) 115–126

Contents lists available at ScienceDirect

Environmental Pollution

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

Bioaccumulation characteristics of mercury in fish in the Three Gorges Reservoir, China☆

Qinqin Xu [a, 1], Lei Zhao [b, 1], Yongmin Wang [a], Qing Xie [a], Deliang Yin [a], Xinbin Feng [b], Dingyong Wang [a, c, *]

[a] College of Resources and Environment, Southwest University, Chongqing 400715, PR China
[b] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, PR China
[c] Chongqing Key Laboratory of Agricultural Resources and Environment, Chongqing 400716, PR China

ARTICLE INFO

Article history:
Received 16 April 2018
Received in revised form
12 August 2018
Accepted 16 August 2018
Available online 21 August 2018

Keywords:
Fish
Mercury
Methylmercury
Biomagnification
Three Gorges Reservoir

ABSTRACT

Newly constructed reservoirs were recognized as hotspot of mercury (Hg) methylation, and then methylmercury (MeHg) accumulation in food chains. The risk of elevated MeHg concentrations in fish is one of the most important concerns in newly constructed reservoirs. The Three Gorges Reservoir (TGR) is one of the largest reservoirs in the world. However, the distribution and bioaccumulation characteristics of Hg species within the food chains and its potential ecological risk in the TGR remain poorly understood. In this study, 264 fish individuals covering 18 species were collected from the TGR. Total mercury (THg) and MeHg concentrations in different organs (gill, heart, liver, muscle and swim bladder) of fish species were analyzed; the values of $\delta^{13}C$ and $\delta^{15}N$ in fish muscle were determined as well to reveal the biomagnification properties of Hg in food chains. Our results showed that concentrations of THg (0.5–272 ng g^{-1}, w.w.) and MeHg (0.1–199 ng g^{-1}, w.w.) in fish muscle from the TGR ubiquitously fall below the safe fish consumption limit on Hg recommended by WHO (500 ng g^{-1}, w.w.) and the US-EPA Water Quality Criterion for MeHg (300 ng g^{-1}, w.w.). The short food web jointly with the limited trophic magnification factor in the TGR explained the relatively low Hg concentrations in predators. Among the five fish organs, muscle represented the highest Hg concentrations, followed by heart, liver, swim bladder, and gill, suggesting that muscle has the highest ability to accumulate Hg compared to the other organs. More importantly, no discernible "reservoir effect" was observed in the TGR within the initial few years after impoundment due to its special eco-environment including: 1) neutral and slightly alkaline pH and low dissolved organic carbon of water, 2) less vegetation coverage in inundated areas, 3) simple food web.

1. Introduction

Mercury (Hg) is one of the most hazardous pollutants in aquatic ecosystem, which is readily transformed to methylmercury (MeHg) that bioaccumulates in aquatic organisms. Within the aquatic food chain, MeHg presents as the primary bioaccumulative form, which causes damage to the renal and central nervous systems (includes motor, sensory deficits and behavioral impairment), reproductive system function and even may lead to fetal death (Beckers and Rinklebe, 2017). The key factor facilitating the transfer of this contaminant from physical to biological compartments is the methylation of inorganic Hg (IHg) to MeHg (Hintelmann, 2010; Kidd et al., 2012). The methylation involves complex relationships between electron donors (such as dissolved organic matter) and electron acceptors including sulfate and ferric iron ions (Marvindipasquale et al., 2014), and Hg bioavailability. It is also mediated by a variety of obligate anaerobic microorganisms (e.g. sulfur and iron reducing bacteria, and methanogens) in anoxic environments (Gilmour et al., 1992; Podar et al., 2015).

Reservoirs may constitute settings for substantial MeHg production, described as "reservoir effects". Numerous studies reported substantially increasing MeHg concentrations in biota after reservoir impoundment (Abernathy and Cumbie, 1977; Hall et al., 2005; St Louis et al., 2004). The flooded soil and vegetation have

☆ This paper has been recommended for acceptance by Prof. W. Wen-Xiong.
* Corresponding author. College of Resources and Environment, Southwest University, Chongqing 400715, PR China.
E-mail address: dywang@swu.edu.cn (D. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.envpol.2018.08.048
0269-7491/

been identified as the primary source of MeHg to the new reservoirs (Ramlal et al., 1986). Nutrient addition from the decomposition of submerged vegetation and organic materials may accelerate microbial methylation in water, and further exercises an impact on Hg bioavailability of fish (Eckley et al., 2015; Gill and Bruland, 1990; Gilmour et al., 1992; Jackson, 1988). Consequently, subjects consuming substantial amounts of fish become vulnerable to MeHg exposure even if the fish MeHg content is moderately elevated (Mailman et al., 2006). The risk of MeHg poisoning is particularly increased in the newly-constructed reservoirs due to the active Hg methylation (James et al., 2002; Stein et al., 1996). Hence, MeHg contamination in hydroelectric reservoirs may have consequences for environmental health.

The Three Gorges Reservoir (TGR) as a newly-formed reservoir completed in 2009, is one of the largest reservoirs in the world. The TGR has occasioned international and domestic debate concerning actual social, environmental, and economic costs involved (Salazar, 2000). Its water table experiences fluctuations from 145 m in summer to 175 m in winter; a zone 30 m in altitude (accounting for 55% of the total flooded area) is, therefore, annually subject to shifting wetness and red-ox conditions that may promote net Hg methylation (Eckley et al., 2015; Wang and Zhang, 2013). Additionally, the TGR which located in central China, experiences a high load of atmospheric Hg deposition (Xu et al., 1999a). Therefore, suitable conditions in flooded soil after impoundment jointly with the high Hg burden in this area may increase the absolute MeHg production and accelerate the MeHg accumulation in fish. Beyond these, the variation of hydrological characteristics (e.g. the water velocity, depth of river, dissolved oxygen, the effective of the light) in Yangtze river and its tributaries fundamentally influenced the habitat variation, food sources, species diversity and community structure of the food chains (Li, 2012). The changes of food web structure and trophodynamics may ultimately alter contaminant (e.g. Hg and other toxic compounds) biomagnification and accumulate rate in biota (Lavoie et al., 2013; Ofukany et al., 2014; Stewart et al., 2003). Moreover, the TGR as an important freshwater fisheries base in China, supplies primary fish resource to local residents. The potential Hg contamination problems with in the food chains should be paid more attention.

Regarding Hg bioaccumulation in fish of the TGR, previous studies reported the Hg concentrations (40–420 ng g^{-1}, w.w.) in fish species in Yangtze River before the TGR being constructed (Jin and Xu, 1997; Xu et al., 1999a, 1999b). These studies further predicted that Hg reactivity within the TGR will increase by 0.4–1.5 times higher than that before the impoundment, and then the Hg concentrations in fish will consequently exceed the safe consumption limitation (Jin and Xu, 1997; Xu et al., 1999a, 1999b). Recently, several studies reported the total mercury (THg) concentrations in limited fish species (Yu et al., 2012; Zhang et al., 2007b) and biomagnification characteristics of Hg within the food chains (Li et al., 2015; Yu et al., 2013) in the tributary of Yangtze River basin. However, previous studies only focused on THg concentration in fish muscle. To date, the data concerning the concentrations of Hg species (THg and MeHg) in different organs (e.g. gill, heart, liver, muscle and swim bladder) and its potential health risk to local residents through fish consumption in the TGR are very limited. The key issue whether the impounding of the TGR will accelerate the MeHg bioaccumulation in aquatic organisms is still unclear. To address these problems, 264 fish samples covering 18 species in the TGR were collected during the periods from 2011 to 2014. Concentrations of THg and MeHg in different fish organs (gill, heart, liver, muscle, swim bladder) were analyzed; the values of $\delta^{13}C$ and $\delta^{15}N$ in fish muscle were determined as well in this study. The primary objectives of this study were to, 1) investigate the concentration and distribution of Hg in fish organs from the TGR; 2) better understand the Hg transfer and bioaccumulation in the fish food web; 3) assess the potential risk for increased Hg reactivity towards methylation in the reservoir.

2. Materials and methods

2.1. Study area and sampling sites

Chongqing is located in the upper reaches of Yangtze River. The Chongqing section of the TGR (N 28°31′-31°44′, E 105°49′-110°12′) encompasses 22 cities and/or counties. Chongqing section has a watershed of 46,158 km^2, which accounts for approximately 85% of total watershed of the TGR. This area experiences a humid subtropical monsoon climate with the annual average air temperature of 15–18 °C. The annual rainfall is approximately 1150 mm but unevenly spatially distributed. Forest covers approximately 22.3% of total area watershed, in which subtropical evergreen broad-leaved forest and warm coniferous forest are the main zonal vegetation. However, the presence of forest vegetation below an elevation of 1000 m was rare prior the construction of the TGR due to increased land reclamation for agriculture over decades (Lu et al., 2010). The mainstream of the TGR was well mixed throughout the water column for the majority of the year, whereas some tributaries were characterized as a stable system (e.g., thermal stratification) and observed more serious trophic state (eutrophic and mesotrophic) (Xu et al., 2011; Zhang et al., 2008). Wastes, especially nutrients from the upper streams of the tributaries accumulated in the bays; thus algal blooming would be inevitable (Zhang and Lou, 2011). Moreover, directly discharged untreated wastewater, as well as a huge amount of legacy toxic sediment from factories, mines and garbage dumping sites increased the internal sources of pollutants (e.g. arsenic, sulfides, cyanides and mercury) to reservoir (Zhang and Lou, 2011).

Four typical sampling stations within the center of the TGR were chosen in this study, including Zhenxi town (“S1”; N29°54′, E107°27′), Shibao village (“S2”; N30°59′, E108°05′), Tujing village (“S3”; N30°21′, E108°03′; is located in Ruxi river) and Hanfeng Lake (“S4”; N31°41′, E108°72′) (Fig. 1). The physical and chemical characteristics of the study sites were listed in Table 1. These sampling sites have large area of water fluctuation zone which are generally used as agricultural land during the lowest water level. Agriculture activities especially the chemical fertilizer usage, largely impacts the water quality of the TGR (e.g. the eutrophication of water body in Hanfeng lake and Ruxi river) (Huang et al., 2015; Xiang et al., 2017). Human being activities especially the tourist, are popular in Shibao village and Hanfeng Lake during our sampling periods. In addition, exogenous inputs from surroundings (such as household waste and industrial discharge) are recognized as primary sources of heavy metals (e.g. mercury) in Shibao Village and Hanfeng Lake (Fu, 2010; Zhang and Lou, 2011). Shibao Village and Zhenxi town have a long history of cage aquaculture activities, which could stimulate the Hg methylation in the reservoir (Meng et al., 2010). However, the cage aquaculture activities gradually decreased after the government polices enforced to reduce the contamination of local ecosystem.

2.2. Sample collection

For each of the sampling stations, fish samples were collected each season during the period from 2011 to 2014. In total, 264 fish samples were collected by fishing net with the help of professional fishermen at the four locations. All specimens were visually inspected for fin and body deformations to avoid selecting cultivated fish. As shown in Table S1, 18 fish species were successfully

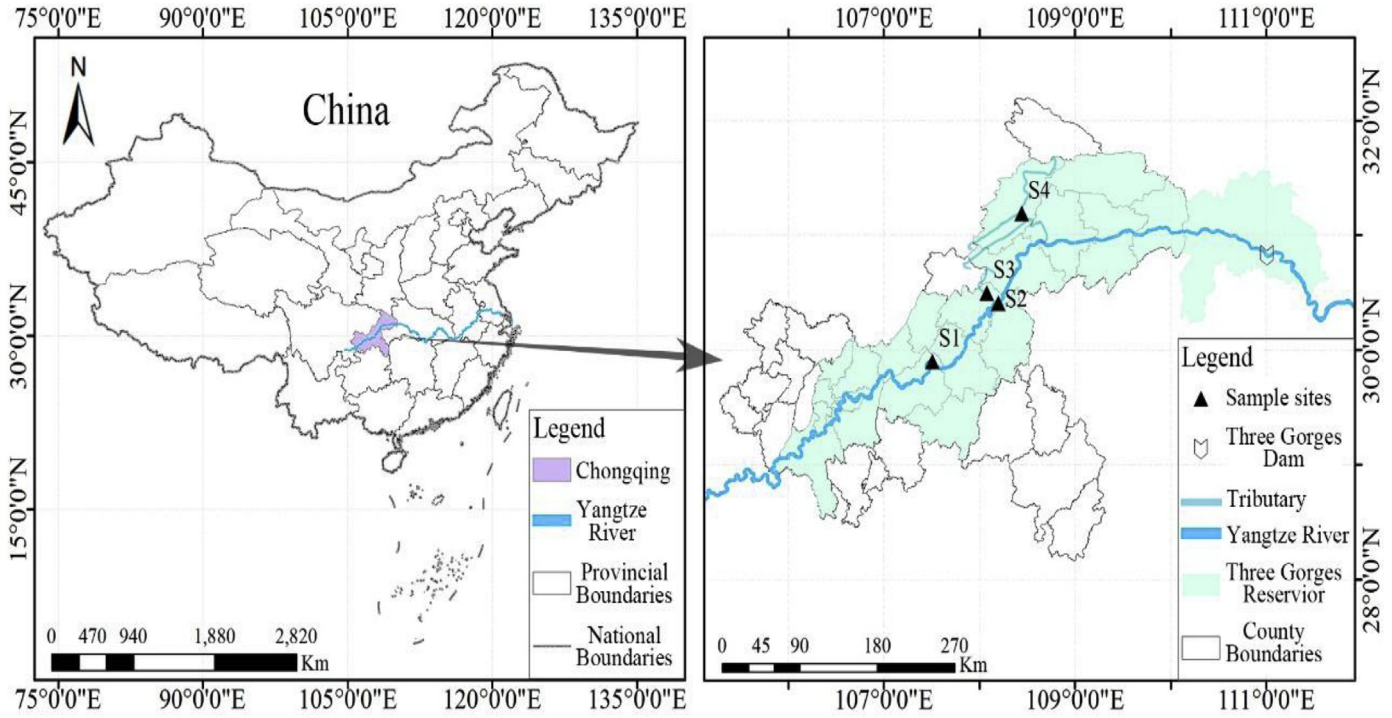

Fig. 1. Map of the study area and sampling locations in the Three Gorges Reservoir.

Table 1
Physical and chemical characteristics of the study sites.

Study sites	Water						Sediment		
	pH*	DO[a] (mg L^{-1})	DOC[a] (mg L^{-1})	MeHg[a] (ng g^{-1})	TN[b] (mg L^{-1})	TP[b] (mg L^{-1})	OM[c] (%)	THg[c] (ng L^{-1})	MeHg[c] (ng L^{-1})
S1	7.55	5.36	2.92	0.21	/	/	0.94	70.38	0.41
S2	7.54	5.40	2.39	0.16	1.35	0.11	/	64.68	0.47
S3	7.96	7.47	2.64	0.27	1.30–3.31	0.02–0.29	/	14.10	0.41
S4	7.69	6.07	4.65	0.33	1.56	0.16	/	57.65	0.54

[a] Data from Li (2013).
[b] Data from Li and Zhang (2010), Xiang et al. (2017) and Huang et al. (2015).
[c] Data from He (2013).

obtained in this study, including 6 Carnivorous species (CV), 8 Omnivorous species (OV), 2 Planktivorous species (PV), and 2 Herbivorous species (HV). Furthermore, the fish species were also classified into two categories based on habitat preference, namely pelagic fish (P) and benthic fish (B). In order to guarantee a diversity of the fish samples (species and size), sampling campaigns were conducted over the seasons from the four sites. Nevertheless, the sampled fish were mixed each time since sampling time and sites are not the major considerations in this study. Although it is impossible to completely collect all the fish species, the most abundant and representative ones within the TGR are included in this study.

The fish samples were stored alive in barrels with water and air purge, or dead on ice in freeze-boxes in the field until further processing in the laboratory. The weight and length of each sample were recorded before dividing and stored frozen for Hg analysis. Fish samples were firstly washed by tap water and deionized water, then a piece of muscle without skin and bone was collected using stainless steel scalpel, so does the gill, heart, liver and swim bladder. All samples were collected and processed following ultraclean sample handling protocols. Subsequently, the freeze-dried fish organs were ground and homogenized to a size of 100 meshes per inch with a mortar for THg and MeHg analysis. The concentration of IHg in fish organs was calculated by the difference between the concentration of THg and MeHg in the sample. Precautions were taken to avoid any cross-contamination during the sample processing. The grinder was thoroughly cleaned after each sample processing. The powdered samples were subsequently packed into plastic dishes, sealed in polyethylene bags, and stored in a refrigerator (4 °C) within desiccators for further laboratory analysis. The water content of the fish organs was also estimated by weight loss. All the data presented in current study including THg and MeHg concentrations in organs of fish samples were shown as ng g^{-1} (wet weight). It should be noted that only 50 fish liver samples were obtained, because some liver samples had too little amount so that it is difficult to test accurately, or some were too fragile to broken after thawing and couldn't be collected.

2.3. Sample analysis

2.3.1. THg and MeHg analyses

All reagents used in this study were at least of analytical grade and were purchased from Shanghai Chemicals Co. (Shanghai, China). For THg analysis, 0.1–0.2 g (dry weight) of fish organs was prepared and digested for 3 h in a concentrated acid mixture (10 ml HNO_3:H_2SO_4 = 7:3 v/v) at 95–140 °C. A suitable volume of aliquot

from digested sample was taken for THg analysis by cold vapor atomic fluorescence spectrometry (CVAFS, Brooks Rand Model III, Brooks Rand Labs, Seattle, WA, USA) following EPA Method 1631 (USEPA, 2002). For MeHg analysis, 0.1–0.2 g (dry weight) of fish organs was digested using 5 mL of 20% KOH in 25 mL fluoropolymer vials. The fluoropolymer bottles were heated at 75 °C in water bath for 3 h (Yan et al., 2005). After completion, an aliquot of digestate was taken for MeHg analysis by aqueous ethylation, Tenax trap, GC-CVAFS (Brooks Rand Model III, Brooks Rand Labs, Seattle, WA, USA) following US EPA Method 1630 (USEPA, 2001a).

2.3.2. Stable isotope analysis

To understand the bioaccumulation characteristics of Hg in the food web of the TGR, 41 muscle samples covering different sizes and species of fish were selected for $\delta^{13}C$ and $\delta^{15}N$ determination. Stable isotope ratio of C ($\delta^{13}C$) and N ($\delta^{15}N$) were determined using stable isotope ratio mass-spectrometer (IRMS, Isoprime 100, Isoprime® UK) coupled with elemental analyzer (Pyrocube, Elementar®-Germany) (Agnihotri et al., 2014). $\delta^{13}C$ and $\delta^{15}N$ values are expressed as the deviation from the standards in parts per thousand (‰):

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

where X is ^{13}C or ^{15}N; R is the ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

On the basis of the $\delta^{15}N$ values in fish muscle and corresponding zooplankton in the TGR ($\delta^{15}N_{zoop}$, $TP_{zoop} = 2$), the trophic position (TP) of the fish species within the food chains was further calculated by using the following equation (Cabana and Rasmussen, 1996):

$$TP_{fish} = \left(\delta^{15}N_{fish} - \delta^{15}N_{zoop}\right)/3.4‰ + 2 \quad (2)$$

where TP_{fish} is the trophic position of the fish species within the food chains; $\delta^{15}N_{fish}$ is the value in fish muscle; the value of $\delta^{15}N_{zoop}$ (8.39) in the TGR is obtained from Li (2012); 3.4‰ is the dietary isotopic discrimination factor at each trophic step.

2.4. Quality assurance/quality control (QA/QC)

Quality control for THg and MeHg determination consisted of method blank, matrix spikes, duplicates, and the use of a certified reference material. Matrix spikes and duplicates were taken regularly (>10% of samples) throughout each sampling session. The method detection limits were determined to 0.002 ng g^{-1} and 0.001 ng g^{-1} for THg and MeHg in organs of fish samples, respectively. The relative standard deviation for duplicate sample analysis was less than 10% for both THg and MeHg in organs of fish samples. Recoveries for matrix spikes ranged from 80% to 122% and from 89% to 124% for THg and MeHg analysis, respectively. The average concentrations of THg and MeHg in the certified reference material (National Research Council Canada, TORT-2, Lobster Hepatopancreas) were 265 ± 22 ng g^{-1} and 137 ± 212 ng g^{-1}, respectively, which were in good agreement with the certified values (THg: 270 ± 60 ng g^{-1}; MeHg: 152 ± 13 ng g^{-1}). The C and N isotopic measurements were mainly calibrated using an international standard, in which the reported Caproic acid ($C_6H_{15}NO_2$) values for $\delta^{13}C$ and $\delta^{15}N$ are 4.6 and −25.3‰. Reference gas tanks were calibrated initially by running several aliquots of this international standard, and then offsets between actual isotopic values from those of measured values were estimated. The relative standard deviation of duplicate samples was less than 0.3‰ for both isotopes.

Statistical analysis was performed using SPSS 15.0 for Windows. Regression analysis was used to evaluate the relationship between Hg concentrations and standard length of analytic specimens. The relationship between Log transformed Hg concentrations and $\delta^{15}N$, as well as the relationship between $\delta^{13}C$ and $\delta^{15}N$. Correlation coefficients (r) and significance probabilities (p) were computed for the linear regression fits. T-test or AVOVA test were employed to compare significant difference between paired or unpaired samples when the data sets following normal distribution. Otherwise, Kruska-Wallis H(K) and Mann-Whitney tests were performed to compare significant differences between independent datasets. Significant differences were all declared at $P < 0.05$. All averaged data sets are arithmetic unless otherwise noted.

3. Results and discussion

3.1. Hg distribution in different fish species

The concentrations and distributions of Hg species (THg and MeHg) and the ratios of MeHg to THg (MeHg/THg) in fish muscle from the TGR are listed in Fig. 2. The average concentrations of THg and MeHg in fish muscle were 54 ng g^{-1} (0.50–272 ng g^{-1}) and 23 ng g^{-1} (0.10–199 ng g^{-1}), respectively, which were much lower than the maximum permissible limit issued by WHO (THg, 500 ng g^{-1}, w.w.) (WHO, 1990) and US-EPA Water Quality Criterion (MeHg, 300 ng g^{-1}, w.w.) (USEPA, 2001b). The species *Ci* represented the lowest Hg concentration (under the US-EPA wildlife Hg threshold of 100 ng g^{-1} w.w (USEPA, 2001b) with ranges of 0.27–16 ng g^{-1} for THg and 0.03–5.8 ng g^{-1} for MeHg, respectively. Relatively higher Hg concentrations almost observed in carnivorous fish species. Hg levels in some individuals from *Ei, Eb, Sc, Sa, Lj, Ca, Ch* and *Pf* species exceeded the wildlife Hg threshold.

MeHg is the main mercury species in fish, normally accounting for 90%. In this study, the proportions of MeHg to THg (%MeHg) in almost of fish species were less than 50%, in which only *Eb, Sc, Mt* and *Pf* exceeded. The mean value for all fish species was 37%, with the range of 3.0–97%, showing significant differences among different fish species (ANOVA test, $F = 3.04$, $p = 0.001$). Carnivorous and omnivorous species exhibited significant higher proportions of %MeHg than planktivorous species (ANOVA test, $p < 0.05$).

The magnitude of Hg concentrations in fish species from the TGR was compared with data from the literature (Table S5). The concentrations of Hg in the TGR represent the similar level when compared with other Chinese lakes/reservoirs, such as the Hongfeng reservoir, Baihua reservoir, Hongjiadu reservoirs, Dongfeng reservoirs, and Wujiangdu reservoirs along Wujiang River basin, Southwest of China (He et al., 2010; Jiang et al., 2010; Liu et al., 2012; Yan et al., 2008; Yao et al., 2010). Studies in North America and northern Europe have confirmed the elevated levels of Hg in fish from newly constructed reservoirs and concluded that the increased Hg levels in fish from reservoirs may last for up to 30 yr after impoundment (Lodenius et al., 1983; Mailman et al., 2006). However, in this study the Hg concentrations in fish observed in the TGR were much lower than those observed in North America and northern Europe. For example, Bilodeau et al. (2018) reported that the Hg concentrations in fish collected from a hydroelectric reservoirs (La Grande hydroelectric reservoirs) in northern Québec (Canada) reached up to 4660 ng g^{-1}.

A strong correlation coefficient ($r = 0.69$, $p < 0.01$) was observed between weight and length within the whole group. When further statistical analyses were conducted with each of the species (Table S2), only the specimens of *Ei*, *Bg*, *Ca* and *A* displayed significant positive relationships between Hg concentrations (THg and

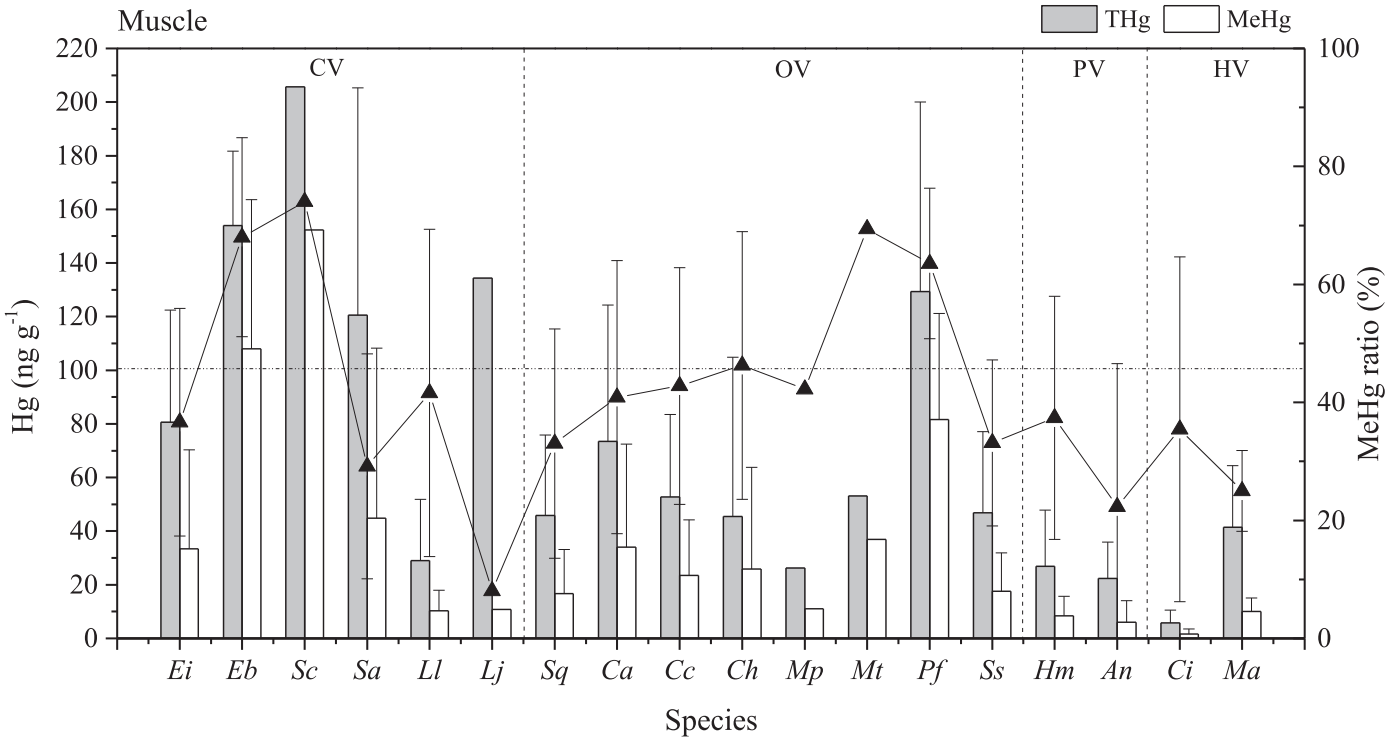

Fig. 2. The concentrations and distributions of Hg species (THg and MeHg) and MeHg/THg ratios in fish muscle (n = 264) covering 18 fish species in the Three Gorges Reservoir (the dashed line is the US-EPA wildlife Hg threshold of 100 ng·g^{-1} w.w.).

MeHg) and fish size, whereas a negative and non-significant correlation between these variables was found for *Sq*, *Hm*, *Cy*, *Ci*, *Pf* and *Ma* ($p > 0.05$). Complex factors such as fish species, trophic levels, seasonal food availability, and watershed features, influence the relationships between fish size and Hg bioaccumulation (Ouédraogo and Amyot, 2013; Silva et al., 2012). Our results showed that concentrations of Hg species in most of the fish species did not linearly scale with the corresponding size, which could be partially attributed to 1) the increased terrestrial nutrients input after the impoundment of the TGR, and then 2) the "grow dilution" pattern in Hg bioaccumulation (Li et al., 2015). However, the direct evidence supporting this hypothesis is unavailable in this study. Thus, to better understand this observation, further work needs to be done.

3.2. Hg distribution in different organs

The concentrations and distributions of THg and MeHg in different organs are displayed in Fig. 3 and summary data are listed in Supplementary Information Table S3. The THg and MeHg concentrations showed statistically significant difference among five organs (Kruska-Wallis H(K), $p < 0.001$). Furthermore, the highest concentrations of THg (54 ± 48 ng g^{-1}) and MeHg (23 ± 32 ng g^{-1}) were generally observed in muscle, which was significantly higher than those in the other organs (gill, heart and swim bladder) (Mann-Whitney, $p < 0.001$). On the opposite, the lowest levels of THg (12 ± 13 ng g^{-1}) and MeHg (5 ± 8 ng g^{-1}) represented in gill. The swim bladder and heart contained medium levels of Hg among all organs. It should be noted that Hg concentrations in liver were limited to 50 individual fish samples. As shown in Fig. 3-b, concentrations of Hg species in liver were all significantly higher than those in gill and swim bladder (ANOVA test, $p < 0.001$). However, MeHg concentrations in liver were significantly lower than those in corresponding muscle (ANOVA test, $p < 0.001$); no significant difference between THg concentrations in liver and heart was observed (ANOVA test, $p = 1.08$). Concentrations of Hg species (THg and MeHg) in fish organs exhibited the following distribution patterns: muscle > heart > liver > swim bladder > gill. Moreover, highly significant positive correlations were observed between MeHg and THg concentrations in gill ($r = 0.83$, $p < 0.01$), heart ($r = 0.95$, $p < 0.01$), liver ($r = 0.93$, $p < 0.01$), muscle ($r = 0.88$, $p < 0.01$) and swim bladder ($r = 0.88$, $p < 0.01$).

Our results suggested that considerably different levels of Hg accumulation exist among organs of fish; muscle has the highest ability to accumulate Hg compared to the other organs. The distribution patterns of Hg species in organs of fish samples are in agreement with the previous studies (Goldstein et al., 1996; Mallory et al., 2018; Squadrone et al., 2013). However, different distribution patterns of Hg level in fish organs was reported previously, such as gill > liver > heart > white muscle in *Brycon amazonicus* (Monteiro et al., 2010), kidney > swim bladder > muscle > gill in fish in a large antimony mining area (Fu et al., 2010), and liver > gill > muscle in pelagic fish species (Hosseini et al., 2013). These may often be shorter-lived species that feed at high trophic levels, and thus comparably high Hg was ingested in their diet (Mallory et al., 2018). After dietary exposure, MeHg may initially be high in organs like liver, but eventually binds to sulfhydryl groups in protein in muscle, and elimination rates of MeHg generally are lower than uptake rates (Wiener et al., 1996). Another explanation may stem from internal redistribution of Hg as the fish degrade protein and catabolise muscle organs for energy. Increased MeHg exposure accelerated demethylation in liver, and the increased produced IHg binding and immobilizing to metallothionine and other proteins containing sulfhydryl groups resulted in higher THg concentration in liver relative to muscle (Atta et al., 2012). This explanation seemed insufficient because intestine was confirmed the important demethylation organ instead of liver (Wang et al., 2017). In this study, a possible speculation for higher

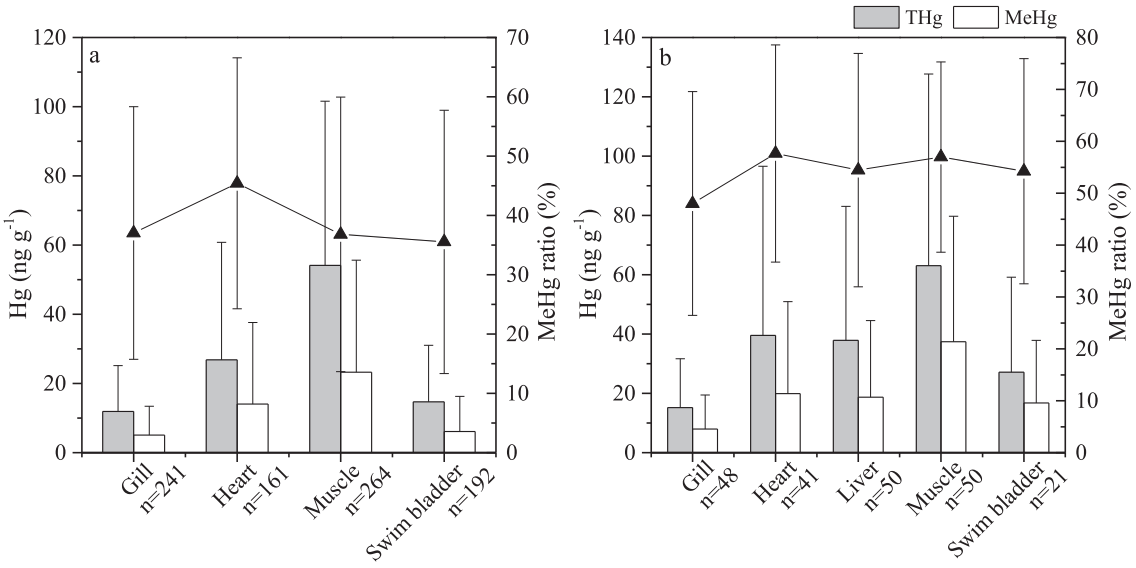

Fig. 3. The concentrations of Hg species (THg and MeHg) and MeHg/THg ratios in gill, heart, muscle, and swim bladder of 264 fish samples (a); the concentrations of Hg species (THg and MeHg) and MeHg/THg ratios in gill, heart, muscle, liver, and swim bladder of selected 50 fish samples (b).

MeHg concentrations in muscle than liver may result from prolonged exposure to low dietary concentrations but lower elimination rates over many years, such that MeHg becomes relatively high in muscle (Wiener et al., 1996). Negligible elimination of MeHg from muscle (<0) and efficient elimination of MeHg from gills (0.12 d^{-1}), liver (0.17 d^{-1}) and intestine (0.20 d^{-1}), as well as efficient transportation of MeHg from other organs into muscle also supported this speculation (Peng et al., 2016). In contrast, IHg was much more slowly distributed into muscle but was efficiently eliminated by the intestine (0.13 d^{-1}) (Peng et al., 2016). Despite of these, MeHg proportions didn't show any clear trend among these organs. Other factors, such as the mode of exposure (e.g. dietary and/or aqueous exposure), body condition and reproductive status, also influence Hg distribution between the different organs (Mallory et al., 2018; Squadrone et al., 2013). Besides, gills in some studies were more often found to have high concentrations of heavy metals (Monteiro et al., 2010). Because it directly exposed water and suspended materials to absorption, osmoregulation and gas exchange, playing remarkable influences on the exchange of toxic metals between a fish and the surrounding environment (Hosseini et al., 2013). In this study, the gills contained relatively lower Hg concentrations comparing with other organs, indicating that Hg uptake in fish organs was not primarily from water, possibly originated from food consumption, mainly absorbing through gastrointestine (Atta et al., 2012).

3.3. Hg distribution in trophic levels

Concentrations of THg and MeHg as well as the ratios of MeHg to THg (MeHg/THg) in five organs (gill, heart, liver, muscle and swim bladder) of different fish species (CV, OV, PV, and HV) are displayed in Fig. 4. Our results showed that concentrations of THg and MeHg in different fish organs among four trophic levels exhibited the following distribution patterns: CV > OV > PV > HV (Fig. 4). Furthermore, the relatively higher MeHg concentrations and MeHg/THg were generally observed in CV and OV for each of the fish organs. However, no discernable difference in the concentration of MeHg in liver, muscle and swim bladder between CV and OV (ANOVA test, $p > 0.05$), as well as in the MeHg concentration in gill and muscle between PV and HV (ANOVA test, $p > 0.05$) were observed. Statistically significant differences in %MeHg in heart (ANOVA test, $F = 8.98$, $p = 0.000$) and muscle (ANOVA test, $F = 4.62$, $p = 0.040$) were observed among four trophic levels. Furthermore, significant difference in THg concentration in gill, heart, and muscle was observed between CV and OV (ANOVA test, $p < 0.05$). However, we failed to observe discernable difference in the THg concentration in gill, muscle and swim bladder between PV and HV (ANOVA test, $p > 0.05$).

Our results showed higher MeHg levels and MeHg/THg in predatory fish than those in non-predatory fish species, which agree with the previous studies (Yu et al., 2012). Current study implied that 1) MeHg levels and MeHg/THg in carnivorous species reflected their position within the food chains, 2) bioaccumulation and biomagnification of MeHg in different fish species was related to diet habit and ability to migrate (Maršálek et al., 2005; Zhang et al., 2007a). Carnivorous species which are located at the top trophic level, feeding on a combination of fish, generally accumulate higher levels of MeHg. On the opposite, the fish species with low level of MeHg and MeHg/THg such as PV and HV, were principally vegetation or invertebrate foragers. In this study, CV and OV roughly contained relatively higher MeHg/THg than other two trophic levels (PV and HV). This results indirectly reflected the higher IHg proportions in PV and HV. It is generally acceptable that the bioaccumulation of IHg and MeHg is related to the primary producer (e.g. macrophytes and phytoplankton). However, MeHg was transferred more efficiently from phytoplankton to herbivores than IHg (Mason et al., 1996; Pickhardt and Fisher, 2007). More important, previous study suggested that the efflux rate constants of MeHg (0.024 d^{-1}) was evidently lower than that for IHg (0.104 d^{-1}) in herbivorous fish (Peng et al., 2016). Therefore, the relatively higher MeHg concentrations and MeHg/THg in predatory fish (CV and OV) but relatively lower MeHg concentrations and MeHg/THg in non-predatory (PV and HV) were jointly observed in this study.

3.4. Bioaccumulation characteristics of food web

Values of $\delta^{13}C$ across 14 fish species ranged from −26.45 to −17.76‰, with a mean value of -22.53 ± 1.94‰ (Table S4 and Fig. 5-a), without any manifest increasing trend from primary producers to consumers. Neither any statistically significant

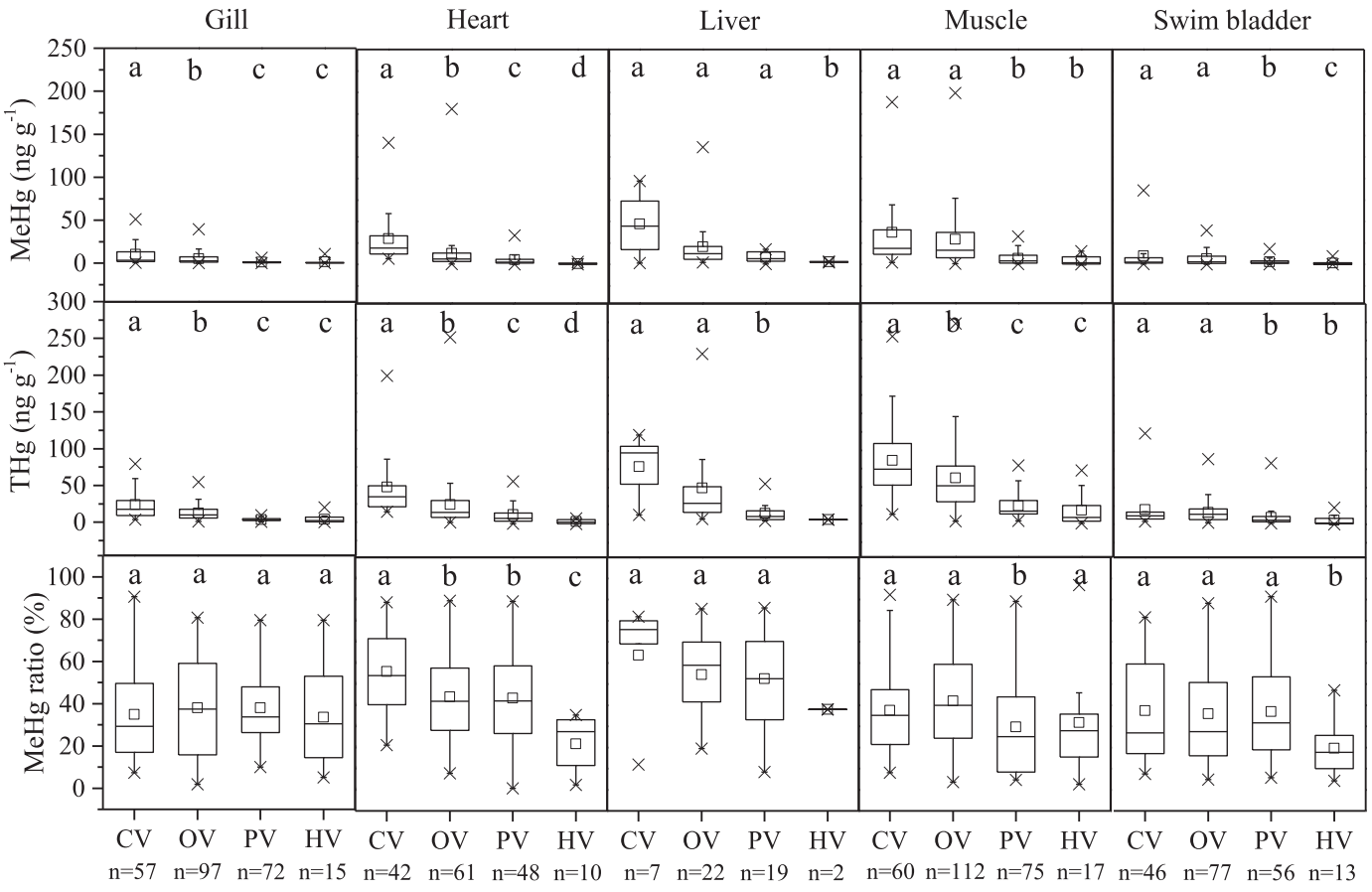

Fig. 4. Concentrations of Hg species (THg and MeHg) and MeHg/THg ratios in five organs in carnivorous (CV, n = 60), omnivorous (OV, n = 112), planktivorous (PV, n = 75) and herbivorous (HV, n = 17) fish species in the Three Gorges Reservoir ("×", the minimum and maximu value; "□", mean value; "-", mid-value; "┬"; "┴", standard deviation; a, b, c, and d show significant levels after ANOVA analysis followed by Tamhane's T2(M) test; the data sets were log-transferred when they were not normal).

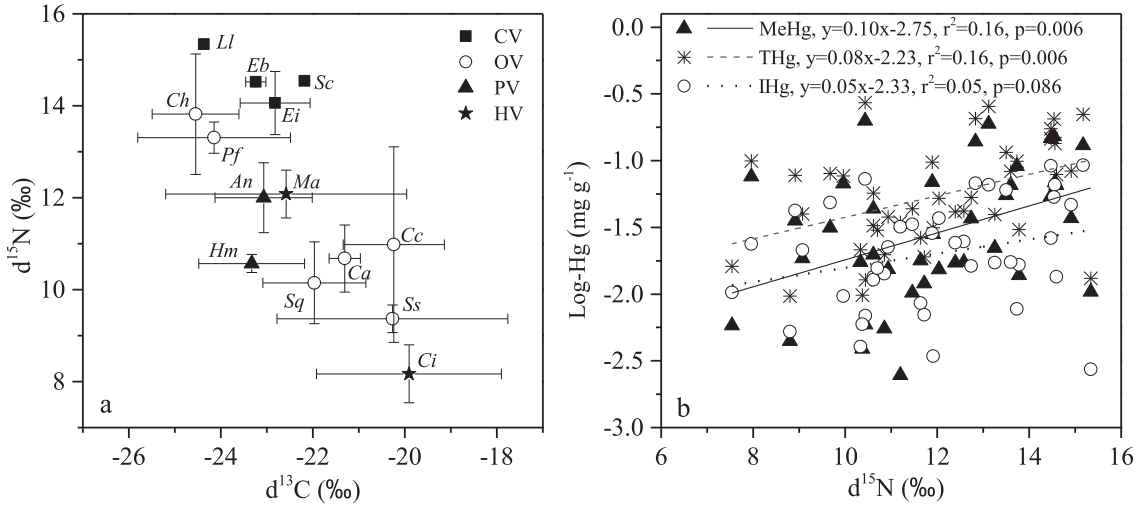

Fig. 5. Correlation between $\delta^{13}C$ and $\delta^{15}N$ in 14 fish species in the Three Gorges Reservoir (a); correlation between log-transformed Hg concentrations and $\delta^{15}N$ value in fish samples in the Three Gorges Reservoir (b).

differences were detected among different trophic levels (ANOVA test, F = 1.112, p = 0.357). The trophic levels of CV (−24 to −22‰) and PV (−24 to −22‰) were observed similar ranges of $\delta^{13}C$ values, suggesting that they might have the same carbon sources. The $\delta^{13}C$ values of OV (−26 to −18‰) and HV (−26 to −18‰) fish also had similar ranges that wider than other two trophic levels, suggesting more diverse food sources. Wang et al. (2014a) reported that the range of $\delta^{13}C$ values in fine particular organic matter, epiphytic

algae, and filamentous algae from middle Yangtze River ranged from −26‰ to −23.8‰, −20‰ to −18‰ and −24‰ to −22‰, respectively. Current study jointly with the previous observations (Wang et al., 2014a) suggested that fine particular organic matter, epiphytic algae, and filamentous algae were the important sources of carbon to OV and HV. However, the primary sources of carbon to CV and PV were limited to fine particular organic matter and epiphytic algae. The $\delta^{13}C$ values of the particulate organic matter (POC) pool were found consistent with the signature of the terrestrial soil organic matter in the Yangtze River, in which the planktonic algal carbon was a minor component of POC pool (Wu et al., 2007). In addition, terrestrial plants, such as some plant parts (seeds and fruits) with high nutritional value, maybe be directly consumed by tropical aquatic animal species, being the dominant carbon sources supporting the consumer taxa (Goulding, 1980; Wang et al., 2014a). Consequently, terrestrial loading of organic matter became an important carbon source to aquatic consumer production.

Notably, $\delta^{13}C$ values decreased with increasing $\delta^{15}N$, showing significant correlations (linear regression, $r^2 = 0.26$, $p < 0.001$). This result was in accordance with previous study (Feng et al., 2018), indicating that food sources for organism at different trophic levels in the food web varied greatly and exhibited a higher degree of bioaccumulation. $\delta^{15}N$ values distributed in the range of 7.5–15‰ with a mean value of 12 ± 2.0‰, increasing with trophic levels. The increase of $\delta^{15}N$ values with trophic levels reflected that ^{15}N is retained preferentially during nutrient assimilation and incorporation into animal tissues (Gu et al., 1996). $\delta^{15}N$ in CV fish species (14 ± 0.64‰) is of substantially higher magnitude than the remaining three trophic levels (ANOVA test, $F = 7.70$, $p < 0.01$), whereas differences between the latter three were insignificant. Nevertheless, $\delta^{15}N$ values of some OV fish were apparently different, for instance, mean $\delta^{15}N$ values of *Ch* and *Pf* were 13–14‰ while *Sq, Ca, Cc* and *Ss* were 9–11‰. These differences attributed to nutritional habits from two groups. Similarly, large difference in $\delta^{15}N$ between two groups of HV fish (*Ma* and *Ci*) were also observed in this study. *Ma* primarily feeds on zooplankton, Vallisneria natans and Hydrilla verticillata (Ke, 1975), whereas *Ci* is known to feed on a wide variety of terrestrial plants with varying fibre content (Hajra et al., 1987; Wen, 1990). Such strong differences in $\delta^{15}N$ among primary producers and in detritus were reported previously (Dominik et al., 2014), which supports our observations.

Regarding relative trophic position (TP) calculated from $\delta^{15}N$ values (Table S4), it was also increasing with trophic levels (CV: 3.8 ± 0.19‰, OV: 2.9 ± 0.58‰, PV: 2.8 ± 0.26‰, HV: 2.6 ± 0.59‰). CV fish species also had significantly higher TP than the other three trophic levels (ANOVA test, $F = 7.70$, $p < 0.01$), while trophic levels of OV, PV and HV showed a high degree of overlap and no significant difference (ANOVA test, $F = 0.69$, $p > 0.05$). The limited variation in TP (2.29) indicated approximately two trophic positions. Thus, short food chains indicated that the fish in the TGR would tend to bioaccumulate less Hg.

Fig. 5-b showed significant correlations between Hg concentrations (THg and MeHg) and $\delta^{15}N$ although they were weak ($r^2 = 0.16$), reflecting Hg biomagnification in a food chain. The regression slopes (0.08 for THg and 0.10 for MeHg) of log-Hg concentration versus $\delta^{15}N$ indicated trophic magnification factor (TMF) in the TGR, which were relatively lower in comparison with other aquatic systems from America and Canada (0.13–0.29) (Clayden et al., 2013; Kidd et al., 2012). Generally, higher slopes were normally reported in lakes that had lower nutrients/MeHg/chloride factors, suggesting higher Hg biomagnification in lakes with lower trophic status (Clayden et al., 2013). This indirectly supports some studies that biotic Hg concentrations can undergo biomass and growth dilution in more nutrient-rich systems (Li et al., 2015). Although no correlations were observed between Hg concentrations and body size for some fish species (e.g. OV, PV and HV) (Table S2), it was not a direct evidence for growth dilution. It should be noted that the fish samples were collected from different sites with different trophic states (tributaries were more nutritious than mainstream) and the length and complexity of their food chains varied greatly. These may partially explain the low slopes of log-Hg concentrations (THg and MeHg) versus $\delta^{15}N$. Furthermore, the simple food web structure which was not favourable to the accumulation of Hg, could be another reason to the low slopes. However, we failed to observe the correlations between IHg concentrations and $\delta^{15}N$ ($p = 0.086$), with the slopes (0.05) lower than that of MeHg, indicating the difference bioaccumulation patterns of IHg versus MeHg. Previous study suggested that MeHg primarily accumulated in the cell cytoplasm of phytoplankton being assimilated by zooplankton, whereas IHg was principally bound in phytoplankton membranes (Mason et al., 1996). Moreover, the assimilated efficiency of MeHg was four times more than that of IHg (Mason et al., 1996). Therefore, the TMF of MeHg was observed to be much higher than that of IHg in this study.

3.5. "Reservoir effect" in the TGR

3.5.1. Concentrations of Hg in fish before and after impoundment of the TGR

The concentrations and temporal distribution patterns of THg and MeHg in fish muscle from the TGR was listed in Fig. 6 and summary data are shown in Supplementary Information Table S5. As summarized in Table S6, the methods for Hg analysis in fish samples employed in this study and literatures were all popular used all over the world. Therefore, we do believe that the results listed in Fig. 6 and Table S5 were trustful and comparable. It should be noted that the TGR started to impound in 2003, with the water level of 135 m; and then, the water level reached up to 155 m in 2006; the TGR was completely created in 2009, with the minimum and maximum water level of 145 m and 175 m, respectively.

In many recently impounded hydroelectric reservoirs, it had been confirmed an elevation of Hg concentrations in predatory and non-predatory fish species (Bodaly et al., 2007; Morrison and Therien, 1995), which can be called as "reservoir effect". As shown in Fig. 6 and Table S5, a slightly descending trend of THg and MeHg concentration in fish muscles during the period from 1986 to 2014. Furthermore, the Hg levels in *Ei, Sa, Ll* and *Ch* all decreased with the time went on, only *Pf* showed a crosscurrent. Therefore, our findings verified that no discernible "reservoir effect" was observed in the TGR within the initial few years after impoundment.

In China, many reservoirs were shown relatively low concentrations of Hg levels and MeHg/THg in fish samples. In Wujiang river, new reservoirs didn't exhibit higher Hg levels than the old ones, thus Hg levels were not fully consistent with the age and evolution of reservoirs (Feng et al., 2018). Even in Baihua Reservoir that affected by industrial wastewater containing Hg, the aquatic food web was found disconnected from the high MeHg zone (Liu et al., 2012). As for the newly TGR, the biggest difference with these reservoirs was the large inundated areas, this probably caused the elevation of Hg methylation. But the fact is our results showed relatively low Hg levels of fish. Contrary to fish from reservoirs in North America and Europe, extremely high Hg concentrations in predators were frequently observed (Bilodeau et al., 2018; Brinkmann and Rasmussen, 2010; Carrasco et al., 2011). In these reservoirs, it has been generally accepted that the high initial MeHg production is related to an initially high availability of fresh organic matter (Bodaly et al., 2007; Silva et al., 2009; Larssen, 2010).

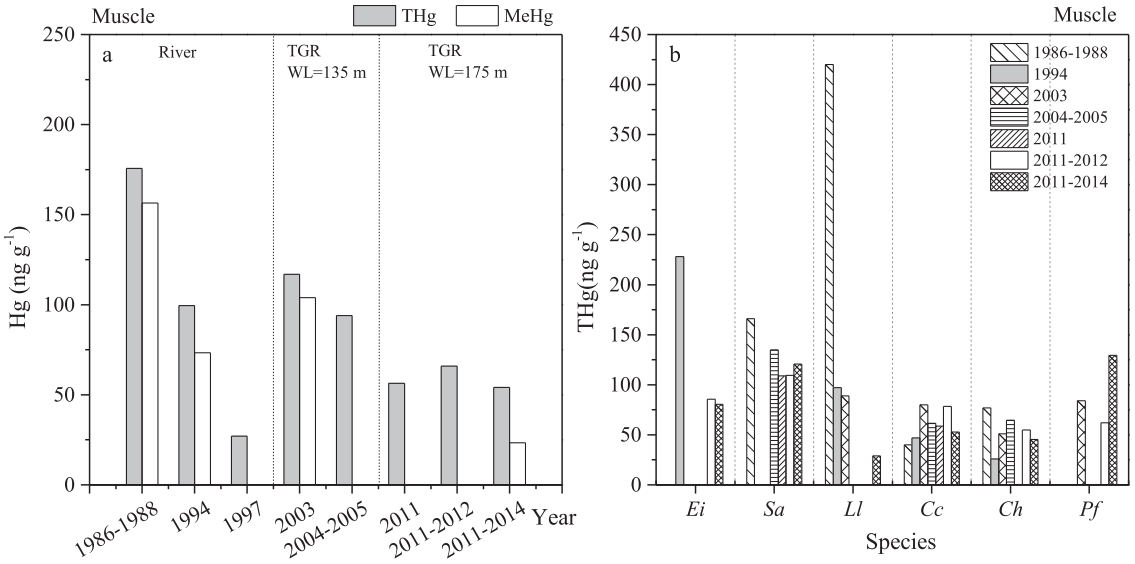

Fig. 6. The concentrations and temporal distribution patterns of THg and MeHg in fish muscle from the Three Gorges Reservoir (WL: water level of the TGR) (a); the concentrations and distribution of THg in muscle of different fish species from the Three Gorges Reservoir (b).

3.5.2. Possible reason of low Hg in fish from the TGR

In the aquatic environment, Hg can be absorbed by organisms through both diffusion and active uptake mechanisms (e.g., by Na^+ and $Ca^{2+)}$. Hg species determined the degree of absorption and biomagnification, which was influenced by organic matter, inorganic ligands, thiol compounds, pH and other chemical factors (Feng et al., 2018). Relatively low Hg levels of the TGR aqueous system (Table 1) could partly explain low Hg concentrations in fish. Under acidic conditions, H^+ may compete with Hg^{2+} at binding site of DOM, thereby favoring the release of Hg^{2+} (Haitzer et al., 2003). The enables inorganic Hg to be activated, absorbed and methylated by organisms. But the relatively low DOC and high pH value of the TGR water may affect Hg bioavailability and methylation, generating comparatively low MeHg concentrations in biota in this aquatic system. In mainstream of the TGR, no seasonal stratification and alga bloom were presented, where less organic matter and nutrients were unfavorable for the methylation and bioaccumulation of mercury in reservoir food chain (Cebalho et al., 2017; Kasper et al., 2014).

In Chinese reservoir, the organic matter of the freshly flooded soil is low and hence the methylation is not driven by the access to fresh material to the some extent (Larssen, 2010). The TGR in particular, it was one of largest reservoir in the world, flooding large areas with different land use. The inundated area was about 350 km^2, primarily composing of bare lands (40%), agricultural lands (30%) and urban areas (10%) before impoundment (Zhang, 2008). The average THg concentration of flooded soil in the TGR was 52 ng g^{-1}, which was in line with the global average level of 50–100 ng g^{-1} (Fadini and Jardim, 2001). But the relatively low organic matter (<1.0%) and the low Hg methylation activity in soils of seasonally drying and flooding alternating areas were observed in the TGR (Xiang et al., 2018). It is reasonable that the overall MeHg level in reservoir surroundings and possibly their catchments are very low.

Submerged flora in the TGR, is dominated by forbs, herbs, shrubs, and dwarf trees, including annuals (*Setaria viridis, Digitaria ciliaris, and Leptochloa chinensis* etc.), flood-tolerant perennials (*Cynodon dactylon, Hemarthria altissima and Capillipedium assimile* etc.) and some woody plants (*Ficus tikoua, Pterocarya stenoptera, and Vitex negundo* etc.) (Lu et al., 2010). Although submerged plant tissues lead to an increase in reservoir Hg mass, they released far more less Hg and nutrients to waters than these in forest inundated reservoirs (Hall and St Louis et al., 2004; Roy et al., 2009). With a long period of impoundment and draw-down, more flood-tolerant perennials tended to survive and annuals gradually faded away, indicating a low rate of plant decomposition unable to boost. Despite flood-tolerant perennials can uptake MeHg into their tissues from soils and enhance Hg methylation in vegetated pots (Windhammyers et al., 2015), it also can decrease Hg methylation by reducing the bioavailable fraction of inorganic Hg possibly through sorption or co-precipitation with the iron plaque formed on the outside of roots or on the root biomass itself (Strickman and Mitchell, 2016; Ullrich et al., 2001; Wang et al., 2014b). In current study, the vegetated terrain were entirely colonized with plant root, which increase the transportation of the subsurface porewater and its associated solutes, as the evapotranspiration drove the movement of porewater MeHg from the bulk to the rhizosphere soil (Windhammyers et al., 2014). This process is an important modulator of MeHg dynamics, inhibiting the release of MeHg to aquatic water from inundated soils in the TGR. As described above, less soil coverage and the more flood-tolerant perennials for Hg storage may decrease the pool of Hg availability for remobilization, and consequently reduce Hg accumulation in fish.

Apart from the low methylation activities of water, inundated soil and plant, the simple food web structure in the TGR also contributed to the low MeHg found in the bio-samples. Food sources and feeding habits have been disturbed, as well as species diversity and community structures of fish have been changed dramatically induced by the TGR constructed. Thus, altered food web structure and trophodynamics may reduce biomagnification and accumulate rate of Hg in biota (Lavoie et al., 2013; Ofukany et al., 2014). Moreover, OV fish (42%) with lower Hg concentrations was the dominant fish species sampled in the TGR. For these factors, it is reasonable to explain the relatively lower Hg concentrations in fish. Despite low Hg levels in fish posed no health risks was widely believed, high Hg intake in riverine groups had great opportunities to exceed the recommended reference dose value (0.1 μg Hg per kg body weight per day for MeHg), thus these communities could be considered at risk (Ceccatto et al., 2016). Therefore, low Hg levels of fish should not be oversight. Fish

consumers should be careful, especially for the vulnerable groups, such as children and pregnant women.

4. Conclusion

This study investigated the bioaccumulation characteristics of THg and MeHg in different organs (gill, heart, liver, muscle and swim bladder) of fish species in the TGR. The values of $\delta^{13}C$ and $\delta^{15}N$ in fish muscle were further analyzed to reveal the biomagnification properties of Hg in food chains. Our results showed that Hg concentrations in the TGR were in relatively low levels, and did not appear to pose a health risk. The highest Hg levels was observed in muscle, followed by heart, liver, swim bladder and gill. Negligible elimination of MeHg from muscle as well as efficient transportation of MeHg from other organs into muscle probably resulted in high Hg levels in muscle. Hg levels increased with the trophic levels, which was accordance with other studies. $\delta^{13}C$ values decreased with increasing $\delta^{15}N$, showing significant correlations (linear regression, $r^2 = 0.26$, $p < 0.001$). This result indicated that food sources for organism at different trophic levels in the food web varied greatly and exhibited a higher degree of bioaccumulation. Nevertheless, the limited variation of TP (2.29) showed a simple food web in the TGR. Uniformly, low trophic magnification factor (TMF) (0.08 for THg and 0.10 for MeHg) was also observed. Apart from these, the low methylation activities of water, inundated soil and plant also contributed to the low Hg levels in predators. Thus, the TGR showed no discernible “reservoir effect” within the initial few years after impoundment.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (Nos. 41173116, 41373113, and 41703130) and Fundamental Research Funds for the Central Universities (XDJK2015C124).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.08.048.

References

Abernathy, A.R., Cumbie, P.M., 1977. Mercury accumulation by largemouth bass (Micropterus salmoides) in recently impounded reservoirs. Bull. Environ. Contam. Toxicol. 17, 595–602.
Agnihotri, R., Kumar, R., Prasad, M.V.S.N., Sharma, C., Bhatia, S.K., Arya, B.C., 2014. Experimental setup and standardization of a Continuous Flow Stable Isotope Mass Spectrometer for measuring stable isotopes of carbon, nitrogen and sulfur in environmental samples. Mapan 29, 195–205.
Atta, A., Voegborlo, R.B., Agorku, E.S., 2012. Total mercury distribution in different tissues of six species of freshwater fish from the Kpong hydroelectric reservoir in Ghana. Environ. Monit. Assess. 184, 3259–3265.
Beckers, F., Rinklebe, J., 2017. Cycling of mercury in the environment: sources, fate, and human health implications: a review. Crit. Rev. Environ. Sci. Technol. 47, 693–794.
Bilodeau, F., Therrien, J., Schetagne, R., 2018. Intensity and duration of effects of impoundment on mercury levels in fishes of hydroelectric reservoirs in northern Québec (Canada). Inland Waters 7, 493–503.
Bodaly, R.D., Jansen, W., Majewski, A., Fudge, R., Strange, N., Derksen, A., Green, D., 2007. Postimpoundment time course of increased mercury concentrations in fish in hydroelectric reservoirs of northern Manitoba, Canada. Arch. Environ. Contam. Toxicol. 53, 379–389.
Brinkmann, L., Rasmussen, J.B., 2010. High levels of mercury in biota of a new Prairie irrigation reservoir with a simplified food web in Southern Alberta, Canada. Hydrobiologia 641, 11–21.
Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Natl. Acad. Sci. Unit. States Am. 93, 10844–10847.
Carrasco, L., Benejam, L., Benito, J., Bayona, J.M., Díez, S., 2011. Methylmercury levels and bioaccumulation in the aquatic food web of a highly mercury-contaminated reservoir. Environ. Int. 37, 1213–1218.
Cebalho, E.C., Díez, S., Filho, M.D.S., Muniz, C.C., Lázaro, W., Malm, O., Ignácio, A.R.A., 2017. Effects of small hydropower plants on mercury concentrations in fish. Environ. Sci. Pollut. Res. Int. 24, 22709–22716.
Ceccatto, A.P.S., Testoni, M.C., Ignácio, A.R.A., Santos-Filho, M., Malm, O., Díez, S., 2016. Mercury distribution in organs of fish species and the associated risk in traditional subsistence villagers of the Pantanal wetland. Environ. Geochem. Health 38, 713–722.
Clayden, M.G., Kidd, K.A., Wyn, B., Kirk, J.L., Muir, D.C., O'Driscoll, N.J., 2013. Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes. Environ. Sci. Technol. 47, 12047–12053.
Dominik, J., Tagliapietra, D., Bravo, A.G., Sigovini, M., Spangenberg, J.E., Amouroux, D., Zonta, R., 2014. Mercury in the food chain of the Lagoon of Venice, Italy. Mar. Pollut. Bull. 88, 194–206.
Eckley, C., Luxton, T., McKernan, J., Goetz, J., Goulet, J., 2015. Influence of reservoir water level fluctuations on sediment methylmercury concentrations downstream of the historical Black Butte mercury mine. OR. Appl. Geochem. 61, 284–293.
Fadini, P.S., Jardim, W.F., 2001. Is the negro River basin (Amazon) impacted by naturally occurring mercury? Sci. Total Environ. 275, 71–82.
Feng, X., Meng, B., Yan, H., Fu, X., Yao, H., Shang, L., 2018. Chapter 9: bioaccumulation of mercury in aquatic food chain. In: Biogeochemical Cycle of Mercury in Reservoir Systems in Wujiang River Basin, Southwest China. Springer, pp. 339–390.
Fu, C., 2010. Temporal and Spatial Distribution of the Heavy Metals in the Water-Level-Fluctuating Zone and The Effects of DOM on the Behaviors of Heavy Metals, Municipal Engineering. Dissertation. Chongqing University, Chongqing, P.R. China.
Fu, Z., Wu, F., Amarasiriwardena, D., Mo, C., Liu, B., Zhu, J., Deng, Q., Liao, H., 2010. Antimony, arsenic and mercury in the aquatic environment and fish in a large antimony mining area in Hunan, China. Sci. Total Environ. 408, 3403–3410.
Gill, G.A., Bruland, K.W., 1990. Mercury speciation in surface freshwater systems in California and other areas. Environ. Sci. Technol. 24, 1392–1400.
Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26, 2281–2287.
Goldstein, R.M., Brigham, M.E., Stauffer, J.C., 1996. Comparison of mercury concentrations in liver, muscle, whole bodies, and composites of fish from the Red River of the North. Can. J. Fish. Aquat. Sci. 53, 244–252.
Goulding, M., 1980. Chapter 12: Interactions of Fishes with Fruits and Seeds, the Fishes and the Forest, Explorations in Amazonian Natural History. University of California press, pp. 217–230.
Gu, B., Schelske, C.L., Hoyer, M.V., 1996. Stable isotopes of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow hypereutrophic lake. J. Fish. Biol. 49, 1233–1243.
Haitzer, M., Aiken, G.R., Ryan, J.N., 2003. Binding of mercury (II) to aquatic humic substances: influence of pH and source of humic substances. Environ. Sci. Technol. 37, 2436–2441.
Hajra, A., Tripathi, S., Nath, D., Chatterjee, J., Karmakar, H., 1987. Comparative digestibility of dietary plant fibre in grass carp Ctenopharyngodon idella (Val). Proc. Natl. Acad. Sci. Unit. States Am. 57, 232–236.
Hall, B., Louis, V.S., Rolfhus, K., Bodaly, R., Beaty, K., Paterson, M., Cherewyk, K.P., 2005. Impacts of reservoir creation on the biogeochemical cycling of methyl mercury and total mercury in boreal upland forests. Ecosystems 8, 248–266.
Hall, B.D., St Louis, V.L., 2004. Methylmercury and total mercury in plant litter decomposing in upland forests and flooded landscapes. Environ. Sci. Technol. 38, 5010–5021.
He, T., Wu, Y., Pan, L.S., 2010. Distribution of mercury species and their concentrations in fish in Hongfeng reservoir. J. Southwest Univ. 32, 78–82.
He, X., 2013. Variation Characteristics of Total Mercury and Methylmercury in Soil (Sediment) in Water-level-fluctuating Zone of the Three Gorges Resevoir Region, Environmental Science and Engineering. Dissertation. Southwest University, Chongqing, P.R. China.
Hintelmann, H., 2010. Chapter 11: organomercurials. Their formation and pathways in the environment. In: Organometallics in Environment and Toxicology. The Royal Society of Chemistry, pp. 365–401.
Hosseini, M., Nabavi, S.M.B., Parsa, Y., 2013. Bioaccumulation of trace mercury in trophic levels of benthic, benthopelagic, pelagic fish species, and sea birds from Arvand River, Iran. Biol. Trace Elem. Res. 156, 175–180.
Huang, Q., He, B., Zhao, X., Wang, Y., 2015. Temporal variation of trophic status in drawdown area of Hanfeng Lake in the storage period of Three Gorges Reservoir in China. Environ. Sci. 36, 928–935.
Jackson, T.A., 1988. The mercury problem in recently formed reservoirs of northern Manitoba (Canada): effects of impoundment and other factors on the production of methyl mercury by microorganisms in sediments. Can. J. Fish. Aquat. Sci. 45, 97–121.
James, G.W., David, P.K., Gray, H.H., Anton, M.S., 2002. Chapter 16: Ecotoxicology of Mercury, Handbook of Ecotoxicology. CRC press, pp. 409–465.
Jiang, H., Feng, X., Yan, H., 2010. Mercury distribution characteristics in fish body in Reservoirs of Wujiang watershed. In: Chinese Environment Science Association Academic Anual Conference Memoir, vol.3, pp. 2557–2562.
Jin, L., Xu, X., 1997. Methylmercury distribution in surface water and fish in the Three Gorges Reservoir area. Pesources & Enuironment in the Yangtza Valley 6, 324–328.
Kasper, D., Forsberg, B.R., Amaral, J.H., Leitã£O, R.P., Py-Daniel, S.S., Bastos, W.R., Malm, O., 2014. Reservoir stratification affects methylmercury levels in river water, plankton, and fish downstream from Balbina hydroelectric dam,

Amazonas, Brazil. Environ. Sci. Technol. 48, 1032–1040.
Ke, H., 1975. An excellent fresh water food fish, Megalobrama amblycephala, and its propagating and culturing. Acta Hydrobiol. Sin. 5, 293–314.
Kidd, K., Clayden, M., Jardine, T., 2012. Chapter 14: Bioaccumulation and Biomagnification of Mercury through Food Webs, Environmental Chemistry and Toxicology of Mercury. Wiley, Hoboken, pp. 455–499.
Larssen, T., 2010. Mercury in Chinese reservoirs. Environ. Pollut. 158, 24–25.
Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environ. Sci. Technol. 47, 13385–13394.
Li, B., 2012. Ecological Studies on the Fish Food Web Structures and Trophic Relationships with Stable Isotope Technology in a Tributary in the Three Gorges Reservoir Areas (TGR), School of Life Science. Dissertation. Southwest University, Chongqing, P.R. China.
Li, J., Zhou, Q., Yuan, G., He, X., Xie, P., 2015. Mercury bioaccumulation in the food web of Three Gorges Reservoir (China): tempo-spatial patterns and effect of reservoir management. Sci. Total Environ. 527, 203–210.
Li, P., 2013. Distribution Characteristics of Mercury in Water in Water Levle Fluctuating Zone of the Three Gorges Reservoir, Environmental Science and Engineering. Dissertation. Southwest University, Chongqing, P.R. China.
Li, R., Zhang, X., 2010. Analysis of water quality in three Gorges zhongxian branch. Environment & Ecology in the Three Gorges 32, 40–43.
Liu, B., Yan, H., Wang, C., Li, Q., Guédron, S., Spangenberg, J.E., Feng, X., Dominik, J., 2012. Insights into low fish mercury bioaccumulation in a mercury-contaminated reservoir, Guizhou, China. Environ. Pollut. 160, 109–117.
Lodenius, M., Seppänen, A., Herranen, M., 1983. Accumulation of mercury in fish and man from reservoirs in northern Finland. Water Air Soil Pollut. 19, 237–246.
Lu, Z., Li, L., Jiang, M., Huang, H., Bao, D., 2010. Can the soil seed bank contribute to revegetation of the drawdown zone in the Three Gorges Reservoir Region? Plant Ecol. 209, 153–165.
Mailman, M., Stepnuk, L., Cicek, N., Bodaly, R.A., 2006. Strategies to lower methyl mercury concentrations in hydroelectric reservoirs and lakes: a review. Sci. Total Environ. 368, 224–235.
Mallory, M.L., O'Driscoll, N.J., Klapstein, S., Varela, J.L., Ceapa, C., Stokesbury, M.J., 2018. Methylmercury in tissues of Atlantic sturgeon (Acipenser oxyrhynchus) from the saint john river, new brunswick, Canada. Mar. Pollut. Bull. 126, 250–254.
Maršálek, P., Svobodová, Z., Randák, T., Švehla, J., 2005. Total mercury and methylmercury contamination of fish from the Skalka reservoir: a case study. Acta Vet. 74, 427–434.
Marvindipasquale, M., Windhammyers, L., Agee, J.L., Kakouros, E., Kieu, I.H., Fleck, J.A., Alpers, C.N., Stricker, C.A., 2014. Methylmercury production in sediment from agricultural and non-agricultural wetlands in the Yolo Bypass, California, USA. Sci. Total Environ. 484, 288–299.
Mason, R.P., Reinfelder, J.R., Morel, F.M., 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. Environ. Sci. Technol. 30, 1835–1845.
Meng, B., Feng, X.B., Chen, C.X., Qiu, G.L., Sommar, J., Guo, Y.N., Liang, P., Wan, Q., 2010. Influence of eutrophication on the distribution of total mercury and methylmercury in hydroelectric reservoirs. J. Environ. Qual. 39, 1624–1635.
Monteiro, D.A., Rantin, F.T., Kalinin, A.L., 2010. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxã, Brycon amazonicus (Spix and Agassiz, 1829). Ecotoxicology 19, 105–123.
Morrison, K., Therien, N., 1995. Changes in mercury levels in lake whitefish (Coregonus clupeaformis) and northern pike (Esox lucius) in the LG-2 reservoir since flooding. Water Air Soil Pollut. 80, 819–828.
Ofukany, A.F., Wassenaar, L.I., Bond, A.L., Hobson, K.A., 2014. Defining fish community structure in Lake Winnipeg using stable isotopes (δ13C, δ15N, δ34S): implications for monitoring ecological responses and trophodynamics of mercury & other trace elements. Sci. Total Environ. 497, 239–249.
Ouédraogo, O., Amyot, M., 2013. Mercury, arsenic and selenium concentrations in water and fish from sub-Saharan semi-arid freshwater reservoirs (Burkina Faso). Sci. Total Environ. 444, 243–254.
Peng, X., Liu, F., Wang, W.X., 2016. Organ-specific accumulation, transportation, and elimination of methylmercury and inorganic mercury in a low Hg accumulating fish. Environ. Toxicol. Chem. 35, 2074–2083.
Pickhardt, P.C., Fisher, N.S., 2007. Accumulation of inorganic and methylmercury by freshwater phytoplankton in two contrasting water bodies. Environ. Sci. Technol. 41, 125–131.
Podar, M., Gilmour, C.C., Brandt, C.C., Soren, A., Brown, S.D., Crable, B.R., Palumbo, A.V., Somenahally, A.C., Elias, D.A., 2015. Global prevalence and distribution of genes and microorganisms involved in mercury methylation. Sci. Adv. 1 e1500675.
Ramlal, P.S., Rudd, J.W., Hecky, R.E., 1986. Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. Appl. Environ. Microbiol. 51, 110–114.
Roy, V., Amyot, M., Carignan, R., 2009. Beaver ponds increase methylmercury concentrations in Canadian shield streams along vegetation and pond-age gradients. Environ. Sci. Technol. 43, 5605–5611.
Salazar, J.G., 2000. Damming the child of the ocean: the Three Gorges project. J. Environ. Dev. 9, 160–174.
Silva, D.S.D., Lucotte, M., Paquet, S., Davidson, R., 2009. Influence of ecological factors and of land use on mercury levels in fish in the Tapajós River basin, Amazon. Environ. Res. 109, 432–446.
Silva, G.S.D., Neto, F.F., De Assis, H.C.S., Bastos, W.R., de Oliveira Ribeiro, C.A., 2012. Potential risks of natural mercury levels to wild predator fish in an Amazon reservoir. Environ. Monit. Assess. 184, 4815–4827.
Squadrone, S., Prearo, M., Brizio, P., Gavinelli, S., Pellegrino, M., Scanzio, T., Guarise, S., Benedetto, A., Abete, M., 2013. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (Silurus glanis) from Italian Rivers. Chemosphere 90, 358–365.
Stein, E.D., Cohen, Y., Winer, A.M., 1996. Environmental distribution and transformation of mercury compounds. Crit. Rev. Environ. Sci. Technol. 26, 1–43.
Stewart, A.R., Stern, G.A., Lockhart, W.L., Kidd, K.A., Salki, A.G., Stainton, M.P., Koczanski, K., Rosenberg, G.B., Savoie, D.A., Billeck, B.N., 2003. Assessing trends in organochlorine concentrations in Lake Winnipeg fish following the 1997 Red River flood. J. Great Lake. Res. 29, 332–354.
St. Louis, V.L., Rudd, J.W.M., Kelly, C.A., Bodaly, R.A., Paterson, M.J., Beaty, K.G., Hesslein, R.H., Andrew, H.A., Majewski, A.R., 2004. The rise and fall of mercury methylation in an experimental reservoir. Environ. Sci. Technol. 38, 1348–1358.
Strickman, R.J., Mitchell, C.P., 2016. Accumulation and translocation of methylmercury and inorganic mercury in Oryza sativa: an enriched isotope tracer study. Sci. Total Environ. 574, 1415–1423.
Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit. Rev. Environ. Sci. Technol. 31, 241–293.
USEPA, 2001a. In: Agency, E.P. (Ed.), Method 1630: Mehtylmercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS. Environmental Protection Agency, U.S., pp. 1–41
USEPA, 2001b. Water Quality Criterion for the Protection of Human Health: Methylmercury. EPA-823-R-01-001. US Environmental Protection Agency, Washington, DC.
USEPA, 2002. In: Agency, E.P. (Ed.), Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. Environmental Protection Agency, U.S., pp. 1–41
Wang, F., Zhang, J., 2013. Mercury contamination in aquatic ecosystems under a changing environment: implications for the Three Gorges Reservoir. Chin. Sci. Bull. 58, 141–149.
Wang, J., Gu, B., Huang, J., Han, X., Lin, G., Zheng, F., Li, Y., 2014a. Terrestrial contributions to the aquatic food web in the middle Yangtze River. PLoS One 9 e102473.
Wang, X., Li, B., Tam, F.Y., Huang, L., Qi, X., Wang, H., Ye, Z., Meng, M., Shi, J., 2014b. Radial oxygen loss has different effects on the accumulation of total mercury and methylmercury in rice. Plant Soil 385, 343–355.
Wang, X., Wu, F., Wang, W.X., 2017. In Vivo mercury demethylation in a marine fish (Acanthopagrus schlegeli). Environ. Sci. Technol. 51, 6441–6451.
Wen, Z., 1990. Studies on aquatic vegetation and rational carrying capacity of grass carp, Ctenopharyngodon idella Val. In: Lake Niushanhu, Institute of Hydrobiology. Dissertation, Academia Sinica Wuhan, P.R. China.
WHO, 1990. Environmental Health Criteria 101: Merthylmercury. World Health Organization, Geneva, pp. 1–144.
Wiener, J.G., Spry, D.J., Wiener, J.G., Spry, D.J., Wiener, J.G., Spry, D.J., Wiener, J.G., Spry, D.J., Wiener, J.G., Spry, D.J., 1996. Chapter 13: Toxicological Significance of Mercury in Freshwater Fish, Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations, pp. 297–340.
Windhammyers, L., Fleck, J.A., Ackerman, J.T., Marvindipasquale, M., Stricker, C.A., Heim, W.A., Bachand, P.A., Eaglessmith, C.A., Gill, G., Stephenson, M., 2014. Mercury cycling in agricultural and managed wetlands: a synthesis of methylmercury production, hydrologic export, and bioaccumulation from an integrated field study. Sci. Total Environ. 484, 221–231.
Windhammyers, L., Marvindipasquale, M., Krabbenhoft, D.P., Agee, J.L., Cox, M.H., Herediamiddleton, P., Coates, C., Kakouros, E., 2015. Experimental removal of wetland emergent vegetation leads to decreased methylmercury production in surface sediment. J. Geophys. Res. Biogeosci. 114, 295–311.
Wu, Y., Zhang, J., Liu, S., Zhang, Z., Yao, Q., Hong, G., Cooper, L., 2007. Sources and distribution of carbon within the Yangtze River system. Estuar. Coast Shelf Sci. 71, 13–25.
Xiang, R., Li, Q., Yu, Y., Zhang, H., Dong, C., Shi, J., Wu, Z., 2017. Functional group characteristics of planktonic diatoms and their relationship with environmental factors in the Ruxi river. Environ. Sci. 38, 3290–3301.
Xiang, Y., Wang, Y., Zhang, C., Shen, H., Wang, D., 2018. Water level fluctuations influence microbial communities and mercury methylation in soils in the Three Gorges Reservoir, China. J. Environ. Sci. 68, 206–217.
Xu, X., Qiu, C., Deng, G., Hui, H., Zhang, Y., 1999a. Chemical-ecological effects of mercury pollution in the Three Gorges Reservoir area. Acta Hydrobiol. Sin. 23, 197–203.
Xu, X., Zhang, X., Jin, L., Qiu, C., 1999b. Effect of mercury reactivity on fish mercury contents in the Three Gorges Reservoir after impoundment. Resour. Environ. Yangtze Basin 8, 198–204.
Xu, Y., Zhang, M., Wang, L., Kong, L., Cai, Q., 2011. Changes in water types under the regulated mode of water level in Three Gorges Reservoir, China. Quat. Int. 244, 272–279.
Yan, H., Feng, X., Liang, L., Shang, L., Jiang, H., 2005. Determination of methyl mercury in fish using GC-CVAFS. J. Instrum. Anal. 24, 78–80.
Yan, H.Y., Feng, X.B., Liu, T., Shang, L.H., Li, Z.G., Li, G.H., 2008. Present situation of fish mercury pollution in heavily mercury-contaminated Baihua reservoir in Guizhou. Chinese Journal of Ecology 27, 1357–1361.
Yao, H., Feng, X.B., Yu, Y.H., Le, Q.G., Hai, S.L., Fang, S.W., 2010. Mercury

concentration in fish body in Hongjiadu reservoir of guizhou province. Chinese Journal of Ecology 29, 1155–1160.
Yu, Y., Wang, Y., Zhou, H., Gao, B., Zhao, G., 2013. Mercury contents in fish and its biomagnification in the food web in Three Gorges Reservoir after 175m impoundment. Acta Ecol. Sin. 33, 4059–4067.
Yu, Y., Wang, Y.C., Gao, B., Hong, Y.N., Wan, X.H., Zhou, H.D., 2012. Assessment on pollution risk of mercury in fishes of Three Gorges Reservoir after 175 m impoundment. Resour. Environ. Yangtze Basin 21, 547–551.
Zhang, H., 2008. Characteristic analyses of the water-level-fluctuating zone in the three Gorges reservoir. Bull. Soil Water Conserv. 28, 46–49.
Zhang, L., Zang, X., Xu, J., Xie, P., Zhu, Z., Su, J., 2007a. Mercury bioaccumulation in fishes of three Gorges reservoir after impoundment. Bull. Environ. Contam. Toxicol. 78, 262–264.
Zhang, Q., Lou, Z., 2011. The environmental changes and mitigation actions in the Three Gorges Reservoir region, China. Environ. Sci. Pol. 14, 1132–1138.
Zhang, S., Li, C., Fu, Y., Zhang, Y., Zheng, J., 2008. Trophic states and nutrient output of tributaries bay in Three Gorges Reservoir after impoundment. Environ. Sci. 29, 7–12.
Zhang, Z., He, L., Li, J., Wu, Z.B., 2007b. Analysis of heavy metals of muscle and intestine tissue in fish - in Banan section of Chongqing from Three Gorges Reservoir, China. Pol. J. Environ. Stud. 16, 949–958.