Differential bioaccumulation of mercury by zooplankton taxa in a mercury-contaminated reservoir Guizhou China

Sheng-Xing Long a, c, Paul B. Hamilton b, Yang Yang a, *, Sai Wang a, Wen-da Huang a, Chuan Chen c, Ran Tao a

a Institute of Hydrobiology, College of Life Science and Technology, JINan University, Guangzhou, PR, 510632, China
b Research and Collections, Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, Ontario, K1P 6P4, Canada
c Guizhou Normal University, Guiyang, Guizhou, 550001, China

ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form 2 March 2018
Accepted 2 April 2018
Available online 10 April 2018

Keywords:
Zooplankton
Mercury (Hg)
Methylmercury (MeHg)
Phytoplankton
Environmental factors

ABSTRACT

Mercury (Hg) contamination in aquatic systems remains a global concern with the biomagnification of methylmercury (MeHg) through primary consumers (zooplankton) to fish and humans. In this study, total mercury (THg) and MeHg concentrations were analyzed in zooplankton collected from Baihua reservoir (Guizhou Province, China). Our results demonstrated that THg and MeHg concentrations were strongly correlated to zooplankton community and biomass composition. The THg concentration was significantly higher in micro-zooplankton compared to meso-zooplankton and macro-zooplankton, and MeHg concentration increased significantly as body size increased. Hg increases in zooplankton were influenced by the numbers of calanoid copepods and Daphnia present relative to phytoplankton and zooplankton biomass. Many zooplankton taxa in the three size-fractions were affected by THg exposure. The biomasses of Bosmina longirostris, Thermocyclops brevifurcatus, Asplanchna priodonta and Cyclops vicinus vicinus were positively correlated with Hg accumulation, while Daphnia hyalina, and Phyllopterygium tunguidus had a negative association. THg and MeHg bioaccumulation factors were correlated with phosphorus and total nitrogen concentration, zooplankton biomass, and chlorophyll-a concentration. Phosphorus loading was associated with increased THg and MeHg accumulation in the zooplankton highlighting biomagnification with eutrophication. Chlorophyll-a levels were not correlated to THg and MeHg accumulation in zooplankton when phytoplankton densities were >10^7 cells L^-1 and chlorophyll-a concentrations <9 µg L^-1. This finding contradicts the idea of MeHg biodilution with increased algae biomass. However, changes in the phytoplankton species and biomass altered the availability of food for zooplankton, particularly micro-zooplankton and macro-zooplankton. Ultimately, the bioaccumulation of MeHg and THg across lower trophic levels was based more on the availability of preferred food resources than on total biological productivity.

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1. Introduction

Public health concerns about neurotoxicity, relating to the trophic transfer and bioaccumulation of methylmercury (MeHg) in aquatic food chains, are increasing around the world (He et al., 2008; Liu et al., 2012; Todorova et al., 2015). Increased food consumption containing unknown levels of Hg has also been a concern, resulting in the development of guidelines for fish consumption in many countries (e.g. Clarkson and Magos, 2006; Todorova et al., 2015). Numerous toxicity studies have shown that MeHg bioaccumulation and trophic transfer in lakes is related to physical, chemical, and ecological factors, including inorganic Hg, dissolved organic carbon, sulfide (S^2^-), sulfate (SO4^2-), dissolved oxygen, temperature, pH, phytoplankton and zooplankton (e.g. Driscoll et al., 2007; Feyte et al., 2012; Yan et al., 2013). Trophic transfer and bioaccumulation of MeHg is often positively related to total mercury (THg) (Long et al., 2017). However, one study, documented that increased eutrophication in the water column had a negative relationship to Hg bioaccumulation; this response was associated with the dilution of Hg through increased phytoplankton densities (Baihua reservoir, in Southwest China) (Liu et al., 2012). Results...
from past research also suggest that eutrophic reservoirs have lower Hg concentrations in fish (top trophic level) compared to oligotrophic reservoirs; this negative relationship has been in part attributed to higher phytoplankton biomass (MeHg dilution per cell) and the partitioning of Hg into biomass size fractions across trophic levels (Pickhardt et al., 2002; Chen and Folt, 2005). Chen et al. (2012) also found that THg concentrations in zooplankton and predators were negatively correlated with chlorophyll-a (Chl-a) concentration because of bio-dilution linked to eutrophication. These results are aligned with observed high Hg burdens observed in fish from more pristine (lower productivity) lakes and reservoirs in North America and northern Europe (Munthe et al., 2007; Larssen, 2010).

Zooplankton are ubiquitous, often abundant in freshwater and can play a key role in the biogeochemical cycling of Hg in freshwater systems (e.g. Todorova et al., 2013; Long et al., 2017). Variability in community composition (including body size) may determine the upward trophic migration of Hg (Chen et al., 2012; Todorova et al., 2015; Gossell et al., 2017), and like phytoplankton, higher zooplankton densities can potentially decrease Hg transfer to fish through zooplankton density dilution (Chen and Folt, 2005). At present, it is not clear how Hg accumulates when there are changes in eutrophic state. This year long temporal and spatial study focused on changes in THg and MeHg concentrations in zooplankton from Baihua Lake reservoir (southeast China). Baihua reservoir has experienced elevated concentrations of Hg due to direct discharges from an acetic acid plant. The reservoir has also received substantial loads of nitrogen and phosphorus as a result of untreated domestic inputs. Recent decreases in nitrogen and phosphorus in the reservoir are attributed to wastewater treatment and reduced loading; wastewater dumping of Hg has been prohibited since 1997. Thus Hg pollution, nutrient loads, and primary production have changed markedly over the last 50 years in Baihua reservoir and documentation of this pollution makes the system a good in situ study case. The objective of the current study is to investigate Hg and MeHg bioaccumulation by zooplankton in relation to Hg availability and phytoplankton biomass. This study tested the hypothesis that differences in biomass and species composition of zooplankton can account for a significant amount of the variation in Hg accumulation. Bioaccumulation theory was used (in the context of the changing nutrient status of karst plateau reservoirs in China) along with changes in the zooplankton community to provide an alternative perspective on the potential effects of food chain composition on bioaccumulation of Hg and MeHg.

2. Materials and methods

2.1. Study sites

Baihua Lake reservoir (26° 35′–26° 42′ N, 106° 27′–106° 34′ E) is approximately 16 km northwest of Guiyang City, Guizhou Province, southwest China. The reservoir was built in 1962 and filled in 1966. Baihua reservoir provides hydroelectric power, water for irrigation, drinking water, and recreational resources to local communities and millions of people in the City of Guiyang. The reservoir is long and narrow with a mean water depth of 13 m and mean water residence time of one month (Liu et al., 2012). The reservoir is in a subtropical karst region with a typical subtropical rainy monsoon climate having an annual mean air temperature of 13.8 °C. The highest water temperature recorded was 28 °C, with a mean water warm season temperature of 23 °C. The lowest winter water temperature recorded was 4 °C, with a mean cold season temperature of 10 °C (Yan et al., 2013). Annual mean precipitation around Baihua reservoir is approximately 1175 mm (Wang et al., 2011). The highest total nitrogen (TN) and total phosphorus (TP) concentrations recorded in the reservoir in 1997 and 1998 were 5.34 and 1.16 mg L⁻¹, respectively. These concentrations were the result of inputs from a fish farming industry. The mean TN and TP concentrations have since decreased in 2012 to 1.70 and 0.03 mg L⁻¹ respectively (Liu et al., 2012; Long et al., 2016).

High THg concentrations in the sediment and water, 12.9 mg kg⁻¹ and 22.4 ng L⁻¹, respectively, were observed due to anthropogenic loading of Hg. Mercury was used as a catalyst for the production of acetic acid between 1971 and 1997 by the Guizhou Organic Chemical plant near the upper part of the reservoir. The chemical plant used 573 t of Hg between 1971 and 1985 (Yan et al., 2008). Untreated wastewater from the plant was discharged directly into the Zhujia River, which flows into Baihua reservoir. This caused the reservoir to become seriously contaminated with Hg (Zhang, 2000). More recently, Liu et al. (2012) reported THg concentrations in the sediment at 3677 ± 2596 ng g⁻¹, and THg in water column at 6.9 ± 4.5 ng L⁻¹. THg concentrations in the water column are currently declining. In the present work, biogeochemical cycling of Hg in zooplankton was investigated at four study sites. S1 Yanjiaozhai is located near the Guizhou Organic Chemical Plant. A large number of zooplankton sample were collected here because of the water depth (8–12 m). S2, Quishuiqo is located near the Maixi River, a source of domestic wastewater (8 m depth), S3, Bengfang is positioned near the water intake for the City of Guiyang (18 m depth) and S4, Daba is found nearby the dam (22 m depth). (S1 Yanjiaozhai, 26° 37′ 55″ N, 106° 29′ 57″ E; S2 Quishuiqo 26° 39′ 08″ N, 106° 32′ 02″ E; S3 Bengfang 26° 20′ 12″ N, 106° 33′ 04″ E; S4 Daba 26° 40′ 59″ N, 106° 32′ 40″ E) (Fig. 1).

2.2. Sample collection

2.2.1. Lake water sampling

Water samples were collected from Baihua reservoir for THg and MeHg analysis. Each water sample (a mixture from 0.5 m below the surface, 6 m deep, and 0.5 m above the bottom) was collected in a borosilicate glass bottle (120 mL). This sample mix allowed for direct correlations between THg and MeHg in water and THg and MeHg in zooplankton which were collected between the surface and 0.5 m above the bottom. Before collection, each bottle was cleaned by soaking in acid, rinsed with ultrapure deionized water, and baked for several hours at 500 °C in a muffle furnace. Each water sample was acidified with 0.5% HCl, placed in a clean zip-closed bag within another zip-closed bag, transported to the laboratory within 24 h, and stored at 4 °C in the dark until analyzed (Long et al., 2017). The water temperature, dissolved oxygen concentration, and pH were measured when each sample was collected using a 6600 multi-sensor probe (Yellow Springs Inc., Yellow Springs, OH, USA). TN and TP concentrations in each sample were determined using the alkaline potassium persulfate oxidation method (Ma et al., 2014). Chlorophyll-a was analyzed by spectrophotometry (UV-2550, Japan) after extraction in 90% acetone (Ma et al., 2014). A phytoplankton sample was collected from the surface water at each sampling site for phytoplankton community composition, abundance, and biomass enumerations. Each phytoplankton sample was preserved with the addition of Lugol’s iodine, then allowed to settle for 48 h and concentrated to a final volume of 30 mL. Cell density was measured using a Sedgwick–Rafter counting chamber with counts at magnifications between 200x and 400x. The species were identified as described by Hu et al. (1980). The total algal biovolume for each species was calculated.
from the number of cells and measured cell sizes. Biomass was determined by converting cell shapes into volumes and then converted to biomass assuming that 1 mm³ was equivalent to 1 mg fresh weight biomass (Papista et al., 2002).

2.2.2. Zooplankton sampling

Zooplankton samples in three size fractions (microzooplankton, meso-zooplankton, and macro-zooplankton) were gathered for Hg and MeHg analysis. The zooplankton was collected by performing multiple tow sampling exercises in the deepest part of the reservoir at each sampling site. Each sample was collected from between the surface and 0.5 m above the bottom. A cone net with 500 μm nylon mesh was used to collect the macrozooplankton; a net with 116 μm nylon mesh was used to collect the meso-zooplankton, and a net with 77 μm nylon mesh was used to collect the micro-zooplankton. Zooplankton in each size fraction were collected until >500 mg dry weight (DW) was accrued. The organisms collected were rinsed with NANOpure water (Millipore Co., Bedford, MA, USA). The 77–116 μm and 116–500 μm fractions were further concentrated by passing the samples through 116 μm and 500 μm mesh filters, respectively, to remove larger zooplankton and particulates. The concentrated zooplankton samples were frozen and transferred to the laboratory for analysis.

In addition, 20 L water samples were collected from the reservoir at each sampling site using a plankton net (64 μm mesh). The concentrated samples were placed in 30 mL bottles for enumerations. Zooplankton were identified using a BX-90B dissecting microscope (Shanghai Bingyu Optical instrument Co., LTD. China) and the body length (in millimeters) was measured (mean of 100 zooplankton) for individual volumes and biomass. The amount of zooplankton biomass was calculated by converting individual measurements into shape related volumes and then adjusted assuming that specific gravity was equivalent to 1 mg fresh weight biomass. Zooplankton species were identified as described by Wang (1961), Jiang and Chu (1979) and Shen and Song (1979).

2.3. Sample analysis

2.3.1. Hg and MeHg analyses

THg and MeHg concentrations in water samples and freeze-dried zooplankton samples were determined at the Key Laboratory of Karst Environment and Geohazard Prevention, Ministry of Education, Guizhou University, Guiyang 550003, China. The Hg speciation methods used for the water samples have been described in detail elsewhere (Long et al., 2017). The THg concentrations in the zooplankton samples were determined by atomic fluorescence spectrometry using a method described in detail by Kainz et al. (2002). Briefly, ~0.1 g of freeze-dried zooplankton was homogenized, and placed into a clean Teflon container along in 40 mL of 16 N HNO₃ and 5 mL of 6 N HCl for 5 h at 121 °C. To ensure that all Hg is converted into and kept in the non-volatile oxidized state (Hg²⁺), an additional oxidant of 0.01% K₂Cr₂O₇ was added to the solution. Subsequently, the digestion tubes (polypropylene Falcon centrifuge tubes) were loosely capped until the reaction terminated, then tightly capped and vortexed (1 min). Thereafter, the caps were loosened to release pressure and the tubes were placed in a water bath (80 °C for 5 h). The digest was diluted (50 mL final volume and weighed) using 0.01% K₂Cr₂O₇ in de-ionized water. Finally, digests were vortexed (1 min) and centrifuged (10 min) to separate the solution from any residue. Each sample was analyzed 3× by cold Vapor Atomic Fluorescence Spectrometry (CV-AFS).

Total MeHg concentration in the zooplankton was determined using methods that have previously been described (Todorova et al., 2015). Briefly, ~0.1 g DW of a zooplankton sample was digested in 3 mL of 25% KOH in methanol for 24 h at 60 °C. Then a 300 μL aliquot of the digest was added to 100 mL of deionized water in a bubblter, adjusted to pH 4.9 with acetate buffer, and ethylated with NaBET₄. The sample was then purged, and the volatilized species were trapped, separated by gas chromatography and detected using a TEKRAN model 2500 cold vapor atomic
2.4. Quality assurance/quality control and statistical analyses

THg and MeHg method detection limits were 0.1 ng L\(^{-1}\) and 0.2 ng L\(^{-1}\), respectively. The accuracy of the method was tested by analyzing National Research Council of Canada fish reference material (TORT-2; certified MeHg concentration 152 ng g\(^{-1}\), certified Hg concentration 270 ng g\(^{-1}\)). The THg recoveries for matrix spike and duplicate matrix spike samples were 94%–99%, and the MeHg recoveries were 91%–104%. The methods used to analyze both THg and MeHg in the water and sediment samples were previously described (He et al., 2008).

Statistical analyses of the data were performed using SPSS 18.0 (PASW), and Origin 7.5 software. The results presented are the arithmetic means with standard errors. Correlation coefficients (r values) and probabilities (p values) were calculated for linear regressions comparing variable interactions. Analysis of variance (ANOVA) was performed to investigate the THg and MeHg concentrations in zooplankton samples from different sites. F-value and probability (p value) were used to indicate the extent of differences between data for samples from different sites. Differences were classed as significant at the p < 0.05 level. Bioaccumulation factors (BAFs) were calculated using the equation BAF = ([Hg or MeHg concentration in biota]/[Hg or MeHg concentration in water]), where biota = zooplankton.

3. Results

3.1. Physical and chemical characteristics of water

Water temperature, pH, dissolved oxygen, Chl-a, TN, and TP concentrations recorded at the sampling sites in Baihua reservoir from October 2015 to September 2016, are shown in Supplement 1. The mean water temperature was 16.8 °C and highest temperatures in August (25 °C). The lowest readings were between January and March 2016, when the mean was 9.0 °C. No discernible changes in dissolved oxygen concentrations were found, indicating that the water was well mixed throughout the year. The mean dissolved oxygen concentration was 7.9 mg L\(^{-1}\), and pH 8.16 (Supplement 1). Chl-a concentrations throughout the seasons varied significantly (p < 0.05), between 3.0 and 13.6 μg L\(^{-1}\). The mean Chl-a concentration was 9.3 μg L\(^{-1}\), which was lower than the euphotic threshold (14.3 μg L\(^{-1}\)) for reservoirs set by the Organization for Economic Cooperation and Development (Long et al., 2016). The trophic status of phytoplankton changed across different months according to Chl-a concentrations (Supplement 1). TP concentration was higher at site S1 than at other sites throughout the sampling period. Overall, TP concentration ranged from 0.02 to 0.046 mg L\(^{-1}\), with a mean of 0.032 mg L\(^{-1}\). Baihua reservoir was mesotrophic according to the Environment Canada trophic status assessment criteria (Environment Canada, 2004) when only TP concentrations were considered. The mean TN concentration was 1.72 mg L\(^{-1}\), slightly higher than the euphotic threshold of 1.5 mg L\(^{-1}\) established by the class IV standard for surface water environmental quality (GB3838-2002). The THg and MeHg concentrations in water samples were 4.34–7.91 ng L\(^{-1}\) and 0.21–0.41 ng L\(^{-1}\), respectively. MeHg concentration was consistently higher in the water samples from site S1 compared to samples from the other sites; the mean concentration at site S1 was 0.37 ng L\(^{-1}\) (Table 1).

3.2. Phytoplankton and zooplankton communities

The prominent phytoplankton in Baihua reservoir were cyanobacteria, diatoms and chlorophytes. Phytoplankton biomass fluctuated during the study period and *Pseudanabaena limnetica* (Lemmermann) Komárek 1974 (cyanobacteria) was significantly higher in biomass in each month except January and February 2016 (Fig. 2a). The *P. limnetica* biomass during the study period was >1.6 mg L\(^{-1}\) at least 67% of the time and did not correlate with Chl-a concentration (p > 0.05). Diatoms were mainly *Aulacoseira* spp, *Ulnaria* spp, *Cycotella* spp (generic complex), and *Fragilariopsis* spp (generic complex). Diatoms comprised on average 27% of the total biomass between October 2015 and September 2016, and formed the second most dominant community. Throughout the winter (December–January) diatoms were the dominant species group. In spring (May) and fall (August–December) diatoms were also a prominent component of the biomass. Pyrrhophytes (predominantly *Peridiniopsis* spp. and *Ceratium hirundinella* (O.F. Müller) Dujardin 1841) were not abundant, but due to their size showed some biomass spikes in November, March–April, and June–August. Chlorophyta were represented by *Scenedesmus* spp, *Tetraedron* spp, *Pediastrum* spp, *Ankistrodesmus* spp, *Spirirogyra* spp, and *Staurastrum* spp. Chlorophytes contributed 1.5–60% (mean 14.6%) of the total biomass between October 2015 and September 2016. The less prominent phytoplankton (<10%) were represented by the cryptophytes (mainly *C. ovata* Ehrenberg, 1838 sensu lato), chrysophytes (e.g. *Distyron diversum* Imhof, 1887) and euglenophytes (e.g. *Euigneea* spp.) (Fig. 2a).

The zooplankton communities contained Copepoda, Cladocera, and Rotifera (Fig. 2b, Supplement 2). Rotifera were represented by Asplancha priodonta Goss, 1850, which size is 300–500 μm (mesozooplankton), Keratella valga Ehrenberg 1834, 102–120 μm (microzooplankton), Keratella cochlearis Goss, 1851, 55–95 μm (microzooplankton), Filinia longiseta Ehrenberg 1834, 125–235 μm (microzooplankton and mesozooplankton), Polyarthra trigla Ehrenberg 1834, 110–165 μm (microzooplankton and mesozooplankton), Brachionus calyciflorus Pallas 1766, 300–350 μm (mesozooplankton) and Brachionus angularis Goss, 1851, 110–205 μm (microzooplankton). Copepod species included Phylodiaptomus tungidus Shen and Tai, 1964, 1460–1960 μm (macrozooplankton), Cyclops vicinus vicinus Uljanin, 1875, 1200–1630 μm (macrozooplankton, Thermocyclops longisetus Kiefer, 1937, 500–960 μm (mesozooplankton and macrozooplankton), Daphnia pulex Claus 1857, 500–1200 μm (mesozooplankton and macrozooplankton). The cladocerans were represented by four dominant species, Bosmina longirostris O.F. Müller 1776, 330–600 μm (mesozooplankton), Diaphanosoma dubium Manulova, 1964, 500–1200 μm (mesozooplankton and macrozooplankton), Ceriodyphium cornutum G.O. Sars, 1885, 300–510 μm (mesozooplankton), and Daphnia hyalina Leydig 1866, 1060–3040 μm (macrozooplankton). Zooplankton community composition and biomass changed between October 2015 and September 2016. Asplancha priodonta was the dominant rotifer between October and November 2015 and between March and June 2016; their biomass was consistently higher than other species (Fig. 2b). The Cladocera *T. brevifurcatus* and *B. longirostris* were present throughout the year, with biomass concentrations of >0.01–0.14 mg L\(^{-1}\) (mean 0.048 mg L\(^{-1}\)) and 0.072–0.56 mg L\(^{-1}\) (mean 0.27 mg L\(^{-1}\)), respectively. *D. hyalina* had the highest density of the cladocerans between December and June, but with a lower biomass compared to *B. longirostris*. *D. dubium* and *C. cornutum* were present in fall, and their biomasses did not change. *P. tungsdivus* dominated the copepod community, contributing between 31% (between October and December 2015) and 46% (between May and September 2016) of the total copepod biomass. The biomass of *M. leuckarti* decreased between June and September 2016 and disappeared by October 2016 while *C. vicinus* vicinus was a
prominent species in the reservoir between November 2015 and June 2016, but disappeared in July.

3.3. THg and MeHg concentrations in zooplankton

Mean THg and MeHg concentrations in zooplankton within different size fractions are presented in Table 1. THg and MeHg concentrations were highest at site S1 (Yanjiaozhai) in all the zooplankton size fractions (Table 1, Fig. 3a and d). MeHg concentrations were significantly higher in the macro-zooplankton (>500 μm) than in the smaller zooplankton at all four sites. THg concentrations in the macro-zooplankton from site S1 (Yanjiaozhai) changed markedly during the sampling period, ranging from 16.5 to 693.8 ng g⁻¹ dry weight, with a mean for the whole year of 186.0 ng g⁻¹ dry weight.
DW (Fig. 3a). THg concentrations in the zooplankton of all three size fractions from site S1 were highest between May and July 2016, (one-way ANOVA, $F = 7.4, p = 0.001$) (Fig. 3a). The lowest THg concentration was found in zooplankton collected from site S2 in February 2016 (Fig. 3b). THg concentrations in the zooplankton of different sizes decreased in the order micro-zooplankton > meso-zooplankton > macro-zooplankton. The bound THg concentrations in the size fractions were significantly different throughout the year ($p < 0.05$) (Fig. 3a–d). THg concentrations in the micro-zooplankton were positively correlated with TP ($R^2 = 0.41, n=48, p<0.001$) and weak correlations were observed with meso-zooplankton and macro-zooplankton (Fig. 5a).
concentrations in the micro-zooplankton were also positively correlated with TN concentration in the water \( (R^2 = 0.4, n = 48, p < 0.0001) \) \( (\text{Fig. 3e}) \). \( \text{THg} \) concentration in the micro-zooplankton were positively correlated with Chl-a and weakly correlated with macro-zooplankton \( (\text{Fig. 5e}) \). \( \text{THg} \) concentrations in the micro-zooplankton were positively correlated with \( T. \ brevifurcatus \) and \( B. \ longirostris \) biomass \( (R^2 = 0.2, n = 48, p < 0.0001) \) and \( A. \ priodonta \) biomass \( (R^2 = 0.6, n = 23, p < 0.0001) \) \( (\text{Fig. 6a and b}) \). \( \text{THg} \) concentrations in the meso-zooplankton were positively, but weakly correlated with \( B. \ longirostris \) biomass \( (R^2 = 0.12, n = 48, p < 0.05) \) \( (\text{Fig. 6c}) \) and \( C. \ vicinus \) biomass \( (R^2 = 0.22, n = 32, p < 0.05) \) \( (\text{Fig. 6d}) \). In contrast, \( \text{THg} \) was negatively correlated with \( D. \ hyalina \) biomass \( (R^2 = 0.23, n = 28, p < 0.05) \) \( (\text{Fig. 6e}) \). \( \text{THg} \) concentration in macro-zooplankton was also negatively correlated with \( P. \ tunguidus \) biomass \( (\text{Fig. 6f}) \), and positively but weakly correlated with total zooplankton biomass \( (R^2 = 0.22, n = 32, p < 0.05) \) \( (\text{Fig. 6g}) \).

\( \text{MeHg} \) concentrations in the macro-zooplankton varied widely between October 2015 and September 2016, ranging from 0.74 to 127.11 ng g\(^{-1}\) \( (\text{annual mean 20.17 ng g}\(^{-1}\) \( S1\) - \( S4\)) \( (\text{Fig. 3e-h}) \). The mean \( \text{MeHg} \) concentration in macro-zooplankton was higher for site \( S1 \) \( (27.68 \text{ ng g}\(^{-1}\)) \( (\text{Fig. 3e}) \). Concentrations of \( \text{MeHg} \) in macro-zooplankton were consistently higher than in meso-zooplankton and for the meso-zooplankton except for November 2015 at sites \( S1 \) and \( S4 \) and September 2016 at site \( S3 \) \( (\text{one-way ANOVA}; \ F = 4.6, p < 0.0001) \) \( (\text{Fig. 4}) \). The mean \( \text{MeHg} \) increased in the order micro-zooplankton < meso-zooplankton < macro-zooplankton for every site. \( \text{THg} \) bioaccumulation factors (BAFs) were significantly higher for the micro-zooplankton than for the meso-zooplankton and macro-zooplankton. \( \text{MeHg} \) BAFs increased markedly in the order micro-zooplankton < meso-zooplankton < macro-zooplankton \( (\text{Fig. 7}) \). The \( \text{THg} \) BAFs for micro-zooplankton were positively correlated with Chl-a concentration \( (R^2 = 0.37, n = 48, p < 0.0001) \), TN concentration \( (R^2 = 0.37, n = 48, p < 0.0001) \), zooplankton biomass \( (R^2 = 0.30, n = 48, p < 0.0001) \) and weakly associated with TP concentration \( (R^2 = 0.11, n = 48, p < 0.0001) \) \( (\text{Fig. 6a}) \). The \( \text{THg} \) BAFs for the meso-zooplankton were weakly, but positively correlated with TP concentration and total zooplankton biomass \( (\text{Fig. 8b}) \). Likewise, \( \text{THg} \) BAFs for the macro-zooplankton were also weakly, but positively correlated with Chl-a and TP \( (\text{Fig. 8c}) \). \( \text{MeHg} \) BAFs for the micro-zooplankton were weakly correlated with TN, TP, and total zooplankton biomass \( (\text{Fig. 8d}) \). Likewise, \( \text{MeHg} \) BAFs for the meso-zooplankton and macro-zooplankton were weakly and positively correlated with TP and TN \( (\text{Fig. 8e and f}) \).
Fig. 5. Correlations between the total mercury (THg) and methylmercury (MeHg) concentrations in zooplankton and total phosphorus (a, b), total nitrogen (c, d), chlorophyll-a (e), and total zooplankton biomass (f), zooplankton biomass and phytoplankton biomass (g).
Fig. 6. Correlations between total mercury (THg) concentrations in zooplankton in different size fractions and the biomasses of different species (a–g). Correlation between methylmercury (MeHg) and total (THg) concentrations in all zooplankton size fractions (h–j).
4. Discussion

In a previous study of Baihua reservoir, Hg concentrations in the zooplankton decreased as chlorophyll concentration increased, suggesting that THg per cell, in the algal bloom, was diluted (Wang et al., 2011). The main reason proposed was high algal densities in eutrophic systems with lower Hg per cell, ultimately decreased Hg bioaccumulation by zooplankton (Pickhardt et al., 2002; Chen et al., 2012). However, we found markedly different Hg bioaccumulation results with higher THg and MeHg concentrations in zooplankton living in higher eutrophication conditions. THg and MeHg BAFs correlated well with the zooplankton community and that higher TN, TP, and Chl-a concentrations were associated with increased Hg concentrations in zooplankton. THg concentrations were significantly higher in micro-zooplankton compared to meso-zooplankton and macro-zooplankton, while concentrations of THg were higher in meso-zooplankton compared to macro-zooplankton. The higher THg concentrations in micro-zooplankton are directly linked to the biomass of specific taxa, B. longirostris, T. brevifurcatus and A. priodonta. THg concentrations in meso-zooplankton and micro-zooplankton also increased as the biomass of C. vicinus vicinus and B. longirostris (smaller form) increased. In contrast, higher biomass levels for D. hyalina and P. tunguidus were related to lower THg concentrations. These results show that Hg accumulation is species specific. Pickhardt et al. (2002) used Daphnia in their studies, a single taxon that is shown here to be negatively associated with Hg uptake compared to other taxa. Therefore as reported here communities with susceptible taxa will show increases in Hg accumulation with eutrophication. Wang et al. (2011) also found taxa specificity showing that some large zooplankton (Calanoids and Copepods) accumulated lower Hg concentration in Baihua reservoir. Masson and Tremblay (2003) found that increased Hg concentrations were positively correlated with the biomass and densities of Holopedium gibberum Zaddach 1855 but decreased when Daphnia spp dominated the macro-zooplankton. Meili (1991) suggested that Hg concentrations in H. gibberum were correlated with dissolved organic carbon (DOC) concentration because the consumption of detrital food increased, possibly favoring the bioaccumulation of Hg. Hessen (1985) reported that H. gibberum had much highest specific filtering rates compared to Daphnia spp, suggesting that filtering capacity allowed Holopedium to ingest more particles (including algae) per unit of time and consequently increase the probability of Hg bioaccumulation. H. gibberum and Daphnia spp have different external structures; Daphnia spp. have a calcium/chitin shell, while H. gibberum is surrounded by a gelatinous muco-poly saccharide mantle (Hessen et al., 1995). These structural differences might have consequences in the bioaccumulation of toxic substances. However this study found that other calcium/chitin shelled cladocerans and copepods accumulated Hg with eutrophication, thus
it appears that wall structure is not significantly influencing Hg accumulation. Results of the present study further indicate that a succession of zooplankton species, mainly *B. longirostris*, *T. brevifurcatus*, *A. priodonta*, *C. vicinus vicinus*, *D. hyalina*, and *P. tunguidus*, affect THg accumulation in the three zooplankton size-fractions in Baihua reservoir. THg concentrations were higher in zooplankton from site S1 nearest the point source of Hg, reflecting contaminant availability and consumption. The Guizhou Organic Chemical plant (GOCP) stopped production in 1997, which means that the current Hg contamination in Baihua reservoir is primarily recycled Hg from benthic sediment. Liu et al. (2012) reported that there were no significant differences in all measured Hg compound concentrations within the oxic layer across Baihua reservoir (where zooplankton was found). However, THg concentration in the solid sediment and MMHg in pore waters tended to decrease with increasing distance from the GOCP (Liu et al., 2012). These results show that the higher THg concentrations in zooplankton at S1 in this study can be positively correlated with THg concentration in the solid sediment/pore waters at S1. The elevated Hg readings at S1 also indicate a long-term compartmental distribution of the contaminant within a single waterbody (Wang et al., 2011). The positive although often weak relationship between MeHg and THg concentrations in the zooplankton supports the well documented finding that MeHg is a transient vector in the bioaccumulation of Hg. The MeHg concentration increased significantly as zooplankton body size increased, from micro-zooplankton to macro-zooplankton, similar to the results of previous studies (Tremblay et al., 1998; Montgomery et al., 2000; Todorova et al., 2015; Gosnell et al., 2017). Hg accumulation is species specific and to

Fig. 8. Correlations between total mercury (THg) and methylmercury (MeHg) bioaccumulation factors (BAF) for zooplankton in different size fractions and environmental factors (the total phosphorus (TP), total nitrogen (TN), chlorophyll-α (Chl-α), and total zooplankton biomass concentrations).
some degree regulated by body size (biomass availability). Larger specimens of selected species can have more Hg accumulation likely due to available absorption mass (Kainz et al., 2002). Further, seasonality played a role in accumulation with the lowest MeHg concentrations in zooplanктон between November 2015 and February 2016; the lowest period of phytoplankton growth. We found that MeHg concentrations in zooplankton increased when total biomass increased and zooplankton biomass was correlated to phytoplankton biomass. However, the relationships between phytoplankton, zooplankton and MeHg were weak (low $R^2$ values), which is in part a function of the variability observed in the MeHg data and the preferential consumption of selected phytoplankton taxa by zooplankton.

The MeHg to THg ratios were significantly higher for macrozooplankton compared to microzooplankton and mesozooplankton, which was similar to the findings of other studies (Kainz et al., 2002; Watras et al., 1998). The result show that lower THg concentration may be linked to lower MeHg concentrations in microzooplankton and mesozooplankton. MeHg bioaccumulation by zooplankton has important toxicological implications for higher trophic levels (Kainz et al., 2006). In this study, we found significantly lower THg concentrations in macrozooplankton than in microzooplankton. In another study, when trophic level of marine zooplankton increased, THg concentrations decreased largely as a result of the decreasing uptake of inorganic Hg, while %MHHg (monomethylmercury) increased because MHHg concentrations remain constant despite decreasing THg concentrations (Foster et al., 2012). This result suggests that higher trophic fish preying on macrozooplankton can have comparatively lower THg and higher MeHg concentrations than expected. Liu et al. (2012) found Hg concentrations in cyprinid fish from Baihua reservoir one order of magnitude lower than typical regulatory values for fish caught for human consumption. The reason for low values may be related to feeding on macrozooplankton, possibly indirect consumption of Daphnia. Liu et al. (2012) also suggest a simple food web (2-orders) could explain selective feeding and the lower observed Hg levels. However, we also found a significant anomaly in May 2016, when MeHg concentrations were higher in microzooplankton compared with mesozooplankton and macrozooplankton. This relationship is possibly influenced by the smaller P. tunguidus and C. vicinus; two species with high biomass accumulating higher MeHg concentrations. Taxa body size has an impact on growth, with C. vicinus (low $R^2$ values), which is in part a function of the variability observed in the MeHg data and the preferential consumption of selected phytoplankton taxa by zooplankton.

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The THg and MeHg concentrations in zooplankton from all three size fractions were positively correlated with TP. THg concentrations in microzooplankton were also correlated with TN. Further MeHg concentrations in the microzooplankton and macrozooplankton were positively, although weakly correlated with TN. Chen et al. (2012) reported that TN and TP concentrations were associated with THg and MeHg bioaccumulation in zooplankton but THg and MeHg concentrations in the large zooplankton (zooplankton size is > 200 m) decreased as nutrient (TP concentration) inputs increased, and increased as TN concentration decreased. Chen et al. (2012) also reported that lower THg and MeHg were present in large zooplankton under eutrophic conditions (higher TP concentration). For example, MeHg and THg. Problems in the reporting of eutrophication data (e.g. Table one in that study, TP presented as mg L$^{-1}$ and low TN concentrations (0.35–1.00 mg L$^{-1}$) make interpretations of the Chen et al. (2012) results uncertain. In the current study TP concentrations in Baihua reservoir were much lower (average 30 µg L$^{-1}$) and TN was higher (average 172 mg L$^{-1}$). We found that increased TP stimulated the growth of phytoplankton and zooplankton. Baihua reservoir has high Hg levels due to past anthropogenic pollution. Under eutrophic conditions THg and MeHg in the water was adsorbed and concentrated by the phytoplankton (particulate matter in the water samples) and zooplankton. In another study, Razavi et al. (2015) suggested that increased TP may enhance THg and MeHg bioaccumulation in zooplankton because inorganic Hg and TP are both adsorbed in suspended particulates (inorganic particles and organic matter). Organic Hg and MeHg have a high affinity for particles and suspended particulate matter is significantly correlated with TP (Lindstrom, 2001). Liu et al. (2012) also noted that MeHg was positively correlated with (TP$^{2} = 0.25, n = 29, P<0.05$) in Baihua reservoir. Therefore it appears that elevated TP concentration leads to enhanced THg and MeHg bioaccumulation in zooplankton by suspended matter transfer.

Some previous studies have reported that high phytoplankton densities decrease THg and MeHg bioaccumulation by zooplankton (Pickhardt et al., 2002; Chen and Folt, 2005; Wang et al., 2011; Chen et al., 2012). This suggests that algal blooms (with high Chl-a concentrations) can dilute Hg levels per cell, or that increased zooplankton biomass can dilute, THg and MeHg concentrations. However, in the current study a positive relationship between THg concentrations in microzooplankton and macrozooplankton with Chl-a was observed. Reason for the conflicting results is the taxon studied (e.g. *Daphnia*) in previous work and changes in the algal community in Baihua reservoir over the last 20 years (Long et al., 2018). Cyanobacteria blooms of *Microcystis*, which are not edible food preferred by zooplankton, have decreased over the last two decades. In this study *P. limnetica* has become dominant and found homogeneously distributed throughout the reservoir with no sudden, explosive blooms. Cell densities of *P. limnetica* were variable but always >10$^5$ cells L$^{-1}$. Chl-a levels were not excessive (3.0–13.6 mg L$^{-1}$) and *P. limnetica* biomass was not correlated with Chl-a. In addition, we found a weak correlation between phytoplankton biomass and zooplankton biomass. Since the prominent species (*P. limnetica*) was not influencing Chl-a levels, it would...
appear that less dominant species were driving the phytoplankton-chl-a relationship. Todorova et al. (2015) reported low Chl-a levels below 9 μg L⁻¹ after 2007 in Onondaga Lake, a mesotrophic system recovering from mercury contamination (1980–2009). They found MeHg concentrations in zooplankton were strongly and positively correlated to phytoplankton biomass ($R^2=0.79$, $P<0.0001$) with the exception of two years (1980, 1987). Todorova et al. (2015) suggested that increased concentrations of phytoplankton were not limiting the bioaccumulation of MeHg in zooplankton. When Chl-a average concentrations are <2.8 μg L⁻¹, some studies have shown lower Hg accumulation rates (Watras et al., 1998; Kainz et al., 2002; Yu et al., 2011). Lower primary productivity (Chl-a concentration or phytoplankton) is usually associated with lower primary consumer (zooplankton) biomass. We hypothesize that zooplankton can accumulate higher Hg because of higher levels of Hg in individual algal cells and a low abundance of selected zooplankton taxa which can take advantage of the available food source. Gosnell et al. (2017) reported the highest concentrations of Hg and MeHg for all phytoplankton in the spring season in Long Island Sound (USA); they suggested low biomass (fewer algae) accumulated most of the burden from the residual Hg and MMHg. Liu et al. (2012) reported that THg and MeHg concentrations in uncontaminated freshwater systems are generally less than 5 ng L⁻¹ and between 0.02 and 0.1 ng L⁻¹, respectively. In the current study, THg and MeHg concentrations in the water were significantly higher, mean THg and MeHg concentrations in water were 6.54 and 0.29 ng L⁻¹ (Table 1), demonstrating that THg and MeHg levels were not limiting. Therefore, higher Chl-a can lead to higher Hg concentrations in zooplankton because of high residual Hg concentrations in the reservoir. Higher phytoplankton densities of preferred “edible” taxa can further enhance the availability of contaminated food for zooplankton. The selectivity of Hg consumption is linked to preferred food availability with herbivorous micro-zooplankton and macro-zooplankton showing the largest Hg incorporation rates. This result has in part been demonstrated by other studies (Beldowska and Cegiolka, 2017; Kainz and Mazumder, 2005; Masson and Tremblay, 2003).

The THg BAFs decreased as the zooplankton size fraction increased from micro-zooplankton to macro-zooplankton, while MeHg BAFs increased as the size fraction increased. Zooplankton taxa can play a selective and important role in Hg bioaccumulation. Elevated phosphorus concentrations during the year caused increased primary production which promoted the growth and reproduction of zooplankton. We therefore suggest that TP concentration affects the THg BAFs of micro-zooplankton, meso-zooplankton, and macro-zooplankton. THg bioaccumulation was at least in part dependent on nutrient concentrations in the water. It is evident that THg bioaccumulation by micro-zooplankton is related to phytoplankton and zooplankton species composition. The current results indicate that eutrophication can increase the risks of supporting phytoplankton taxa prone to Hg bioaccumulation and trophic magnification. Increased phytoplankton consumption will promote MeHg bioaccumulation by selected zooplankton taxa.

5. Conclusions

THg and MeHg concentrations and THg and MeHg BAFs for zooplankton were positively correlated with the zooplankton community. THg concentrations were significantly higher in micro-zooplankton than in meso-zooplankton and macro-zooplankton. The biomasses of selected species influenced the degree to which THg bioaccumulation occurred. THg concentrations in the micro-zooplankton were influenced by B. longirostris and T. brevifurcatus biomass. Concentrations of THg in meso-zooplankton and macro-zooplankton were positively linked to P. turgidus, C. vicinus, and negative associated with T. brevifurcatus and D. hyalina. The positive relationship between MeHg and THg in the zooplankton indicates that THg in the three zooplankton size-fractions is linked to increased MeHg bioaccumulation. The MeHg concentration increased as zooplankton body size increased, from micro-zooplankton to macro-zooplankton, although the correlation was weak. TP concentration may increase the risks posed to aquatic ecosystems because increases in TP promote selective phytoplankton taxa growth and MeHg bioaccumulation. With enhanced transfer of Hg in the lower trophic levels, fish at a higher trophic level will show increased bioaccumulation both in MeHg and THg.

Acknowledgments

We are grateful to Professor Henri J. Dumont, who helped us through reviewing this manuscript. This work was supported by the National Natural Science Foundation of China-Guangdong United Foundation (U1501235). PBH was supported by a RAC grant from the Canadian Museum of Nature (2017–2019). We extend our thanks to the Great Lakes Sea Grant Network and specifically to the NOAA Great Lakes Environmental Laboratory (GLERL) (https://www.glerl.noaa.gov/) for access to the Asplancha priodonta rotifer image.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.04.008.

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