



Improving the qualities of the trophic magnification factors (TMFs): A case study based on scaled $\Delta^{15}\text{N}$ trophic position framework and separate baseline species

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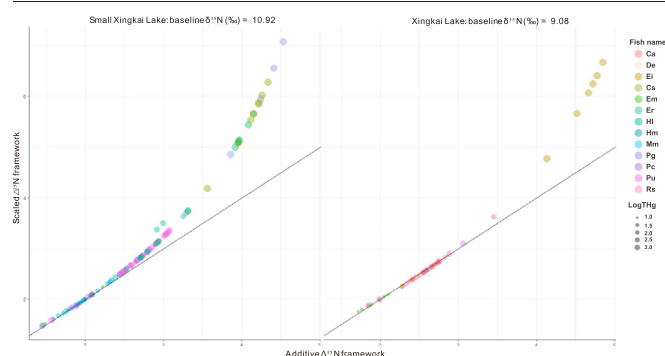
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HIGHLIGHTS

- An improved trophic position framework is crucial for correct assessment of TMFs.
- We recommend using independent baseline species even in the connected ecosystems.
- Scaled $\Delta^{15}\text{N}$ framework shows higher TPs, and hence lower TMFs.

GRAPHICAL ABSTRACT



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ABSTRACT

Scientific understanding of trophic magnification factors (TMFs) is conducive to formulating environmental management measures. Trophic position (TP) of species is the key parameter in TMFs assessment. Nitrogen stable isotopes ($\delta^{15}\text{N}$) provide a powerful tool to estimate TP. However, some limitations could introduce considerable uncertainty into TP and TMFs assessment which mainly includes: 1) determination of $\Delta^{15}\text{N}$ between two adjacent trophic positions; 2) determination of baseline species. Different from the widely used constant $\Delta^{15}\text{N}$ (3.4 ‰) between two adjacent trophic positions, which is called additive $\Delta^{15}\text{N}$ framework, $\Delta^{15}\text{N}$ gradually decreases as trophic position increases under scaled $\Delta^{15}\text{N}$ framework, which has been confirmed by more and more laboratory studies and meta-analyses. In this study, we sampled in two similar littoral ecosystems separated by one natural dam, which is called Small Xingkai Lake and Xingkai Lake, analyzed the $\delta^{15}\text{N}$ and total mercury (THg) of each species. On the one hand, we compared the TP of species under the additive $\Delta^{15}\text{N}$ framework and scaled $\Delta^{15}\text{N}$ framework with the White shrimp (*Exopalaemon modestus*) as baseline species in two lakes respectively. On the other hand, we explored the possible changes in TMFs based on TP. Our results show, under the scaled $\Delta^{15}\text{N}$ framework, the trophic position of the same species is higher, while TMFs is lower compared with the additive $\Delta^{15}\text{N}$ framework; even if in the two interconnected lakes, distributed the same baseline species, in the similar ecosystem, separate baselines should also be used. In this study, two frameworks of the food chain were compared in two interconnected freshwater ecosystems for the first time. The difference between TMFs of two lakes was obvious under scaled framework but not under additive framework. We also recommend that future TMFs assessments should be based on the scaled $\Delta^{15}\text{N}$ framework because it has improved the accuracy of trophic position assessment.

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1. Introduction

Trophic magnification factors (TMFs) could describe food web biomagnification, especially in the field, as it integrates the whole bioaccumulation processes across the entire food chain and under realistic environmental conditions. It is suggested as a reliable tool for bioaccumulation worldwide (Kosfeld et al., 2021; Borgå et al., 2012; Riyadi et al., 2015; Gobas et al., 2009). TMFs is the factor that reflects the change in concentrations per trophic position, and is estimated from the antilog of the log-normal regression, which has been widely applied to assess the trophic magnification of pollutants through food webs (Borgå et al., 2012; Broman et al., 1992; Fisk et al., 1998; Walters et al., 2016). Trophic position (TP) is a key parameter in determining TMFs, because the correct assessment of TMFs is calculated based on the relationship between the TP of an organism and the concentration of a pollutant in the organism (Walters et al., 2011). The theory of trophic dynamics provides a basic theoretical framework (Lindeman, 1942). And relative abundances of naturally occurring stable isotopes $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$, referred to as $\delta^{15}\text{N}$) have been used as a routine method to quantify the trophic position (TP). From an evaluation point of view, TMFs >1 indicate biomagnification (Mackay et al., 2016; Simmonet-Laprade et al., 2019). Although TMFs is increasingly used to describe the trophic dynamics of pollutants, some uncertainties and limitations also exist in its estimation (Borgå et al., 2012; Mackay et al., 2016).

Ecologists have been committed to improving trophic position estimation, thereby TMFs. In the last decade, two approaches have been widely discussed in various related studies. One is the probability distribution method to estimate species specific $\Delta^{15}\text{N}$ and TP, based on the food web $\delta^{15}\text{N}$ and binary dietary matrix. It is a way to reduce the uncertainty in TMFs calculation by using a Markov chain Monte Carlo method to model the probability distributions of trophic relationships and $\Delta^{15}\text{N}$ (Starrfelt et al., 2013; Rossi et al., 2015; Borgå and Ruus, 2019). The probability distribution of TP due to this approach will only affect the confidence interval of the regression (uncertainty) but not the mean TMFs estimate itself (Starrfelt et al., 2013). The other is separating the bulk $\delta^{15}\text{N}$ into the baseline and trophic enriched amino acids for a more detailed understanding of what part of the changes in $\delta^{15}\text{N}$ is due to the trophic aspect. Compound-specific isotope analysis of amino acids (CSI-AAAs) is a technique which has the potential to reduce the limitations of $\delta^{15}\text{N}$ analysis for estimating TP on bulk tissue (Chikaraishi et al., 2009; McClelland and Montoya, 2002). Both methods improve the accuracy of the assessment to a certain extent, whether from the perspective of a more sophisticated algorithm or more advanced technology. However, a trophic framework with constant $\Delta^{15}\text{N}$ is still be used while assessing the trophic position of organisms. As the fundamental problem of trophic position evaluation, the improvement of the framework based on the baseline and $\Delta^{15}\text{N}$ is the most critical which should not be ignored.

On the one hand, $\Delta^{15}\text{N}$ (trophic enrichment factor), which reflects the enrichment from prey to predator, is known shows a great variation in different species, trophic ecology and physiology (Robbins et al., 2010; Vander Zanden and Rasmussen, 2001). Most commonly, a fixed value of $\Delta^{15}\text{N}$ 3.4 ‰ is used to estimate relative species worldwide (Lavoie et al., 2013; Post, 2002; Walters et al., 2011; Borgå et al., 2013; Xing et al., 2021; Jia et al., 2015), which is the mean of 56 case studies (SD = 0.98, $n = 56$) (Post, 2002), that is what we called additive $\Delta^{15}\text{N}$ framework used to evaluate the trophic position of consumers and thereby TMFs. However, with the deepening of research, researchers have found that the $\Delta^{15}\text{N}$ shows a high variability across species, nutrient ecology, and physiology (Robbins et al., 2010; Vander Zanden and Rasmussen, 2001). Numerous studies have shown that significant variability in $\Delta^{15}\text{N}$ (from 0.6 to 5.5 ‰) produces uncertainty estimation in TP (Vander Zanden and Rasmussen, 2001; Post, 2002; Dubois et al., 2007; Won et al., 2020). For example, Caut et al. (2009) conclude that the overall mean estimate of $\Delta^{15}\text{N}$ is 2.75 ‰ (SE = 0.10) after concluding 268 animal-diet nitrogen discrimination factors from 66 publications. The average value of $\Delta^{15}\text{N}$ in the study of Li et al. (2022) in Amur pike with the highest trophic position is 1.01 ‰.

More studies have suggested that discrimination is a process of dynamic, rather than a constant, equilibrium process (Olive et al., 2003; Hussey et al., 2014). Meanwhile, the fact that $\Delta^{15}\text{N}$ decreases with the increasing dietary $\delta^{15}\text{N}$ between trophic levels has also been demonstrated in meta-analyses and laboratory studies (Caut et al., 2008; Overmyer et al., 2008). Therefore, Hussey et al. (2014) established the scaled $\Delta^{15}\text{N}$ framework, which estimates more reliable TPs with known feeding relationships. This approach is considered to have provided more robust estimates of trophic position (Hussey et al., 2014; Nawrocki et al., 2020). Based on a meta-analysis, it is found that $\Delta^{15}\text{N}$ decreases as the trophic position increases. Since the effects of $\Delta^{15}\text{N}$ are bound to influence the assessment of trophic position and, therefore, the TMFs, studies have shown that the reduction of $\Delta^{15}\text{N}$ will make the TMFs closer to 1 (Starrfelt et al., 2013), which means the TMFs for the assessment of biomagnification will be smaller under the scaled $\Delta^{15}\text{N}$ framework compared to the additive $\Delta^{15}\text{N}$ framework. Previous research has shown the scaled $\Delta^{15}\text{N}$ framework is considered to significantly reduce the bias of trophic position estimates (Hussey et al., 2014), based on which we think the TMFs can be improved.

On the other hand, the baseline of the food web can also influence the assessment of the trophic position (Lavoie et al., 2013; Lorrain et al., 2015), and then affect the TMFs. The natural distribution of nitrogen isotope is influenced by location, pollution, etc., so each food chain has its own baseline (Camin et al., 2007; Fisk et al., 2001). For the selected baseline species, they may have different $\delta^{15}\text{N}$ due to factors like distribution, seasonal and spatial variations, even if they are the same species (Jennings and Van Der Molen, 2015). Earlier studies have shown that hydrological connectivity strongly affects the biodiversity and stability of interconnected aquatic habitats (Pringle, 2003; Yuan et al., 2018; Casanova et al., 2009). It also affects the trophic positions by affecting the trophic interactions of fishes (Roach et al., 2009). Therefore, it is critical to assess the TMFs by establishing a more scientific method for assessing the trophic position of species in a food web (Borgå et al., 2012; Kidd et al., 2001).

In this study, under the premise that the assessment under the scaled $\Delta^{15}\text{N}$ framework can better reduce the bias in the assessment of trophic position, which is considered to be in closer conformity to the situation in the natural food web, we compared the differences between the two methods in the assessment of trophic positions of the two adjacent and connected lakes (with similar littoral ecosystems separated only by a natural dam) sharing the same baseline species, and then further evaluated their impact on the TMFs. In short, the following two points have been the focus of this paper: 1) comparison of trophic positions assessment based on different trophic frameworks and baselines; 2) comparison of TMFs assessment based on different trophic frameworks and baselines. The TMFs should represent their respective food web, both in terms of $\Delta^{15}\text{N}$ and $\delta^{15}\text{N}$ of baseline species. In this study, two frameworks of the food chain were compared in two interconnected freshwater ecosystems for the first time, on whose basis, the effects of different frameworks on TMFs were compared as well. We think a more scientific understanding of the TMFs is conducive to the effective quantitative assessment and comparison of biomagnification among different ecosystems (Burkhard et al., 2013), which helps to formulate relevant environmental management measures.

2. Materials and methods

2.1. Research site

Xingkai Lake (45°20'N, 132°40'E) is a shallow lake on the border between China and Russia located in the north temperate zone, with maximum and average water depths of 10 m and 4.5 m, respectively (Sun et al., 2018); it is the fourth largest freshwater lake in the world (including Xingkai Lake and Small Xingkai Lake) and a Sino-Russian border lake which covers an area of 4380 km². The lake has only one out-flowing river in the northeast, the Songacha River, which flows into the Ussuri River (Yang et al., 2021). The climate in Xingkai Lake is characterized by a typical temperate continental Monsoon with a mean annual temperature of 2.9–3.1 °C.

Xingkai Lake and Small Xingkai Lake, with a natural sand dam about 90 km long and 1 km wide in between, are connected hydrologically through sluices. During the wet season, the flood will flow over the dam, connecting the two lakes. Xingkai Lake is a semi-enclosed shallow lake, which has a $175 \times 10^8 \text{ m}^3$ water storage capacity; Small Xingkai Lake is also a shallow lake, whose water storage capacity is $3.3 \times 10^8 \text{ m}^3$ (Yuan et al., 2018). Overall, Xingkai Lake and Small Xingkai Lake are separated by only one natural dam. While Xingkai Lake primarily has a sandy bottom with less water bottom vegetation and higher dissolved oxygen in the water. Small Xingkai Lake has been in a state of severe swamping and mild eutrophication (Yu et al., 2015).

2.2. Sample collection

On 18th–20th July 2018, we collected fish samples both in the littoral zone of Xingkai Lake and Small Xingkai Lake, which means our sampling of the two lakes was carried out both in the littoral ecosystem. The main reasons for choosing littoral zone as the sampling site include: 1) littoral zones which have the similar carbon source is an important part of two lakes in terms of structure and function; 2) littoral zones are generally the most productive area of lakes with a relatively complete food chain. The basic information of sampling points is provided in the supporting information (Fig. S1). We used two types of fishing nets, ground cage nets, and gill-net nets. There are mainly 55 species of fish in Xingkai Lake where Topmouth culter (*Erythroculter ilishaeformis*) is a typical top consumer, and White shrimp (*Exopalaemon modestus*) is the dominant species and the main food of many fish (Li, 2014). There are 123 species of fish in Small Xingkai Lake, where White shrimp is also the dominant one, and small swamp fishes are rich in number as well, with Northern snakehead (*Channa argus*) and Amur pike (*Esox reicherti*) as top consumers (Tang et al., 2011; Li, 2014). White shrimp and Clearhead icefish are dominant species in both lakes (Li, 2014; Tang et al., 2011; Wang and Yu, 2013). In 2010, Clearhead icefish accounted for 36.4 % of the catch in Xingkai Lake (Tang et al., 2011), and 44.7 % of the catch in 2012 was Clearhead icefish, the highest catch compared with other fish species (Wang and Yu, 2013). See the basic information of samples in Table 1. The number and total length of fish can be found in supporting information (Table S1).

2.3. Stable isotope and total mercury (THg) analysis

2.3.1. Stable isotope analysis

We identified all samples for isotope analysis to species. For fish samples, we took 2 g of back muscle tissue from fish as an individual sample; for insects, the collected insects were cleaned with deionized water to remove the dirt adhering to the surface, after which the surface water was absorbed by filter paper, and the shell of the Diving beetle was removed; for White shrimp, we removed the shell and the gastrointestinal tract to analyze the soft tissue of White shrimp. We then dried them at 60 °C for 48 h. Ethics Committee of Laboratory Animal Welfare, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences provided

ethics approval for this research (20220513). We ground the samples to a refined powder using a mortar and pestle or a SPEX Certiprep 6750 freezer mill. Isotope analysis was taken with a Thermo Finnigan Delta Plus Advantage stable isotope mass spectrometer at the Public Technology Service Center of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. Stable isotope values were represented by the δ notation, parts per thousand (‰). The standard reference materials were based on Vienna Pee Dee Belemnite (V-PDB) for ^{13}C and atmospheric N_2 for ^{15}N . The formula was as follows:

$$\delta X(\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (1)$$

X represents ^{13}C or ^{15}N , R represents the stable isotope ratio ($^{13}\text{C}/^{12}\text{C}$ for carbon; $^{15}\text{N}/^{14}\text{N}$ for nitrogen). Replicate measurements by using standard materials showed that the analytical errors were $\delta^{13}\text{C} < 0.2 \text{ ‰}$ and $\delta^{15}\text{N} < 0.3 \text{ ‰}$.

2.3.2. Total mercury (THg) analysis

In the laboratory, all the samples were digested by using $\text{H}_2\text{SO}_4\text{-HNO}_3\text{-V}_2\text{O}_5$ so that the Hg in all samples was converted into Hg^{2+} , which was reduced to elemental Hg by adding 20 % SnCl_2 solution afterward. These procedures were taken for Hg analysis using a Cold Vapor Atomic Absorption spectrophotometer (Model F-732, Jintan, China; detection limit is 0.05 ng/mL). Moreover, the total Hg concentrations of all the samples were on a wet-weight base.

In addition, blank samples were also included in the analysis. The blank samples in each batch were analyzed in duplicates to ensure their reproducibility to a great extent. The standard reference material of human hair (GBW-07601) was used for analysis as a part of the quality control (accuracies within 100 % \pm 20 %).

2.4. Data analysis

The TMFs is usually used to evaluate the amplification degree of pollutants in the food chain. We calculated the TMFs under the additive $\Delta^{15}\text{N}$ framework and scaled $\Delta^{15}\text{N}$ framework, respectively. The formula for calculating the TMFs is as follows:

$$\text{LogC} = a + b * \text{TP} \quad (2)$$

$$\text{TMFs} = 10^b \quad (3)$$

C is the concentration of pollutants; TP is the trophic position of species in the food chain; a indicates the intercept of the linear regression equation, and b represents the slope. In this study, we calculated the trophic position under the two frameworks, and adopted the following formula when calculating the additive $\Delta^{15}\text{N}$ framework:

$$\text{TP}_{\text{additive}} = \frac{(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})}{\Delta^{15}\text{N}} + \lambda \quad (4)$$

Table 1

Basic information of samples in Xingkai Lake and Small Xingkai Lake.

	Scientific name	Common name	Abbreviation	Feeding type	
Xingkai Lake	<i>Dytiscidae</i>	Diving beetle	De	Omnivorous	
	<i>Rhodeus sericeus</i>	Amur bitterling	Rs	Omnivorous	
	<i>Erythroculter ilishaeformis</i>	Topmouth culter	Ei	Carnivorous	
Small Xingkai Lake	<i>Misgurnus mohoity</i>	Amur weatherfish	Mm	Omnivorous	
	<i>Channa argus</i>	Northern snakehead	Cs	Carnivorous	
	<i>Esox reicherti</i>	Amur pike	Er	Carnivorous	
	<i>Perccottus glenii</i>	Amur sleeper	Pg	Carnivorous	
	<i>Pseudobagrus ussuriensis</i>	Ussuri catfish	Pu	Omnivorous	
	<i>Hemibarbus maculatus</i>	Spotted steed	Hm	Carnivorous	
	<i>Hemiculter leucisculus</i>	Sharpbelly	Hl	Omnivorous	
	Common species of Xingkai and Small Xingkai Lakes	<i>Carassius auratus</i>	Crucian carp	Ca	Omnivorous
		<i>Protosalanx chinensis</i>	Clearhead icefish	Pc	Carnivorous
		<i>Exopalaemon modestus</i>	White shrimp	Em	Omnivorous

Where $\delta^{15}\text{N}_{\text{consumer}}$ is the nitrogen isotope of consumers, and $\delta^{15}\text{N}_{\text{baseline}}$ is the nitrogen isotope of the baseline species, both $\delta^{15}\text{N}_{\text{consumer}}$ and $\delta^{15}\text{N}_{\text{baseline}}$ are determined by experiment. λ is the trophic level of the baseline species in the food chain ($\lambda = 2$). Primary consumers provide a great isotopic baseline for estimating $\text{TP}_{\text{consumers}}$ in lake ecosystems (Post, 2002). Therefore, researchers aim to select primary consumers as baseline species (Vander Zanden et al., 2003; Cabana and Rasmussen, 1996). Among them, long-lived primary consumers provide a more suitable baseline for quantitative estimation trophic position of species across ecosystems (Post et al., 2000). White shrimp, feeding primarily on microalgae, is the long-lived primary consumer, with relatively stable eating habits; it is also the dominant species and the primary food source for carnivorous fish in Xingkai Lake (Yuan, 2018). Therefore, we selected the White shrimp distributed in both Xingkai Lake and Small Xingkai Lake as the baseline organism (TP = 2). $\Delta^{15}\text{N}$ is the enrichment value in the process of trophic transfer, generally at 3.4 ‰ under the additive $\Delta^{15}\text{N}$ framework (Post, 2002).

We also estimated TP of species under the scaled $\Delta^{15}\text{N}$ framework. The approach is proposed by Hussey et al. (2014), who calculate the trophic position of organisms by integrating a meta-analytical model of $\Delta^{15}\text{N}$ vs. dietary $\delta^{15}\text{N}$ values into a dietary $\delta^{15}\text{N}$ value-dependent enrichment model. The calculation formula is as follows:

$$TP_{\text{scaled}} = \frac{\log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{base}}) - \log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{TP}})}{k} + TP_{\text{base}} \quad (5)$$

Where $\delta^{15}\text{N}_{\text{lim}}$ is the saturating isotope limit as TP increases, $\delta^{15}\text{N}_{\text{base}}$ represents the isotope value for the baseline organism, $\delta^{15}\text{N}_{\text{TP}}$ is the consumer isotope value at a given TP, k is the rate at which $\delta^{15}\text{N}_{\text{TP}}$ approaches $\delta^{15}\text{N}_{\text{lim}}$ per TP step. TP_{base} is the trophic position of the consumer used to define $\delta^{15}\text{N}_{\text{base}}$ (i.e., TP = 2 in this study). The model to solve TP needs to calculate the values of $\delta^{15}\text{N}_{\text{lim}}$ and k , which are concluded by a meta-analysis by Hussey et al. (2014) of the following formula:

$$k = -\log\left(\frac{\beta_0 - \delta^{15}\text{N}_{\text{lim}}}{-\delta^{15}\text{N}_{\text{lim}}}\right) \quad (6)$$

$$\delta^{15}\text{N}_{\text{lim}} = \frac{-\beta_0}{\beta_1} \quad (7)$$

Intercept β_0 and slope β_1 characterize the change in $\Delta^{15}\text{N}$ as dietary $\delta^{15}\text{N}$ values increase. After the meta-analysis by Hussey et al. (2014), $\beta_0 = 5.92$ [4.55, 7.33], $\beta_1 = -0.27$ [-0.41, -0.14] were used to calculate TP (See Supplementary Materials S4 and S5 in Hussey et al., 2014). The TP of species was calculated by the above two methods, respectively, and the influence of their differences on the TMFs was analyzed.

The experiment results were analyzed using SPSS 19.0, R 4.1.2, and the independent-samples *T*-Test was carried out to test the difference of nitrogen isotopes between the same species in Xingkai Lake.

3. Results

3.1. Comparison of trophic positions assessment based on two different trophic frameworks and baselines

3.1.1. Isotope values for different trophic levels based on two frameworks and curve fitting

Based on the additive $\Delta^{15}\text{N}$ framework and scaled $\Delta^{15}\text{N}$ framework, we calculated values of $\delta^{15}\text{N}$ for different trophic positions, and further performed curve fitting of isotopes and trophic positions under different frameworks. The overall trend and correlation of the results of the two frameworks for each lake were similar (Fig. 1). The White shrimp, as a typical primary consumer, was the dominant species in both Xingkai Lake and Small Xingkai Lake. The trophic position of White shrimp was set as 2.00. According to the isotope analysis, the $\delta^{15}\text{N}$ value of the White shrimp was 10.92 ± 0.90 in Small Xingkai Lake, and 9.08 ± 0.87 in Xingkai Lake. The difference between the two groups was significant ($t = -4.41$, $p = 0.00$). In general, since the $\delta^{15}\text{N}$ value of the baseline species in Small Xingkai Lake was higher than that of Xingkai Lake, the nitrogen isotope value corresponding to the same trophic position (TP > 2) of Small Xingkai Lake were higher than the corresponding ones of Xingkai Lake no matter under which framework.

Even in the same lake, the nitrogen isotope values corresponding to the same trophic position under different frameworks were not the same. In Small Xingkai Lake, the $\delta^{15}\text{N}$ value of TL3-TL6 under scaled $\Delta^{15}\text{N}$

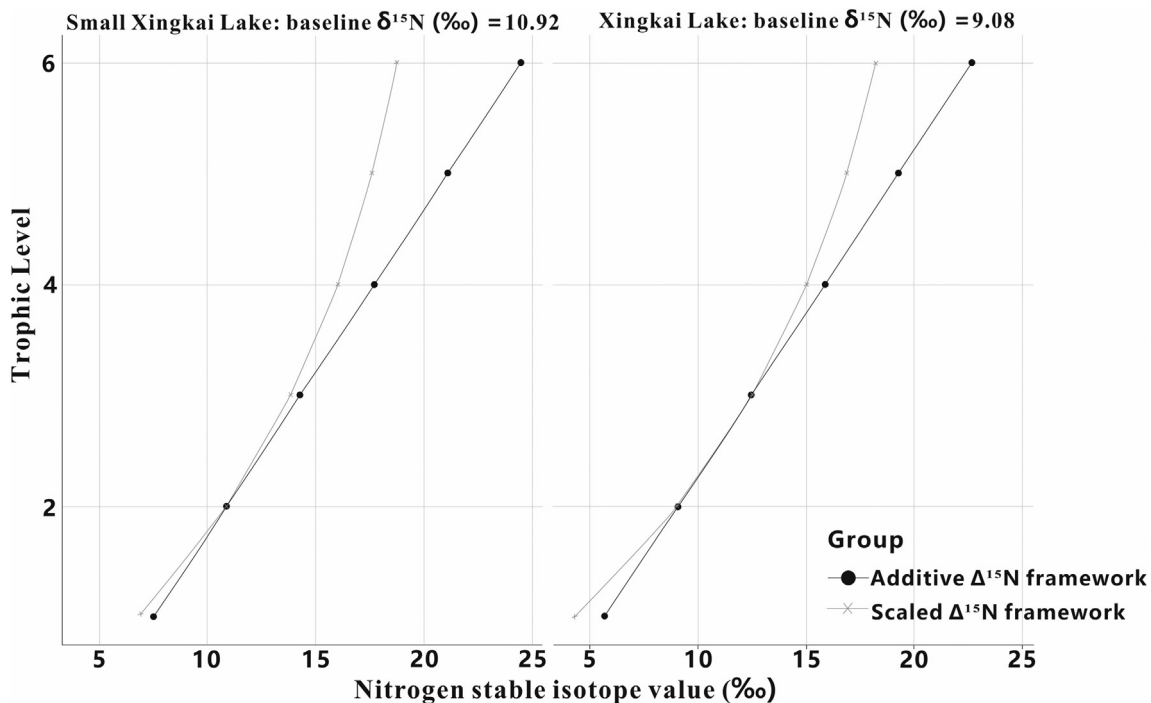


Fig. 1. Comparison of the nitrogen isotope of different trophic levels in Small Xingkai Lake and Xingkai Lake under two frameworks.

Table 2
Trophic position and total mercury content of fish species in Small Xingkai Lake.

Species (Number)	Abbreviation	TP _{scaled}	TP _{additive}	THg(ppb)
White shrimp (9)	Em	2.00 ± 0.09	2.00 ± 0.88	15.94 ± 1.52
Amur weatherfish (26)	Mm	2.09 ± 0.07	2.08 ± 0.59	34.95 ± 7.98
Spotted steed (5)	Hm	2.39 ± 0.18	2.34 ± 0.16	126.54 ± 22.06
Clearhead icefish (6)	Pc	2.49 ± 0.19	2.42 ± 0.16	119.04 ± 5.38
Ussuri catfish (34)	Pu	2.79 ± 0.08	2.66 ± 0.07	197.27 ± 36.46
Crucian carp (7)	Ca	2.83 ± 0.41	2.70 ± 0.32	175.86 ± 43.40
Sharpbelly (17)	Hl	2.88 ± 0.15	2.69 ± 0.12	99.20 ± 25.99
Amur pike (7)	Er	5.03 ± 0.23	3.91 ± 0.10	922.86 ± 163.14
Northern snakehead (8)	Cs	5.56 ± 0.23	4.10 ± 0.09	1404.36 ± 408.31
Amur sleeper (4)	Pg	6.11 ± 0.48	4.26 ± 0.15	755.00 ± 36.46

framework was smaller than the corresponding $\delta^{15}\text{N}$ value under additive $\Delta^{15}\text{N}$ framework. In Xingkai Lake, the $\delta^{15}\text{N}$ value of the TL4-TL6 under scaled $\Delta^{15}\text{N}$ framework was smaller than that of the additive $\Delta^{15}\text{N}$ framework. The deviation of the $\delta^{15}\text{N}$ value of the same trophic position between the two frameworks increased with the trophic position (Fig. 1). The stable nitrogen isotope values of fish in Small Xingkai Lake and Xingkai Lake is also provided in the supporting information (Fig. S2).

3.1.2. Trophic position and total mercury content of species in small Xingkai Lake and Xingkai Lake under two different frameworks

In Small Xingkai Lake, the range of TP_{scaled} was 2.00–6.11 and the range of TP_{additive} was 2.00–4.26 (Table 2). Amur sleeper had the highest trophic position in Small Xingkai Lake, but the content of THg was not the highest; its average TP_{scaled} was 6.11 ± 0.48 , which was higher than that of TP_{additive} (4.26 ± 0.15). Northern snakehead and Amur pike, which are also carnivorous fish, had relatively high trophic positions and also a high content of total mercury. Sharpbelly, Crucian carp, and Ussuri catfish, which are omnivorous fish, had relatively low trophic positions and also a relatively low content of total mercury. In Small Xingkai Lake, all TP_{scaled} values were greater than TP_{additive} values except for the baseline species (White shrimp). Individuals with higher trophic positions might not necessarily have higher content of total mercury.

In Xingkai Lake, the range of TP_{scaled} was 2.00–5.97, the range of TP_{additive} was 2.00–4.61 (Table 3), and the trophic position of individuals between TP2 to TP3 were closer under the two frameworks. Topmouth culter was the top carnivorous and also had the highest content of THg; its average TP_{scaled} was 5.97 ± 0.28 , which was higher than the corresponding TP_{additive} (4.61 ± 0.11). However, for fishes higher than TP2, not all TP_{scaled} was greater than TP_{additive} in Xingkai Lake. In terms of total mercury, the content of total mercury of the same species in Xingkai Lake was lower than that of Small Xingkai Lake.

3.2. Comparison of TMFs assessment based on different trophic frameworks and baselines

As shown in Fig. 2, we compared the TMFs of Small Xingkai Lake and Xingkai Lake under two different frameworks, using the baselines of Small Xingkai Lake and Xingkai Lake, respectively, both of which are White shrimp but with different values of nitrogen isotope. Except for the TMFs c_2 (1.70, TMFs of Small Xingkai Lake) and TMFs d_1 (2.40, TMFs of Xingkai Lake), which were recommended TMFs based on their respective baseline and scaled $\Delta^{15}\text{N}$ framework, the rest were assumed scenarios,

Table 3
Trophic position and total mercury content of fish species in Xingkai Lake.

Species (number)	Abbreviation	TP _{scaled}	TP _{additive}	THg(ppb)
White shrimp (9)	Em	2.00 ± 0.08	2.00 ± 0.86	12.07 ± 2.93
Diving beetle (5)	De	2.31 ± 0.10	2.33 ± 0.11	37.23 ± 0.83
Clearhead icefish (5)	Pc	2.52 ± 0.18	2.52 ± 0.18	104.72 ± 9.18
Amur bitterling (7)	Rs	2.56 ± 0.13	2.57 ± 0.13	98.54 ± 24.40
Crucian carp (23)	Ca	2.62 ± 0.05	2.63 ± 0.04	45.01 ± 14.49
Topmouth culter (6)	Ei	5.97 ± 0.28	4.61 ± 0.11	900.42 ± 119.57

including the misuse of the trophic baseline (e.g.: TMFs a_1 ; TMFs b_2 ; TMFs c_1 ; TMFs d_2), that is, although the two lakes are connected, the baselines are indeed different. Under the additive $\Delta^{15}\text{N}$ framework, there was basically no difference in TMFs between the two lakes. In comparison, the contrast of TMFs in the two lakes by using the baseline of Small Xingkai Lake ($\delta^{15}\text{N} = 10.92$) under the scaled $\Delta^{15}\text{N}$ framework was significant, which can be seen in Fig. 2C. However, as shown in Fig. 2, the TMFs are clearly above 1 which indicates biomagnification of mercury of in both lakes, and the TMFs of Xingkai Lake was greater than that of Small Xingkai Lake in any scenario.

4. Discussions

The results have shown that the determination of trophic position is an essential basis for TMFs determination, including: 1) determination of the baseline species; 2) determination of $\Delta^{15}\text{N}$. This study mainly explored the impact of baseline and $\Delta^{15}\text{N}$ on the assessment of trophic position, and then discussed the possible changes of TMFs for different baselines and $\Delta^{15}\text{N}$. We selected the White shrimp distributed in both Xingkai Lake and Small Xingkai Lake as the baseline organism (TP = 2). As the $\delta^{15}\text{N}$ value of the White shrimp (baseline organism) was different in the two lakes, we assessed trophic position based on different $\delta^{15}\text{N}$ values of baselines of the two lakes. As to the $\Delta^{15}\text{N}$, we compared the results of the trophic position between the scaled $\Delta^{15}\text{N}$ framework and the additive $\Delta^{15}\text{N}$ framework, then compared the TMFs based on the total mercury bioaccumulation. We have suggested the complete and scientific expression of TMFs, which will facilitate comparison among related studies.

4.1. Recommending independent and suitable baseline species for the evaluation of TMFs, even for the same species in connected ecosystems

The selection of baseline organisms and the accuracy of baseline isotopes are crucial to infer and analyze ecological issues at different levels and scales (Vander Zanden and Fetzer, 2007; Vander Zanden et al., 1999). For this study, changes in baseline directly affected trophic position, which in turn affected the assessment of TMFs. The selection of baseline species requires several factors to be satisfied simultaneously, which include having a stable diet, widespread existence, and no apparent temporal and spatial fluctuations (Post, 2002).

Early studies mostly used the $\delta^{15}\text{N}$ of zooplankton as the baseline for lake planktonic food webs (Post, 2002; Cabana and Rasmussen, 1994; Matthews and Mazumder, 2005). However, the stable isotope composition of different zooplankton species varies significantly (Kling et al., 1992; Grey et al., 2001); there were also significant seasonal variations of isotopes in different zooplankton species (Matthews and Mazumder, 2005; Leggett et al., 2000). Some scholars suggested that the $\delta^{15}\text{N}_{\text{base}}$ and $\delta^{13}\text{C}_{\text{base}}$ should be quantified by using long-lived primary consumers in the aquatic ecosystem. The reason is that the temporal variance of long-lived primary consumers' isotopic signature is much lower (Cabana and Rasmussen, 1996). Long-lived primary consumers provide a more suitable baseline for the need to have quantitative estimates of trophic position (Post et al., 2000). Therefore, in this study, we chose the White Shrimp as the baseline species,

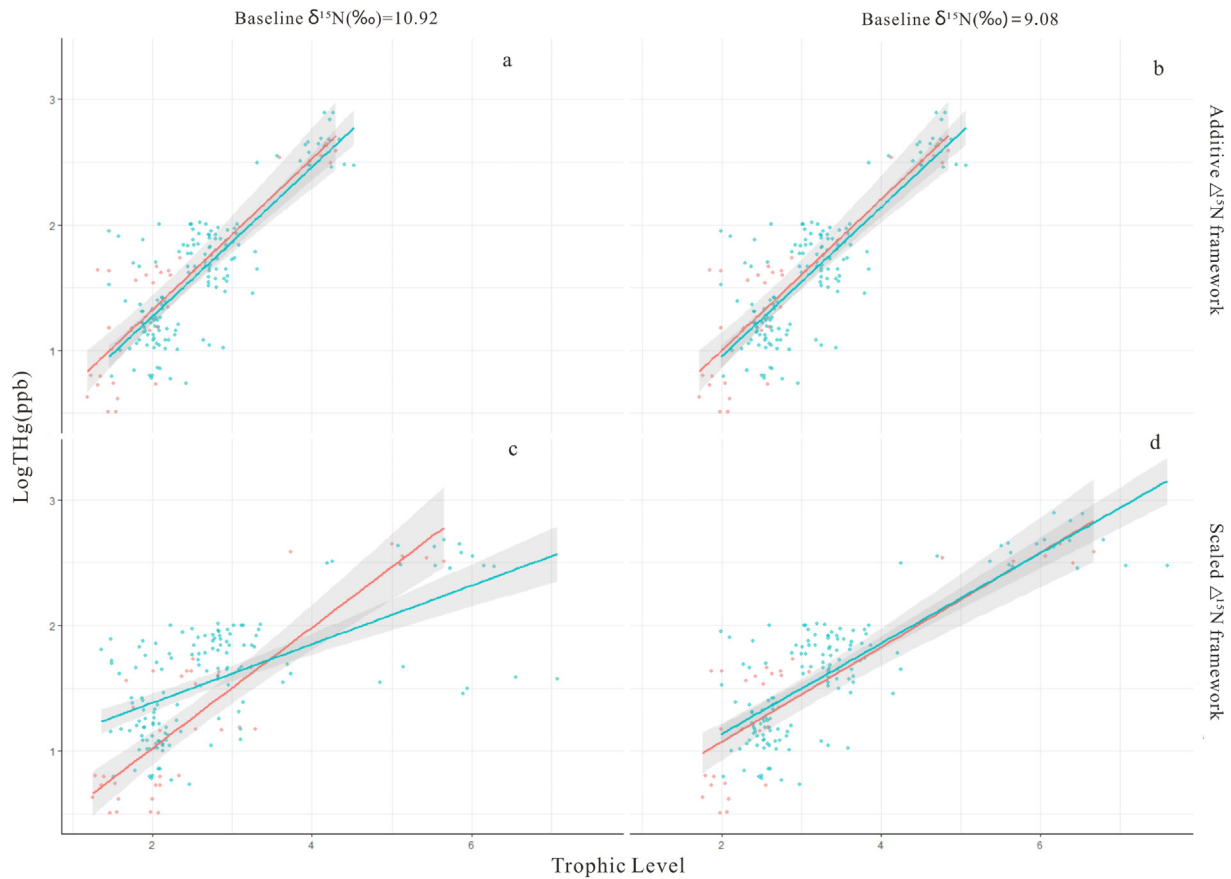


Fig. 2. TMFs of Small Xingkai Lake (Green line) and Xingkai Lake (Red line) in different scenarios (Two different baseline isotope values and two different trophic position frameworks). (a: $F = 0.41, p = 0.66$; b: $F = 0.41, p = 0.66$; c: $F = 14.40, p = 0.00$; d: $F = 0.35, p = 0.70$; Based on scaled $\Delta^{15}\text{N}$ framework and separate baseline species: $F = 7.24, p = 0.00$).

Hypothetical Scenario a₁: TMFs a₁ (Red line of facet 'a') = 3.98, $R^2 = 0.78, F = 108.80, p = 0.00$; TMFs of Xingkai Lake with Small Xingkai Lake baseline under additive $\Delta^{15}\text{N}$ framework;

Hypothetical Scenario a₂: TMFs a₂ (Green line of facet 'a') = 3.89, $R^2 = 0.67, F = 292.70, p = 0.00$; TMFs of Small Xingkai Lake with Small Xingkai Lake baseline under additive $\Delta^{15}\text{N}$ framework;

Hypothetical Scenario b₁: TMFs b₁ (Red line of facet 'b') = 3.98, $R^2 = 0.78, F = 108.80, p = 0.00$; TMFs of Xingkai Lake with Xingkai Lake baseline under additive $\Delta^{15}\text{N}$ framework;

Hypothetical Scenario b₂: TMFs b₂ (Green line of facet 'b') = 3.89, $R^2 = 0.67, F = 292.70, p = 0.00$; TMFs of Small Xingkai Lake with Xingkai Lake baseline under additive $\Delta^{15}\text{N}$ framework;

Hypothetical Scenario c₁: TMFs c₁ (Red line of facet 'c') = 3.02, $R^2 = 0.74, F = 100.20, p = 0.00$; TMFs of Xingkai Lake with Small Xingkai Lake baseline under scaled $\Delta^{15}\text{N}$ framework;

Scenario c₂: TMFs c₂ (Green line of facet 'c') = 1.70, $R^2 = 0.39, F = 87.95, p = 0.00$;

TMFs of Small Xingkai Lake with Small Xingkai Lake baseline under scaled $\Delta^{15}\text{N}$ framework;

Scenario d₁: TMFs d₁ (Red line of facet 'd') = 2.40, $R^2 = 0.74, F = 86.39, p = 0.00$;

TMFs of Xingkai Lake with Xingkai Lake baseline under scaled $\Delta^{15}\text{N}$ framework;

Hypothetical Scenario d₂: TMFs d₂ (Green line of facet 'd') = 2.29, $R^2 = 0.66, F = 283.60, p = 0.00$; TMFs of Small Xingkai Lake with Xingkai Lake baseline under scaled $\Delta^{15}\text{N}$ framework.

which is the best choice. On the one hand, from the point of view of feeding, White shrimp in Xingkai Lake is different from other predatory shrimp, as its feeding habits are relatively simple. Being an omnivorous animal, the White shrimp feeds on zooplankton, plant detritus, and algae all its life (Niu, 2018). According to the latest literature records, the white shrimp in the Xingkai Lake area mainly feeds on microalgae, with no complicated feeding situation (Yuan, 2018). On the other hand, White shrimp exists as a dominant species on both sides of Xingkai Lake dam all year round, which is the main food for many predatory fish (Yuan, 2018). So from this point of view, White shrimp is more practical as the baseline species (TP = 2). More importantly, studies have confirmed that White shrimp can be a suitable baseline species for the food web in the Xingkai Lake area (Yuan, 2018). From these aspects, although there is no perfect TP2 in aquatic ecosystems because of its complexity and changeability, the white shrimp is the closest and the best option for baseline species in Xingkai Lake. However, although

we believe that the selection of the White Shrimp as baseline species is already the optimal choice, in other words, the nitrogen isotope value of the White Shrimp being low enough, there were still cases where the TP of several fish is less than 2 in this study. We acknowledge the fact, and at the same time, we know that because of the complex omnivorous problem in the aquatic ecosystem, when we choose natural organisms as the baseline species, such issues will arise, which is difficult to take both into account. We believe the 'selection of baseline species in the food chain' is a scientific issue well worth discussing, which will also serve as a crucial starting point for our subsequent research.

The baseline is essential for assessing the trophic position, as an increase of a baseline means a rise of $\delta^{15}\text{N}$ for each trophic position and vice versa. Therefore, the baseline determination affects the assessment of trophic position and, thus, the TMFs. It can also be drawn from several hypothetical scenarios in this study that the results of TMFs calculated by using different

baseline nitrogen isotopes under the same trophic framework and in the same lake are not the same. In the process of calculating the trophic positions and the TMFs of each species in these two lakes, we used different $\delta^{15}\text{N}$ of White Shrimp from Small Xingkai Lake and Xingkai Lake separately as the baseline. Since the nitrogen isotope of baseline species can be influenced by many environmental conditions like eutrophication, freshwater or saltwater ecosystems (Bryan et al., 2012), and the concentration of some pollutants (Anderson and Cabana, 2005), etc., the nitrogen isotope value of baseline species in Small Xingkai Lake (10.92) was significantly greater than that of Xingkai Lake (9.08). According to related research, Small Xingkai lake is more eutrophic than Xingkai lake (Yu et al., 2015). Small Xingkai Lake has been in a state of mild eutrophication and severe swamping (Yu et al., 2015). The total nitrogen in the water of Small Xingkai Lake is higher than that of Xingkai Lake (Ji et al., 2013; Yu et al., 2015), which may be the reason of White shrimp in Small Xingkai Lake has a higher nitrogen isotope than that in Xingkai Lake. There is also similar phenomenon in southern China. Taihu Lake is more eutrophic than Chaohu Lake. And those fish of the same species from Taihu Lake have significantly elevated $\delta^{15}\text{N}$ values (4.3 ‰) compared with those from the less eutrophic Chaohu Lake, indicating that the isotopic signature might reflect the trophic situation of their habitats (Xu et al., 2008). In addition, although the two lakes are separated by only a natural dam, hydrological connectivity strongly influences biodiversity and the stability of the interconnected aquatic habitats (Pringle, 2003; Yuan et al., 2018; Casanova et al., 2009). Lavoie et al. (2013) emphasize that organisms from the disconnected system, migratory ones, or those that are not linked to similar carbon sources should not be pooled together. Therefore, even if Small Xingkai Lake and Xingkai Lake have a hydrological connection and have the same dominant species, White shrimp, the nitrogen isotope values of their respective baselines should be used separately for different food chains as the eutrophication may influence the nitrogen isotope of baseline species and will have an impact on assessing the TMFs in our hypothetical scenario. That is, when representing biomagnification in terms of the TMFs, eutrophication did affect biomagnification. In addition, we think eutrophication is a factor that deserves more attention for its influences on the TMFs. Few studies have explored the effect of trophic status on contaminant biomagnification in lakes (Poste et al., 2015), and relevant studies have produced contradictory results, leading to a heated debate (Verburg et al., 2014; Clayden et al., 2014). Researchers who have used chlorophyll *a* as an indicator of trophic status in water, have shown that in these tropical African lakes, which have high levels of primary productivity and phytoplankton biomass, the biomagnification is relatively lower. That indicates, mercury biomagnification may become moderate by trophic status of lakes (Poste et al., 2015). Therefore, in the subsequent research, it is recommended to strengthen the relevant research on the effects of eutrophication on TMFs assessment in aquatic ecosystems.

4.2. Recommending scaled $\Delta^{15}\text{N}$ framework rather than additive $\Delta^{15}\text{N}$ framework for TMFs assessment

Our results have methodological implications for determining trophic positions and TMFs in polluted environments, where elevated $\delta^{15}\text{N}$ values will translate into over or under estimated trophic positions and biased estimated TMFs (Ek et al., 2015).

Considering the two types of frameworks, we recommend using the scaled $\Delta^{15}\text{N}$ framework for calculating TMFs. There are four main reasons: 1) the scaled $\Delta^{15}\text{N}$ framework has improved the accuracy of trophic estimates (Hussey et al., 2014), so it will help improve the quality of TMFs; 2) the additive $\Delta^{15}\text{N}$ framework was concluded from 56 case studies (Post, 2002), while the scaled $\Delta^{15}\text{N}$ framework was confirmed by not only laboratory studies but also meta-analysis, which is more reliable (Caut et al., 2008; Overmyer et al., 2008; Hussey et al., 2014); 3) based on our results, as can be seen from Table S2 in the supporting information, TP3-TP4 started to be less than 3.4 ‰, $\Delta^{15}\text{N}$ decreases with the increasing $\delta^{15}\text{N}$. This means that for the same organism, especially high-trophic organisms, TP_{scaled} was higher than TP_{additive}. For mercury bioaccumulation, due

to scaling and resulting higher estimated TP for a given $\delta^{15}\text{N}$, the resulting TMFs will be lower under the scaled $\Delta^{15}\text{N}$ framework, than in addition, as the additive is an exponential function. It is a massive improvement in accurately evaluating TMFs, which is similar to the study of Li et al. (2022); 4) under the additive $\Delta^{15}\text{N}$ framework, there was basically no difference in TMFs between the two lakes. However, differences in TMFs between the two lakes were better assessed under the scaled $\Delta^{15}\text{N}$ framework. On the whole, the scaled $\Delta^{15}\text{N}$ framework respects the regular variation of $\Delta^{15}\text{N}$ and improves on the original basis. Therefore, we think it is a significant improvement by taking into account a lot of factors that have not been considered before. Considering the complexity of the carbon sources issues, we collected samples both in the littoral zone of Xingkai Lake and Small Xingkai Lake (on both sides of natural dams between lakes), which means our sampling of the two lakes was carried out both in the littoral ecosystem, have the similar carbon source. In the meantime, studies have shown that the scaled method was not sensitive to the influence of incorporating carbon source in the model (Nawrocki et al., 2020). Therefore, the issue of carbon source may have little effect on this study. Anyway, $\delta^{13}\text{C}$ is used to determine the sources of carbon for different consumers. As an important aspect, carbon sources is also a key question which we need to focus on in our future research.

According to the TMFs of these two Lakes, the bioaccumulation of THg of Xingkai Lake is higher than that of Small Xingkai Lake. The TMFs of Small Xingkai Lake and Xingkai Lake were 1.70 and 2.40, respectively. Considering the factors mentioned above, we suggest that the comprehensive expression is ‘TMFs of THg of Xingkai lake = 2.40, baseline ($\delta^{15}\text{N}$) = 9.08, trophic position range (2.00-5.97)’. This way, TMFs of different regions or times can be compared and summarized effectively.

As the interaction between the food chain and pollution is complicated (Garay-Narváez et al., 2014), more research are needed to explore factors that influence TMFs. There are two main aspects in the study, factors of organism itself and environmental factors. For example, lipid-water partition coefficients will affect the fractionation of nitrogen isotope; besides, the form of biodiversity and the complexity of interaction networks are essential to understand the effects of pollution and other ecosystem threats (Garay-Narváez et al., 2013). Therefore, to have a better knowledge of the improvement of TMFs, a deep understanding of the food web complexity is needed.

Factors like species, individual feeding habits, and latitude will influence the THg accumulation of individuals inevitably (Coelho et al., 2013; Lavoie et al., 2013) and then affect the evaluation of TMFs. Some top predators, such as sharks or bass, reside and migrate in multiple ecosystems, which may make the determination of trophic position and bioaccumulation uncertain (Ackerman et al., 2015; Ramos and González-Solís, 2012; Post, 2002; Kobayashi et al., 2015). The possible reasons include individual sharks feeding as generalists, high overlap in THg among shark prey, and differences in turnover time between ecological tracers and THg (McMeans et al., 2015). In Small Xingkai Lake, the trophic positions of predators also could not wholly explain intra-species or inter-species THg accumulation. The possible reasons may be similar to that of Greenland sharks, such as individual feeding habits and the complexity of the food web. In addition, organism size, ability to biotransform chemicals, reproductive status, and other factors could also influence the TMFs (Borgå et al., 2012). For example, the thermal group has always been viewed as an essential factor affecting the regression of TMFs. TMFs calculated from regressions including the entire food web consisting of both poikilotherms and homeotherms may overestimate the biomagnification of poikilotherms, and underestimate that of homeotherms (Fisk et al., 2001; Hop et al., 2002). So to better understand the THg accumulation of different individuals in the future, it is essential to strengthen the research of food chain structure based on individuals (Forero et al., 2005).

In terms of environmental factors that influence TMFs (Kidd et al., 2012), bioaccumulation of mercury of total mercury and methylmercury is positively correlated with latitude (Lavoie et al., 2013; Walters et al., 2016). THg and MeHg concentrations at the base of the food chain are higher in lakes than in marine environments (van der Velden et al.,

2013). So, we suggest that TMFs reports are necessary to provide a description of related environmental conditions and even biological physiological conditions in the future.

CRedit authorship contribution statement

Qiang Wang: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition. **Xingchun Li:** Writing – review & editing, Visualization. **Xuehong Zhou:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.160095>.

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