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Mercury bioaccumulation in fish in an artificial lake used to carry out cage culture

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ABSTRACT

As a global toxic pollutant, mercury (Hg) bioaccumulation within food chain could be influenced by human disturbance. Ten typical fish species were collected from Changshou Lake, an artificial lake used to carry out cage fish culture, to investigate the C/N isotopic compositions and Hg bioaccumulation in fish. The results showed that the total Hg (THg) and methylmercury (MeHg) levels in fish muscles (56.03 ± 43.96) and (32.35 ± 29.57) ng/g, wet weight), comparable with those in most studies in China, were significantly lower than the international marketing limit (0.5 mg/kg). Past human input for cage culture in this lake led to abnormal ^{15}N enrichment in food chain, as the quantitative trophic levels based on $\delta^{15}\text{N}$ were different with that classified by feeding behaviors. This phenomenon subsequently demonstrated that it should be considered thoughtfully with respect to the application of the traditional method for understanding Hg bioaccumulation power by the slope of $\log_{10}[\text{Hg}]$ with $\delta^{15}\text{N}$ regression in specific water body (i.e., Changshou Lake). In addition, no significant linear correlation between Hg and body weight or length of some fish species was observed, suggesting that the fish growth in the eutrophic environment was disproportionate with Hg bioaccumulation, and fish length or weight was not the main factor affecting Hg transfer with food web. The occurrence of human disturbance in aquatic system presents a challenge to a better understanding of the Hg bioaccumulation and biomagnification within the food chain.

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Introduction

Mercury (Hg), a toxic pollutant, is present in ecosystems globally. It becomes of greater toxicological concern after transformation to the methylated form (methylmercury, MeHg), which is the most toxic form of Hg with potent neurovirulence (Li et al., 2010; Shao et al., 2016). In particular, MeHg is well known to be readily bioaccumulated and biomagnified in higher trophic levels along aquatic food chains (Mergler et al., 2007; Stein et al., 1996), leading to Hg levels in

predatory fish increasing by a million times compared to that in surrounding water (Gandhi et al., 2014). This sufficiently high Hg concentration in some species could pose a potential threat to wild life and human health (Li et al., 2012), and Hg associated with fish and seafood consumption has been confirmed to be the primary exposure pathway for this toxicant in most people (Harris et al., 2003; Pickhardt et al., 2006). Numerous advisories on consumption of fish caught from lakes, rivers, and coastal areas in North America and Europe have been issued, particularly for pregnant women and young children (Unep,

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2002). However, Hg concentrations in fish in China have been reported by many works to be generally below the international marketing limit (0.5 mg/kg) despite of the elevated Hg levels in environment matrixes (Cheng and Hu, 2012; Peng et al., 2015). It is plausibly reasoned that the sharply decrease of wild fish over the past few decades caused by overfishing, habitat destruction and other human activities makes the farmed freshwater fish as the dominant fish in market (Cheng and Hu, 2012). Whereas, farmed fish are typically excessively fed by inputting more nutrients into water body, making fish fast growing and short-lived, which significantly reduce the Hg biomagnification (Cheng and Hu, 2012). From this, it can be speculated that human disturbances play a critical role in the Hg bioaccumulation in aquatic ecosystems. For example, overfishing has been reported in Bohai Sea to be the primary reason for the shorten food chain, and the large fish with high economic value was gradually replaced by small fish and invertebrates (Xu et al., 2010). Additionally, complicated nitrogen input has been reported to increase the $\delta^{15}\text{N}$ values in water and subsequently disturb the normal trophic levels of food chain (Feng et al., 2018).

Changshou Lake, a man-made reservoir lake, is the largest freshwater lake in Chongqing, China. It plays an important role in local economy through freshwater aquaculture and tourism. Since the in-lake commercial cage fish farming was carried out in 1989, extensive amount of fish feed or nutrients (to simulate the algal growth) have been inputted into lake that lead to serious eutrophication of this lake. Although the official ban for cage culture was carried out during 2004–2005, the remaining pollutants in sediment still aggravated the deterioration of water quality. Multi-proxy evidences of sediment in Changshou Lake also have reliably recorded its environmental evolution from natural evolution to eutrophication due to human activities (Zhu et al., 2016). While the biophysicochemical changes of environmental factors accompanying with eutrophication have been previously reported to have influences on the Hg distribution, Bai et al. (2015) found that the highest average total Hg (THg) and MeHg levels in water both occurred in aquaculture area of Changshou Lake. However, little is known about the pollution status of THg and MeHg in fish, and influences of changes of environmental conditions on the bioaccumulation and biomagnification of Hg in Changshou Lake.

Hg study coupled with analysis of stable carbon and nitrogen isotopes in aquatic biota has been considerably used to better understand the food web transfer of Hg in aquatic system (Senn et al., 2010). Among them, stable carbon isotopes ratio ($^{13}\text{C}/^{12}\text{C}$ or $\delta^{13}\text{C}$) are mainly employed to provide information on dietary carbon sources and composition of organisms, and about 1‰ of increase in $\delta^{13}\text{C}$ sometimes occurs with increasing trophic levels (Hecky and Hesslein, 1995). Stable nitrogen isotopes are the most popular tools for quantifying trophic levels (TLs) since the ratio of $^{15}\text{N}/^{14}\text{N}$ (expressed as $\delta^{15}\text{N}$) consistently fractionate between 3‰ and 4‰ per TL through the food chain (Kidd et al., 1995).

Therefore, the primary objective of this work was to study the Hg distribution in fish in Changshou Lake, investigate the food structure by $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysis in fish, and subsequently assess the bioaccumulation and biomagnification of both THg and MeHg along the food chain in this human disturbed lake.

1. Materials and methods

1.1. Sampling region and fish samples

Changshou Lake, a man-made reservoir lake with watershed of 6667 hm^2 , is located in Changshou District in Chongqing, China. Longxi River is its main water source, and is a primary tributary into Yangze River (Fig. 1). Typical fish species in Changshou Lake were collected: Topmouth culter, Mandarin fish, Yellow catfish, Catfish, Bighead carp, Silver carp, Common carp, Crucian carp, Wuchang bream and Grass carp. Among them, carnivorous, omnivorous, plankivorous and herbivorous fish are all covered, and their basic parameters (including weight, total length and feeding habits) are shown in Table 1. These fishes were caught from Changshou Lake by local fishermen or obtained from surrounding residents fishing, and then shipped into lab in buckets with water.

1.2. Samples preparation and analysis

The total length and weight of sampled fish were measured immediately upon arrival at the laboratory. After recording the total length and mass, fish was dissected to get muscle tissues under clean conditions. The muscle samples were washed using ultrapure water (18.2 M Ω cm at 25°C), freeze dried (−60°C) and then ground to fine powder for further chemical analysis. Water content was estimated from weight loss after freeze-drying samples.

Dry samples of fish tissues (0.05–0.1 g) were placed in nickel boats, and sent into Direct Mercury Analyzer 80 (DMA80, Milestone, Italy) for measuring THg by thermal combustion method according to EPA 7473 (Epa, 1998). The detection limit of this method is approximately 0.5 ng/g. Certified reference materials (CRMs) GBW07428 from National Research Centre for Certified Reference Materials (Peking, China) were analyzed every 10 samples, and the average recovery was (93 ± 10)%. Duplicate samples were determined every 10 samples with relative standard deviation (RSD) of 7%.

For MeHg, about 0.1 g homogenized fish samples were digested in 50 mL Teflon tubes by 5 mL KOH solution of 20 g/L in water bath (75°C) for 3 hr, followed by ethylation, gas chromatographic (GC) separation and cold vapor atomic fluorescence spectroscopy (CVAFS, Brooks Rand model III, Brooks Rand Laboratories, USA) determination. The method detection limit for MeHg is about 0.02 ng/g. Similar to THg measurement, CRM TORT-2 (lobster hepatopancreas) from National Research Council Canada (Ottawa, ON, Canada) was analyzed every approximately 20 samples with average recovery of (90 ± 15)%. Duplicate samples were also determined every 20 samples and the mean RSD was about 5%. All the THg and MeHg concentrations in fish presented in current study were shown as ng/g wet weight (ww), which can be calculated from the water content of fish and the directly measured Hg concentrations in dry weight.

^{13}C and ^{15}N isotopes were determined by isotope ratio mass spectrometry (IRMS) (Isoprime 100, Germany) coupled with the elemental analyzer (Elementar Pyro Cube, Germany). 1–3 mg fish muscle samples were placed into tin capsules and then were introduced into elemental analyzer with autosampler.

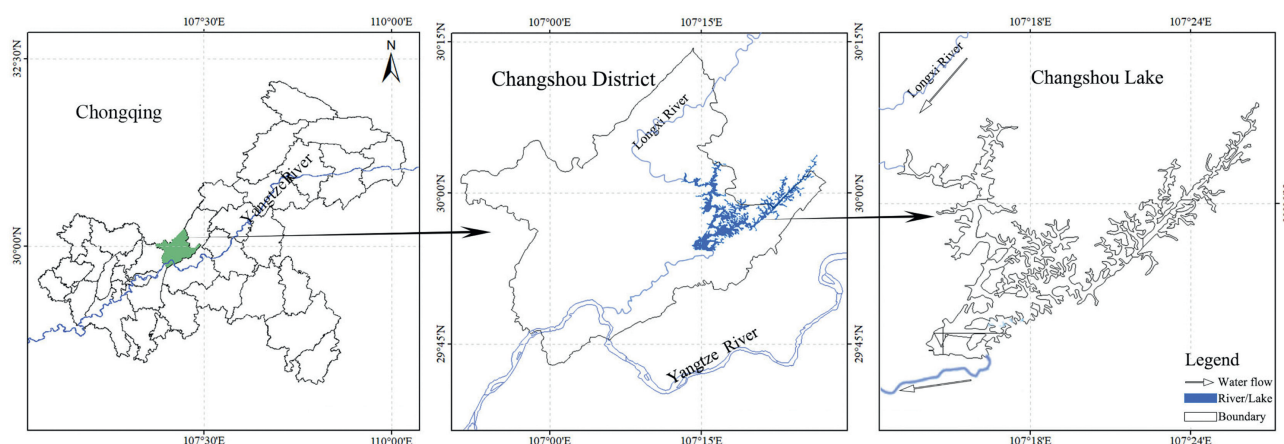


Fig. 1 – Map showing the study region located in Chongqing, China.

The C and N in fish were transformed into CO₂ and N₂ by combustion at 1000°C following determination by IRMS. The stable isotope abundances were expressed by δ notation as the deviation from standards in parts per thousand (‰) according to Eq. (1):

$$\delta^{13}\text{C} (\text{‰}) \text{ or } \delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

where, R_{sample} and R_{standard} are the ratios of the heavy to light isotopes ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) in the sample and standard. $^{13}\text{C}/^{12}\text{C}_{\text{standard}}$ and $^{15}\text{N}/^{14}\text{N}_{\text{standard}}$ values were based on the international standards of Vienna Pee Dee Belemnite (VPDB) and the atmospheric AIR.

The blanks were analyzed every 20 samples, and no signals were detected throughout the analysis. The measurement precision was 0.2‰ and 0.3‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

1.3. Data analysis

Hg biomagnification could be quantified by trophic level (TL) based on $\delta^{15}\text{N}$ in Eq. (2) (Lavoie et al., 2013; Post, 2002).

$$\text{TL}_{\text{consumer}} = \frac{\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}}{\Delta^{15}\text{N}} + \lambda \quad (2)$$

where, $\text{TL}_{\text{consumer}}$ is the TL for a given consumer, λ is the TL of the baseline organism (primary consumer, $\lambda = 2$), $\delta^{15}\text{N}_{\text{consumer}}$

and $\delta^{15}\text{N}_{\text{baseline}}$ are the $\delta^{15}\text{N}$ values of the given and primary consumer, $\Delta^{15}\text{N}$ is the trophic enrichment factor which is equal to 3.8 (Fisk et al., 2001; Yuan et al., 2012).

2. Results and discussion

2.1. Hg in fish

THg concentrations in fish (muscle) in Changshou Lake varied widely between species ranging from 2.48 to 187.47 ng/g (all the Hg values in fish tissues were described in wet weight, ww). The average THg level (\pm standard deviation, SD) of all tested fishes was (56.03 \pm 43.96) ng/g. MeHg levels were in the range of 0.30 to 112.91 ng/g (ww) and averaged (32.35 \pm 29.57) ng/g. No sample for THg and MeHg in our study approached or exceeded the critical thresholds of 500 ng/g for THg established by the World Health Organization (WHO) (WHO, 1991) and the maximum level of contaminant in food (500 ng/g for MeHg in fish) (GB2762-2012). This finding is supported by many previous studies where the Hg concentrations in most fish samples in China (except for some marine fish samples) were reported to be below the international marketing limit (0.5 mg/kg) (Cheng and Hu, 2012; Liu et al., 2012; Zhang and Wong, 2007).

Table 1 – Weight and total length of fish species collected from Changshou Lake.

Species	Common name	N	Weight (g)	Total length (cm)	Feeding habits *
<i>Erythroculter ilishaeformis</i>	Topmouth culter	17	92.53–1410 (474.7 \pm 397.2)	21.7–54.4 (36.6 \pm 1.0)	Carnivores
<i>Siniperca chuatsi</i>	Mandarin fish	4	87.9–524.9 (304.7 \pm 189.1)	15.8–31.0 (25.1 \pm 6.6)	
<i>Silurus asotus</i>	Catfish	2	551.3–1081.8 (816.6 \pm 375.2)	45.0–49.7 (47.4 \pm 3.3)	
<i>Aristichthys nobilis</i>	Bighead carp	3	2359–3011.6 (2665.2 \pm 328.2)	53.5–63.4 (59.7 \pm 5.4)	Planktivores
<i>Hypophthalmichthys molitrix</i>	Silver carp	3	1380–3024.4 (2099.8 \pm 841.1)	52.1–57.7 (55.8 \pm 3.2)	
<i>Pelteobagrus fulvidraco</i>	Yellow catfish	1	207.72	30.5	Omnivores
<i>Cyprinus carpio</i>	Common carp	1	1288.9	45.5	
<i>Carassius auratus</i>	Crucian carp	9	51.2–737.3 (212.64 \pm 241.1)	14.5–30.7 (19.3 \pm 5.8)	
<i>Ctenopharyngodon idellus</i>	Grass carp	4	384.6–2202.0 (929.6 \pm 854.0)	41.5–57.0 (41.9 \pm 10.8)	Herbivores
<i>Bluntnose black bream</i>	Wuchang bream	2	695.3–1186.9 (941.4 \pm 347.1)	30–44.9 (37.5 \pm 10.5)	

* Feeding habits were based on the published study (Xu et al., 2018).

Hg concentrations varied among the different tested fish species (Fig. 2), and a significant positive correlation ($p < 0.01$) between THg and MeHg was found. The average THg and MeHg concentrations in *Silurus asotus* (one kind of carnivorous fish) were the highest, and the lowest appeared in *Blunt-snout bream* (herbivores). Feeding behavior is well-known to be a major factor influencing Hg bioaccumulation in fish (Li et al., 2009). When fish species in Changshou lake were classified into four groups (herbivore, planktivore, omnivore and carnivore) according to their feeding behaviors (Xu et al., 2018), both highest THg and MeHg contents appeared in carnivorous fish and followed by planktivore, omnivore and herbivore (Fig. 3), indicating that the Hg transferred via the food chain. However, there was no significant difference in THg and MeHg between carnivorous and planktivorous fish ($p = 0.226$ for THg, $p = 0.615$ for MeHg), which was supported by a previous study where similarity in Hg levels was reported between piscivorous and planktivorous fish (Azevedo-Silva et al., 2016).

MeHg has been previously reported to be the primary species in fish due to its more efficient retention and slower excretion rates than Hg^{2+} (Kidd et al., 2011). The MeHg could account for about 90% of THg reported in the America or Europe aquatic system (Khaniki et al., 2005; Perrot et al., 2010). However, in the present study, the percentages of MeHg against THg (MeHg%) were ranged from 2% to 99% with an average of $(53 \pm 26)\%$ (Fig. 3), significantly lower than that reported of 90%. Most of the ratios were less than 50% in this study (only two ratios over 90% occurred in crucian and silver carp), and the MeHg% was even less than 30% in some herbivorous and omnivorous fish. This finding is similar to that studied in Wujiang River Basin with this ratio of less than 50% in most samples (Feng et al., 2018).

The MeHg% has been reported to be increased with increasing trophic level in an aquatic web (Bowles et al.,

2001; Kidd et al., 2011; Mason et al., 1996; Žižek et al., 2007), as fish obtained most (>90%) of the Hg from their food (Kidd et al., 2011). Whereas, the highest MeHg% ($73\% \pm 22\%$) appeared in planktivorous fish in this study, which could be plausibly due to its food sources not only including plankton but also including some organic detritus (such as carcass). This also could explain why no significant difference in THg and MeHg was observed between carnivorous and planktivorous fish.

2.2. Fish isotopic compositions in the Changshou Lake

$\delta^{13}C$ values potentially can be used as tracers of nutrient and food sources in food webs (Hecky and Hesslein, 1995). The $\delta^{13}C$ signatures in sampled fish were in the range of -26.54‰ to -17.90‰ , broader than that in the Three Gorges Reservoir (-26.87‰ to -23.11‰) (Yu et al., 2013), exhibiting more multiple food sources in Changshou Lake. Considering the feature of the range of $\delta^{13}C$ values representing the variable food sources, planktivorous fish here had a narrower dietary than other fishes, as it had a smaller variation range of $\delta^{13}C$ values (-23.79‰ to -26.54‰) than those of other kinds of fishes (-17.9‰ to -23.58‰ for herbivores; -20.59‰ to -24.38‰ for omnivores; -20.41‰ to -24.94‰ for carnivores). In addition, considerable differences ($p < 0.01$) of $\delta^{13}C$ were observed among fish species (except carnivores and omnivores), which probably associated with the different food sources for these fishes. Just as the bighead carp and silver carp (filter feeders, planktivorous fish) mainly consume microalgae, zooplankton and other fish bait with low $\delta^{13}C$, and omnivorous fish feed on higher $\delta^{13}C$ bait (such as the epicalst and mollusk), resulting in a significant lower average $\delta^{13}C$ value ($-25.31\text{‰} \pm 1.04\text{‰}$) for planktivorous fish than that of omnivorous fish ($-22.39\text{‰} \pm 1.07\text{‰}$).

$\delta^{15}N$ is used to characterize trophic relationship in aquatic food webs (Cabana and Rasmussen, 1994; Post, 2002). Fish in study area showed a broader $\delta^{15}N$ range from 6.52‰ to 18.15‰ (with an average of $(13.48 \pm 3.49)\text{‰}$), equivalent to about three trophic levels. This average $\delta^{15}N$ value was significantly higher compared with the studies in Natural

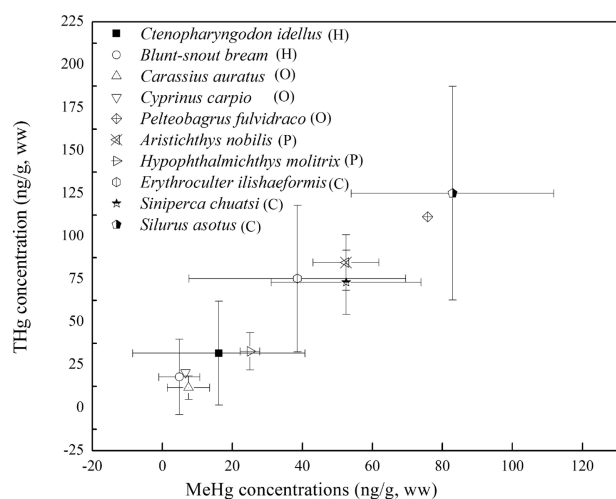


Fig. 2 – Isotope bi-plot diagram of tested fish. Values are means of THg (total mercury) and MeHg (methylmercury) \pm standard deviation. C, P, O and H in legend represent the different fish feeding habits of Carnivores, Planktivores, Omnivores and Herbivores. ww: wet weight.

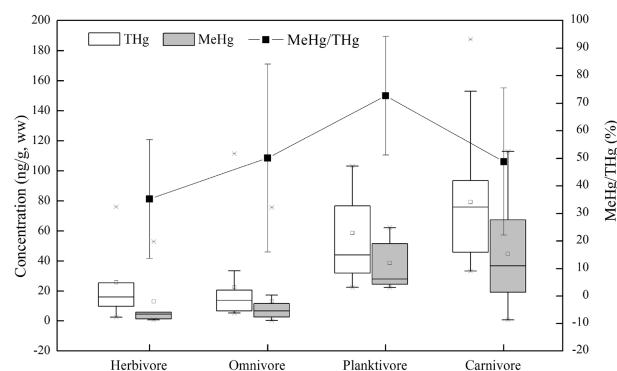


Fig. 3 – Distribution of THg, MeHg and the proportion of MeHg against THg in fish. Box plot showing the median (solid line), mean (small square), 25th and 75th percentiles (box boundary), 1st and 99th percentiles (whiskers).

Reserve (Wang et al., 2009) or in oligotrophic lake (Arcagni et al., 2017). It is well-known that $\delta^{15}\text{N}$ is a valid descriptor of eutrophication (Lake et al., 2001), and nutrient-enriched lakes display increased $\delta^{15}\text{N}$ due to the increased loading of nitrate from the watershed and the enhanced denitrification states in the lake sediment (Havens et al., 2003; Ogawa et al., 2001; Xu and Xie, 2004). Although the nutrients throwing into Changshou Lake has been banned for over 10 years, the levels of nutrients in water from the re-emission of cumulative animal waste and fertilizer in sediment were measured to be 1.74–2.63 mg/L for total nitrogen and 0.15–0.20 mg/L for total phosphorus during sampling period. This result confirmed that the Changshou Lake is in a mid-eutrophication status. Thus, the increased $\delta^{15}\text{N}$ could probably be attributed to the nutrient-enriched condition being of Changshou Lake. In addition, the past inputted fish feed should be another possible reason for increased $\delta^{15}\text{N}$ here, as the fish feed is well-known as a mixture mainly including bean cake, rice husk, fish meal and bone meal which have high $\delta^{15}\text{N}$ (especially for the latter two). Once excessive fish feed deposited into sediment, it would put the sediment or even the whole water body in a higher $\delta^{15}\text{N}$ environment. On the other hand, the decomposed fish feed could be the food sources for benthic organisms or fish by resuspension, subsequently disturbing the normal $\delta^{15}\text{N}$ enrichment with the food chain over the long-term.

We have calculated, from $\delta^{15}\text{N}$ results, that herbivorous, omnivorous, planktivorous and carnivorous fish exhibited average TL of 2.39 ± 1.00 , 2.40 ± 0.73 , 3.87 ± 0.44 and 3.51 ± 0.7 , respectively. Analysis of variance (ANOVA) indicated that no significant difference of TL was observed between planktivorous and carnivorous fish. These quantitative TLs based on $\delta^{15}\text{N}$ were different with those classified by feeding behaviors, as the TL of planktivorous was higher than that of carnivorous fish. This observation supported the above speculation of the occurrence of abnormal $\delta^{15}\text{N}$ enrichment in this human-disturbed lake. As shown in Fig. 4, the $\delta^{15}\text{N}$ enrichment in planktivore was the greatest with $\delta^{15}\text{N}$ of $16.23\text{‰} \pm 1.69\text{‰}$, and a gradient in $\delta^{15}\text{N}$ of the order of 1.4‰ was observed between planktivorous and carnivorous fish. Similar abnormal $\delta^{15}\text{N}$ fractionation in fish was found in one polluted lake (Dianchi Lake) (Wei and Zhou, 2014) where $\delta^{15}\text{N}$ value of planktivorous fish was also higher than that of other fishes located in higher positions of food chain (such as yellow catfish and whitefish). Jing et al. (2017) observed that $\delta^{15}\text{N}$ enrichment in plankton in Hongfeng Lake could reach up to over 20‰, even significantly higher than $\delta^{15}\text{N}$ in benthic organism, which was attributed to the nitrogen pollution. Eutrophication could influence the $\delta^{15}\text{N}$ of primary food source and subsequently affect the $\delta^{15}\text{N}$ of consumers through predatory relation (Lake et al., 2001). We thus reasoned that the highest $\delta^{15}\text{N}$ in planktivorous fish in this study was plausibly due to the major contribution of its food with increased $\delta^{15}\text{N}$, as the planktivorous fish is known to have a narrow dietary and lives chiefly on the microcystis (accounting for over 90% to the total food volume) which probably also have higher $\delta^{15}\text{N}$ enrichment as described by aforementioned study in Hongfeng Lake. Furthermore, consumption the residual fish feed also could increase the $\delta^{15}\text{N}$ of planktivorous fish. Nonetheless, it is worth conducting further

studies to verify the abnormal $\delta^{15}\text{N}$ enrichment in such human-disturbed lake by investigating the $\delta^{15}\text{N}$ in the whole food chain including sediment, particulate organic matters, plankton and other environmental media. Although Hg bioaccumulation has been studied in eutrophic environment, the mechanisms are still unclear. As such, we cannot give reasonable explanations for this phenomenon now, and further work is also needed to understand the Hg bioaccumulation in human-disturbed lake particularly for the lake with cage fish farming.

2.3. Bioaccumulation of Hg with trophic levels

Biomagnification power of Hg was always assessed using slope of the relationship between logarithm transformed (to the base 10) Hg concentration ($\log_{10}[\text{Hg}]$) and $\delta^{15}\text{N}$ according to the following Eq. (3) (Campbell et al., 2005; Lavoie et al., 2013). A significant and positive slope ($b > 0$) indicates Hg biomagnification in food web (Lavoie et al., 2013).

$$\log_{10}[\text{Hg}] = b \times \delta^{15}\text{N} + \text{constant} \quad (3)$$

However, considering that $\delta^{15}\text{N}$ in this studied eutrophic lake of which showed an abnormal enrichment with the food chains, the application of the slope of the $\log_{10}[\text{Hg}]$ with $\delta^{15}\text{N}$ regression probably could bring impacts on precisely understanding of Hg bioaccumulation rate in aquatic food webs. Thus, we investigated the Hg bioaccumulation with trophic level from two different scenarios, i.e., considering (1) taking all fish species into account or (2) removing planktivorous fish from the food chain, for better understanding the Hg bioaccumulation with food chains in this human disturbed lake. Linear regressions (Fig. 5) indicated that THg biomagnified in the food web ($r^2 = 0.11$; $p < 0.05$; $n = 46$) with a biomagnification power of 0.05 ($\log_{10}[\text{THg}] = 0.05 (\delta^{15}\text{N}) + 0.96$), when taking all the fish species into consideration. Similarly, MeHg also showed a significant correlation with $\delta^{15}\text{N}$ ($\log_{10}[\text{MeHg}] = 0.07 (\delta^{15}\text{N}) + 0.32$; $r^2 = 0.10$;

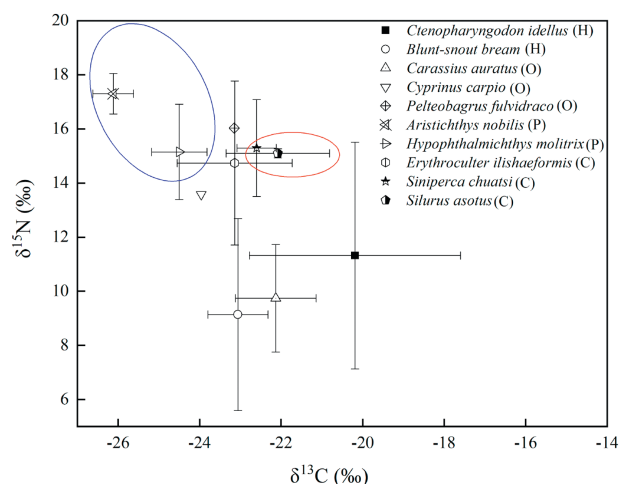


Fig. 4 – Isotope bi-plot diagram of tested fish. Values are means of $\delta^{13}\text{C}$ and $\delta^{15}\text{N} \pm$ standard deviation. The symbols in blue and red circles represent the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of planktivores and carnivores, respectively.

$p < 0.05$; $n = 46$), and the slope was higher than that for THg. When considering removing planktivorous fish from the food chain, an increase was observed for Hg biomagnification power (0.07 for THg and 0.09 for MeHg) with trophic level ($\log_{10}[\text{THg}] = 0.07 (\delta^{15}\text{N}) + 0.73$, $r^2 = 0.31$, $p < 0.01$, $n = 40$; $\log_{10}[\text{MeHg}] = 0.09 (\delta^{15}\text{N}) + 0.13$, $r^2 = 0.19$, $p < 0.01$, $n = 40$). Since the sample number of the planktivorous fish is small ($n = 6$), we found that removing six samples from the food chain with n of 46 cannot significantly influence the slopes of log-linear regression between these two scenarios (t-test, $p = 0.35$ for THg and $p = 0.61$ for MeHg). However, the abnormal ^{15}N enrichment in eutrophic lake should still be considered to fully understand the Hg bioaccumulation with food webs, and the slope of the \log_{10} -transformed [Hg] with $\delta^{15}\text{N}$ regression is supposed to use thoughtfully in the presence of abnormal ^{15}N enrichment. If this regression cannot be a good indicator of bioaccumulation rate in aquatic food webs of eutrophic lake, more precise indicators free from influence of human disturbance are further needed to be found to confirm the trophic level, and to improve the biomagnification power equation used in similar water bodies. Nevertheless, these bioaccumulation rates (b) were comparable with those from other [Hg]- $\delta^{15}\text{N}$ studies published in China. Strong linear relationships were found between $\log_{10}[\text{Hg}]$ and $\delta^{15}\text{N}$ in the Three Gorge reservoir ($b = 0.08$ for THg and $b = 0.10$ for MeHg) (Xu et al., 2018), Wujiang River ($b = 0.06$ for THg) (Feng et al., 2018), and Lake Taihu ($b = 0.052$ for THg and $b = 0.118$ for MeHg) (Wang et al., 2012). However, the biomagnification power (b) is significantly lower than the average value worldwide (0.16 ± 0.11 for THg and 0.24 ± 0.08 for MeHg, respectively) summarized by Lavoie et al. (2013) from 205 aquatic food webs worldwide including the whole food web and only fish-only proportion of the whole food web (Lavoie et al., 2013).

Actually, many studies have been investigated the reasons why low levels or lower potential for bioaccumulation of Hg in fish in freshwater occurred in China. The given reasons include

(1) carnivorous wild fish at high trophic positions employed in most studies conducted in Northern European and North America water systems; (2) farmed freshwater fish replacing the wild fish as the dominant fish in market caused by overfishing, pollution and habitat destruction; (3) low MeHg production due to the poor organic content in some water bodies; (4) growth dilution induced by excessive nutrients from human activities. The environmental conditions in Changshou Lake were confirmed to be beneficial to Hg methylation, since the proportions of MeHg against THg in water in Changshou Lake have been found to be in the range of 25%–36% (Bai et al., 2015). Considering this, the growth dilution caused by eutrophication possibly could influence the Hg levels and bioaccumulation of fish in this study. It is well-known that weight and length are important variables determining levels of accumulation of Hg by fish of the same species (Tuomola et al., 2008). Therefore, in natural aquatic systems without human disturbance, the bioaccumulation of Hg in fish is not only influenced by the trophic level, but also the age and the body size of the fish (Cheng and Hu, 2012; Chumchal et al., 2010). Shao et al. (2016) found that the THg levels in fish in remote rivers across the Tibetan Plateau have significantly positive correlations with length ($p < 0.05$) and weight ($p < 0.05$) of fish (Shao et al., 2016). Due to the lack of suitable sample numbers of some fish species in this study, only the relationship between body weight or length of Topmouth culter and Crucian carp and their Hg levels were analyzed. No significant linear correlation ($p > 0.05$) was observed, and the disproportionate Hg accumulation of these two fish species according to the fish growth could be ascribed that the natural growth has been disturbed in Changshou Lake influenced by providing abundant nutrients for fish. Therefore, it also preliminarily indicated that body length or weight may not be the main factors affecting Hg concentrations of some fish species in eutrophic lake. But anyway there was no direct evidence for growth dilution as mentioned above.

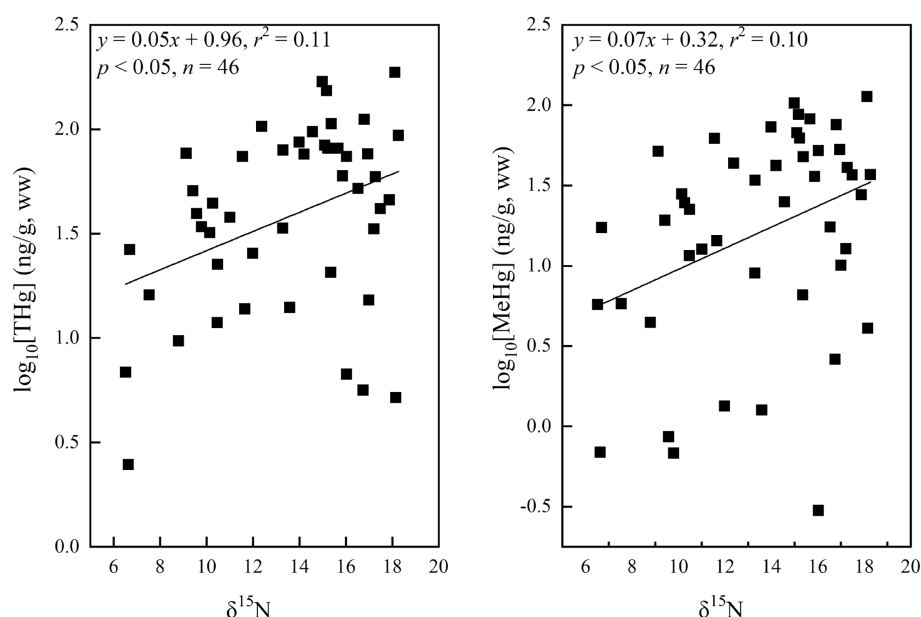


Fig. 5 – Correlation between $\log_{10}[\text{Hg}]$ and $\delta^{15}\text{N}$ (the scenario with taking the all the fish species into consideration).

We thereby speculated that the Hg bioaccumulation in fish is rather complicated in an artificial lake used to carry out cage culture, and the occurrence of human disturbance in freshwater presents a challenge to a better understanding of the Hg bioaccumulation and biomagnification with food chain. First, the normal food structure could be disorganized by excessive human input, leading to inadaptability of the conventional method for estimating the bioaccumulation rate in eutrophic water. And the influences were still existed, even though the abundant fish feed, fowl manure and fertilizer throwing has been prohibited for over 10 years in Changshou Lake. Second, Hg chemical speciation and bioavailability would be affected by these human behaviors for cage fish culture, however, little is known about how it will influence its bioaccumulation by organisms. Further studies are warranted to examine the mechanisms of the influence of this human disturbance on Hg transformation, bioaccumulation and cycling in aquatic systems.

3. Conclusions

This study investigated Hg biomagnification in one artificial lake used to carry out cage culture. The THg and MeHg levels (56.03 ± 43.96 ng/g for THg and 32.35 ± 29.57 ng/g for MeHg) in fish are in agreement with previous reports of low fish Hg concentrations in other fresh and coastal waters in China. Average MeHg% ($53\% \pm 26\%$) was as well lower than that reported in the most America or Europe aquatic systems (about 90%). Both THg and MeHg could biomagnify through food webs, and MeHg biomagnified at a higher rate than THg. The ^{15}N appeared abnormal enrichment in this lake with highest values observed in plankivorous fish, probably due to the major contribution of its food with increased $\delta^{15}\text{N}$. This phenomenon influenced quantitatively the Hg biomagnification power (b), as this value when taking all fish species into consideration was 0.05 and 0.07 for THg and MeHg lower than that removing plankivorous fish from food chain (0.07 for THg and 0.09 for MeHg). The slope of the \log_{10} -transformed [Hg] with $\delta^{15}\text{N}$ regression probably is not a good indicator of Hg bioaccumulation rate in aquatic food webs of lake for fish cage culture. Therefore, it can be speculated that the human activity could partly affect the Hg biomagnification, and further studies are warranted to examine the mechanisms of Hg transfer in the whole food chain in human disturbed lake.

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