Chemosphere 225 (2019) 320–328

Contents lists available at ScienceDirect

Chemosphere

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

Primary amino acids affect the distribution of methylmercury rather than inorganic mercury among tissues of two farmed-raised fish species

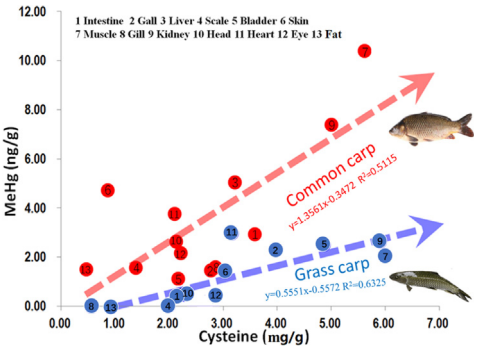
Yi Man [a, b, c], Runsheng Yin [a, *], Kai Cai [d], Chongyang Qin [b, c], Jianxu Wang [b], Haiyu Yan [b, **], Miling Li [e]

[a] State Key Laboratory of Ore Deposit Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, Guizhou Province, China
[b] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, Guizhou Province, China
[c] University of Chinese Academic of Sciences, Beijing, China
[d] Guizhou Academy of Tobacco Science, Guiyang, Guizhou Province, China
[e] Department of Earth, Ocean and Atmospheric Sciences, University of British Columbia, Vancouver, British Columbia, Canada

HIGHLIGHTS

- Methylmercury is rich in protein-rich fish tissues.
- Cysteine and its related/derived amino acids have a high biological association with methylmercury.
- The formation of MeHg-Cys complexes can be an important driving force for methylmercury distribution in fish bodies.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 December 2018
Received in revised form
16 February 2019
Accepted 10 March 2019
Available online 11 March 2019

Handling Editor: Martine Leermakers

Keywords:
Mercury
Methylmercury
Primary amino acid
Cysteine
Fish

ABSTRACT

The distributions of primary amino acids, MeHg and IHg in body tissues of two commonly farm-raised fish species (common carp: *Cyprinus carpio*; grass carp: *Ctenopharyngodon idellus*) in Guizhou Province, SW China, were investigated to understand the effects of primary amino acids on MeHg and IHg metabolism in farm-raised fish. The primary amino acids were classified into four groups: (1) essential and polar amino acids; (2) essential and non-polar amino acids; (3) non-essential and polar amino acids; and (4) non-essential and non-polar amino acids. For both fish species, groups (1, 2 and 3) were enriched in muscle and kidney, whereas group (4) was enriched in scale. The two fish species showed low MeHg concentrations (grass carp: 0.5–3.9 ng/g; common carp:1.0–7.4 ng/g) and low MeHg proportions (grass carp: 2–45%; common carp: 6–37%) in their tissues, which are mainly due to the simple food web structures and the fast growth of the farm-raised fish. Positive correlations ($r = 0.342$ to 0.472; $p < 0.01$; $n = 78$) were observed between MeHg and several primary amino acids (cysteine, threonine, phenylalanine, leucine, valine, glutamate serine and tyrosine) in fish tissues, which may be driven by the formation of MeHg-Cys complexes within fish body. However, no significant correlations were observed between IHg and any primary amino acids, indicating the metabolic processes of IHg and MeHg are

* Corresponding author
** Corresponding author
E-mail addresses: yinrunsheng@mail.gyig.ac.cn (R. Yin), yanhaiyu@mail.gyig.ac.cn (H. Yan).

https://doi.org/10.1016/j.chemosphere.2019.03.058

different. This study advances our understanding that cysteine and its related/derived amino acids may be an important driving force for MeHg distribution and translocation in fish.

1. Introduction

Mercury (Hg) has received great public concern due to its high toxicity to organisms (Ullrich et al., 2001). Since industrialization, anthropogenic activities (e.g., coal burning and nonferrous metal refining) have significantly increased the amount of mercury cycling in the environment. In aquatic ecosystems, inorganic Hg (IHg) can be readily methylated to methylmercury (MeHg) (Hsu-Kim et al., 2013). MeHg has a strong capability to accumulate along aquatic food chains to reach concentrations of 4–5 magnitude higher in fish than the surrounding water (Lepak et al., 2018; Li et al., 2016). Mercury pollution therefore poses human health risks when people consume fish contaminated with methylmercury (Kwon et al., 2014).

Mercury metabolism in biological systems is believed to be regulated by primary amino acids, because primary amino acids are not only the basic substance synthesizing protein, but also can mediate the metabolism of both toxic and essential trace elements in organisms (Sakami and Harrington, 1963; Ballantyne, 2001; Chong et al., 2004; Cerneia et al., 2014). The interactions between Hg and primary amino acids are believed to be largely affected by the functions and structures of primary amino acids. Primary amino acids can be classified to polar and non-polar amino acids (Creighton, 1993). Polar amino acids contain polar groups (e.g., -OH, -COOH, -SH,-NH_2) in side chains, and are hydrophilic, whereas non-polar amino acids contain hydrocarbons side chains (e.g., −H or −C/-H) and are hydrophobic. Compare to the affinity constants for Hg bonding to oxygen- or nitrogen-containing ligands, the affinity constants of Hg bonding to thiol anions (-SH) is about 10 orders of magnitude higher (Zalups, 2000; Wang et al., 2012). Among the 20 primary amino acids, only cysteine contains thiol in its side chain (-CH_2-SH). Studies have reported the close relation between MeHg and cysteine in organisms, mainly in mammals (Roos and others, 2010; Yin et al., 2008; Aschne and Clarkson, 1988; Mason et al., 1996; Boado et al., 1999; Duelli et al., 2000; Killian and Chikhale, 2001). Previous studies demonstrated MeHg is transported into the mammalian cells mainly as the MeHg-cysteine conjugate (MeHg-Cys), which allows a better absorption and uptake of Hg by body tissues (Wang et al., 2012; Atmaca, 2004; Roos and others, 2010). MeHg-Cys can be readily transported across cell membranes and the blood-brain barrier (BBB) in mammals (Clarkson, 1993). Experimental studies suggested that the neutral amino acid transport system L is a significant route for MeHg-Cys transmembrane movement (Atmaca, 2004; Yin et al., 2008; Aschne and Clarkson, 1988), since MeHg-Cys complexes are thought to mimic structurally methionine, a substrate for amino acid carriers such as the L-type large neutral amino acid transporters (LATs) (Daniel et al., 2011). MeHg in mammalian cells is demonstrated to be mainly transported as a MeHg-Cys complex by the ubiquitous L-type large neutral amino acid transporter 1 (LAT1) (Yin et al., 2008). Besides cysteine, it is still unclear whether other primary amino acids could affect the metabolism of Hg in organisms.

Fish consumption is regarded as the major MeHg exposure sources for global population. To date, however, the effects of primary amino acids on the metabolism of Hg in fish remain poorly understood. Although MeHg-cysteine has been measured in fish muscle proteins, suggesting MeHg-Cys complex may play an important role in the metabolism of MeHg (Lemes and Wang, 2009; Harris et al., 2003; Leaner and Mason, 2002; Andrahennadi et al., 2007), it is unclear whether the similar mechanism occurs for inorganic Hg (IHg). To our knowledge, there is by far no study documented the interactions between IHg and cysteine in fish, considering IHg fraction is usually low in fish (note that MeHg usually accounts for more than 90% of total Hg in wild fish). In recent studies, farm-raised fish have been shown higher IHg fractions compared to wild fish, due to the simple food web structures and the fast growth of the fish (Liu et al., 2012; Meng et al., 2016). The choose of farm-raised fish may therefore provide the opportunity to look at the possible relationship between IHg and amino acids.

Here, the concentrations of primary amino acids, MeHg and IHg among tissues of two commonly farm-raised fish species in China (common carp: *Cyprinus carpio*; grass carp: *Ctenopharyngodon idellus*) in Guizhou Province, SW China were investigated. The aims of this study were (1) to understand the distribution patters of primary amino acids, MeHg and IHg in fish tissues; (2) to verify the relations between primary amino acids (especially cysteine) and MeHg; and (3) to check if primary amino acids can affect the IHg distribution in fish tissues.

2. Methods

2.1. Sample collection and preparation

In August 2017, common carps and grass carps were bought from a local market in Guiyang, SW China. Each fish species contained three individuals so that the representative of this study can be guaranteed. To minimize the variability of individuals, fish with similar lengths (common carp: 37.3 ± 3.8 cm; grass carp: 48.0 ± 1.0 cm) and weights (common carp: 626.7 ± 98.5 g; grass carp: 1184.3 ± 149.9 g) were chosen for each species. According to the fish seller, both fish species were artificially fed in a small local pond using the same feed. This allows us to minimize the variability resulting from dietary intake in distributions of Hg and amino acids.

The fish samples were placed in a cooler and delivered to the laboratory as soon as possible. These fish were killed by cervical section, cleaned by 18.2 MΩ cm water (Milli-Q), and used for histological processing. The fish individuals were dissected in the laboratory under contaminant-free conditions. Fish tissues (muscle, kidney, liver, heart, gill, head, intestine, scale, skin, gall, eye, air bladder and fat) were carefully separated from each other, using a pre-cleaned (i.e., rinsed with alcohol and 18.2 MΩ cm water) stainless steel knife. The removed organs were rinsed with 18.2 MΩ cm water and stored in polyethylene bags to avoid cross contamination. A total number of 78 tissue samples were sampled, and the samples were freeze-dried (−78 °C) until moisture is completely removed. Freeze-dried samples were powered and homogenized using a pre-cleaned grinder (i.e., cleaned with alcohol), and were transferred into new polyethylene bags and stored in a refrigerator at 4 °C, prior to the following analysis.

2.2. Amino acids analysis

The hydrochloric acid hydrolysis method was used to prepare

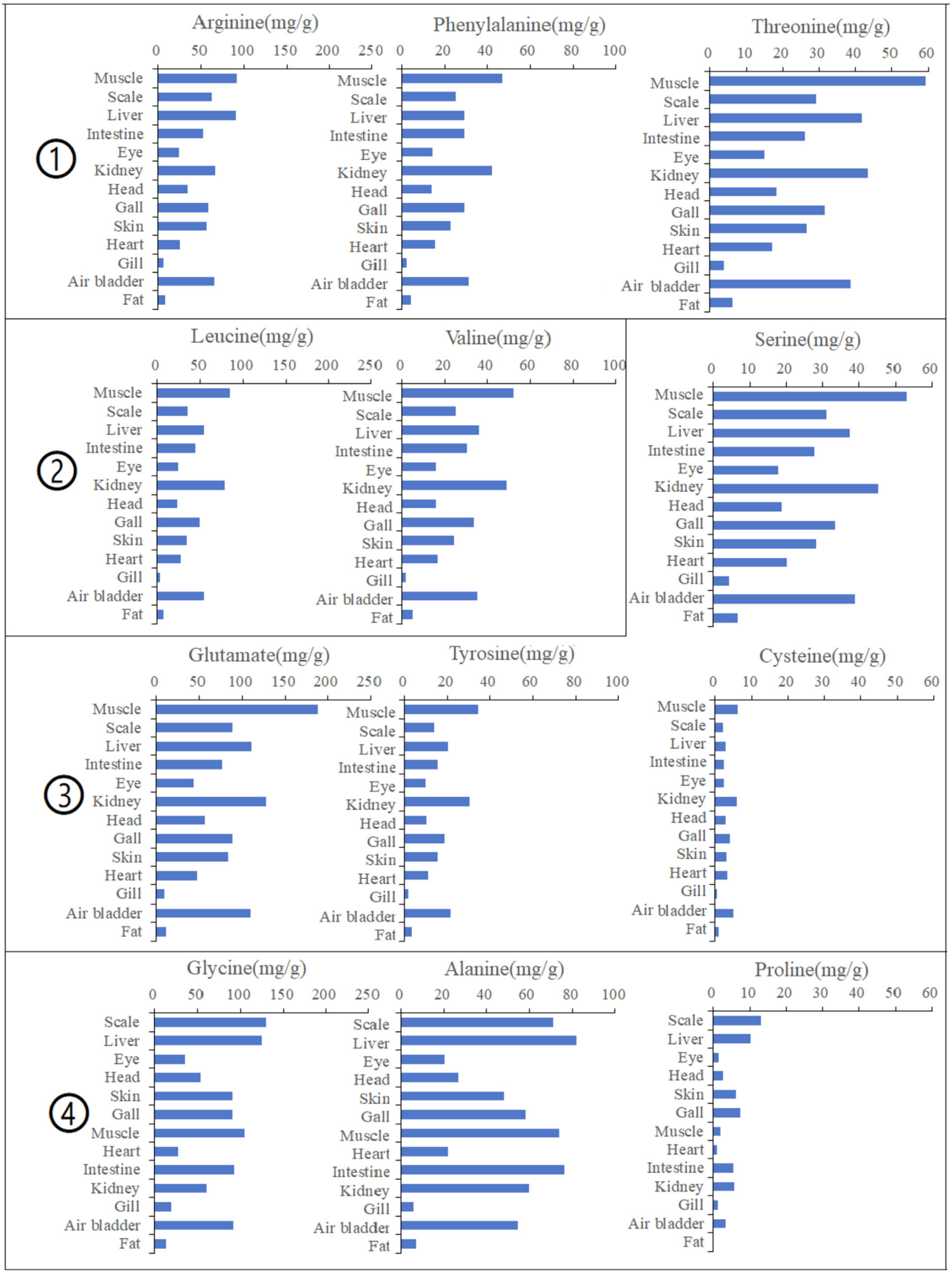


Fig. 1. Distribution pattern of amino acids for grass carp (*Ctenopharyngodon idellus*, n = 3). ① essential and polar amino acids; ② essential and non-polar amino acids; ③ non-essential and polar amino acids; ④ non-essential and non-polar amino acids. Coefficients of variation for analyses of the three individuals of each fish species were <20%.

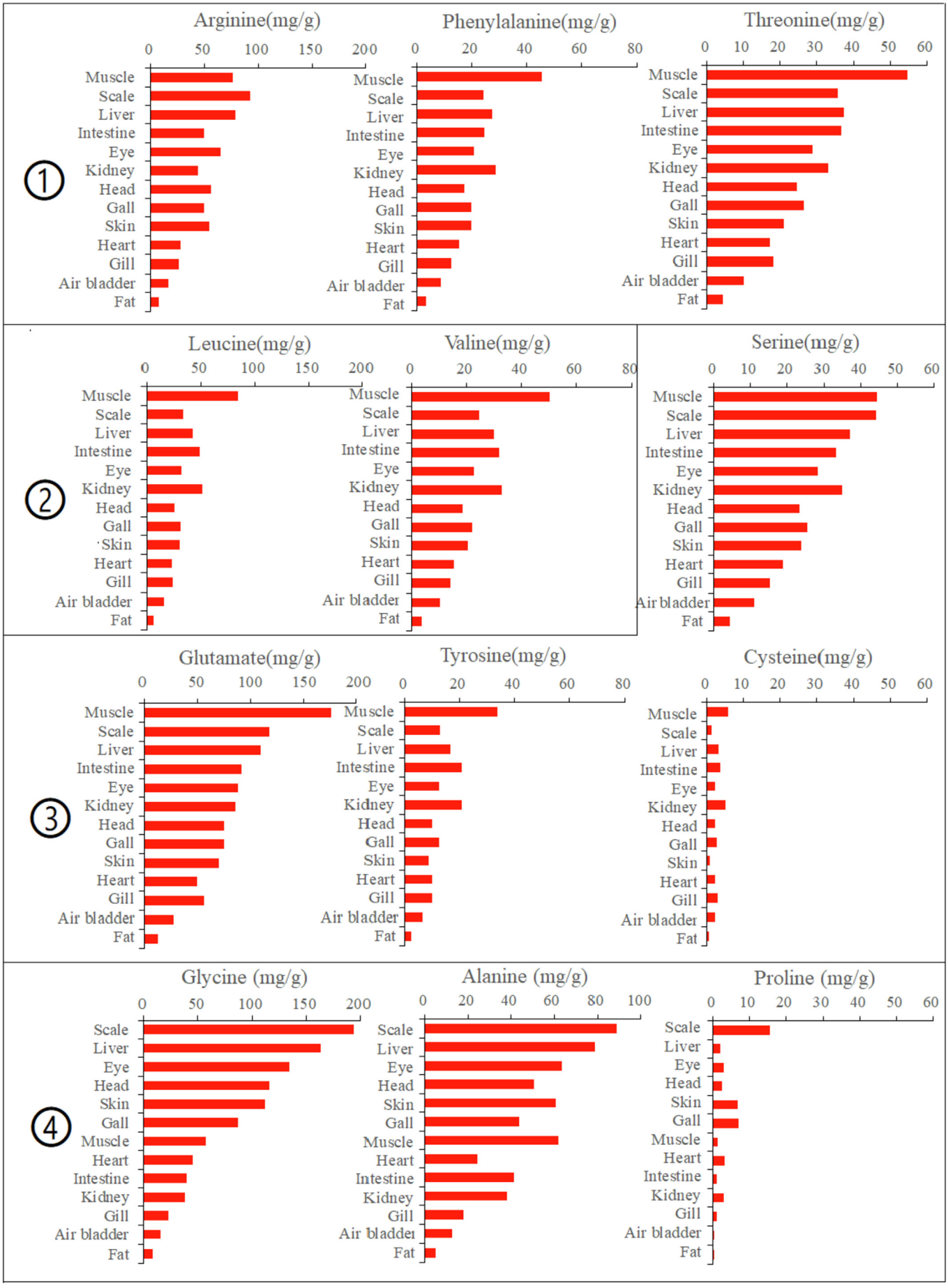


Fig. 2. Distribution pattern of amino acids for common carp (*Cyprinus carpio*, n = 3). ① essential and polar amino acids; ② essential and non-polar amino acids; ③ non-essential and polar amino acids; ④ non-essential and non-polar amino acids. Coefficients of variation for analyses of amino acids for the three individuals of each fish species were <20%.

the fish samples (Zheng and Zheng, 2013). Briefly, 0.05 g samples and 40 μL amino acid internal solutions (*DL*-2-aminoadipic acid and *DL*-norvaline) were digested using 3 mL 6 M HCl for 24 h at 110 °C in extraction vessel. After cooling to room temperature, the digests were filtered through 0.45 μm nylon membrane and subsequently diluted up to 15 mL with ultrapure water and stored at −20 °C until further analysis. The solutions were measured by ultra-high performance liquid chromatography (UHPLC), following a previous method (Cai et al., 2017). The detection limit of the method was 0.01 μg/mL (3σ). According to the results, 12 primary amino acids (arginine, glycine, valine, leucine, phenylalanine, tyrosine, glutamate, serine, threonine, cysteine, alanine, proline) were determined. However, we failed to measure the other 8 primary amino acids (histidine, methionine, glutamine, lysine, isoleucine, aspartate, tyrptophan, asparaginate), due to their low concentration levels in our samples and poor peak shapes during analysis. Recoveries of 88–107% were observed for the spiked samples (n = 5). Coefficients of variation for analyses of amino acids for the three individuals of each fish species were <20%.

2.3. Mercury concentration analysis

For THg analysis, about 0.5 g of each sample was digested with 5 mL of HNO_3 at 95 °C for 3 h until the sample was completely dissolved. 0.5 mL of BrCl was added to the digest for overnight, to convert all Hg species to Hg (II). Excessive BrCl was reduced by 0.2 mL of $NH_2OH{\cdot}HCl$. The solution was analyzed by cold vapor atomic fluorescence spectrometry (CVAFS) (Tekran 2500, Canada) (USEPA, 2002). For MeHg analysis, about 0.5 g of each sample was digested with 5 mL of 25% KOH at 75 °C for 3 h. Then the extract was buffered with sodium acetate at pH 4.9 and ethylated in a borate glass bottle with a Teflon-lined cap. Quantification of MeHg was performed by gas chromatographic separation and pyrolysis, followed by CVAFS detection (MERX, Brooks Rand) (USEPA, 2002). IHg concentrations were estimated by THg minus MeHg. Unfortunately, the IHg and MeHg of grass carp's liver samples were not measured, due to limited sample masses.

The QA/QC of THg and MeHg analysis was assessed using standard reference material TORT-2 from National Research Council of Canada, method blanks, and sample duplicates. The method detection limits (3σ) were 10 pg g^{-1} for THg and 2 pg g^{-1} for MeHg. The relative standard deviations for duplicate samples were within 6% and 8% for THg and MeHg, respectively. The recoveries of THg and MeHg for TORT-2 were 98–103% (n = 3) and 95–106% (n = 3), respectively. Coefficients of variation for analyses of THg and MeHg for the three individuals of each fish species were <16%.

2.4. Statistical analyses

Data were analyzed in SPSS (version 19.0). Correlation coefficients (r) and significance probabilities (p) were computed for the linear fits according to Pearson correlation analysis. One-way analysis of variance (ANOVA) was carried out to compare whether concentrations vary significantly between fish species, and among different tissues.

3. Results and discussion

3.1. Amino acids distribution

The distributions of amino acids in common carp and grass carp were shown in Figs. 1 and 2, respectively. According to their structures and properties, amino acids were classified into four groups (1) essential and polar amino acids (arginine, threonine and phenylalanine); (2) essential and non-polar amino acids (leucine and valine); (3) non-essential and polar amino acids (glutamate, cysteine, serine and tyrosine); and (4) non-essential and non-polar amino acids (glycine, proline and alanine). For tissues of both fish species, in general, glutamate has the highest concentrations, followed by glycine, arginine, alanine, leucine, serine, threonine, valine, phenylalanine, tyrosine, proline, cysteine (Figs. 1 and 2). Zheng and Zheng (2013) measured the primary amino acids in fish muscles from Xiamen Bay China, and also observed that glutamate has the highest concentration (~110 mg/g, dry wet) and cysteine had the lowest concentration (~10 mg/g, dry wet). Essential amino acids cannot be synthesized by the organism, and thus have to be obtained from diet, while non-essential amino acids can be produced in body (Wu et al., 2013). Glutamate, which is a non-essential amino acid and a major constituent of a wide variety of proteins, can be easily synthesized under normal conditions from the diet via numerous bioreactions (Grabowska et al., 1981). Cysteine is uncommonly found on the surface of a protein, due to oxidation to form a disulfide bond (Reisner, 1951). In organisms, sulfur has much lower abundance than other basic elements for amino acids (i.e., carbon, hydrogen and nitrogen), which may be another reason for the lowest abundance of cysteine in fish tissues. For both grass carp and common carp, the highest concentrations of amino acids in groups (1), (2) and (3) were generally found in protein-rich tissues, such as muscle and kidney, whereas the lowest concentrations were found in low-protein tissues such as fat and gill. Similarly group (4) amino acids have the lowest concentrations in fat and gill, but scale showed the highest concentrations.

Significantly positive correlations ($p < 0.01$, Table 1) were observed among essential amino acids [groups (1) and (2)] for all fish tissues. Essential amino acids mainly come from dietary intake

Table 1
Pearson's correlation matrix among amino acids for all fish tissues (n = 78) investigated in this study.

	Glu	Arg	Ser	Thr	Tyr	Cys	Leu	Val	Phe	Gly	Ala	Pro
Glu												
Arg	0.891**											
Ser	0.943**	0.899**										
Thr	0.974**	0.889**	0.965**									
Tyr	0.811**	0.620**	0.798**	0.848**								
Cys	0.705**	0.480**	0.748**	0.769**	0.822**							
Leu	0.886**	0.714**	0.886**	0.920**	0.920**	0.853**						
Val	0.895**	0.766**	0.912**	0.933**	0.901**	0.841**	0.598**					
Phe	0.916**	0.783**	0.916**	0.927**	0.878**	0.821**	0.974**	0.987**				
Gly	0.558**	0.806**	0.582**	0.497**	0.224*	0.054ns	0.238*	0.329**	0.916**			
Ala	0.734**	0.876**	0.735**	0.704**	0.458**	0.299**	0.598**	0.677**	0.783**	0.842**		
Pro	0.207ns	0.368**	0.233*	0.184ns	0.044ns	−0.082ns	0.084ns	0.142ns	0.190ns	0.556**	0.504**	1.000**

Significance level: **($p < 0.01$), *($p < 0.05$), ns($p > 0.05$).

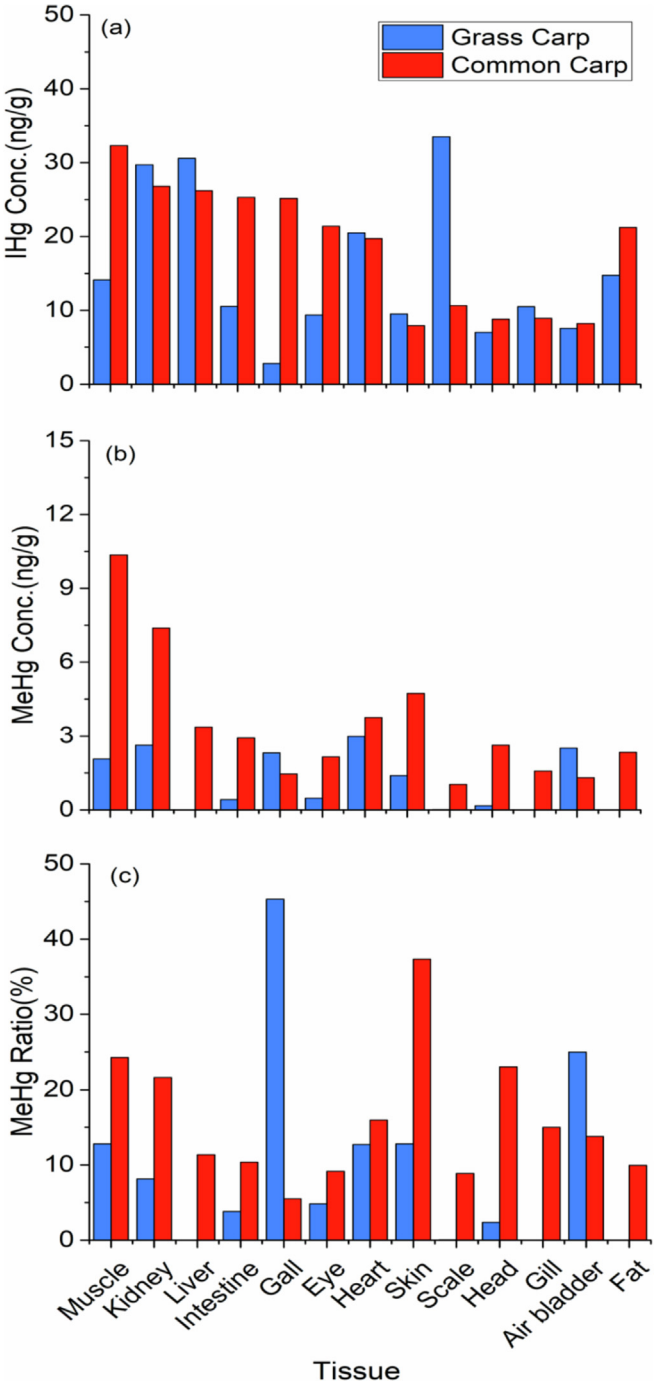


Fig. 3. Distribution patterns of (a) IHg, (b) MeHg and (c) MeHg proportions for grass carp (*Ctenopharyngodon idellus*, n = 3) and common carp (*Cyprinus carpio*, n = 3). Coefficients of variation for analyses of IHg and MeHg for the three individuals of each fish species were <16%. Note: MeHg for grass carp liver was not measured due to limited sample masses.

as fish itself cannot synthesize in body (Wu et al., 2013). For both fish species, the similar distribution patterns of groups (1) and (2) can be explained by the same dietary source, as they were artificially fed in a small pond using the same feedstuff. Essential amino acids are mainly used to synthesize protein, and more than half of the essential amino acids consumed by fish are deposited into body protein (Andersen et al., 2016). The highest concentrations of groups (1) and (2) in muscle were consistent with the fact that muscle is rich in protein and accounts for over 50% of the body weight of most fish species (Weatherley and Gill, 1987).

For non-essential amino acids [groups (3) and (4)], biological processes may play important roles in their distribution because they can be endogenously synthesized in fish body. Essential amino acids are important material sources for non-essential amino acids synthesis. For instance, tyrosine and cysteine can be synthesized endogenously from phenylalanine; glutamate can be synthesized endogenously from histidine (Hoffer, 2016). As shown in Table 1, it is interesting to note that amino acids in group (3) were also positively correlated ($p < 0.01$) with that in groups (1) and (2). Due to their hydrophilic characteristics, the correlations may be explained by the fact that amino acids in group (3) can form hydrogen bonds with the protein's substrate which are rich in amino acids in groups (1) and (2)(Branden and Tooze, 1991). Interestingly, less positive or no correlations were observed between amino acids in group (4) and other groups. Amino acids in group (4) have hydrophobic side chains, which are composed mostly of carbon and hydrogen, and have very small dipole moments, and tend to be repelled from water (Eisenberg et al., 1982). The enrichment of group (4) amino acids in fish scales, as shown in Figs. 1 and 2, may play a crucial role in fish skin protection and isolating fish body from the surrounding water. For instance, glycine protects the damage of skin from the UV rays, oxidation, and free radical; proline is essential to skin health; alanine can combine with the epidermal cells to fill up creases and provide skin with the building blocks (i.e., to make collagen) (Kitazawa and Iwasaki, 1999; Murakami et al., 2012; Richter et al., 1987).

3.2. Interactions between Hg and amino acids

Overall, the THg concentrations of the fish muscles (grass carp: 7.2–41.6 ng/g; common carp: 6.6–42.7 ng/g, dry weight) were much lower than the guideline THg values (e.g., 300 ng/g) (USEPA, 2001). Unlike wild fish which contain the majority of Hg (>90%) in the form of MeHg (Lepak et al., 2018; Li et al., 2016), all our fish tissue samples showed higher IHg concentrations (grass carp: 2.8–33.5 ng/g; common carp: 7.9–33.2 ng/g) than MeHg concentrations (grass carp: 0.5–3.9 ng/g; common carp:1.0–7.4 ng/g), as shown in Fig. 3a and b. The proportions of MeHg in our fish tissues are 2–45% and 6–37% for grass carp and common carp, respectively (Fig. 3c), which are much lower than previous results of wild fish (Lepak et al., 2018; Li et al., 2016). The low MeHg concentrations and MeHg/THg ratios in artificial fed fish species from Guizhou provinces have been previously reported and explained by the simple food web structures and the fast growth of fish (Liu et al., 2012).

Table 2
Correlations between amino acids and MeHg for all fish tissues (n = 78) investigated in this study.

Group (1)	MeHg	Group (2)	MeHg	Group (3)	MeHg	Group (4)	MeHg
Arginine	0.233*	Leucine	0.432**	Glutamate	0.342**	Glycine	−0.010^{ns}
Threonine	0.461**	Valine	0.415**	Serine	0.360**	Alanine	0.158$^{ns.}$
Phenylalanine	0.411**			Tyrosine	0.461**	Proline	−0.103^{ns}
				Cysteine	0.472**		

Significance level: **($p < 0.01$), *($p < 0.05$), ^{ns}($p > 0.05$).

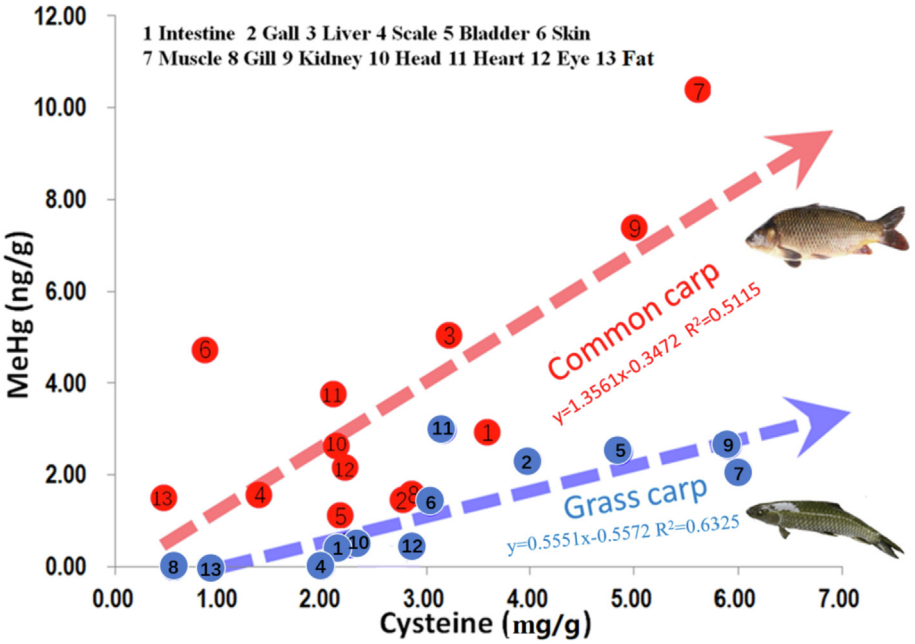


Fig. 4. The correlation between cysteine and methylmercury in grass carp (*Ctenopharyngodon idellu*, n = 3) and common carp (*Cyprinus carpio*, n = 3).

No clear difference in IHg concentrations in fish tissues can be found between the two fish species (Fig. 3a). However, common carps in general have higher MeHg concentrations in their tissues, compared to grass carp (Fig. 3b). Dietary differences may not be the main reason for the MeHg difference as the two fish species were artificially fed in a small pond using the same feedstuff. Here, we attribute the observed MeHg differences between two fish species to different feeding habitats. The common carp lives mainly in deeper waters and above sediment where Hg methylation rates was higher than the surface water where grass carp lives (Meng et al., 2016; Liu et al., 2012). The biodilution effect due to the higher growth rates or the higher photodemethylation rates in the surface water may be other reasons for the lower MeHg concentrations in grass carp tissues. Given that both fish species were fed in the same pond within the same period, the higher growth rate of grass carp can be easily proven by their higher weights.

The large variations of MeHg and IHg concentrations in tissues of both fish species allow us to compare the effects of primary amino acids to both MeHg and IHg metabolism. Muscle and kidney showed higher MeHg levels than other tissues for both species ($p < 0.01$, ANOVA), as shown in Fig. 3b. As shown in Table 2, MeHg concentrations in body tissues of both fish species were significantly positively correlated ($p < 0.01$) with amino acids in groups (1), (2) and (3), but not with group (4). The correlation coefficient ($r = 0.472$) between cysteine and MeHg was the highest among all the correlations in Table 2. We infer that the correlations are driven by the formation of MeHg-Cys complexes in fish bodies, or increased uptake of MeHg-Cys complexes. The affinity constant for Hg bonding to −SH is in the order of 10^{15} to 10^{20}, whereas the affinity constants for Hg bounding to O/N-containing ligands (e.g., carbonyl or amino groups) are about ten orders of magnitude lower (Zalups, 2000; Zhang et al., 2004). Cysteine is the only one containing -SH group among the 20 primary amino acids, and it has been reported that −SH groups in organisms are present mainly in cysteine and cysteine residues of proteins and enzymes (Wang et al., 2012). MeHg can be readily across cell membranes through the formation of MeHg-Cys complex, which allows a rapid absorption and uptake of MeHg, and a long half-life of MeHg in animal bodies (Mason et al., 1996). LAT1 is expressed in many tissues and is the major carrier for MeHg-Cys complex in multiple tissues in mammals (Boado et al., 1999; Duelli et al., 2000; Killian and Chikhale, 2001). LAT1 preferentially also transports the branched and aromatic amino acids (e.g., leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, histidine and methionine, etc) (Kanai et al., 1998; Yanagida and others, 2001), which may explain the positive correlations between MeHg and these amino acids in Table 2.

As shown in Fig. 4, both fish species showed significantly positive correlations between MeHg and cysteine concentrations, indicating that cysteine is an important driver in the distribution of MeHg for both fish species. However, common carp has higher MeHg/cysteine ratios than grass carp. The differences in MeHg/cysteine ratios result from elevated MeHg levels in common carp compared to grass carp, note that the cysteine levels are comparable between the two fish species.

As shown in Table 3, no significant correlations ($p < 0.05$) were

Table 3
Correlations between IHg and amino acids for all fish tissues (n = 78) investigated in this study.

Group (1)	IHg	Group (2)	IHg	Group (3)	IHg	Group (4)	IHg
Arginine	0.054^{ns}	Leucine	0.269^{ns}	Glutamate	$0.198^{ns.}$	Glycine	$-0.169^{ns.}$
Threonine	0.159^{ns}	Valine	0.234^{ns}	Cysteine	0.140^{ns}	Proline	$-0.164^{ns.}$
Phenylalanine	0.230^{ns}			Serine	0.213^{ns}	Alanine	$0.073^{ns.}$
				Tyrosine	0.240^{ns}		

Significance level: $^{**}(p < 0.01)$, $^{*}(p < 0.05)$, $^{ns}(p > 0.05)$.

observed between amino acids [groups (1), (2), (3) and (4)] and IHg, indicating the metabolic processes of IHg and MeHg were different in fish bodies. While both IHg and MeHg have strong affinities for -SH groups, MeHg is readily transported across cell membranes in the form of MeHg-Cys via the LAT1 transporter (Yin et al., 2008). IHg-thiol complexes, however, are bulky and polar and have a tendency to be charged. Owing to the repulsive interaction of phospholipid cell membranes, IHg-thiol complexes cannot enter the cell membranes by passive diffusion (Ndu et al., 2012). According to our results, the highest IHg concentrations were observed in kidney and liver. When Hg(II) ions are bound to -SH ligands possessing a net negative charge (Cheesman et al., 1989), the mercuric conjugates of these molecules are not easy to absorb on the lumen membrane, but are excreted in the urine (Zalups, 1995; Cannon et al., 2000). The elimination of IHg by urine results in short half-life of IHg in animal bodies (Roos and others, 2010), which may also be a reason for the lack of correlations between IHg and amino acids.

4. Conclusions and implications

Our study showed that cysteine and its related/derived amino acids [groups (1), (2) and (3)] have a high biological association with MeHg, but not with IHg. The association with MeHg may be driven by the formation of MeHg-Cys complexes within fish body. The close interactions between MeHg and cysteine have been mainly observed in mammalian systems, and our study suggests cysteine plays a similar role during MeHg metabolism in fish, albeit the dissimilarity in the capacity of MeHg demethylation between fish and mammals. The metabolic difference in primary amino acids leads to their variable abundances in fish tissues, and the formation of MeHg-Cys complexes can be an important driving force for MeHg distribution and translocation in fish tissues. Of particular environmental and human toxicological significance, the formation of MeHg-Cys complexes can be better transported in different organs and tissues than MeHg alone. It is still unclear whether MeHg has a close relation to other primary amino acids (histidine, methionine, glutamine, lysine, isoleucine, aspartate, trytophan, asparaginate), especially methionine which contains sulfur in its structure and could be transformed to cystine and cysteine. However, we failed to measure these amino acids in our samples, due to their low concentration levels. Further studies are therefore needed to rule out whether these primary amino acids could influence the metabolism of MeHg and IHg in fish bodies.

Acknowledgement.

Runsheng Yin was funded by the Chinese Academy of Sciences through the Pioneer Hundred Talent Plan. Haiyu Yan was funded by the National Natural Science Foundation of China (41273099). Xiangdong Li from the Hong Kong Polytechnic University and two anonymous reviewers are acknowledged for their constructive comments that have largely improved the quality of this paper.

References

Andersen, S.M., Waagbo, R., Espe, M., 2016. Functional amino acids in fish health and welfare. Front. Biosci. 8, 143–169.
Andrahennadi, R., Wayland, M., Pickering, I.J., 2007. Speciation of selenium in stream insects using X-ray absorption spectroscopy. Environ. Sci. Technol. 41, 7683–7687.
Aschne, M., Clarkson, T.W., 1988. Uptake of methylmercury in the rat brain: effect of amino acids. Brain Res. 462, 31–39.
Atmaca, G., 2004. Antioxidant effects of sulphur-containing amino acids. Yonsei Med. J. 45, 776–788.
Ballantyne, J.S., 2001. Amino acid metabolism. Fish Physiol. 20, 77–107.
Boado, R.J., Li, J.Y., Nagaya, M., Zhang, C., Pardridge, W.M., 1999. Selective expression of the large neutral amino acid transporter at the blood-brain barrier. Proc. Natl. Acad. Sci. U.S.A. 96, 12079–12084.
Branden, C., Tooze, J., 1991. Molecular biology in three dimensions. Book Reviews: introduction to protein structure. Science 253, 685–686.
Cai, K., Xiang, Z.M., Li, H.Q., Zhao, H.N., Lin, Y.C., Pan, W.J., Lei, B., 2017. Free amino acids, biogenic amines, and ammonium profiling in tobacco from different geographical origins using microwave-assisted extraction followed by ultra high performance liquid chromatography. J. Sep. Sci. 23, 4477–4660.
Cannon, V.T., Barfuss, D.W., Zalups, R.K., 2000. Molecular homology and the luminal transport of Hg_2 in the renal proximal tubule. J. Am. Soc. Nephrol. 11, 394–402.
Cerneia, N., Hegera, Z., Zítka, O., Adama, V., Kizeka, R., 2014. Amino acids and their interactions with heavy metals. J. Metallomics Nanotechnol. 1, 49–52.
Cheesman, B.V., Arnold, A.P., Rabenstein, D.L., 1989. Nuclear magnetic resonance studies of the solution chemistry of metal complexes. Part 25. $Hg(thiol)_3$ complexes and HG(II)-thiol ligand exchange kinetics. ChemInform 20, 6359–6364.
Chong, A.S., Hashim, R., Ali, A., Hara, K., 2004. Amino acid profile of various body tissues and eggs of discus fish, Symphysodon aequifasciata. J. Appl. Aquac. 16, 157–168.
Clarkson, T.W., 1993. Molecular and ionic mimicry of toxic metals. Annu. Rev. Pharmacol. Toxicol. 32, 545–571.
Creighton, T.E., 1993. Proteins: Structures and Molecular Properties, second ed., vol. 323. W. H. Freeman and Company, New York, p. 294.
Daniel, H.R., Robson, L.P., Marcelo, F., Michael, A., Denise, B., João, B., Rocha, T., de Vargas Barbosa, N.B., 2011. Modulation of methylmercury uptake by methionine: Prevention of mitochondrial dysfunction in rat liver slices by a mimicry mechanism. Toxicol Appl. Pharmacol. 252, 28–35.
Duelli, R., Enerson, B.E., Gerhart, D.Z., Drewes, L.R., 2000. Expression of large amino acid transporter LAT1 in rat brain endothelium. J. Cereb. Blood Flow Metab. 20, 1557–1562.
Eisenberg, D., Weiss, R.M., Terwilliger, T.C., Wilcox, W., 1982. Hydrophobic moments and protein structure. Faraday Symp. Chem. Soc. 17, 109–120.
Grabowska, A., Nowicki, M., Kwinta, J., 1981. Glutamate dehydrogenase of the germinating triticale seeds: gene expression, activity distribution and kinetic characteristics. Acta Physiol. Plant. 33.
Harris, H.H., Pickering, I.J., George, G.N., 2003. The chemical form of mercury in fish. Science 301, 1203-1203.
Hoffer, L.J., 2016. Human protein and amino acid requirements. J. Parenter. Enteral Nutr. 40, 460–475.
Hsu-Kim, H., Kucharzyk, K.H., Zhang, T., Deshusses, M.A., 2013. Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review. Environ. Sci. Technol. 47, 2441–2456.
Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., Endou, H., 1998. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J. Biol. Chem. 273, 23629–23632.
Killian, D.M., Chikhale, P.J., 2001. Predominant functional activity of the large, neutral amino acid transporter(LAT1) isoform at the cerebrovasculature. Neurosci. Lett. 306, 1–4.
Kitazawa, M., Iwasaki, K., 1999. Reduction of ultraviolet light-induced oxidative stress by amino acid-based iron chelators. Biochim. Biophys. Acta 1473, 400–408.
Kwon, S.Y., Blum, J.D., Chen, C.Y., Meattey, D.E., Mason, R.P., 2014. Mercury isotope study of sources and exposure pathways of methylmercury in estuarine food webs in the Northeastern US. Environ. Sci. Technol. 48 (17), 10089–10097.
Leaner, J.J., Mason, R.P., 2002. Methylmercury accumulation and fluxes across the intestine of channel catfish, Ictalurus punctatus. Comp. Biochem. Physiol., C 132, 247–259.
Lemes, M., Wang, F.Y., 2009. Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. J.Anal.Atom.Spectrom. 24, 663–668.
Lepak, R.F., Janssen, S.E., Yin, R., Krabbenhoft, D.P., Ogorek, J.M., DeWild, J.F., Hurley, J.P., 2018. Factors affecting mercury stable isotopic distribution in piscivorous fish of the laurentian great lakes. Environ. Sci. Technol. 52, 2768–2776.
Li, M., Schartup, A.T., Valberg, A.P., Ewald, J.D., Krabbenhoft, D.P., Yin, R., Balcom, H.P., Sunderland, E.M., 2016. Environmental origins of methylmercury accumulated in subarctic estuarine fish indicated by mercury stable isotopes. Environ. Sci. Technol. 50, 11559–11568.
Liu, B., Yan, H., Wang, C., Li, Q., Guédron, S., Spangenberg, J.E., Dominik, J., 2012. Insights into low fish mercury bioaccumulation in a mercury-contaminated reservoir, Guizhou, China. Environ. Pollut. 160, 109–117.
Mason, R.P., Reinfelder, J.R., Morel, F.M.M., 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. Environ. Sci. Technol. 30, 1835–1845.
Meng, B., Feng, X.B., Qiu, G.L., Li, Z.G., Yao, H., Shang, L.H., Yan, H.Y., 2016. The impacts of organic matter on the distribution and methlylation of mercury in a hydroelectric reservoir in Wujiang river, Southwest China. Environ. Toxicol. Chem. 35, 191–199.
Murakami, H., Shimbo, K., Inoue, Y., Takino, Y., Kobayashi, H., 2012. Importance of amino acid composition to improve skin collagen protein synthesis rates in UV-irradiated mice. Amino Acids 42, 2481–2489.
Ndu, U., Mason, R.P., Zhang, H., Lin, S., Visschera, P.T., 2012. Effect of inorganic and organic ligands on the bioavailability of methylmercury as determined by using a mer-lux bioreporter. Appl. Environ. Microbiol. 78, 7276–7282.
Reisner, D.B., 1951. Sulfur-containing amino acids. J. Am. Chem. Soc. 78, 2132–2135.
Richter, K., Egger, R., Kreil, G., 1987. D-alanine in the frog skin peptide dermorphin is

derived from L-alanine in the precursor. Science 238, 200–202.
Roos, D.H., others, 2010. Complex methyl-mercury–cysteine alters mercury accumulation in different tissues of mice. Basic Clin. Pharmacol. Toxicol. 107, 789–792.
Sakami, W., Harrington, H., 1963. Amino acid metabolism. Annu. Rev. Biochem. 32, 355–398.
Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit. Rev. Environ. Sci. Technol. 31, 241–293.
USEPA, 2001. Water Quality Criterion for the Protection of Human Health: Methylmercury. Highlights of EPA-823-R-01E001. Protection Agency, Washington, DC.
USEPA, 2002. Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry; USEPA:WA, USEPA. 2002. Method 1630, Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. USEPA, WA.
Wang, F., Lemes, M., Khan, M., 2012. Metallomics of mercury: role of thiol- and selenol-containing biomolecules. In: Environmental Chemistry and Toxicology of Mercury. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 517–544.
Weatherley, A.H., Gill, H.S., 1987. The Biology of Fish Growth. Academic Press, London.
Wu, G.Y., Wu, Z.H., Dai, Z.L., Yang, Y., Wang, W.W., Liu, C., Wang, B., Wang, J.J., Yin, Y.L., 2013. Dietary requirements of "nutritionally non-essential amino acids" by animals and humans. Amino Acids 44, 1107–1113.
Yanagida, O., others, 2001. Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. Biochim. Biophys. Acta 1514, 291–302.
Yin, Z., Jiang, H., Syversen, T., Rocha, J.B.T., Farina, M., Aschner, M., 2008. The methylmercury-L-cysteine conjugate is a substrate for the L-type large neutral amino acid transporter. J. Neurochem. 107, 1083–1090.
Zalups, R.K., 1995. Organic anion transport and action of r-glutamyl transpeptidase in kidney linked mechanistically to renal tubular uptake of inorganic mercury. Toxicol. Appl. Pharmacol. 132, 289–298.
Zalups, R.K., 2000. Molecular interactions with mercury in the kidney. Pharmacol. Rev. 52, 113–143.
Zhang, J., Wang, F., House, J.D., 2004. Thiols in wetland interstitial waters and their role in mercury and methylmercury speciation. Limnol. Oceanogr. 49, 2276–2286.
Zheng, C.Y., Zheng, B., 2013. Analysis of amino acid in muscle of lateolabrax japonicus. J. Zhejiang Univ. Sci. Technol. 15, 73–75.