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RESEARCH ARTICLE

Mercury bioaccumulation in zooplankton and its relationship with eutrophication in the waters in the karst region of Guizhou Province, Southwest China

Cong Yao[1] · Tianrong He[1] · Yiyuan Xu[1] · Shu Ran[1] · Xiaoli Qian[2] · Shengxing Long[1]

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Abstract

Zooplankton play an important role in the transfer of mercury (Hg) from the lower to upper trophic positions in the food chain. In this study, total mercury (THg) and methylmercury (MeHg) levels were measured in three size fractions of zooplankton collected from three reservoirs (Hongfeng, Baihua, and Aha Reservoir) and one wetland in karst areas to understand mercury accumulation in zooplankton from alkaline environments. The results showed that the alkaline waters had lower zooplankton MeHg levels (0.1 to 66.8 ng g^{-1}) than most of the acidic waters reported. However, the zooplankton THg levels (6.3 to 494.9 ng g^{-1}) were comparable. The macro-zooplankton (> 500 μm) had significantly higher THg and MeHg levels than meso-zooplankton (116 to 500 μm) in the three reservoirs at all seasons, which showed biomagnification of mercury in the food chain. The correlation between Hg in water and zooplankton and Hg in zooplankton of different sizes indicated that THg bioaccumulation in zooplankton was related to the THg levels in water; however, MeHg bioaccumulation in zooplankton was controlled by many other factors, such as their feeding and living habits. In the three reservoirs, the THg and MeHg concentrations in zooplankton decreased with increasing eutrophication. However, compared with the three reservoirs, Caohai Wetland, with large amounts of aquatic plants, had a much lower trophic level and higher MeHg content in water but much lower zooplankton MeHg levels and bioaccumulation factors (BAFs). The large amounts of plant residue might dilute mercury in the food chain, revealing that high primary production could result in lower Hg bioaccumulation, rather than only being influenced by nutrient levels.

Keywords Zooplankton · Mercury · Methylmercury · Alkaline · Eutrophication · Bioaccumulation

Introduction

The biogeochemical cycles of mercury (Hg) in aquatic environments have been a worldwide environmental concern since its methylation product (methylmercury, MeHg) is readily bioaccumulated along food chains (Driscoll et al. 1994; Mergler et al. 2007). MeHg levels in fish and invertebrates in some lakes or marine systems have been found to exceed state, federal, or international health guidelines (Chase et al. 2001).

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✉ Tianrong He
hetianrong@139.com

[1] Key Laboratory of Karst Environment and Geohazard Prevention, Guizhou University, Guiyang 550003, China

[2] The School of Resources and Environment Engineering, Guizhou University, Guiyang 550003, China

It is well known that MeHg in fish is mostly derived from their food (Hall et al. 1997) and that the concentration, speciation, and distribution of MeHg in those hydrobionts, such as zooplankton at low trophic positions, play an important role in MeHg bioaccumulation in fish (Rolfhus et al. 2011; Guimarães et al. 1999, 2000; Verburg 2014; Yu et al. 2011). Therefore, the study of mercury accumulation in zooplankton is very important for the assessment of ecological health risks in aquatic environments.

Hg concentrations in zooplankton have previously been shown to be correlated with a number of environmental factors, such as water mercury levels, eutrophication, catchment area, water pH, organic carbon, and water color (Tremblay et al. 1995; Back and Watras 1995; Westcott and Kalff 1996; Tsui and Wang 2004; Driscoll et al. 2007; Chen et al. 2012). Among those factors, water eutrophication was believed to be one of most important factors determining the bioaccumulation of Hg in zooplankton (Yu et al. 2011; Watras et al. 1998;

Chen et al. 2012; Razavi et al. 2015). Some researchers (Gantner et al. 2010; Yu et al. 2011; Chen et al. 2012) observed higher zooplankton mercury content in water with lower nutrients, and some researchers (Watras et al. 1998; Wang et al. 2011; Razavi et al. 2015) found that the average total mercury (THg) concentration of zooplankton decreased with increasing chlorophyll A (Chla) content.

Although there have been many investigations on mercury accumulation in zooplankton, most have focused on acidic waters (Yu et al. 2011; Kainz and Mazumder 2005; Watras and Bloom 1992; Watras et al. 1998; Surette et al. 2006; Masson and Tremblay 2003), and few have focused on alkaline waters. The karst waters, widely distributed in Guizhou Province, present significant differences from the acidic water regarding the migration and transformation of pollutants. Guizhou Province is located in the Pacific Rim mercury mineralization zone (Gustin et al. 1999; Qiu et al. 2006). A large number of mining and industrial activities have resulted in high mercury loads in some natural water bodies in Guizhou (Tan et al. 2000; Feng et al. 2002; Tang et al. 2007; Yan et al. 2008; He et al. 2015). Additionally, with the development of the economy and urbanization, many water bodies have tended to be artificially disturbed, and eutrophication has been a phenomenon of wide concern in Guizhou (Long et al. 2012; Guo et al. 2015). These alkaline, mercury-rich, and eutrophic water characteristics may affect the migration and transformation of mercury in aquatic ecosystems. Although there have been many studies on the geochemical cycle of mercury in water bodies in Guizhou, most of the studies focused on mercury distributions in water, sediment, and fish, and few focused on those distributions in zooplankton. To determine the mercury accumulation characteristics in zooplankton in lakes and reservoirs experiencing mercury pollution and eutrophication in karst areas of Guizhou Province, we investigated the mercury accumulation characteristics of zooplankton in different ecological types with different nutrient levels (Hongfeng Reservoir, Aha Reservoir, Baihua Reservoir, and Caohai Wetland) in the karst area of Guizhou Province and discussed their response to eutrophication. It is of practical and scientific significance to provide more substantial basic data for the biogeochemical cycle of mercury in karst areas facing mercury pollution and eutrophication.

Materials and methods

Study sites

Hongfeng Reservoir, Baihua Reservoir, Aha Reservoir, and Caohai Wetland were selected as the research object in this study. These water bodies have historically suffered from a variety of chemical wastewater and domestic sewage and have shown different degrees of eutrophication; with the control of pollution sources around water bodies, the water quality in some areas of the water bodies has improved. More details are provided in Table 1.

According to the geographical and environmental characteristics of the study areas, 6, 4, 2, and 3 sampling points were selected in Hongfeng Reservoir, Baihua Reservoir, Aha Reservoir, and Caohai Wetland, respectively (Fig. 1). The layout of sampling points is shown in Fig. 1.

Sample collection

Water sampling

In October 2015 and February, May, and August 2016, water samples were collected from Hongfeng Reservoir, Baihua Reservoir, Aha Reservoir, and Caohai Wetland. Each water sample (a mixture from 0.5 m below the surface, 3 or 6 m deep, and 0.5 m above the bottom) was collected in borosilicate glass bottles (100 mL) and acidified using a 0.5% HCl solution, placed in the clean double zipped-type bags, transported to the laboratory within 24 h, and stored at 3–4 °C in dark until analysis. Before collection, all the bottles were cleaned by acid leaching, rinsing with ultrapure deionized water and heating for several hours in a muffle furnace at 500 °C. The filtered samples were collected by filtering with a 0.45-μm filter (Millipore) on site (He et al. 2008). Total nitrogen (TN) and total phosphorus (TP) concentrations in water were analyzed using the alkaline potassium persulfate oxidation method (Environmental Protection of China 1990a, b). Chlorophyll a (Chla) was analyzed by spectrophotometry (UV-2550, Japan) after extraction in 90% acetone (Pápista et al. 2002).

Zooplankton sampling

In October 2015 and February, May, and August 2016, zooplankton samples in three size fractions (micro-zooplankton, meso-zooplankton, and macro-zooplankton) were collected from Hongfeng Reservoir, Baihua Reservoir, and Aha Reservoir for THg and MeHg analyses. Caohai Wetland was not sampled in October 2015, and only macro- and meso-zooplankton were collected. The zooplankton samples were collected as described by Long et al. (2018). The macro-zooplankton, meso-zooplankton, and micro-zooplankton were collected by using 500 μm, 116 μm, and 77 μm nylon mesh, respectively. Each sample was collected by dragging the nylon net from the bottom 0.5 m to the surface of the water repeatedly until > 500 mg dry weight (DW) was accrued. The zooplankton collected by the 77 μm nylon net were further filtered by 116 μm nylon mesh to remove zooplankton larger than 116 μm, thereby obtaining zooplankton of 77–116 μm. The zooplankton of 116–500 μm were also collected in a similar way. The collected samples were rinsed with ultrapure

Table 1 Basic information of the three reservoirs and wetland studied

Information	Hongfeng Reservoir	Baihua Reservoir	Aha Reservoir	Caohai Wetland
Location	Qingzhen City, a suburb of Guiyang City, Guizhou Province	Northwest of Guiyang City, Guizhou Province	Guiyang City, Guizhou Province	Weining County, Guizhou Province
Coordinates	26° 24′–26° 34′ N 106° 20′–106° 26′ E	26° 35′–26° 42′ N 106° 27′–106° 34′ E	26° 30′–26° 33′ N 106° 03′–106° 39′ E	26° 45′–27° 00′ N 104° 10′–104° 25′ E
Surface area (km^2)	57.2	14.5	4.5	46.5
Max depth (m)	45	45	24	5
Mean depth (m)	12.5	12.5	13	2
Hydraulic retention time (days)	118.6	72	160.6	85.6
Pollution situation	Once polluted by waste water from more than 20 surrounding factories.[a]	Has been seriously mercury contaminated by chemical plants.[b]	Once polluted by coal mine, industrial, and domestic wastewater.[c]	Once polluted by lead, zinc smelting, and domestic wastewater.[d]

[a] Data obtained from Zhang (1999); [b] data obtained from Yan et al. (2008); [c] data obtained from Feng et al. (2018a); [d] data obtained from Li (2004) and Zhang and Lei (2010)

deionized water (Millipore Co., Bedford, MA, USA). The resulting zooplankton samples were transferred to the laboratory and freeze-dried for analysis.

In addition, 20 L water samples were collected at each sampling point using a plankton net (64 μm mesh) for zooplankton counting and species identification. Zooplankton species were identified using a BXT-90B dissecting microscope (Shanghai Bingyu Optical instrument Co., Ltd., China) as described by Jiang and Chu, Shen and Song, and Long et al. (Long et al. 2018; Jiang and Chu 1979; Shen and Song 1979).

Sample analysis

Hg and MeHg analyses

The THg and MeHg concentrations in the water samples were determined by cold vapor atomic fluorescence spectrometry (CVAFS) using a method described in detail by Bloom and Fitzgerald (1988), Bloom (1989), Horvat et al. (1993), US EPA (2001, 2002), and He et al. (2008). For THg concentrations in the water samples, all mercury was oxidized to divalent mercury, then reduced to Hg0, trapped in gold tube, and analyzed by CVAFS. MeHg concentrations in the water samples were determined using the standard distillation-ethylation-GC-CVAFS technique.

THg and MeHg concentrations in zooplankton were determined using methods that have been previously described by Yan et al. (2005a, b) and Feng et al. (2018b). Briefly, THg was analyzed by acid digestion, $SnCl_2$ reduction, gold trap collection, and CVAFS, and MeHg was analyzed by KOH digestion, aqueous ethylation, Tenax trap collection, and GC-CVAFS.

Quality assurance/quality control and statistical analyses

Quality assurance and quality control of the analytical process were carried out using duplicates, method blanks, matrix spikes, and standard reference materials (TORT-2, fish reference material). Method blanks and duplicates were taken regularly (> 10% of samples) throughout each sampling campaign. The THg recoveries for standard reference material were 92~108% (n = 10), the MeHg recoveries were 90~109% (n = 10), and the relative deviation of parallel samples was less than 10%.

Statistical analyses of the data were performed using SPSS 20.0 (PASW) and Origin 9.0 software. The equations for calculating the trophic state index (TSI) in the waters are as follows (Carlson 1977; Kratzer and Brezonik 1981):

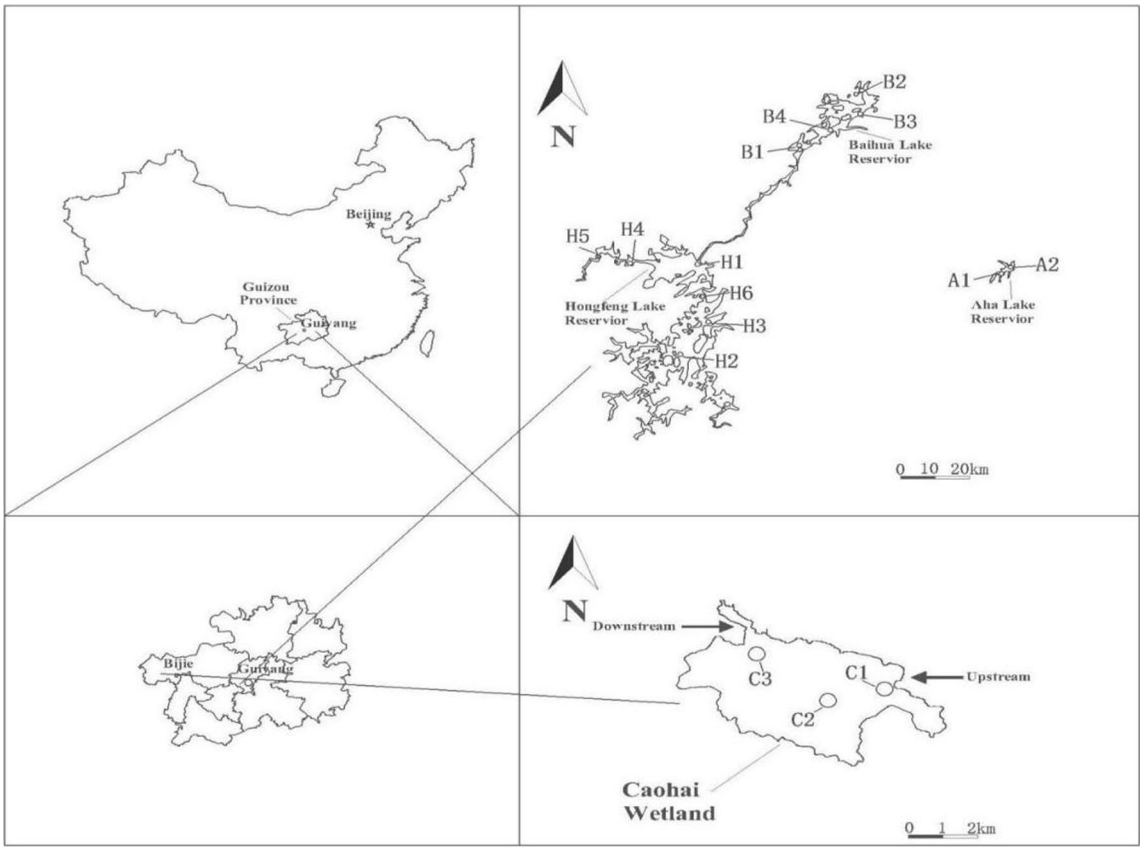

Fig. 1 Locations of the study area and sampling sites in the three reservoirs and wetland, Guizhou Province, China

$$TSI_{CHla} = 30.6 + 9.81\ In(CHla, \mu g\ L^{-1})$$
$$TSI_{TP} = 4.15 + 14.42\ In(TP, \mu g\ L^{-1})$$
$$TSI_{TN} = 54.45 + 14.43\ In(TN, mg\ L^{-1})$$

The formula for calculating bioaccumulation factors (BAFs) is as follows:

$$BAF = Biota_{THg\ or\ MeHg}/Water_{THg\ or\ MeHg}$$

where biota = zooplankton (Razavi et al. 2015; Long et al. 2016)

Results

Water characteristics

Table 2 shows the water characteristics and mercury concentrations in the water. The water was alkaline in all the waters studied. The dissolved organic carbon in the water of CW was significantly higher than that in the water of HR, AR, and BR. The THg concentrations in the water of HR were significantly higher than those in the water of CW, AR, and BR (ANOVA, $P < 0.05$), but no significant difference was found between CW, AR, and BR (ANOVA, $P > 0.05$). The MeHg concentrations in the water of CW were significantly higher than those in the water of HR and AR (ANOVA, $P < 0.05$), and no significant difference was observed between HR, AR, and BR (ANOVA, $P > 0.05$).

Overall, TN concentrations in HR, BR, AR, and CW ranged from 1.15 to 2.56 mg L^{-1} (mean ± standard deviation, 1.66 ± 0.37 mg L^{-1}), 1.47 to 2.19 mg L^{-1} (1.77 ± 0.20 mg L^{-1}), 2.17 to 2.88 mg L^{-1} (2.66 ± 0.26 mg L^{-1}), and 1.23 to 2.06 mg L^{-1} (1.65 ± 0.34 mg L^{-1}), respectively (Fig. 2a). The TP concentrations in HR, BR, AR, and CW ranged from 10 to 56 μg L^{-1} (31 ± 14 μg L^{-1}), 21 to 43 μg L^{-1} (31 ± 11 μg L^{-1}), 35 to 83 μg L^{-1} (50 ± 15 μg L^{-1}), and 13 to 43 μg L^{-1} (26 ± 9 μg L^{-1}), respectively (Fig. 2b). The CHla concentrations in HR, BR, AR, and CW ranged from 4.57 to 26.56 μg L^{-1} (9.08 ± 4.48 μg L^{-1}), 6.80 to 12.20 μg L^{-1} (9.23 ± 3.2 μg L^{-1}), 10.15 to 20.21 μg L^{-1} (13.51 ± 3.49 μg L^{-1}), and 5.57 to 11.14 μg L^{-1} (8.01 ± 1.93 μg L^{-1}), respectively (Fig. 2c). It can be seen from Fig. 2 that the seasonal variation of TP and TN was not obvious, but the CHla in autumn was obviously higher than that in other seasons (ANOVA < 0.05). There were significant differences in the trophic indexes among different sampling sites in each water body, which indicated that the water quality of different water inlets of the water bodies was quite different; however, in general, the trophic indexes of each sampling point in Aha Lake were significantly higher than those of the other water bodies ($P < 0.01$). For

the three reservoirs HR, BR, and AR, negative correlations were observed between TP and THg ($r = -0.661$, $P < 0.05$, $n = 12$), as shown in Fig. 5a, and a negative correlation was observed between the THg and CHla concentrations ($r = -0.604$, $P < 0.05$, $n = 12$, Fig. 5b). Although no clear correlation was observed between TP and MeHg ($r = -0.369$, $P > 0.05$, $n = 12$, Fig. 5a), AR, which showed the most serious eutrophication, had significantly lower MeHg concentrations than did HR and BR (ANOVA, $P = 0.033$).

According to the TSI values (Table 3), among the 15 sampling sites that we investigated, six sites were likely limited by P ($TSI_{CHla} - TSI_{TP} > 0$), whereas no sampling sites were limited by N ($TSI_{CHla} - TSI_{TN} > 0$). According to the TSI_{CHla} values, AR is eutrophic, and HR, BR and CW are mesotrophic. The trophic states showed the following order from eutrophic to mesotrophic: AR, BR, HR, and CW (Razavi et al. 2015; Long et al. 2016; Matthews et al. 2002). If the trophic state was evaluated using TP concentrations alone according to the EC (Environment Canada 2004), the same trophic state would be classified (eutrophic water, 35~100 μg L^{-1} TP; mesotrophic water, 10~20 μg L^{-1} TP).

Zooplankton communities

The zooplankton in HR, BR, AR, and CW mainly consist of copepods, cladocerans, and rotifers. The dominant species during different sampling periods (October 2015, February 2016, May 2016, and August 2016) are summarized in Table 4.

Total Hg concentration in zooplankton

The temporal variations of THg concentrations in zooplankton (with different body sizes) collected from HR, BR, AR, and CW are shown in Fig. 3(a–d). The concentration ranges of THg in zooplankton from HR, BR, AR, and CW were 13.3 to 494.9 ng g^{-1} (138.8 ± 104.7 ng g^{-1}), 6.3 to 361.8 ng g^{-1} (139.1 ± 91.4 ng g^{-1}), 20.7 to 254.6 ng g^{-1} (102.0 ± 62.8 ng g^{-1}), and 15.4 to 385.7 ng g^{-1} (100.1 ± 109.1 ng g^{-1}), respectively. The THg concentrations of zooplankton in the waters that we studied were comparable to those reported in acidic waters (e.g., Watras and Bloom 1992; Watras et al. 1998; Back and Watras 1995; Paterson et al. 1998). No clear differences in the mean THg concentrations were observed for zooplankton collected from HR, BR, AR, and CW.

According to their body sizes, the zooplankton collected in our study were classified into three groups: macro-zooplankton (> 500 μm), meso-zooplankton (116 to 500 μm), and micro-zooplankton (77 to 116 μm). For the three reservoirs during the four seasons, in addition to two sites in HR and BR (963.1 ng g^{-1} and 628.86 ng g^{-1}, respectively) showing anomalously high THg concentrations, significant differences in THg concentrations were observed between zooplankton with different body sizes (ANOVA, $P = 0.006$). The THg concentration decreased in the following order: micro-zooplankton (159.4 ± 93.4 ng g^{-1}) > macro-zooplankton (136.2 ± 103.8 ng g^{-1}) > meso-zooplankton (99.0 ± 70.4 ng g^{-1}). In CW, however, no difference in THg concentration between meso-zooplankton and macro-

Table 2 Water characteristics and BAF zooplankton mercury in the reservoirs and wetland studied (mean (±SD)[a] and range)

	Hongfeng Reservoir	Baihua Reservoir	Aha Reservoir	Caohai Wetland
pH	8.3 ± 0.46a[b]	8.29 ± 0.53a	7.69 ± 0.35a	8.52 ± 0.91a
	(7.92–9.14)	(7.85–9.07)	(6.67–8.1)	(7.52–9.62)
THg (ng L^{-1})	4.61 ± 0.797a	2.32 ± 0.369b	1.98 ± 0.03b	1.67 ± 0.357b
	(3.97–5.99)	(1.88–2.69)	(1.96–2.00)	(1.34–2.05)
DTHg (ng L^{-1})[c]	1.66 ± 0.568a	1.80 ± 0.258a	1.76 ± 0.015a	-[d]
	(0.96–2.69)	(1.59–2.17)	(1.75–1.77)	
MeHg (ng L^{-1})	0.077 ± 0.02a	0.135 ± 0.026ab	0.097 ± 0.0249a	0.201 ± 0.119b
	(0.048–0.107)	(0.102–0.160)	(0.079–0.115)	(0.125–0.338)
DMeHg (ng L^{-1})[e]	0.058 ± 0.03b	0.115 ± 0.0428a	0.074 ± 0.006ab	-
	(0.024–0.093)	(0.055–0.154)	(0.069–0.078)	
DOC (mg L^{-1})	2.70 ± 0.22a[f]	2.67 ± 0.39a[g]	1.67 ± 0.35b[h]	10.76 ± 3.30c[i]
	(2.36–2.92)	(1.97–3.46)	(1.33–2.35)	(7.61–18.82)
BAF zooplankton THg (× 10^4)	3.73 ± 2.13a	5.81 ± 2.66a	3.77 ± 0.61a	4.00 ± 2.48a
	(1.58–6.64)	(3.62–9.63)	(3.34–4.20)	(1.14–5.57)
BAF zooplankton MeHg (× 10^4)	13.6 ± 4.27a	13.04 ± 3.65a	7.39 ± 0.42a	0.51 ± 0.27b
	(8.25–19.69)	(8.77–17.29)	(7.11–7.70)	(0.23–0.77)

[a] ± standard deviation (SD); [b] the different lowercase letters in a row indicate significant differences among different treatments at $P < 0.05$ level; [c] DTHg, dissolved total mercury; [d] -, mean data not monitored; [e] DMeHg = dissolved methylmercury; [f] data obtained from Lu et al. (2007); [g] data obtained from Xu et al. (2014); [h] data obtained from Wang et al. (2016); [i] data obtained from Qian et al. (2009)

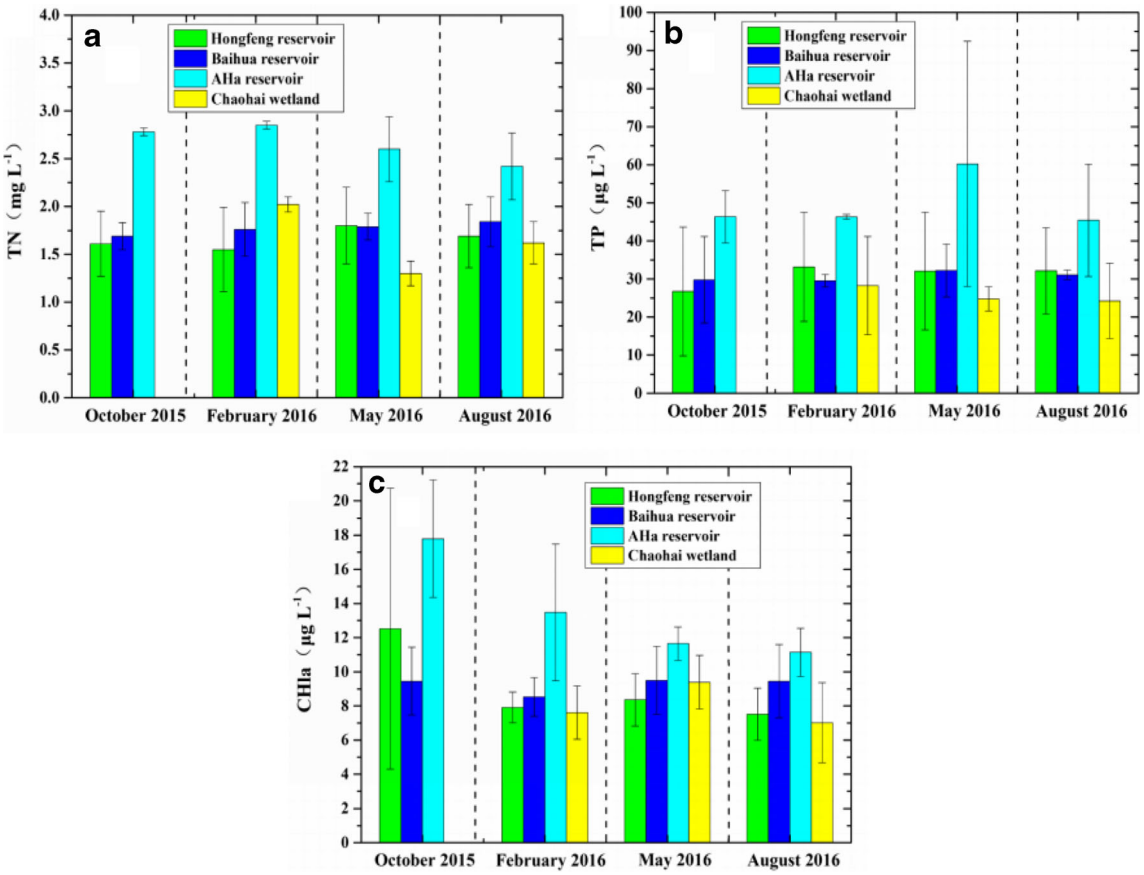

Fig. 2 Total nitrogen (TN), total phosphorus (TP), and chlorophyll a (CHla) concentrations in the reservoirs and wetland studied

zooplankton was observed. In the three reservoirs, no clear correlations were observed between the THg concentrations in zooplankton with different body sizes ($P > 0.05$, Fig. 4a, b). In our study, aside from one site in HR (5.99 ng L^{-1}) that showed anomalously high THg concentrations in the water body, there was a significantly positive correlation between THg concentrations in water and zooplankton ($r = 0.665$, $P < 0.01$, $n = 14$, Fig. 4e).

For zooplankton collected from HR, BR, and AR, their THg concentrations showed no temporal differences. In CW, however, zooplankton collected in August (217.7 ng g^{-1}) showed significantly higher THg concentrations (ANOVA, $P = 0.008$) than those collected in February (35.2 ng g^{-1}).

MeHg concentration in zooplankton

The temporal variations of MeHg concentrations in zooplankton (with different body sizes) collected from HR, BR, AR, and CW are shown in Fig. 3(e–h). The concentration ranges of MeHg in zooplankton from HR, BR, AR, and CW were 0.2 to 66.8 ng g^{-1} (14.1 ± 14.6 ng g^{-1}), 0.8 to 57.3 ng g^{-1} (14.6 ± 13.7 ng g^{-1}), 1.2 to 32.1 ng g^{-1} (9.6 ± 9.4 ng g^{-1}), and 0.1 to 21.1 ng g^{-1} (3.2 ± 5.1 ng g^{-1}), respectively, which were lower than those reported in zooplankton in most of the acidic waters (Watras and Bloom 1992; Watras et al. 1998; Westcott and Kalf 1996; Back and Watras 1995; Paterson et al. 1998; Garcia et al. 2007; Hall et al. 2009). For example, Westcott and Kalf (1996) reported that MeHg concentrations in zooplankton ranged from 19 to 448 ng g^{-1} in acidic waters. In most of the acidic waters, there were higher levels of MeHg in zooplankton than in the alkaline waters that we studied, but the same levels of THg in zooplankton were observed (Watras and Bloom 1992; Watras et al. 1998; Back and Watras 1995; Paterson et al. 1998). This result may be related to the higher methylation rate in acidic waters (Miskimmin et al. 1992; Kelly et al. 2003; Golding et al. 2008).

The zooplankton collected from the three reservoirs (HR, BR, and AR) consistently showed significantly higher MeHg levels than those collected from CW (ANOVA, $P = 0.007$). For the three reservoirs during the four seasons, aside from two sites in HR (98.10 ng g^{-1} and 52.62 ng g^{-1}), which showed anomalously high MeHg concentrations, macrozooplankton showed significantly higher MeHg levels than did meso-zooplankton (ANOVA, $P = 0.021$). The MeHg concentration decreased in the following order: macrozooplankton (15.7 ± 15.6 ng g^{-1}) > micro-zooplankton (14.5 ± 12.9 ng g^{-1}) > meso-zooplankton (9.5 ± 10.0 ng g^{-1}). However, no difference in MeHg concentration was observed

Table 3 Trophic state index (TSI) using mean CHla (μg L^{-1}), TP (μg L^{-1}), and TN (mg L^{-1}) in the reservoirs and wetland studied

Reservoir/wetland	Site	TSI_{CHla}	TSI_{TP}	TSI_{TN}	$TSI_{CHla} - TSI_{TP}$	$TSI_{CHla} - TSI_{TN}$	Trophic state[a]	Possible limiting factor[b]
Hongfeng Reservoir	H1	51.32	52.71	60.94	− 1.39	− 9.62	Mesotrophic	-
	H2	52.35	51.13	60.28	1.22	− 7.93	Mesotrophic	P
	H3	55.45	55.00	66.64	0.45	− 11.19	Eutrophic	P
	H4	50.33	48.72	58.84	1.61	− 8.51	Mesotrophic	P
	H5	53.05	55.42	60.43	− 2.37	− 7.38	Mesotrophic	-
	H6	49.78	56.52	62.21	− 6.74	− 12.43	Mesotrophic	-
	Mean	52.05	53.25	61.56	–	–	-	-
Baihua Reservoir	B1	51.98	55.00	63.47	− 3.02	− 11.49	Mesotrophic	-
	B2	52.53	53.29	63.52	− 0.76	− 10.99	Mesotrophic	-
	B3	53.46	54.16	62.71	− 0.7	− 9.25	Mesotrophic	-
	B4	51.54	51.41	60.87	0.13	− 9.33	Mesotrophic	P
	Mean	52.38	53.46	62.64	–	–	-	-
Aha Reservoir	A1	57.00	58.39	68.66	− 1.39	− 11.66	Eutrophic	-
	A2	55.20	62.45	68.49	− 7.25	− 13.29	Eutrophic	-
	Mean	56.10	60.42	68.58	–	–	-	-
Caohai Wetland	C1	50.48	48.05	61.75	2.43	− 11.27	Mesotrophic	P
	C2	50.39	49.36	61.73	1.03	− 11.34	Mesotrophic	P
	C3	52.07	55.00	61.54	− 2.93	− 9.47	Mesotrophic	-
	Mean	50.98	50.80	61.67	-	-	-	-

[a] TSI_{Chla} values between 45 and 55 are associated with mesotrophic systems and > 55 indicates eutrophic systems (Razavi et al. 2015)

[b] Phosphorus (P) limitation is indicated by $TSI_{Chla} - TSI_{Tp} > 0$, nitrogen (N) limitation is indicated by $TSI_{Chla} - TSI_{TN} > 0$ (Matthews et al. 2002)

between macro-zooplankton and meso-zooplankton collected from CW. In the three reservoirs, significant positive correlations were observed between the MeHg concentrations in zooplankton with different body sizes ($P < 0.05$, Fig. 4c, d). However, no clear correlation between MeHg concentrations in water and zooplankton was observed ($r = -0.309$, $P > 0.05$, $n = 15$, Fig. 4f), and there was no correlation between the THg and MeHg concentrations of zooplankton ($P > 0.05$, Fig. 4g).

The zooplankton MeHg concentrations in HR, BR, and AR showed significant temporal differences (ANOVA, $P = 0.001$). For the three reservoirs, the MeHg levels in zooplankton collected in October were slightly higher than those collected in May or August but much higher than those collected in February. In CW, the MeHg levels in zooplankton collected in August (7.89 ng g^{-1}) were significantly higher (ANOVA, $P = 0.002$) than those collected in May (1.02 ng g^{-1}) and February (1.4 ng g^{-1}).

In the three reservoirs, no clear difference in MeHg fractions (%MeHg) could be observed among the seasons, reservoirs, or size fractions. The MeHg fractions ranged from 0.3 to 56.7%, with a mean value of 12.1% ± 12.0%, in the reservoirs during the four seasons. Our values were comparable with those reported for Lake Champlain, USA (4 to 53%) (Chen et al. 2012), and for some lakes in eastern China (30.8 to 60%) (Razavi et al. 2015) but lower than those reported for lakes in the Adirondacks, New York, USA (0 to 74%) (Yu et al. 2011), and lakes in Wisconsin, USA (11 to 83%) (Watras et al. 1998). In CW, zooplankton with different body sizes also showed no differences in %MeHg; the MeHg fractions ranged from 0.2 to 12.8%, with a mean value of 3.4% ± 4.1%, much lower than that in the three reservoirs (ANOVA, $P = 0.009$).

The bioaccumulation factor values of THg and MeHg in zooplankton ranged from 1.14×10^4 to 9.63×10^4 ($4.34 \times 10^4 \pm 2.21 \times 10^4$) and 0.23×10^4 to 1.96×10^5 ($1.00 \times 10^5 \pm 6.15 \times 10^4$), respectively; these values were similar to those of Razavi et al. (2015), who reported 10^3~10^4 for the BAF of THg in zooplankton collected from subtropical reservoirs, but lower than those of Stewart et al. (2008), who reported 10^4~10^6 for the BAF of MeHg in zooplankton from a temperate reservoir. Overall, the BAF of MeHg was relatively higher than that of THg (ANOVA, $P < 0.01$). The BAF of MeHg in CW was significantly lower than that in the three reservoirs (ANOVA, $P < 0.05$) (Table 2).

Discussion

Seasonal distribution of mercury in zooplankton

In the three reservoirs, the zooplankton THg concentrations showed no temporal differences. However, the zooplankton MeHg concentrations showed significant temporal differences (ANOVA, $P = 0.001$), and those collected in October had slightly higher levels than those collected in

Table 4 The dominant species and density of zooplankton in the reservoirs and wetland studied

Reservoir/ wetland	Month	Dominant species and density (ind L^{-1})
Hongfeng Reservoir	October 2015	*Asplanchna priodonta* (767), *Daphnia hyalina* (66.4), *Bosmina longirostris* (9.8), *Phyllodiaptomus tunguidus* (30), *Mesocyclops leuckarti* (23.5), *Thermocyclops brevifurcatus* (7.1)
	February 2016	*Asplanchna priodonta* (360), *Keratella cochlearis* (360), *Daphnia hyalina* (26.8), *Bosmina longirostris* (42.9), *Phyllodiaptomus tunguidus* (16.2), *Thermocyclops brevifurcatus (4.8)*
	May 2016	*Asplanchna priodonta* (330), *Daphnia hyalina* (11.8), *Keratella cochlearis* (180), *Bosmina longirostris* (8.7), *Phyllodiaptomus tunguidus (5.7)*
	August 2016	*Asplanchna priodonta* (630), *Daphnia hyalina* (52.8), *Bosmina longirostris* (13.9), *Phyllodiaptomus tunguidus* (7.6)
Baihua Reservoir	October 2015	*Brachionus angularis* (330), *Keratella valga* (240), *Asplanchna priodonta* (480), *Brachionus calyciflorua* (150), *Bosmina longirostris* (22.9), *Phyllodiaptomus tunguidus* (3.2)
	February 2016	*Daphnia hyalina* (8.6), *Bosmina longirostris* (9.6), *Cyclops vicinus vicinus* (7.3), *Themocyclops mongolicus* (8.1)
	May 2016	*Asplanchna priodont* (780), *Daphnia hyalina* (15.3), *Bosmina longirostris* (5.9), *Phyllodiaptomus tunguidus* (3.5), *Thermocyclops brevifurcatus* (4.6), *Mesocyclops leuckarti* (3), *Cyclops vicinus vicinus* (4)
	August 2016	*Keratella cochlearis* (420), *Bosmina longirostris* (17.2), *Phyllodiaptomus tunguidus* (6.8), *Thermocyclops brevifurcatus* (19.6), *Mesocyclops leuckarti* (3.1)
Aha Reservoir	October 2015	*Asplanchna priodont* (270), *Daphnia hyalina* (23.3)
	February 2016	*Daphnia hyalina* (56.1)
	May 2016	*Asplanchna priodonta* (290), *Daphnia hyalina* (57.9), *Bosmina longirostris* (11.7)
	August 2016	*Asplanchna priodonta* (170), *Keratella cochlearis* (390), *Daphnia hyalina* (31)
Caohai Wetland	February 2016	*Asplanchna priodonta* (290), *Daphnia hyalina* (62.9), *Bosmina longirostris* (16.7), *Phyllodiaptomus tunguidus* (10.6)
	May 2016	*Asplanchna priodonta* (420), *Daphnia hyalina* (12.2), *Bosmina longirostris* (5.8), *Phyllodiaptomus tunguidus* (8.8)
	August 2016	*Asplanchna priodonta* (120), *Daphnia hyalina* (4.6), *Bosmina longirostris* (2.6), *Phyllodiaptomus tunguidus* (3.4)

May or August but much higher levels than those collected in February. Perhaps this pattern reflects the temporal variations of MeHg levels or zooplankton communities in the water body. In spring and summer, MeHg concentrations in waters are expected to be higher due to the higher temperature, stratification, and anoxic conditions of the water bodies (He et al. 2010). Compared with those in the other two reservoirs, methylmercury levels in Baihua Reservoir increased significantly in May and August, which was related to the serious mercury pollution in the history of Baihua Reservoir. In the anoxic environment in spring and summer, Hg and MeHg in sediment were released into the water body, which increased the risk of mercury enrichment in aquatic organisms. However, in CW, the THg levels in zooplankton in August were significantly higher (ANOVA, $P = 0.008$) than those collected in February, and the MeHg levels in zooplankton in August were significantly higher (ANOVA, $P = 0.002$) than those collected in May and February. The seasonal distribution of mercury in zooplankton in CW was different from that in the three reservoirs, which may be due to the totally different hydraulic and water quality characteristics of CW, as well as the totally different growth cycle of zooplankton.

Relation between zooplankton Hg levels and biological characteristics

In addition to micro-zooplankton, it is clear that macro-zooplankton had significantly higher THg and MeHg levels than meso-zooplankton (ANOVA, $P < 0.05$) in all seasons of the three reservoirs. Previous studies have reported similar results that the mercury levels in zooplankton with larger body sizes were higher than those in zooplankton with smaller body sizes, and this usually was explained by the biomagnification of mercury in the food chain (Masson and Tremblay 2003; Wang et al. 2011; Kainz et al. 2002, 2006). In our study, micro-zooplankton did not follow the rule that the Hg levels increase with body size (Surette et al. 2006; Todorova et al. 2015; Gosnell et al. 2017). Perhaps this is because the way that we define the micro-zooplankton is different from previous studies (Wang et al. 2011; Kainz et al. 2006), which defined micro-zooplankton as having larger body sizes (100~200 μm). Due to the smaller body sizes, it was possible that some suspended materials were mixed in our micro-zooplankton samples and led to higher THg and MeHg levels.

As shown above, MeHg concentrations between different body sizes were significantly correlated, while not for THg.

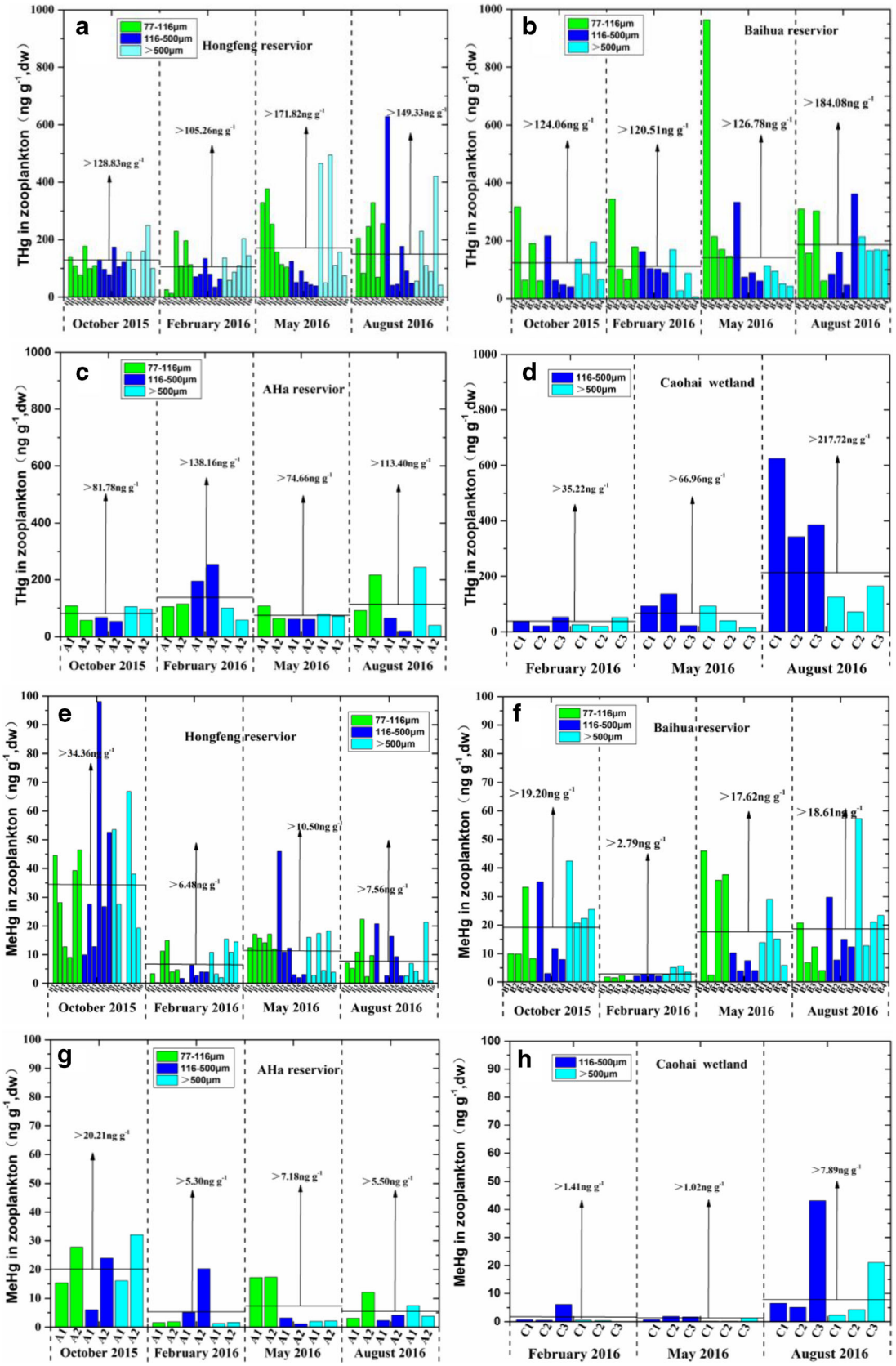

Fig. 3 The temporal variations of total mercury (THg) (**a**, **b**, **c**, **d**) and methylmercury (MeHg) (**e**, **f**, **g**, **h**) concentrations in zooplankton in the reservoirs and wetland studied (lines with an arrow and a value indicated mean value of mercury in zooplankton at all points in each month)

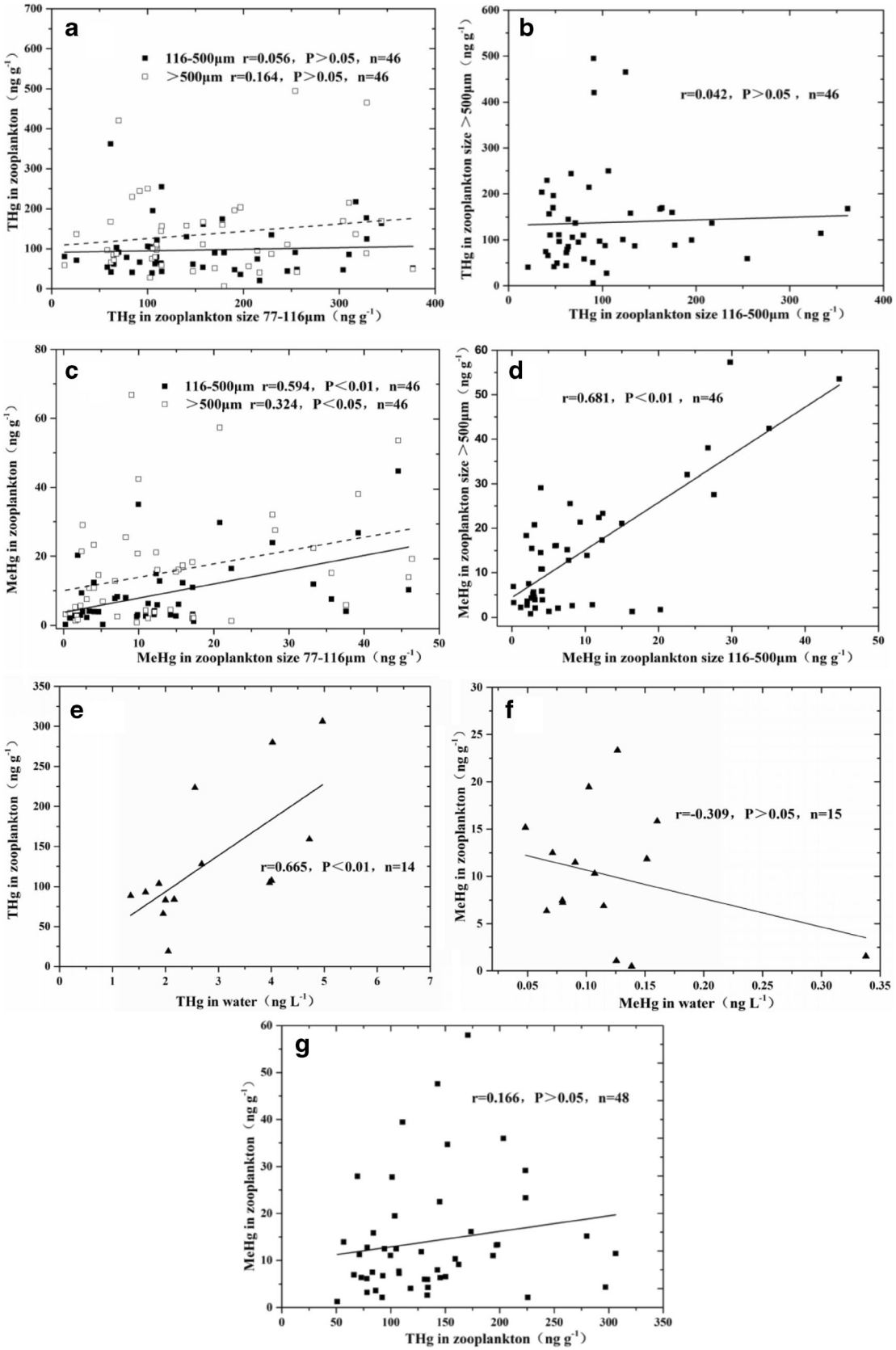

Fig. 4 The relationship between total mercury (THg) in zooplankton with different body sizes (**a**, **b**), the relationship between methylmercury (MeHg) in zooplankton with different body sizes (**c**, **d**), total mercury (THg) (**e**) and methylmercury (MeHg) (**f**) concentrations in water (unfiltered) versus concentrations in zooplankton, and total mercury (THg) concentration vs methylmercury (MeHg) concentration in zooplankton (**G**)

This result indicates that the biomagnification of MeHg with increasing body size is more significant than that of THg. According to previous studies, zooplankton mainly receive their MeHg from diet (Tsui and Wang 2004; Monson and Brezonik 1999). The MeHg levels in zooplankton were closely related to their habitat, food ration, and food type (Kainz and Mazumder 2005; Kainz et al. 2002). As shown above, THg concentrations between water and zooplankton were significantly correlated, while not for MeHg. Our results indicated that THg bioaccumulation in zooplankton was related to the THg level in water but that MeHg bioaccumulation in zooplankton was controlled by many other factors, such as their feeding and living habits. This finding is consistent with previous observations (Tsui and Wang 2004; Morel et al. 1998). Research by Tsui and Wang (2004) showed that in *Daphnia magna*, dietary exposure was important for MeHg bioaccumulation, but water exposure was important for Hg(II) bioaccumulation. Morel et al. (1998) demonstrated that Hg(II) was bound chiefly to particulate cellular material (membranes) of diatoms that were excreted rather than absorbed by zooplankton. In contrast, MeHg was associated with the soluble fraction of the diatom cell and was efficiently assimilated by zooplankton. Therefore, the bioaccumulation of MeHg in zooplankton was mainly affected by the MeHg levels in their prey. There was no correlation between the THg and MeHg concentrations of zooplankton, which further indicated that the bioaccumulation pathways for THg and MeHg in zooplankton were different. The relatively higher BAF of MeHg than of THg (ANOVA, $P < 0.01$) indicates that MeHg is more readily bioaccumulated than THg.

Some researches have found that the zooplankton density also affects the mercury content of zooplankton (Chen and Folt. 2005; Chen et al. 2005, 2012). Chen and Folt (2005) found a negative correlation between zooplankton density and Hg concentrations in zooplankton and in both herbivorous and predatory fish. In our study, rotifers had a much higher density than that of copepods and cladocerans. However, the density of zooplankton appears to have a limited effect on the Hg levels in zooplankton. No clear correlations were observed between the density and Hg concentrations in zooplankton, consistent with previous studies showing that the Hg levels in zooplankton had no relationship with zooplankton density (Razavi et al. 2015).

Influence of trophic state on Hg bioaccumulation in zooplankton

As shown above, TP and THg were negative correlations in the three reservoirs. AR, which showed the most serious eutrophication, had significantly lower MeHg concentrations than did HR and BR, while no clear correlation between TP and MeHg (Fig. 5a). Our results clearly showed that the increase in eutrophication could result in decreased THg and MeHg levels in zooplankton, which agrees well with the observations of previous studies. For instance, Chen and Folt (2005) investigated the Hg and trophic states in 20 lakes in the northeastern US and found that zooplankton collected from eutrophic lakes showed much lower Hg levels than those of other lakes (which have low productivity). Similar results were also observed by Razavi et al. (2015), who investigated 7 lakes in eastern China. Chen et al. (2012) compared the THg and MeHg levels in two lakes during a 4-year period and found that in the eutrophic lake, THg (24 to 65 ng g^{-1}) and MeHg (3 to 33 ng g^{-1}) concentrations were lower than those in the oligotrophic lake (THg, 70 to 330 ng g^{-1}; MeHg: 18 to 99 ng g^{-1}).

The eutrophication could accelerate algal biomass growth, which further increases the biomass of zooplankton. This phenomenon also leads to low Hg concentrations in zooplankton due to the biodilution effect (Pickhardt et al. 2002; Chen and Folt 2005; Wang et al. 2011; Gosnell et al. 2017). As shown above, a negative correlation was observed between the THg and CHla concentrations (Fig. 5b), showing that increasing the Chla levels can reduce THg accumulation in zooplankton.

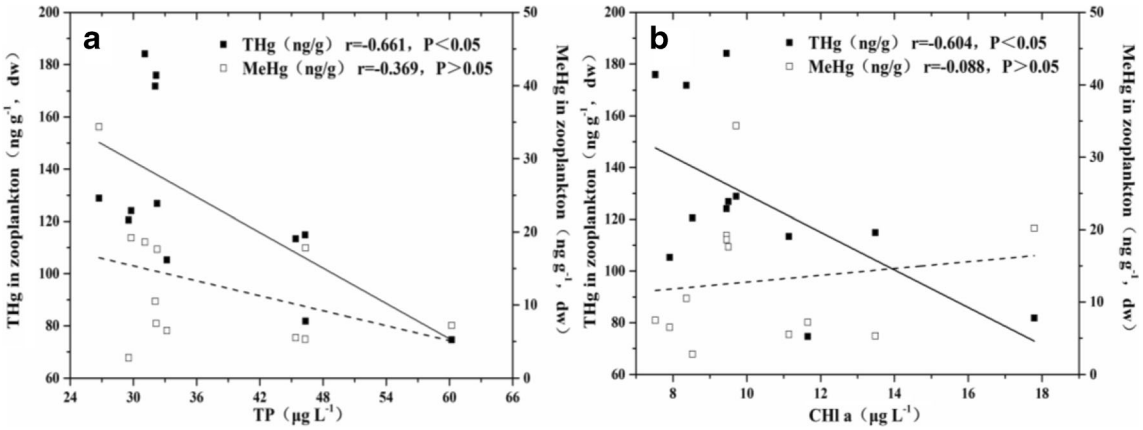

Fig. 5 Total phosphorus (TP) and chlorophyll a (CHla) in water versus total mercury (THg) and methylmercury (MeHg) in zooplankton in the reservoirs and wetland studied

The highest CHla levels and the lowest THg and MeHg levels in zooplankton from AR are consistent with previous observations that suggested that Hg can be biodiluted (Watras et al. 1998; Pickhardt et al. 2002; Chen et al. 2012; Razavi et al. 2015; Chen and Folt 2005; Wang et al. 2011). For instance, Watras et al. (1998) investigated zooplankton in 15 lakes in the USA and found that the THg concentration reached 289 ng g^{-1} when that of CHla was 4.55 μg L^{-1}, while the THg concentration decreased to 224 ng g^{-1} when that of CHla increased to 8.20 μg L^{-1}. Chen and Folt (2005) found that phytoplankton density was negatively correlated with Hg concentrations in phytoplankton and their consumers (zooplankton). It is obvious that this rule applies for the three reservoirs that we investigated.

In Caohai Wetland, however, the THg and MeHg concentrations in zooplankton did not increase as the trophic level decreased. In the waters we studied, CW had the lowest trophic level and CHla levels but also the lowest zooplankton Hg concentrations and MeHg bioaccumulation factors, although there were significantly higher MeHg concentrations in the water of CW than in the water of HR and AR. Many studies have shown that environmental factors in wetlands are more conducive to mercury methylation (Louis et al. 1994; Driscoll et al. 1998; Liu et al. 2002; Schäfer et al. 2010; Bates and Hall 2012). The lower zooplankton Hg concentrations and BAF in CW may be explained by the abundant species of aquatic animals and plants and the high primary productivity. There are more than 40 common plant species in Caohai, including *Alternanthera philoxeroides* (Mart.) Griseb., *Potamogeton malaianus* Miq., *Scirpus validus* Vahl, *Potamogeton crispus* L., *Myriophyllum verticillatum* L., *Ceratophyllum demersum* L., and so on. Some aquatic plant residues can enter the food web through the detritus, which is one of the food sources for zooplankton (Poulet 1976; Swift et al. 1979; Steinberg et al. 1998). These large amounts of plant debris may dilute mercury in the biological chain, similar to the large amount of algae triggered by eutrophication. This consideration indicates that higher primary production of the freshwater ecosystem may be more decisive for lower Hg bioaccumulation in the food chain, rather than only looking at nutrient levels.

Conclusions

In the three reservoirs, the relationship between mercury in zooplankton and the trophic state in water suggests that the bioaccumulation of Hg in zooplankton is closely related to the trophic state of the water body. Compared with the three reservoirs, CW has a much lower trophic state and higher MeHg levels in water but much lower zooplankton MeHg levels and BAF, which may be because large amounts of plant detritus might dilute mercury in the food chain.

The results of the analysis of THg and MeHg in zooplankton show that the biomagnification of MeHg with increasing zooplankton body size is more significant than that of THg and further show that MeHg is more readily bioaccumulated in zooplankton. The correlation between Hg in water and zooplankton and Hg in zooplankton of different sizes indicated that THg bioaccumulation in zooplankton was related to the THg levels in water, but MeHg bioaccumulation in zooplankton might be controlled by many other factors.

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