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Mercury bioaccumulation in the food web of Three Gorges Reservoir (China): Tempo-spatial patterns and effect of reservoir management

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HIGHLIGHTS

- Hg concentrations were measured in biota of the main stem of 3 Gorges Reservoir.
- Fish Hg concentration post-flood period > pre-flood period > flood period.
- Fish Hg concentrations were the highest farthest from the dam.
- THg in fish 2 years after inundation were the same as before impoundment.
- Low biomagnification was ascribed to low DOC content in the sediment.

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ABSTRACT

Tempo-spatial patterns of mercury bioaccumulation and tropho-dynamics, and the potential for a reservoir effect were evaluated in the Three Gorges Reservoir (TGR, China) from 2011 to 2012, using total mercury concentrations (THg) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of food web components (seston, aquatic invertebrates and fish). Hg concentrations in aquatic invertebrates and fish indicated a significant temporal trend associated with regular seasonal water-level manipulation. This includes water level lowering to allow for storage of water during the wet season (summer); a decrease of water levels from September to June providing a setting for flood storage. Hg concentrations in organisms were the highest after flooding. Higher Hg concentrations in fish were observed at the location farthest from the dam. Hg concentrations in water and sediment were correlated. Compared with the reservoirs of United States and Canada, TGR had lower trophic magnification factors (0.046–0.066), that are explained primarily by organic carbon concentrations in sediment, and the effect of "growth dilution". Based on comparison before and after the impoundment of TGR, THg concentration in biota did not display an obvious long-term reservoir effect due to (i) short time since inundation, (ii) regular water discharge associated with water-level regulation, and/or (iii) low organic matter content in the sediment.

1. Introduction

Mercury (Hg) is an ubiquitous pollutant released to the environment from natural and anthropogenic sources (Feng and Qiu, 2008; Fitzgerald et al., 1998; Tartu et al., 2013; US Environmental Protection Agency, 2001). Hg bioaccumulates and bioamplifies within the food web (Watras et al., 1998), and poses a potential threat to fish, wildlife and human health (Hammerschmidt and Fitzgerald, 2006; Sandheinrich and Wiener, 2011).

Concentrations of Hg in fish in newly-flooded reservoirs have been found to be affected by the quality of the water (Snodgrass et al., 2000), reservoir size (Hakanson et al., 1988), the time since impoundment (Hylander and Goodsite, 2006), water residence time (Montgomery et al., 2000), and bacterial methylation of Hg found in flooded soils (Blum et al., 2013). Seasonal variation of Hg concentrations in reservoirs is influenced by stratification/de-stratification patterns of the reservoir (Canavan et al., 2000; Dijkstra et al., 2013), but the spatial pattern of Hg in inundated environments can be dominated by internal processes as a result of the availability of organic matter (Kasper et al., 2012). The accumulation of Hg by biota varies widely across reservoirs as shown by the biomagnification factor (BMF). Observed variations of BMF in American and Canadian reservoirs were explained by differences in location (Lavoie et al., 2010), trophic position (Tuomola et al., 2008),

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food web structure (Liu et al., 2012), and eutrophication status (Zhang et al., 2012).

To understand Hg bioaccumulation in aquatic ecosystems, carbon and nitrogen stable isotopes are used as ecological tracers (Hobson et al., 2012; Jardine et al., 2006). The $\delta^{13}C$ and $\delta^{15}N$ signatures of consumers reflect the isotopic composition of food ingested and assimilated (Deniro and Epstein, 1978). The $\delta^{15}N$ value is effective in quantifying the trophic position, because the enrichment of nitrogen isotope occurs incrementally across trophic levels at a constant rate (3–4‰) (Vander Zanden and Fetzer, 2007). In contrast, for $\delta^{13}C$, the enrichment of carbon isotope is not obvious (approx. 1‰) between two successive trophic positions (Persic et al., 2004; Vander Zanden and Rasmussen, 1996). Stable isotope analyses are widely used in ecotoxicological studies to elucidate contaminant behavior (e.g., bioconcentration and biomagnification) through the whole trophic chains (Lavoie et al., 2013).

Three Gorges Dam (TGD), located on the main stem of the Yangtze River (China), is one of the biggest hydroelectric dams in the world, measuring 2309 m long and 181 m high (China Three Gorges Project Corporation, CTGPC, http://www.ctgpc.com.cn, 2010). Construction of TGD has formed a giant subtropical reservoir (Three Gorges Reservoir, TGR). TGR experienced three impoundment stages (Jun. 2003, Oct. 2006 and Nov. 2010 for water level of 135 m, 156 m and 175 m, respectively) (Xu et al., 2011). Since the impoundment on Nov. 2010, TGR inundates a total area of 1080 km^2 and forms a water-level-fluctuation region with a total area of 350 km^2 (Zhong and Qi, 2008). Water velocity and the potential for natural purification are reduced downstream toward the dam. The dam is operated to generate electricity, provide water for irrigation, and control flooding. Thus, the water levels fluctuate seasonally. The water level is lowered from the fall (175 m) to spring (155 m) to the early summer (145 m). Flood storage is important during the summer. During this time the reservoir is filled again to 175 m. Great concern has arisen regarding environmental pollution in this region in recent years (Hu and Cai, 2006). TGR is located within a zone of high Hg concentration due to natural enrichment and human activities, with two key anthropogenic Hg emission sources being coal combustion and metal smelting (Feng and Qiu, 2008; Xu et al., 1999). Thus, more attention has been paid to the ecological and environmental safety of aquatic products induced by Hg contaminations before and after the impoundment of TGR (Jin and Xu, 1997; Li et al., 2009; Xu et al., 1999; Yu et al., 2013; Zhang et al., 2007). Many studies have been carried out to measure the Hg concentration of the water column and soil (Ran et al., 2008; Tian et al., 2013), sediment (Ye et al., 2011) and Hg bioaccumulation of fish (Li et al., 2009; Yu et al., 2013; Zhang et al., 2007). However, little research has been conducted to clarify the Hg trophodynamics from water column, to primary producers to higher trophic level consumers in TGR.

Since TGR is a newly-formed reservoir on the main stem of the Yangtze River and experiences regular water discharge associated with water-level regulation, we hypothesized that TGR is not subjected to the reservoir effect of Hg pollution after the maximal depth impoundment (175 m) on Nov 2010, but Hg tropho-dynamics can vary seasonally and spatially due to regular water level regulations and the difference of Hg background level across sites. The objectives of the present study are primarily to (i) clarify seasonal (different hydrological periods within one year) and spatial patterns of Hg bioaccumulation and trophodynamics in the food web of TGR and, (ii) assess the potential reservoir effect of Hg pollution after the maximal depth impoundment.

2. Materials and methods

2.1. Study site description

TGR (29°16′ to 31°25′ N, 106° to 111°50′ E) covers a 600 km valley from Zigui (Yichang, Hubei Province) to Chongqing Province in the mainstream of Yangtze River, China (Fig. 1). The reservoir contains 39.3 km^3 of water and has a total surface area of 1080 km^2. Climate in this region belongs to southeast subtropic monsoon. Annual temperature averages to 16.519 °C and annual precipitation is approximately 1100 mm with 80% occurring from April to October (Ye et al., 2013). Water level close to TGD reached its designed maximum of 175 m on Nov 2010 (Ye et al., 2013). Water level regulation in TGR is carried out through anti-season scheduling (see Supplementary Fig. S1). Considering the massive water flow from the upstream of Yangtze River, the water level (close to TGD) needs to 145 m at the start of the flood season (June to August). Due to water storage, the level in TGR rises gradually to the maximal (175 m) and remains at this height during dry season (October to January). It drops gradually in the winter and spring (February to April), and is maintained at 155 m from April to May, then falls to the lowest height (145 m) in June to prepare for the coming of the flood season.

Riverbeds of the original reaches in TGR exhibit different elevations and the maximal water level impoundment results in an actual mean water depth of 70 m and maximal depth of 170 m. According to the geographical and hydrological characteristics of TGR, we selected three types of habitat in TGR as study stations (Fig. 1). Zigui reach is close to Three Gorges Dam (TGD), and Wanzhou and Fuling reaches (Chongqing Province) are situated at the midstream and upstream of TGR, respectively. The geographic, hydrological and climatic characteristics of three sampling stations are shown in Table 1. For each station, three typical study sites (including the main channel and estuary of tributaries) were established for the collection of water and biological samples.

2.2. Sampling and laboratory processing

In accordance with the water level regulations of TGR (Supplementary Fig. S1), sampling was conducted in July (pre-flooding), November (post-flooding) in 2011 and May (pre-flooding) and, August (post-flooding) in 2012. This was done to assess the effect of water-level regulations within one year on Hg bioaccumulation and tropho-dynamics. The actual water levels close to TGD were 140 m, 175 m, 158 m and 145 m on the sampling dates of July 2011, November 2011, May 2012 and August 2012, respectively. Seston, water sample, aquatic invertebrate and fish were collected from different stations at each sampling date.

Strict "clean hands-dirty hands" methods were followed for water sample collection (Gill and Fitzgerald, 1985). Because of known differences between Hg concentration in anoxic and oxic layers in the water column (Liu et al., 2012), water samples were collected from the surface and bottom layers using a 5-L modified Patalas's bottle sampler. The mixed water samples were transferred to PETG bottles after rinsing the bottles two times with sample water. The bottles were double-bagged and transported by a portable icebox, immediately back to the laboratory. Water samples were filtered through acid-washed glass fiber filter (0.45 μm, Whatman GF/C) using a vacuum pump. Three glass fiber filters were utilized for water sample analyses at each site. Particulate matter kept on the filter was analyzed as a seston sample and filtered water was used for the determination of dissolved THg concentration in the water column. Water samples for THg analyses were preserved through the addition of guaranteed reagent hydrochloric acid (final concentration of 0.7% in the water) and potassium permanganate (final concentration of 0.5% in the water), and then refrigerated in dark bags until analyses (Sinclair et al., 2012).

Due to the water depth of 70–170 m and water velocity in the mainstream, the riverbed was not sampled. Benthic organisms were collected primarily from the water-level-fluctuation region of TGR using a Peterson grab sampler (grab surface area = 0.0625 m^2), and then filtered through a sieve of 380 μm. Freshwater shrimp were mainly acquired from fishermen who used trawl nets. Aquatic invertebrates were identified to the lowest practical taxon. Invertebrate samples were stored in either acid-cleaned plastic bags or acid-cleaned teflon vials (for smaller organisms) and frozen. Later, frozen samples were

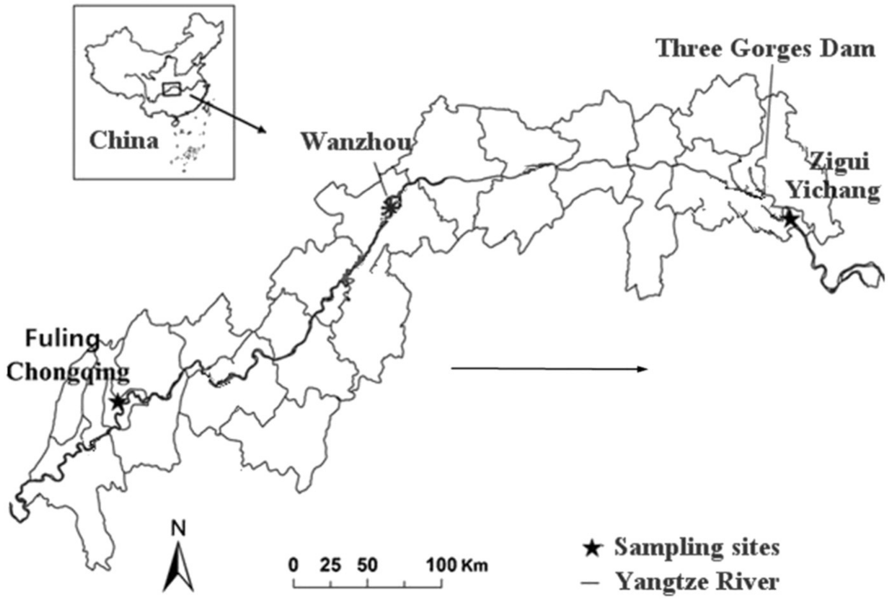

Fig. 1. Map of Three Gorges Reservoir (TGR) showing the locations of water column and biological sampling. The black line in the middle of the map represents the main stem of TGR and the small asterisks represent the study locations. The polygons around the main stem represent the cities and counties in the TGR region. Arrow represents the direction of water flow in TGR.

thawed, rinsed with ultra-clean water, weighed, and freeze-dried. Molluscs (e.g., snail) were removed from their shells, rinsed with ultra-clean water, and frozen at −80 °C in an ultra-low temperature freezer.

Fish were caught using gill net and trawl net from Zigui, Wanzhou and Fuling reaches along the main channel of Yangtze River. A total of 908 individuals representing 52 aquatic species were selected from all the locations for this study. Each fish was measured for body weight and body length, but aquatic invertebrates were measured for body weight only (see Supplementary Table S1). In general, a filet of 5–20 g of muscle tissue was cut from the dorsal area. For small-sized fish and aquatic invertebrates (e.g., freshwater shrimp), muscle tissues of some individuals were usually incorporated into one sample due to the small sample amount. Seston and fish muscle samples were immediately wrapped in acid-cleaned plastic bags and placed in an ice box, transported to the laboratory and kept in a freezer. In the laboratory, samples were freeze-dried for Hg and stable isotope analyses.

2.3. Analysis of mercury

Total mercury (THg) analyses of filtered water samples was done by cold vapor atomic fluorescence spectrometry (CVAFS) using a Series 2600 Hg Analysis System (Tekran, Knoxville, US) following US EPA Method 1631 (US EPA, 2002) modified by Tekran. Details of the analyses are described in Lavoie et al. (2010). Procedural banks, field banks, equipment banks, and field replicates were used routinely with each batch of samples analyzed to evaluate and maintain quality assurance/quality control (QA/QC). Blanks were tested every three samples and, two samples were spiked to calculate the recovery of the method. Each sample was run in duplicate and coefficients of variation were below 15%. The procedural, field, and equipment blanks were less than the detection limits in any case. The method's detection limit was 0.5 ng L^{-1}.

Seston, aquatic invertebrate, and fish samples were analyzed by means of cold vapor atomic absorption spectroscopy (CVAAS) according to the methods described by McIntyre and Beauchamp (2007). Approximately 1.0–3.0 g of aquatic invertebrate and fish tissues was digested in nitric and sulfuric acid mixed with potassium permanganate and potassium persulfate at 100 °C for 2.0–2.5 h. For seston, each glass fiber filter with particulate matter was torn into pieces by gloved hand and then digested the same way as the tissue samples. Hydroxylamine hydrochloride was added after digestion, and stannous chloride was added immediately before analysis. THg concentrations in samples were expressed in nanogram per gram wet weight (ng g^{-1} ww), but analyses were executed on frozen-dried samples. Moisture content for each sample was assessed by weighing the fresh tissue and freeze-dried sample on an analytical balance (±0.1 mg). The instrument limit of quantification for seston, aquatic invertebrate and fish samples was 1 ng g^{-1} ww. All seston, aquatic invertebrate and fish samples analyzed for THg in this study were above detection limits.

Table 1
Selected characteristics of three sampling stations in Three Gorges Reservoir (Yu, 2006; Li et al., 2002).

Variables	Reaches		
	Fuling	Wanzhou	Zigui
Longitude/latitude	E 107°23′/N 29°42′	E 108°22′/N 30°50′	E 110°57′/N 30°52′
Flooded area (km^2)	87,920	228.6	113
Length (km)	65	30.6	24
Flow (m^3/s)	1650	4.4	2.5
Precipitation (mm)	1096.3	1162.5	1200.5
Evaporation (mm)	1048	1096	1601
Wind speed (m/s)	1.4	0.5	0.9
Channel depth (m)	77	92	168
Bank width (m)	1605	1302	1693
Distance from TGD (km)	484	277	1

2.4. Stable isotope analysis and determination of trophic position

Prior to measuring, seston samples for stable isotope analysis were acidified with 1 N HCl overnight to remove carbonate and dried to a constant weight at 60 °C, then ground to a fine powder using an acidic clean mortar and pestle. Freeze-dried biota samples for $\delta^{13}C$ analysis were treated with 1 N HCl to remove carbonate, followed by treatment with 1% dichloromethane in methanol to eliminate lipids (Sweeting et al., 2006). Samples for $\delta^{15}N$ analysis did not undergo any further treatment after lyophilization. Homogenized biota samples were weighed in a tin foil cup, wrapped, and analyzed with a Flash EA-1112 HT elemental analyzer accompanied by a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., USA). Isotopic ratios were expressed relative to international standards (Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). Delta values were defined as:

$$\Delta R = \left[\left(X_{sample} - X_{standard}\right)/X_{standard}\right] \times 10^3 (‰)$$

where R = ^{13}C or ^{15}N and X is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The analytical precision was within 0.1‰ and 0.2‰ for carbon and nitrogen isotope measurements, respectively.

Trophic positions (TPs) were determined for all common species at three study stations of TGR and used to compare the $\delta^{15}N$ data across sites. Estimates of TP were calculated based on the $\delta^{15}N$ values in fish muscle and primary consumer (baseline of food web). Freshwater snail, *Turbo fluctuosa*, is a sedentary primary consumer species with long lifespan and was used as baseline indicator (TP = 2). TPs of fish and aquatic invertebrate were assessed by an equation as follows:

$$TP_{consumer} = 2 + \left(\delta^{15}N_{consumer} - \delta^{15}N_{baseline}\right)/V\delta^{15}N$$

where $\delta^{15}N_{consumer}$ and $\delta^{15}N_{baseline}$ were the nitrogen isotope values for aquatic consumers (fish and aquatic invertebrate) and baseline indicator, respectively. The discrimination factor ($\Delta\delta^{15}N$) at each trophic step was defined as 3.4‰ (Post, 2002; Jardine et al., 2006).

2.5. Statistical analysis

Statistical analyses were conducted through SPSS Ver 19.0 (Chicago, IL, USA). Correlation analyses between THg concentrations and growth factors (body length and body weight) were examined by Pearson's rank correlation test. We evaluated the interspecific difference, spatial and temporal trends in Hg concentrations using multi-factor analysis of variance (MANOVA), with species, season and sites as fixed factors, and Hg concentration as dependent variable. The ANOVA result was followed by a Tukey post hoc test. One-way ANOVA analyses were conducted to evaluate the effects of study stations on the trophic positions of aquatic consumers. P value less than 0.05 was defined as statistically significant. Biomagnification of Hg was evaluated through the regression slope of log-transformed Hg vs. $\delta^{15}N$ (Jardine et al., 2006). The linear regression equation was expressed by Log_{10} [Hg] = (a × $\delta^{15}N$ + b) with a 0.95 confidence interval and the regression slope.

3. Results and discussion

3.1. Food web structure

Since the exposure to Hg occurred primarily through diet (Eagles-Smith et al., 2008), the carbon source and trophic position (TP) of aquatic consumers determined by $\delta^{13}C$ and $\delta^{15}N$ analyses help to understand THg trophodynamics in TGR. Based on the common species at three study stations, the $\delta^{13}C$–$\delta^{15}N$ plot of food web components (seston, aquatic invertebrate and fish) revealed different trophic relationships (resource use and trophic position) (Fig. 2). Greater min–max ranges of $\delta^{13}C$ in the upstream than that in Zigui reach (Fig. 2) might exhibit more diverse food resources (Persic et al., 2004; Vander Zanden and Rasmussen, 1996) or more TPs (i.e., longer food chain length) in the upstream parts of the TGR than Zigui reach (Vander Zanden and Fetzer, 2007). Although there were similar min–max ranges of TP across stations (1.84, 1.75 and 1.85 TPs for Fuling, Wanzhou and Zigui reaches, respectively), TPs of aquatic consumers exhibited significant differences across three reaches (One-way ANOVA, $F = 4.482$, $p < 0.05$; Supplementary Table S3). Furthermore, aquatic consumers exhibited relatively lower trophic position in Fuling (3.66 TPs) and Wanzhou (3.16 TPs) reaches than that in Zigui reach (3.75 TPs). After the impoundment of TGR to the maximal depth, hydrological properties (e.g., flow velocity) of Fuling and Wanzhou reaches were closer to river status than those of Zigui reach (Table 1 and Fig. 1). Fish in Fuling and Wanzhou reaches of TGR had access to more allochthonous food source (e.g., suspended organic matter) from the upstream of the Yangtze River than those in Zigui reach. Accordingly, food web upstream showed more multiple food resources than those in the downstream parts of TGR, but showed a little shorter food chain length than that in the region near the dam of the TGR. To be specific, *Carassius auratus*, *Silurus asotus* and *Siniperca chuatsi* exhibited higher trophic positions in Fuling and Wanzhou reaches than those in Zigui reach (Supplementary Table S3).

3.2. THg concentrations in water column, seston, aquatic invertebrate and fish

THg concentrations in the water column, seston, and biota of TGR were measured to evaluate the bioaccumulation and potential biomagnification through aquatic food web of TGR, and potential exposure of humans to Hg (Tables 2 and 4). Overall, no individual data in biota exceeded the standard of 300 ng g^{-1} ww (UNEP Chemicals, 2002) and the WHO (or FAO) guideline of 500 ng g^{-1} ww (UN-FAO/WHO, 1991). The water column usually shows a very low THg concentration

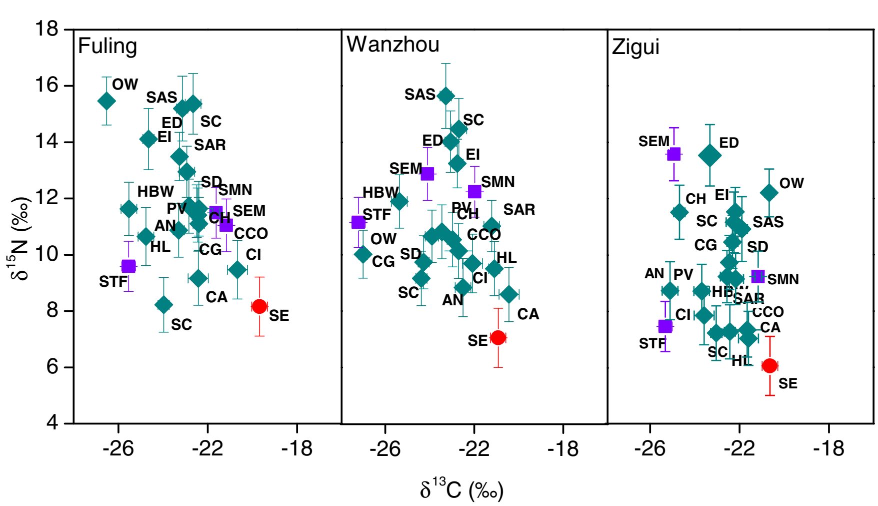

Fig. 2. Stable isotope diagram of aquatic food web at three sampling sites in Three Gorges Reservoir. (The species code was in accordance to Supplementary Table S1; Fish: green diamond; aquatic invertebrate: blue square; Seston: red circle; Each diamond or square represents one species). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Total mercury concentration (mean ± SD) of water column and seston in Fuling, Wanzhou and Zigui reaches of TGR.

Station	THg of seston ($ng\ g^{-1}$ dw)				THg of water column ($ng\ L^{-1}$)			
	Jul. 2011	Nov. 2011	May 2012	Aug. 2012	Jul. 2011	Nov. 2011	May 2012	Aug. 2012
Fuling	13.0 ± 0.3	7.5 ± 0.5	5.7 ± 0.5	5.3 ± 0.5	43 ± 7.3	30 ± 4.2	33 ± 2.1	37 ± 7.1
Wanzhou	11.7 ± 0.4	4.2 ± 0.3	5.2 ± 0.2	4.9 ± 0.2	31 ± 6.4	28 ± 6.2	24 ± 4.8	25 ± 5.7
Zigui	8.5 ± 0.5	5.9 ± 0.2	4.4 ± 0.1	3.8 ± 0.2	20 ± 5.1	16 ± 3.4	14 ± 5.1	10 ± 2.4

(Rogowski et al., 2009), but this can bioamplify along the aquatic food chain (Watras et al., 1998). Averaged THg concentration of seston (6.7 $ng\ g^{-1}$ dw, n = 27), aquatic invertebrate (34.6 $ng\ g^{-1}$ ww, n = 71) and fish (65.6 $ng\ g^{-1}$ ww, n = 810) were two-to-three orders of magnitude higher compared to that in water column (0.026 $ng\ g^{-1}$). These data show strong bioaccumulation of Hg from the water column to the higher trophic level organisms. In addition, aquatic invertebrate and fish exhibited 5.2 and 9.8 times of THg concentration of seston, respectively, suggesting a trophic shift from primary producer to higher trophic level consumer (Kwon et al., 2012).

Our study indicated low concentration of Hg in fish, with values similar to those during the initial impoundment of TGR (Zhang et al., 2007). In contrast, comparison of concentrations pre-impoundment of TGR showed THg concentration of *Coreius heterodon*, *Cyprinus carpio* and *S. asotus* were slightly increased (Jin and Xu, 1997), but were significantly lower than the values predicted by Xu et al. (1999) (Table 3). THg concentrations in the water column were comparable to the result from the pre-impoundment of TGR (Ye et al., 2010). Sediments are often considered as the place of Hg storage and Hg methylation in aquatic systems (Muir et al., 2009). THg concentrations of carp in TGR were comparable to those from a variety of aquatic systems (Table 3), except for those found in Babeni Reservoir, which had higher sediment concentrations of Hg. Therefore, relatively low Hg concentrations in fish in TGR might be explained by the physical characteristics of sediment, e.g., poor organic matter at rock substrate (Supplementary Table S4).

THg concentrations of fish in TGR were lower compared with those in other Chinese hydroelectric reservoirs. Our study revealed higher THg concentrations in the water column relative to those in Baihua Reservoir (China) (Tables 2 and 3). Baihua Reservoir has been inundated for 13 years, and THg concentrations of omnivorous and carnivorous fish ranged from 4 to 254 $ng\ g^{-1}$ ww (Liu et al., 2012), whereas THg concentrations of fish in our study (inundated for two years) ranged between 31.5 and 117 $ng\ g^{-1}$ ww. Elevated Hg concentrations in fish were found in hydropower reservoirs of North America and northern Europe in the late 1970s and early 1980s (Abernathy and Cumbie, 1977; Lodenius et al., 1983). Typically, several-fold increases in fish Hg concentration were detected at the first 5–10 years after the filling of reservoirs (Verdon et al., 1991; Bodaly et al., 2007). The sampling in this study was conducted just two years after TGR reached its maximum depth of impoundment and the "reservoir effect" of Hg contamination in TGR was not significant.

3.3. Temporal-spatial patterns of Hg bioaccumulation

THg concentrations in aquatic consumers showed no significant spatial difference across stations (MANOVA, F = 1.332, p = 0.291, Supplementary Table S5), but the overall trend was that Fuling reach (78 $ng\ g^{-1}$ ww, n = 218) > Wanzhou reach (63 $ng\ g^{-1}$ ww, n = 285) > Zigui reach (57 $ng\ g^{-1}$ ww, n = 377). Ye et al. (2011) demonstrated higher Hg concentrations in the soil of the water-level-fluctuation region in the upstream than in the downstream region of TGR, suggesting the importance of Hg background concentrations on the water column and sediment of TGR (Table 2 and Supplementary Table S4). Our study stations were impacted by anthropogenic activities (e.g., industrial discharge and shipping) to different degrees. In addition water samples reflect the hydrological conditions during periods of sampling (Horvat et al., 2003). Seston is composed of detrital and tiny organisms thus can contain sediment. Hg concentrations in water column, seston and sediment were higher in the upstream region than near the dam as revealed in Table 2 and Supplementary Table S4. THg concentrations in water column decreased gradually from Fuling, to Wanzhou and Zigui reach. THg concentrations of seston were significantly different across stations (Two-Way ANOVA, F = 6.372, $p < 0.01$), with significantly higher Hg values in the Fuling reach as compared to the Zigui reach. The highest Hg concentration of most fish species occurred in Fuling reach. This reach has the largest flooded area and the highest average flow velocity of the three reaches (Table 1) and receives a large amount of waste water from various local industries. This Hg is further passed on to primary producers and higher trophic level consumers (Pizarro-Barraza et al., 2014). So, the Hg level at the base of food web played a key role in Hg bioaccumulation of aquatic consumers in TGR.

In our study, there was relatively stable water level at each sampling season. THg concentrations in aquatic organisms exhibited significant difference across sampling seasons (MANOVA, F = 11.93, $p < 0.01$, Supplementary Table S5), and the trend was Nov. 2011 > May 2012 > Jul. 2011 > Aug. 2012. We observed the highest Hg concentration of aquatic organisms in Nov. 2011 (post-flood period, highest water level) and

Table 3
Comparison of THg concentration (mean or mean ± SD) in the water column, sediment and fish from selected studies (The species code was in accordance to Supplementary Table S1).

Location	Water ($ng\ L^{-1}$)	Sediment ($ng\ g^{-1}$ dw)	Fish species ($ng\ g^{-1}$ ww)						Reference
			CI	AN	CCO	CA	CH	SAS	
Baihua Reservoir, China	1–20	724 ± 410	14 ± 2	49 ± 33	51 ± 62	83 ± 47		41 ± 16	Liu et al. (2012)
Hong Kong, Pearl River Delta, China		57–1670	26 ± 7	34 ± 7	19 ± 3	14 ± 5			Zhou and Wong (2000)
Babeni Reservoir, Romania	3–9	700–3300				460–550			Bravo et al. (2010)
Illinois & Mississippi Rivers, US	2–8		28–64		35–129				Rogowski et al. (2009)
TGR, China	24	70			77	47	40	166	Jin and Xu (1997), Ye et al. (2010)
(pre-impoundment)			140	84	110			240	Xu et al. (1999)
					61.6		64.7	124.8	Zhang et al. (2007)
TGR, China	26 ± 10	55	18 ± 8	47 ± 14	81 ± 17	55 ± 12	56 ± 12	117 ± 10	He, 2013
(post-impoundment)									Present study

Table 4
Averaged THg concentrations (ng g^{-1} wet weight) of invertebrate and fish at different study sites and seasons in TGR.

Category	Code	Fuling	Wanzhou	Zigui	Jul. 2011	Nov. 2011	May 2012	Aug. 2012
Invertebrates								
Snail *Turbo fluctuosa*	STF	24.5 ± 3.2	26.2 ± 1.3	18.0 ± 5.7	19.8 ± 5.8	36.1 ± 8.1	25.5 ± 3.6	19.9 ± 5.3
Shrimp *Macrobrachium nipponense*	SMN	34.8 ± 6.4	45.2 ± 11.4	32.6 ± 6.9	31.4 ± 11.7	58.1 ± 12.6	54.4 ± 12.6	33.9 ± 3.1
Shrimp *Exopalaemon modestus*	SEM	45.9 ± 5.7	40.3 ± 9.4	36.1 ± 9.4	28.3 ± 6.7	47.4 ± 12.2	34.4 ± 9.4	32.5 ± 5.2
Fish								
Herbivore								
Ctenopharynodon idellus	CI	20.2 ± 3.2	17.8 ± 5.3	17.5 ± 6.2	20.2 ± 2.6	23.5 ± 5.8	19.1 ± 5.2	14.6 ± 2.7
Planktivore								
Hypophthalmichthys molitrix	HM	34.9 ± 4.7	32.1 ± 6.9	30.3 ± 3.2	35.3 ± 8.4	36.3 ± 2.6	28.2 ± 4.3	22.3 ± 6.9
Aristichthys nobilis	AN	42.3 ± 15.8	45.3 ± 10.4	37.6 ± 2.6	37.7 ± 12.9	48.1 ± 10.5	41.3 ± 11.6	32.6 ± 2.5
Omnivore								
Hemiculter leucisculus	HL	56.7 ± 12.4	50.0 ± 9.4	44.0 ± 6.4	53.7 ± 8.4	61.5 ± 13.5	52.9 ± 21.6	36.7 ± 11.5
Hemiculter bleekeri	HBW	80.2 ± 24.7	79.4 ± 13.1	75.3 ± 8.1	73.8 ± 9.9	85.2 ± 22.7	73.8 ± 4.8	68.4 ± 14.1
Cyprinus carpio	CCO	89.5 ± 16.2	80.1 ± 5.8	65.3 ± 12.8	77.9 ± 4.9	74.3 ± 21.7	82.9 ± 10.7	70.6 ± 6.6
Carassius auratus	CA	63.8 ± 12.6	40.5 ± 9.0	54.5 ± 9.8	55.1 ± 14.4	61.4 ± 7.3	61.3 ± 13.8	54.6 ± 7.1
Coreius heterodon	CH	57.2 ± 11.7	54.3 ± 7.9	53.2 ± 12.5	55.7 ± 7.2	59.5 ± 13.6	45.9 ± 7.5	41.9 ± 15.7
Coreius guichenoti	CG	81.3 ± 4.7	70.2 ± 4.7	63.2 ± 9.7	65.7 ± 10.2	68.4 ± 21.6	78.2 ± 3.5	70.5 ± 5.7
Saurogobio dabryi	SD	55.9 ± 6.3	53.7 ± 7.9	45.7 ± 11.3	65.6 ± 13.6	34.0 ± 9.6	81.7 ± 21.7	28.1 ± 2.6
Squalidus argentatus	SAR	56.3 ± 7.1	53.4 ± 2.8	42.0 ± 6.2	46.1 ± 9.2	61.5 ± 7.5	55.3 ± 3.9	48.6 ± 13.5
Pelteobagrus vachelli	PV	49.1 ± 2.8	82.2 ± 14.7	54.5 ± 6.8	76.5 ± 4.5	67.9 ± 9.3	65.9 ± 10.5	71.5 ± 15.6
Oriental weatherfish	OW	76.3 ± 23.6	70.2 ± 21.6	67.1 ± 13.7	77.1 ± 5.6	85.6 ± 65.	57.1 ± 12.8	63.7 ± 13.8
Carnivore								
Erythroculter dabryi	ED	63.8 ± 17.5	50.9 ± 5.9	66.5 ± 13.8	62.4 ± 8.5	72.4 ± 6.4	64.7 ± 19.5	52.2 ± 16.6
Erythroculter ilishaeformis	EI	88.2 ± 6.4	90.4 ± 10.9	78.1 ± 14.7	75.5 ± 10.4	94.7 ± 9.5	81.5 ± 8.9	74.9 ± 152.
Silurus asotus	SAS	93.9 ± 9.2	120.9 ± 26.8	113.8 ± 13.6	114.6 ± 21.6	123.9 ± 18.5	104.5 ± 13.6	93.3 ± 12.5
Siniperca chuatsi	SC	114.2 ± 5.3	102.7 ± 18.9	90.5 ± 6.2	92.3 ± 22.7	111.6 ± 10.6	111.9 ± 21.5	87.0 ± 16.2

they were approximately 9 times that observed in August 2012 (flood period, lowest water level), suggesting a strong impact of water level regulation on Hg bioaccumulation in aquatic consumers. The inundation of alluvial soil and large discharge of tributaries may contribute to high Hg concentrations in Nov. 2011 as revealed by previous studies (Snodgrass et al., 2000; Sorensen et al., 2005). In Nov. 2011, the water-level-fluctuating zone with external organic matter was inundated, which could activate microbial activity to increase methylmercury production (Sorensen et al., 2005). On the other hand, temporal variation of Hg level could be associated with the frequency of inundation (Ye et al., 2011). In August, the water level decreases and then, the exposed sulfides in the sediment oxidize to sulfate; when water levels rise again in November, the mobilized sulfate provides fuel for sulfate-reducing bacteria, which can activate the Hg methylation and increase the MeHg production (Selch et al., 2007). After the impoundment to the highest depth for three months (October to January, Supplementary Fig. S1), TGR drops to a lower water level because of downstream water flow during spring. Then the water level drops to the lowest level in June to prepare for the ensuing flood period. In this way, regular water discharge induced by water level regulation within one year probably removed excessive Hg accumulated in the water and sediment of TGR and thus, prevented the occurrence of a reservoir effect on Hg pollution.

3.4. Hg biomagnification through aquatic food web

Regression slopes of log-Hg concentration versus $\delta^{15}N$, indicate trophic magnification factor (TMF) in Fuling, Wanzhou and Zigui reaches (Fig. 3); They were comparable to the study (0.04–0.05) by Yu et al. (2013) in TGR, but were lower compared with the studies in reservoirs of America and Canada (0.17–0.29) (Lavoie et al., 2010; Zhang et al., 2012).

Indeed, the biomagnification values of most aquatic systems in China are relatively low (Zhang et al., 2007; Liu et al., 2012). This could be explained mainly by the physical characteristics of sediment, especially organic matter content. It has been observed that organic carbon plays an important role in the transport and bioavailability of both organic and inorganic Hg from sediment to water (Driscoll et al., 1994), and affects Hg uptake by fish. Unlike the reservoirs in North America and Canada, the organic carbon content of submerged soil in freshly-flooded areas was quite low in Chinese reservoirs (Table 5), and therefore methylation production was low compared with those in reservoirs of America and Canada (Larssen, 2010). Soil in TGR is composed primarily of purple sand shale with

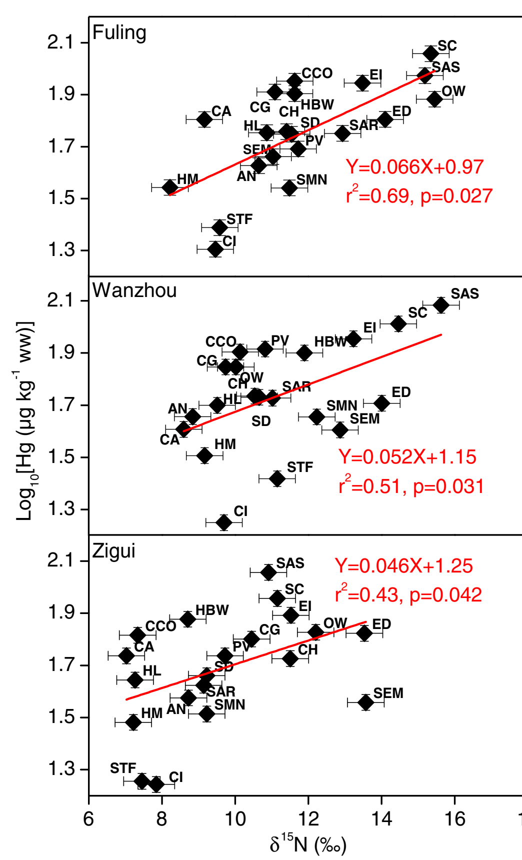

Fig. 3. Relationship between Hg concentrations and trophic positions (measured by $\delta^{15}N$) of fish in Fuling, Wanzhou and Zigui reaches of TGR (The species code was in accordance to Supplementary Table S1).

Table 5
Comparison of dissolved organic carbon (DOC) in sediment in reservoir of selected studies.

Study site	County	DOC (mg C/L)	Reference
Piney Creek reservoir	United States	5.7–6.6	Castro et al. (2002)
EMAP (105 lakes in total)	North America	2–18.2	Chen et al. (2005)
Rio Casca	Central West Brazil	6.2 ± 0.4	Tuomola et al. (2008)
Galt Creek	Canada	9.5	Klinck et al. (2005)
Grand River	Canada	8.7	Klinck et al. (2005)
Boreal lakes	Norway	11.4	Braaten et al. (2014)
Hongfeng Reservoir	China	1.74–3.23	He et al. (2008)
Three Gorges Reservoir	China	2.44–3.83	Yu et al. (2011)

relatively poor nutrients and organic matter content in most areas (Du, 1999). Inundated soil has been identified as the primary source of Hg to new reservoirs (Bodaly et al., 1984) although other sources are possible (Cox et al., 1979). Poor organic content in these areas may weaken microbial activity and hence, reduce methylmercury production. Another important factor may be related to growth dilution. We detected significant correlation between THg concentration and body size (body length and body weight) just for *Erythroculter dabryi* and *S. asotus* among seven selected species (Supplementary Table S6). Larssen (2010) reported that fishes in Chinese reservoirs were mainly fast-growing harvested fish, and had lower Hg concentration relative to size through growth dilution.

On the other hand, the trend of TMF at three study stations was consistent with the pattern of Hg concentration in the water column and seston (Table 2), suggesting that the biomagnification of Hg may be governed by the Hg level at the base of food web in TGR. Furthermore, the biomagnification of Hg was also reflected in the magnitude of Hg bioaccumulation in the seston, which supports the previous finding that the magnitude of Hg biomagnification is strongly linked to the accumulation or dilution of Hg by primary producers (Pickhardt et al., 2002).

4. Conclusions

Our study indicated no obvious increase in Hg concentrations of fish from that measured before impoundment and at initial impoundment of Three Gorges Reservoir. This may be explained by three factors: (1) short time since inundation (just 2 years); (ii) regular water discharge associated with water-level regulation; and (iii) low organic matter content in the sediment of the water-level-fluctuation region. THg concentration of biota indicated a temporal trend that was associated with water level regulations: post-flood period > pre-flood period > flood period. The higher Hg concentrations in fish most upstream of the dam were caused by the Hg concentrations in water and sediment. The trophic magnification factors (TMF) of Hg ranged from 0.046 to 0.066 across stations. In contrast with the reservoirs of United States and Canada, TGR organisms exhibited a lower TMF and this was explained mainly by the low concentration of dissolved organic carbon in the sediment, and “growth dilution”.

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Appendix A. Supplementary data

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