



Importance of accurate trophic level determination by nitrogen isotope of amino acids for trophic magnification studies: A review

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ARTICLE INFO

Article history:

Received 13 December 2017

Received in revised form

13 March 2018

Accepted 14 March 2018

Keywords:

Biomagnification

Trophic magnification factors

Metals

POPs

Trophic levels

CSIA-AAs

ABSTRACT

During the last several decades, persistent organic pollutants and metals cause great concern for their toxicity in organisms as well as for their bioaccumulation and/or trophic transfer through the food chains in ecosystems. A large number of studies therefore have focused on the trophic levels of organisms to illustrate food web structure, as a critical component in the study of pollutant dynamics and biomagnification. The trends in biomagnification of pollutants in food webs indeed provide fundamental information about the properties and fates of pollutants in ecosystems. The trophic magnification supports the establishment of a reliable trophic structure, which can further aid the understanding of the transport and exposure routes of contaminants in accumulation and risk assessments. Recently, efforts to interpret the food web structure using carbon and nitrogen stable isotope ratios have contributed to better understanding of the fate of pollutants in the ecosystem. However, it is known that this isotope analysis of bulk ones has many weaknesses, particularly for uncertainties on the estimate of trophic levels and therefore of magnification factors for studied organisms, enough to support a regulatory interpretation. In this review, we collate studies that investigated biomagnification characteristics of pollutants in aquatic ecosystems, along with calculated trophic magnification factors. Moreover, we introduce a novel approach, compound-specific stable isotope analysis of nitrogen in amino acids, to establish reliable food web structures and accurate trophic levels for biomagnification studies. This method promises to provide sound results for interpreting the influence of the pollutant in organisms, along with their bioaccumulation and magnification characteristics, as well as that in ecosystem.

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1. Introduction

One of the significant issues relating to the environmental pollution studies would be characterizing the risks of pollutants to organisms, particularly humans and other biota at the top of the food chain (Mackay et al., 2016; Ross and Birnbaum, 2003; Zenker et al., 2014). Those concerns are based on the fact that pollutants or xenobiotics can accumulate in organisms and be transferred through the food chain. In the middle of the 20th century, the cases observed in the book *Silent Spring* by Rachel Carson (dichloro

diphenyl trichloroethane, DDT) and the presence of Minamata disease in Japan (mercury, Hg) showed the repercussions of bioaccumulation and magnification as xenobiotics were transferred to the top predators in a food web. With increasing recognition of the dietary pathways that determine body burden, bioavailability, and the effects of pollutants in marine organisms, estimates of bioaccumulation of pollutants and their transfer in the food web (trophic transfer) have received much attention (Wang and Fisher, 1999; Wang and Rainbow, 2008). Biomagnification is the process by which organisms with high trophic levels (TLs) show greater concentrations of a pollutant than those seen at the source (Russell et al., 1999; Walters et al., 2011; Wang et al., 2017). The behavioral properties of pollutants in the environment and in organisms are important because accumulation and trophic transfer might enhance the risks to an ecosystem by increasing bioavailability,

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even if not all forms of a pollutant are directly associated with toxic results. Thus, it is generally considered important to explain the fate of pollutants in the environment by describing their properties, particularly on bioavailability, persistence, and degradability (Alava and Gobas, 2012; Zenker et al., 2014). The results of some studies on pollutants that have transferred through a food chain are used as data to predict hazards to the ecosystem and humans (Alava and Gobas, 2012; Franklin, 2016; Ross and Birnbaum, 2003; Zenker et al., 2014). Furthermore, whether a particular pollutant is magnified or diluted in the food chain is a significant factor in setting management priorities, although uncertainties in those determinations remain an issue (Mackay et al., 2016).

Many studies have shown a clear route for pollutants in a food chain by showing the trophic relationships with accumulated concentrations, and they have done so using several approaches (Barwick and Maher, 2003; Fortibuoni et al., 2013; Munoz et al., 2017; Walters et al., 2011; Watanabe et al., 2008; Zhou et al., 2016). The biomagnification factors presented in those studies are based on information about structures between predator and prey relations, which allow predictions about whether or not a particular pollutant can be transferred and magnified through a particular food chain (Connolly and Pedersen, 1988; Oliver and Niimi, 1988). Thus, reliable food chains and the TL of each organism are the most important background information needed to demonstrate biomagnification in an ecosystem. In most biomagnification studies, the accumulation of exogenous materials (e.g., pollutants) is determined based on concentrations in various organisms and the TL of those organisms in their ecosystem (Barwick and Maher, 2003; Fortibuoni et al., 2013; Munoz et al., 2017; Walters et al., 2011; Watanabe et al., 2008; Zhou et al., 2016). In an early study on the food web structures in aquatic environments, Issacs (1973) suggested that little biomagnification was observed in marine ecosystems because the food chain was more unstructured than that in terrestrial ecosystems, say with a wide range of available prey of various species and ages and with relatively great variations in concentrations and distributions of pollutants in a dynamic aquatic system. However, more recently, many examples of the biomagnification of pollutants in marine environments have been increasingly evidenced from the TL analyses; such as Hg (Atwell et al., 1998), hexachlorobenzene (HCB), DDTs, and polychlorinated biphenyls (PCBs) (Corsolini and Sara, 2017), butyltins (Fortibuoni et al., 2013), organochlorine compounds (Hoekstra et al., 2003), Cu and Zn (Jara-Marini et al., 2009), and polybrominated diphenyl ethers (PBDEs) (Kelly et al., 2008).

One classic methodology for estimating TL analyzes the concentrations of cesium (Cs) and potassium (K), because K, an essential element, is fairly constant in tissue, whereas Cs occurs in organisms only by accumulation (Campbell et al., 2005; Young and Mearns, 1979). Other studies used an approach that recognizes nutritional levels using the stable isotope ratio for nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) (Peterson and Fry, 1987). The method using $\delta^{15}\text{N}$ for trophic analysis was extended and strengthened researchers' ability to estimate TL in complex ecosystems (Peterson and Fry, 1987). Since the 1980s, the analysis of $\delta^{15}\text{N}$ value has become a powerful tool for estimating prey-predator relationships in ecology and has been widely utilized to determine biomagnification. For example, one previous study reported that the results based on $\delta^{15}\text{N}$ values could reveal more reliable connections among the organisms in pelagic ecosystem compared to other methods (Hansson et al., 1997). They also demonstrated that $\delta^{15}\text{N}$ -based interpretation can determine the complex diets of zooplankton and mysids (Hansson et al., 1997). This approach has broadened to include stable isotope ratio and specific compounds in organisms that can provide more specific interpretations when establishing TLs (Chikaraishi et al., 2014; Sackett et al., 2015).

Here, we reviewed recent progress in studying the bioaccumulation and biomagnification of pollutants in aquatic environments. The parameters affecting bioaccumulation and trophic transfer are also discussed for further understanding. In particular, we consider research on trophic position estimates by use of ^{15}N stable isotope ratio, emphasizing the implications of TL analyses when studying biomagnification in a diverse ecosystem.

2. Case studies on the biomagnification of organic chemicals and metals

Biomagnification is the increasing concentration of toxic chemicals (e.g., persistent organic pollutants (POPs) and metals) in the tissues of organisms at successively higher TLs in a food web (Fig. 1). A biomagnification potential of pollutants is one of the important indices when evaluating its biological persistence and prioritizing chemicals of concern in the environment (Borgå et al., 2012). The biomagnification factor (BMF) and trophic magnification factor (TMF) are commonly used to assess the biomagnification potential of toxic organic chemicals (Franklin, 2016). The BMF represents the relative increase in concentration of a contaminant in single predator–prey relationships, whereas the TMF is the average factor of change in concentration of a contaminant per TL across a whole food chain. TMF is generally held to be the most reliable tool for assessing the magnitude of biomagnification (Borgå et al., 2012; Gobas et al., 2009). TMF values can be calculated from the regression slope (b) between TLs and the log concentrations of contaminants in the organisms ($\text{TMF} = 10^b$) (Borgå et al., 2012; Franklin, 2016) (Fig. 1). In the respect to organic pollutants, Gobas et al. (2009) demonstrated that if the TMF value > 1 , the corresponding chemical is biomagnified through the food chain. Bioaccumulative (B) characteristic is one of the significant factor for categorizing priority substances together with persistent (P) and toxic (T) features. In the Stockholm Convention on POPs and other national risk assessment programs (e.g., European Commission), many thousands of commercial chemicals have been evaluated based on P, B, and T-assessment (UNEP, 2001). In this context, the TMF value can be used as a useful criterion for assessing whether a substance is “bioaccumulative” in the outline of B-assessment framework (Gobas et al., 2009). However, TMFs should be used and applied with caution because their values are affected by various abiotic and biotic factors in the given system (Borgå et al., 2012; Franklin, 2016).

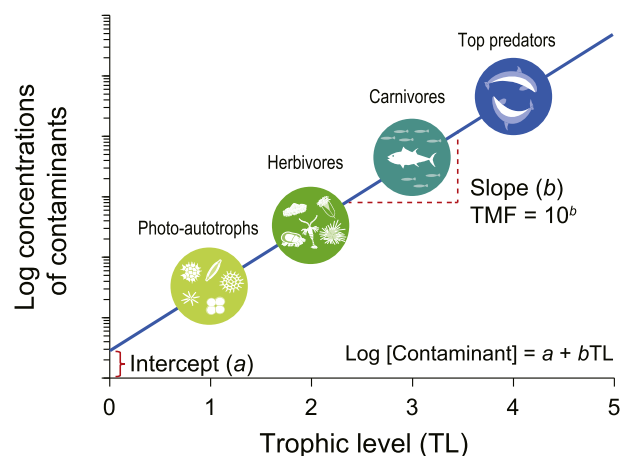


Fig. 1. A scheme of biomagnification and calculation of the trophic magnification factor (TMF) (modified from Borgå et al., 2012).

2.1. Biomagnification of toxic organic chemicals in aquatic food webs

For this section, we collected and reviewed 20 previously reported biomagnification studies that focused on determining the TLs of organisms and the TMF values of various toxic organic chemicals in aquatic ecosystems (Corsolini and Sara, 2017; Fortibuoni et al., 2013; Gu et al., 2016; Helm et al., 2008; Hoekstra et al., 2003; Houde et al., 2006, 2008; Kelly et al., 2008; Kobayashi et al., 2015; McGoldrick et al., 2014; Munoz et al., 2017; Powell et al., 2017; Shao et al., 2016; Su et al., 2017; Sun et al., 2017; Verhaert et al., 2017; Walters et al., 2011; Wang et al., 2012, 2017; Zhou et al., 2016) (Table 1 and Tables S1–S2 of the Supplementary Materials). All of these studies measured TLs using the $\delta^{15}\text{N}$ values, which is based on the principle of isotopic fractionation; the heavier nitrogen isotope (^{15}N) is generally enriched by a consumer's diet (Peterson and Fry, 1987). The $\delta^{15}\text{N}$ values in the tissues of organisms were converted to TLs using trophic discrimination factors (TDFs), which vary between 3.0 and 3.8‰ of $\delta^{15}\text{N}$ (Minagawa and Wada, 1984). The calculated TLs were then used as x-axis values to measure the TMF values (Fig. 1). In some case studies, the $\delta^{15}\text{N}$ values were used directly to measure the TMF values without calculating the TL (Helm et al., 2008; Houde et al., 2008). As concentration units of the contaminants, lipid weight or wet weight was used as the y-axis values in calculating the TMF values (Table 1), which varied from 0.1 to 6.3 among contaminants. Perfluorooctanoic acid (PFOA), *trans*-nonachlor, perfluorooctane sulfonamide (PFOSA), *p,p'*-DDE, dibutyltin (DBT), and hepta-chlorinated biphenyls (CBs) showed the greatest TMF values (TMF > 4) (Fortibuoni et al., 2013; Hoekstra et al., 2003; Houde et al., 2006; Verhaert et al., 2017; Walters et al., 2011), indicating that those chemicals appear to have great biomagnification potential in food webs. Chemicals with smaller TMFs (TMF < 1), such as *cis*-chlordane, γ -hexachlorocyclohexane (γ -HCH), perfluorododecanoic acid (PFDoA), perfluorohexane sulfonic acid (PFHxS), penta- and hexa-brominated diphenyl ethers (BDEs), volatile methylsiloxanes (VMSSs), and short- and medium chain chlorinated paraffins (SCCPs and MCCPs) (Hoekstra et al., 2003; Houde et al., 2006, 2008; Kelly et al., 2008; McGoldrick et al., 2014; Sun et al., 2017) were also found, suggesting that those chemicals did not biomagnify in food webs.

The octanol/water (K_{OW}), octanol/air (K_{OA}), and air/water (K_{AW}) partition coefficients of toxic organic chemicals (Table 1) were collected and compared to the corresponding TMF values (Fig. 2 and Table S2). The results indicate that chemicals with about a $\log K_{OA} > 6$ and a $\log K_{OW}$ between 4 and 8 generally biomagnify in aquatic food webs (TMF > 1). In particular, high TMF values (TMF > 5) were found for chemicals with a $\log K_{OA}$ between 8 and 12 and a $\log K_{OW}$ between 6 and 8, except for PFOA (Borgå et al., 2012; Gobas et al., 2009; Kelly et al., 2007; Thomann, 1989). These results are generally comparable to those of previous studies, which suggested that chemicals with $\log K_{OW} < 5$ do not biomagnify in food webs, whereas chemicals with a $\log K_{OW} > 5$ do biomagnify (Verhaert et al., 2017). Superhydrophobic chemicals ($\log K_{OW} > 7$) showed relatively small TMF values because of their limited bioavailability (Shao et al., 2016). In addition, toxic organic chemicals with a $\log K_{OW} > 2$ and a $\log K_{OA} \geq 6$ showed inherent biomagnification potential in marine-mammalian and terrestrial food webs (Kelly et al., 2007). Overall, the partition coefficients of organic chemicals are good indicators of their biomagnification potentials in aquatic food webs. However, there is a limit to the determination of biomagnification by the partition coefficients alone. In fact, among the chemicals with a $\log K_{OA} > 6$ and a $\log K_{OW}$ between 4 and 8, non-biomagnifiable compounds are frequently found (Fig. 2, Table 1, and Table S2). Thus, the biomagnification potential of toxic organic chemicals is affected by their chemical

properties, such as partition coefficients, and also by other parameters in combination.

The biomagnification potentials and TMFs of toxic organic chemicals are generally influenced by the following factors: 1) chemical properties, 2) biological characteristics, 3) food web and geographical characteristics, and 4) TL measurements. Many of these main factors and potential sources of variability in TMF values of organic chemicals were partly reviewed in the previous papers (Borgå et al., 2012; Franklin, 2016). First, chemical properties, including hydrophobicity, water solubility, molecular mass, and chemical structure, can affect the biomagnification potentials of toxic organic chemicals (Borgå et al., 2012). For example, the biomagnification potentials of polychlorinated biphenyls (PCBs) strongly depend on the number and/or position of chlorine atoms attached to the biphenyl rings. Lower chlorinated PCB congeners (e.g., PCB-28 and -52) are biomagnified through the food web less than higher chlorinated PCBs (Walters et al., 2011). In addition, non-ortho PCB congeners (e.g., PCB-77, -126, and -151) did not biomagnify at all due to their high rates of metabolic clearance (Metcalf and Metcalf, 1997).

Second, biological characteristics such as age, size, sex, reproduction, metabolic capacity, lipid content, growth rate, feeding type, and respiration processes are likely to greatly affect the biomagnification of chemicals (Borgå et al., 2012; Shao et al., 2016; Su et al., 2017). For example, the age of organisms was identified as an especially important factor for biomagnification of recalcitrant chemicals because the net accumulation flux of chemicals was found to increase with age (Ren et al., 2017). In recent study, the mobilization of maternal lipid and protein in breeding stages associated with transfers of pollutants can be also considered as one of significant factors that regulates bioaccumulation and biomagnification (Verreault et al., 2006). However, the TL of organisms does not change over time; consequently, higher TMF values could be found in older individuals than in younger ones. In addition, biodilution occurs in relation to increasing lipid content, so the growth rates of organisms and accumulation rates of chemicals can simultaneously affect TMF values (Borgå et al., 2012). Thus, no methodological issues are necessarily implied when various studies report significantly different TMF values for the same chemicals (e.g., PCBs) (Kobayashi et al., 2015; Villa et al., 2011; Walters et al., 2011) because the differences could stem from biological characteristics. However, up to the present, those biological characteristics have not been considered in calculating TMF values.

Third, TMF values could be affected by the specific food web and its geographical characteristics, such as food-chain length, energy (carbon) flow through food web, and seasonal and spatial variabilities of contamination levels (Corsolini and Sara, 2017; Sun et al., 2017; Verhaert et al., 2017). Borgå et al. (2012) suggested that at least 3 TLs of organisms are needed to obtain reliable TMF values for given chemicals. Previous biomagnification studies showed, however, that only few cases met that standard (Table 1). In addition, previous studies generally measured TLs using $\delta^{15}\text{N}$ values; the carbon sources of predators and energy flow (e.g., using $\delta^{13}\text{C}$) were considered less often (Sun et al., 2017). Various ecological parameters could also contribute to variability in biomagnification of contaminants (Corsolini and Sara, 2017). Altogether, thus, future biomagnification studies would need to consider the transfer and fate of contaminants through the entire food web of an ecosystem. Finally, determining the TL of an organism can cause errors in TMF calculations for contaminants through a lack of species-specific TDF values, seasonal and spatial variability of background $\delta^{15}\text{N}$ values (discussed further below), difficulty in sampling organisms to fully represent the food web, and difficulty in sorting low-level micro-organisms (e.g., planktons).

Table 1
Summary of biomagnification studies of toxic organic chemicals in aquatic ecosystems.

Region (Country)	Survey year(s)	Food web or diet	TL ^a (min–max)	TL measured by	TDF ^b	Target compounds ^c	TMF ^d		References
							Mean ± SD (or min–max)	Based on ^e	
Barrow, AK & Pt. Lay, AK (USA)	1999–2000	Zooplankton	2.0	$\delta^{15}\text{N}$	3.8	cis–chlordanes	0.72 ± 0.07	Lipid weight	Hoekstra et al., 2003
		Fish	2.3–3.5			trans–nonachlor	5.21 ± 0.09		
Charleston Harbor (USA)	2004	Mammals	2.8–4.1	$\delta^{15}\text{N}$	3.4	γ –HCH	0.65 ± 0.07	Wet weight	Houde et al., 2006
		Fish	3.4–4.3			PFOA	6.3 ± 6.7		
		Mammals	4.4			PFNA	2.4 ± 3.1		
			PFDA			2.2 ± 2.4			
			PFUA			2.3 ± 2.5			
PFDoA	0.6 ± 0.8								
Eastern Hudson Bay (Canada)	1999–2003	Algae	1.0	$\delta^{15}\text{N}$	3.8	PFOSA	5.0 ± 7.0	Lipid weight	Kelly et al., 2008
		Zooplankton	2.0			PFOS	1.8 ± 1.2		
						tri–BDE	0.96		
						tetra–BDE	0.44–1.60		
						penta–BDE	0.76–0.96		
Bivalves	2.3	hexa–BDE	0.81–0.88						
Gironde estuary (France)	2012	Fish	3.1–3.6	$\delta^{15}\text{N}$	3.4	PFTeDA	0.96	Wet weight	Munoz et al., 2017
		Birds	3.5–3.9			Br–PFOS	1.20		
		Mammals	4.0–5.5			L–PFOS	0.94		
		Invertebrates	N.A.			MeFOSAA	0.18		
						FOSA	1.20		
Lake Dianshan, Shanghai (China)	2014	Fish	N.A.	$\delta^{15}\text{N}$	3.4	BDE 28	1.59	Lipid weight	Zhou et al., 2016
		Crab	N.A.			BDE 47	1.58		
						BDE 99	1.81		
						BDE 100	1.35		
						BDE 153	1.51		
						BDE 154	1.41		
						BDE 183	1.73		
						6–MeO–BDE	1.27		
						47			
						2′–MeO–BDE	0.73		
68									
Lake Erie (Canada)	2009	Plankton	2.0	$\delta^{15}\text{N}$	3.4	cVMS D4	0.74	Lipid weight	McGoldrick et al., 2014
		Mayfly	2.2			cVMS D5	0.75		
Lake Erie (Canada)	2010	Fish	3.1–4.2	$\delta^{15}\text{N}$	3.4	cVMS D6	0.71	Lipid weight	Su et al., 2017
		Fish	N.A.			pTBX	0.36		
Lake Hartwell (USA)	2005, 2006	Invertebrates	1.6–2.1	$\delta^{15}\text{N}$	3.4	BB–101	0.42	Lipid weight	Walters et al., 2011
		Fish	2.5–4			BB–153	0.28		
						HBCDD	1.08		
						di–CB	1.95		
						tri–CB	1.46–2.68		
Carnivores	2.5–3.4	tetra–CB	1.91–3.57						
Omnivores	2.5–3.6	penta–CB	3.10–5.07						
Piscivores	3.5–4.1	hexa–CB	3.90–6.24						
Planktivores	2.5–3.5	hepta–CB	4.38–6.63						
Lake Ontario (Canada)	1999, 2001, 2004	Plankton	N.A.	$\delta^{15}\text{N}$ values ^f		Σ MCCPs	0.22 ± 0.10	Lipid weight	Houde et al., 2008
		Invertebrates	N.A.			MCCPs	0.06–0.36		
Lake Ontario (Canada)	2002, 2003	Fish	N.A.	$\delta^{15}\text{N}$ values ^f		tri–CN	0.90–0.95	Lipid weight	Helm et al., 2008
		Invertebrates	N.A.			tetra–CN	0.82–1.23		
						penta–CN	0.81–1.34		
						hexa–CN	1.12–1.42		
						hepta–CN	1.04–1.28		
Forage fish	N.A.	octa–CN	0.90						
Lake trout	N.A.								

Table 1 (continued)

Region (Country)	Survey year(s)	Food web or diet	TL ^a (min–max)	TL measured by	TDF ^b	Target compounds ^c	TMF ^d		References	
							Mean ± SD (or min–max)	Based on ^e		
Lake Taihu (China)	2009	Herbivorous fish Omnivorous fish Carnivorous fish	1.06–3.40	$\delta^{15}\text{N}$	3.4	2–3 ring PAHs	1.00–1.35	Lipid weight	Wang, 2012	
			2.02–3.14			4–6 ring PAHs				0.91–1.90
			1.97–3.45			α -HCH				1.00
			β -HCH			1.25				
			p,p'-DDE			1.96				
			p,p'-DDD			1.68				
			p,p'-DDT			1.62				
			α -endosulfan			1.80				
			β -endosulfan			1.04				
			Endosulfan sulfate			1.76				
			α -chlordane			0.84				
			β -chlordane			0.83				
			Aldrin			1.91				
	Dieldrin	2.49								
	Endrin aldehyde	0.83								
Lake Taihu (China)	2015	Plankton	1.83–2.00	$\delta^{15}\text{N}$	3.8	BPAF	2.52	Wet weight	Wang et al., 2017	
		Invertebrates	2.44–3.15			BPC				2.69
Longtang Town (China)	2014	Fish	3.17–4.79	$\delta^{15}\text{N}$	3.4	BPZ	1.71	Lipid weight	Sun et al., 2017	
		Invertebrates	N.A.			\sum SCCPs				0.17
Northern Adriatic Sea (Italy)	2008, 2010	Fish	N.A.	$\delta^{15}\text{N}$	3.4	SCCPs	0.130–0.376	Lipid weight	Fortibuoni et al., 2013	
		Crustaceans	3.1–3.2			TBT				3.98
		Cephalopods	3.2–3.42			DBT				4.62
		Osteichthyes	2.8–4.4			\sum BTs				3.88
North Bohai Bay (China)	2014	Elasmobranchs	2.8–3.02	$\delta^{15}\text{N}$	3.4		–	Lipid weight	Shao et al., 2016	
		Mammals	4.5							–
		Zooplankton	1.7	$\delta^{15}\text{N}$	3.4	tri-BDE	2.34	Lipid weight		
		Invertebrates	2.0–3.1			tetra-BDE				2.34–3.31
		Fish	2.5–3.1	$\delta^{15}\text{N}$	3	penta-BDE	2.57–3.09	Lipid weight	Verhaert et al., 2017	
			N.A.			hexa-BDE				1.74
Olifants Gorge (South Africa)	2012	Fish	N.A.	$\delta^{15}\text{N}$	3	hexa-CB	2.0	Lipid weight	Kobayashi et al., 2015	
						hepta-CB				1.9–3.0
						octa-CB				2.6
						nona-CB				2.3
						p,p'-DDE				4.9
						p,p'-DDD				3.1
						p,p'-DDT				2.8
						α -HCH				1.3
						β -HCH				1.4
						mono-CB				1.88
						di-CB				2.81
						tri-CB				1.75
						tetra-CB				1.25–1.51
penta-CB	1.26–1.44									
hexa-CB	1.06									
hepta-CB	1.18									
octa-CB	1.25									
deca-CB	2.00									
BDE 28, 33	1.03									
BDE 47	1.65									
BDE 66	1.37									
BDE 100	1.66									
BDE 99	0.98									
BDE 154	1.66									
BDE 153	1.27									
BDE 183	0.46									
HCB	>0.4									
Ross Sea (Antarctic) & Iceland (Sub-Arctic)	2004–2006	Invertebrates	3.07–3.17	$\delta^{15}\text{N}$	3.4		>0.6	Wet weight	Corsolini and Sara, 2017	
		Fish	2.49–4.77			p,p'-DDE				
		Invertebrates	3.06–3.69			HCB				>0.7
		Fish	3.60–5.17			p,p'-DDE				>0.3

(continued on next page)

Table 1 (continued)

Region (Country)	Survey year(s)	Food web or diet	TL ^a (min–max)	TL measured by	TDF ^b	Target compounds ^c	TMF ^d		References	
							Mean ± SD (or min–max)	Based on ^e		
Tokyo Bay (Japan)	2011	Fish	2.6–4.4	$\delta^{15}\text{N}$	3.4	cVMS D4	0.60	Lipid weight	Powell et al., 2017	
Yangtze River Delta (China)	2013	Mollusks	N.A.	$\delta^{15}\text{N}$	3.8	4–OP	cVMS D5	0.60	Wet weight	Gu et al., 2016
							cVMS D6	0.70		
							2.93			
							4–n–NP	1.22		
		Prawn	N.A.			4–t–OP	0.90			
		Fish	2.0–4.5			4–NP	0.72			

^a TL: trophic level of organisms. N.A. not available.

^b TDF: trophic discrimination factor of $\delta^{15}\text{N}$ values.

^c Target chemicals. All acronyms of target chemicals are presented in Table S1 of the Supplementary Materials.

^d TMF: Trophic magnification factor was calculated using TL and log concentration of the chemical. Bold indicates TMF > 1.

^e Calculation of TMF using TL and chemical concentration based on wet weight or lipid weight.

^f TMF was determined based on the relationship between $\delta^{15}\text{N}$ value (not TL) and chemical concentration.

2.2. Trace metal bioaccumulation and trophic transfer in aquatic food webs

The biomagnification and trophodynamics of trace metals in aquatic environments have caused great socio-ecological concern in many aspects, especially for human consumption of fish and human health (Castro-González and Méndez-Armenta, 2008; Solomon, 2008). For example, the outbreak of Minamata disease in Japan raised awareness about metal accumulation and magnification within the food web and its associated risks to the health of humans and ecosystems (Harada, 1995; Mergler et al., 2007). Such risk associated with biotransference of metals has been reported, although fewer studies are available for trace metals than for POPs. This is because the most elements have relatively great bioavailability and thus can be controlled in organisms (Gray, 2002).

Of note, the mechanisms of biological storage and/or elimination of trace metals vary cross the species and taxa as well as chemical properties of target compounds (Wang and Rainbow, 2010). For this reason, many studies on metals have focused on

the mechanisms associated with accumulation and elimination in an individual basis (Wang, 2002; Wang and Fisher, 1999; Wang and Rainbow, 2008, 2010). With respect to accumulation, exposure routes through trophic transfer have received consideration as a dominant pathway in aquatic organisms (Wang, 2002; Wang and Fisher, 1999; Wang and Rainbow, 2008). The quantitative importance of accumulation depends on the biological availability of metals, which differs with the specific type of food being ingested (Luoma, 1983). In the accumulation trends of metals from prey to predator, accumulated concentrations show a high association with the corresponding prey concentrations. The trophic transfer factors, defined as the ratio of concentration in predators to that in prey, are calculated using assimilation efficiency (AE), ingestion rate (% of body weight), and an efflux rate constant. Therefore, studies on the biodynamics of trace metals associated with bioaccumulation and magnification are generally conducted using radioisotope elements, which enables to address kinetics and develop predicted concentration factors from their uptake and loss in organisms (Luoma and Rainbow, 2005; Wang et al., 1996). For example, the

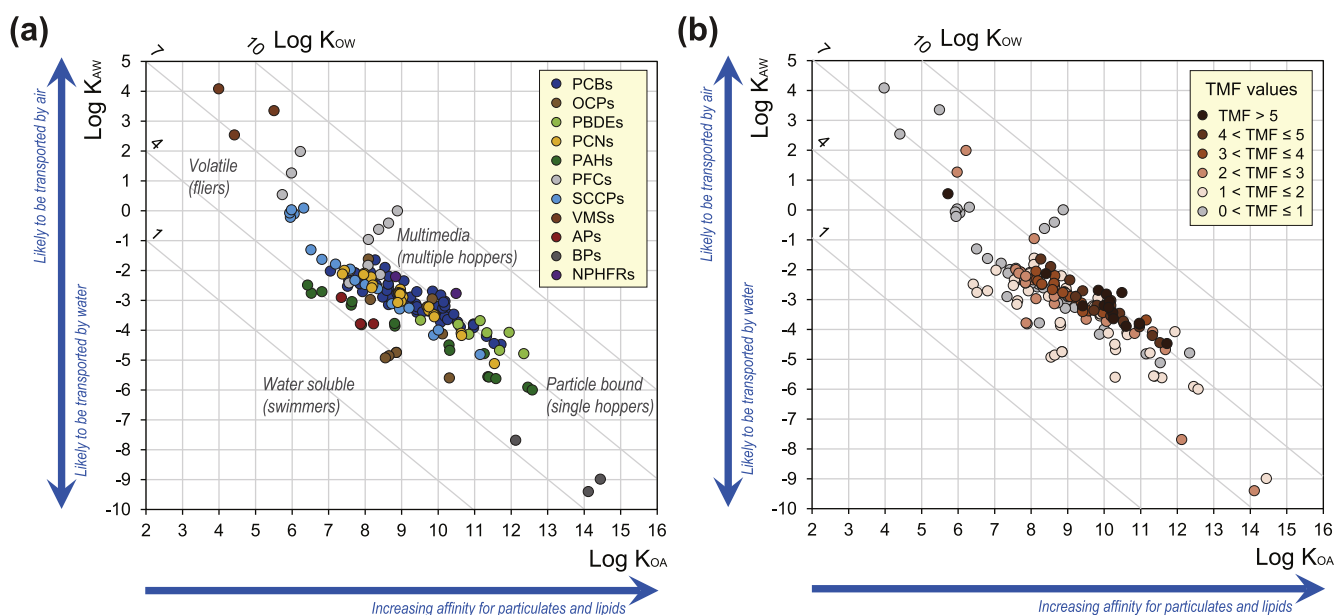


Fig. 2. (A) Chemical space map of toxic organic chemicals based on partition coefficients ($\text{Log } K_{\text{OA}}$, $\text{Log } K_{\text{AW}}$, and $\text{Log } K_{\text{OW}}$) and (b) distributions of the TMF values of toxic organic chemicals obtained from the previous studies presented in Table 1. (The partition coefficients used in this figure are presented in Table S2 of the Supplementary Materials.)

review by Wang (2012) collated the AE results of Hg for different prey organisms measured using a radiotracer methodology to understand accumulation and trophic transfer (Lawson and Mason, 1998; Mathews and Fisher, 2008; Pickhardt et al., 2006; Wang and Wong, 2003). Also, the predicted potential on biomagnification using these AE and efflux constants in copepods and bivalves was emphasized (Reinfelder et al., 1998; Wang, 2002).

The bioaccumulation of trace metals is a good proxy to estimate the integrated outcome of exposed organisms in polluted environments because all organisms take up and accumulate trace metals, whether they are essential or not (Luoma and Rainbow, 2005). However, many previous studies have evidenced and highlighted the uncertainty associated with characterizing bioaccumulation of metals because of its great variabilities cross elements, taxa, and also surrounding environments. In anyhow, the chemical concentrations reach a balance between intake and excretion, which depends on various assimilation and excretion rates specific to each organism's physiological and environmental conditions (Calbet et al., 2016; Wang, 2002; Wang and Rainbow, 2008).

One important feature in accumulation of metals would be bioavailability, for example, the bioavailable fractions of non-essential metals (such as Cd or Pb) in sediment showed elevated accumulation compared to essential metals in several aquatic organisms, including polychaetes (a detritus feeder) and bivalves (a filter feeder), regardless of feeding type (Chan, 1988; Wang, 2002). It is also noteworthy that the metabolism, storage, release of metals are influenced by specific physiology whether the element is necessary or non-essential (Rainbow et al., 2002). Comparative results have been documented for the elements that can be biomagnified, although most studies have been limited to specific chemicals such as Hg, Se, and Zn (Cui et al., 2011; Ikemoto et al., 2008; Tu et al., 2012).

In the kinetic models for bivalves with a high assimilation rate for metals (mussels *Mytilus edulis* and *Perna viridis*, clam *Ruditapes philippinarum*, and oysters *Crassostrea rivularis* and *Saccostrea glomerata*), the predicted biomagnification of Zn, Cd, Se, Co, Se, Ag, Am, and Cr was significant, even considering a low ingestion rate (10%) (Chong and Wang, 2001; Ke and Wang, 2001; Wang et al., 1996; Wang, 2002). However, many practical studies on biomagnification have demonstrated that most elements in organisms are not seemingly related to their trophic levels; the exceptions are Hg and Zn (Wang, 2002). In a study based on a complex food web, only one link among 35 trophic interactions showed a biomagnified trend for Pb (Barwick and Maher, 2003). Gray (2002) demonstrated that about half of studies on trace metals conducted during the past 30 years (48 papers studies for 1970–2000) showed biomagnification of Hg, and none of them showed biomagnification of other elements in a marine ecosystem. Furthermore, Gray (2002) found no evidence of biomagnification of Cd. In our review of studies on the biomagnification of metals, Hg was found to be the most studied element, and it is classified as metalloid, which has somehow different characteristics from others (Kim et al., 2012; Wang, 2012). Previous studies also demonstrated that biomagnification is generally accepted, but that of Hg in biota depends on trophic level (Atwell et al., 1998; Cabana and Rasmussen, 1994). In a Pearl River estuary, Zeng et al. (2013) demonstrated that, when the whole food web was the focus, the different transfer patterns of Cu, Pb, Zn, and Cd observed within and between different habitat communities could be explained by the hiding transfer patterns of those metals in specific communities.

The excretion and elimination mechanisms for bioavailable forms of elements also account for the complex results on bioaccumulation and magnification (Wang and Fisher, 1999; Wang, 2012). Several processes control the food chain transfer of metals

from prey to high TLs, including sequestration (metal-rich granules, metallothionein, and sulfide), which complicates the understanding of trophic transfer (Wang, 2002). In fact, many studies have shown that most elements appear to be excreted or regulated in organisms (Luoma and Rainbow, 2005; Templeman and Kingsford, 2015). In an accumulation and excretion study, the rapid uptake and excretion of metals in the jellyfish *C. maremetens* suggest that it could be an efficient scavenger of Cu and Zn (Templeman and Kingsford, 2015).

Among the factors that affect biomagnification, growth significantly regulates the results; fast growth might lead to less biomagnification with large body size (lower surface: mass ratio), resulting in reduced uptake of elements, even Hg, in some regions, say tropical region Hg biodilution (Poste et al., 2015; Ward et al., 2011). A recent study conducted in an area contaminated by drainage from an abandoned mine demonstrated that biodilution can depend on pollution status and level, along with the route of direct or indirect exposure, which regulates the ratio of accumulation (Watanabe et al., 2008). Thus, studies on biomagnification of metals have focused on whether it can be observed in certain organisms in specific areas, whereas research on POPs has suggested the magnification factors for each compound (Table 1). Some studies on metals have compared their results using simple regression curves for the concentrations of accumulated metals in organisms at particular trophic positions (Barwick and Maher, 2003; Ciesielski et al., 2010; Zeng et al., 2013).

The results of earlier studies showed rough comparisons based on information from *in situ* organisms, whose food web relations can be estimated using gut contents, feeding strategies, and habitat preferences (Barwick and Maher, 2003). For instance, the Se concentrations measured in organisms collected from the Lake Macquarie estuary in Australia showed significant accumulation and magnified results, but Cd showed no such trends because of high concentrations of Cd in detritivores, the fourth level of a food web based on feeding properties (autotrophs, herbivores, detritivores, planktivores, omnivores, and carnivores) (Barwick and Maher, 2003). Results from a different study supposed that the trophic position of organisms can be altered within a single species according to feeding habits, which can vary with body size (Torres-Rojas et al., 2014). However, Layman et al. (2005) found no relationship between trophic position and body size across all taxa in diverse food webs characterized by a broad range of primary consumer body size.

To establish reliable TLs, stable isotope analysis of nitrogen has been adopted for *in situ* samples (Hobson and Welch, 1992). The enrichment of ^{15}N , following a previous study that found that $\delta^{15}\text{N}$ values increased on average 3–5‰ per trophic level, was used to interpret the trophic position of each sample (DeNiro and Epstein, 1981; Hobson and Welch, 1992; Minagawa and Wada, 1984). Many studies on bioaccumulation of metals and their transference through food webs have used this parameter to compare the TLs of target organisms (Table 2). Briefly, studies about the food web and metal biomagnification showed results using biplots for the slope of metal concentrations and stable isotope values (Cui et al., 2011; Ikemoto et al., 2008; Kim et al., 2012; Tu et al., 2012). The trends for the concentrations of metals drawn on the plots represent the possibilities of biomagnification in *in situ* samples. Some researchers calculate TLs by differentiating $\delta^{15}\text{N}$ values between prey and predators with enrichment factors. As shown in Table 2, most data on metals and trophic positions are based on TLs calculated with nominal values (3.4 or 3.8) as an enrichment factor, following Hobson and Welch (1992). Furthermore, BMFs, corrected for differences in trophic position (based on $\delta^{15}\text{N}$) (Dehn et al., 2006), can also be calculated for selected predator–prey scenarios by estimating the numeric TLs in an ecosystem and interpreting the

Table 2
Recent studies on metals and metalloids regarding magnification in food chains.

Region (Country)	Samples (number of species)	TL analysis		Biomagnification			References	Remarks (Area, sampling date, etc.)
		Method	TL (TP) ^a	Targets	TMF ^b (BMP ^c , BMF ^d , TTF ^e)	Magnified element		
Arctic system	Particulate organic matter through polar bears (<i>Ursus maritimus</i>) and seals (<i>Phoca hispida</i>). (27 species)	$\delta^{15}\text{N}$	1.50 3.80	Hg	0.2 (BMP) ^c	Hg	Atwell et al., 1998	Lancaster Sound, Northwest Territories
Lake Macquarie Estuary (Canada)	Sources (zoopl./detritus) Autotrophs (3) Herbivores (2) Planktivores (2) Detritivores (4) Omnivore (1) Carnivores (fish, 4)	Feeding strategies (including gut content and habitat)	N.A. ^{**}	Se, Cu, Cd, Zn, As, Pb	N.C ^{***}	As Pb (one link from zooplankton-crab-fish)	Barwick and Maher, 2003	Polluted seagrass ecosystem, 13–17 Mar. 1999
Mining District (Cooke City) (USA)	Trophic Level 2 Trophic Level 3	$\delta^{15}\text{N}$ (EF: 2.1–4.9, avg. 3.5)	N.A.	Fe, Cu, Zn	N·C	Zn	Quinn et al., 2003	Mine area, Sep. 1999.–May 2000 Diminution element: Fe
Franks Tract, Ca (USA)	Epiphyte-based food web invertebrates Epiphyte-based food web fish Epiphyte algae (1) Insects (2) Bivalve (1) Gastropods (2) Annelida (5) Crustaceans (3)	$\delta^{15}\text{N}$	Inferred by $\delta^{15}\text{N}$ values	Cu, Cd	N·C	Cd (two trophic links: epiphyte-based food web and fish food web)	Croteau et al., 2005	Freshwater
Arctic area	Zooplankton-Bowhead Whale Zoopl.- Arctic Cod Zoopl. - Ringed Seal Arctic Cod-Ringed Seal Shrimp-Bearded Seal Herrings-Spotted Seal Ringed Seal-Polar bear	$\delta^{15}\text{N}$	N.A. (Inferred by $\delta^{15}\text{N}$ values)	Cd, THg, Ag	Hg: 1.4 (BMF)	Hg	Dehn et al., 2006	Tissue-specific accumulation (kidney and liver)
Mekong Delta, (Vietnam)	Phytoplankton POM Gastropod (1) Crustaceans (5) Fish (15)	$\delta^{15}\text{N}$	N.A. (Inferred by $\delta^{15}\text{N}$ values)	V, Cr, Mn, Co, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Hg, Tl, Pb, and Bi	–	Se, Rb, Hg	Ikemoto et al., 2008	Diminution element: Mn
Ginzan Creek (Japan)	POM (fine and coarse) Epilithon Leaf Macroinvertebrates (16–28) Producers (16) Primary consumers (11–17) Second. consumers (5–9)	$\delta^{15}\text{N}$	N.A. (Inferred by $\delta^{15}\text{N}$ values).	Pb, Ag, Zn, Hg, Cu, As	–	–	Watanabe et al., 2008	Mine area 1 Dec. 2004 Number of taxa is different at each site
Arctic system	Marine food webs Polar bear (<i>Ursus maritimus</i>)	$\delta^{15}\text{N}$ EF: 2–4 (avg. 3.8)	4.1–5.5	Hg	–	Hg	Horton et al., 2009	Hair samples of polar bear
Estero de Urías lagoon (Mexico)	Primary producers (6) Primary consumers (9) Secondary consumers (4) Tertiary consumers (1)	$\delta^{15}\text{N}$ (EF: 3.4)	1–5	Cd Cu Pb Zn	Cd: 1.9 –2.1 Pb: 2.1 –3.0	–	Jara-Marini et al., 2009	Mangrove area

Cd, Zn, Hg

Table 2 (continued)

Region (Country)	Samples (number of species)	TL analysis		Biomagnification			References	Remarks (Area, sampling date, etc.)
		Method	TL (TP) ^a	Targets	TMF ^b (BMP ^c , BMF ^d , TTF ^e)	Magnified element		
Yellow River Delta (China)	Primary producer (1) Invertebrates (4) Fish (6) Water birds (4)	$\delta^{15}\text{N}$ (EF:3.8)	1.5 1.88–2.11 2.18–3.65 2.85–3.80	As Cd Cr Cu Mn Ni Pb Zn Hg	As: 0.41 Cd: 3.12 Cr: 0.91 Cu: 0.76 Mn: 0.26 Ni: 0.75 Pb: 0.98 Zn: 1.40 Hg: 2.82		Cui et al., 2011	Diminution elements: As, Cr, Cu, Pb, Mn, Ni
Masan bay (Korea)	Benthic food web	$\delta^{15}\text{N}$ (EF:3.4)	N.A. (Inferred by $\delta^{15}\text{N}$ values)	Hg (Total) Hg (Me)	0.119	Hg	Kim et al., 2012	
Shrimp farm in Ba Ria Vung Tau (Vietnam)	Sediment SPM Sipuncula (Worm) (1) Crustaceans (6) Cephalopod (1) Fish (13)	$\delta^{15}\text{N}$	N.A. (Inferred by $\delta^{15}\text{N}$ values)	23 elements (including Zn, Se, Hg, Co, Cu, Ag)	–	Zn, Se, Hg	Tu et al., 2012	Integrated shrimp mangrove farm
Iténez River (Bolivia)	Fish 4 trophic levels (8)	$\delta^{15}\text{N}$ (E:2.8)	TP 1.9–2.99	Hg	–	Hg	Pouilly et al., 2013	
Pearl river estuary (China)	Zooplankton (1) Fish (7) Mollusks (5) Crustaceans (7)	$\delta^{15}\text{N}$ (EF:3.4)	2–4.5	Cu Pb Zn Cd	Cu:1.18/ 1.52 Pb: 1.14/ 1.18 Zn:0.62/ 0.81/ 0.90 Cd: 0.19/ 0.27		Zeng et al., 2013	TMFs differ by food chain Diminution elements: Cd, Zn
Southwest Florida (USA)	Invertebrates (18) Fish (40)	$\delta^{15}\text{N}$ (EF:3.4)	1.50–4.15	Hg	5.05	Hg	Thera and Rumbold, 2014	
Lake Winnipeg (Canada)	Fish (17)	$\delta^{15}\text{N}$ (EF:3.4)	TP 3.7–4.3	Hg and 14 other elements		Hg	Ofukany et al., 2014	
Burkina Faso (Africa)	Water, sediment Fish Zooplankton Mollusks	$\delta^{15}\text{N}$ (EF:3.4)	N.A. (Inferred by $\delta^{15}\text{N}$ values)	Hg, Se	THg: 2.9 –6.5 MeHg: 2.9–6.6		Ouédraogo et al., 2015	Fluvial reservoirs Rainy season, 2010
African Lake (8 sites) (Africa)	Fish at 8 different sites	$\delta^{15}\text{N}$ (EF:3.4)	1.5–4.9	Hg	1.9–5.6		Poste et al., 2015	
Lake Bikal (Siberia)	Water Plankton Invertebrates (9) Fish (14, 6 pelagic and 8 benthic)	Feeding strategies	Estimated from feeding strategy and metal conc.	As, Cd, Pb, Hg, Se	–	Hg	Ciesielski et al., 2016	Diminution elements: As, Cd, Pb No correlation: Se
Laboratory	Diatom Chlorophyte (diatom-chlorophyte) Cryptophyte (diatom-cryptophyte) Copepod (diatom-copepod)	–	N.A.	MeHg	1.04 –1.35 (TTF) ^d 1.37 –1.78 1.40 –1.83	MeHg	Lee and Fisher, 2017	Biological samples were collected in the field and acclimated for lab-based experiments

^aEF: enrichment factor.

^{**}N.A. not available.

^{***}N.C. not calculated in the literature.

^a TL (TP): trophic level (position) of organisms.

^b TMF: trophic magnification factor.

^c Biomagnification power (BMP): slope of the regression line of $\delta^{15}\text{N}$ against concentration in an organism in the food web.

^d BMF: Biomagnification factor.

^e Trophic transfer factor (TTF) = (assimilation efficiency × ingestion rate)/(efflux rate constant + growth).

dynamics of metals through relationships between predators and prey.

3. Application of nitrogen isotope ratio for TL estimation in TMF study

TMFs are determined by the relationship between concentration of a chemical and the TLs of organisms in a food web. Thus, determining accurate TLs for organisms is one of the most important factors in TMF estimation. The nitrogen stable isotope ratio is largely applied for TL estimation in TMF study because of the enrichment caused in the consumer from excreting nitrogenous waste (e.g., ammonia, urea, and uric acid) (McCutchan et al., 2003). As shown in Tables 1 and 2, many studies have estimated TLs to show the magnification of pollutants using various values of the trophic discrimination factor (TDF) between predators and prey. This approach provides time-integrated trophic information, whereas the other traditional methods (e.g., gut content analysis or direct observation) give only snapshot data. However, the nitrogen isotope ratio of consumers cannot provide reliable TLs because the nitrogen isotope ratio reflects both trophic enrichment and the nitrogen isotope ratio of the source nitrogen (diet). For instance, different $\delta^{15}\text{N}$ values among primary consumers in a food web results in different TLs for consumers, even though different consumers have similar $\delta^{15}\text{N}$ values (Fig. 3a). In fact, some previous studies to calculate TL in aquatic food web showed caution in using nitrogen isotope value of bulk tissues because of temporal and spatial variations in nitrogen isotope baseline (Vander zanden and Rasmussen, 1999; Sackett et al., 2015).

3.1. Variability of TDF and basal nitrogen in TL estimation

TDF, the enrichment in ^{15}N according to increasing TL, is an essential factor in TL estimation. Most TMF studies have applied a representative TDF (i.e., 3.4‰) using the nitrogen isotope ratio of bulk tissue (Tables 1 and 2). However, recent studies have pointed out the defects of that method (Vander zanden and Rasmussen, 2001; Post, 2002; Robbins et al., 2005; Dubois et al., 2007; Reich et al., 2008). For example, significantly large ranges of TDF among species (from 0.6‰ to 5.5‰) reported in the previous studies (DeNiro and Epstein, 1981; Post, 2002; Vander zanden and Rasmussen, 2001) demonstrated that applying an average TDF can cause incorrect estimation of TL, emphasizing the importance of an independent TDF for each species in a food web. As shown in Fig. 3b, estimating trophic positions by applying a consistent TDF can cause misunderstanding in the TLs because TDF can vary by

species and environment.

TDF is also variable by tissue type within an organism and diet quality. Yokoyama et al. (2008) reported a larger range of nitrogen isotopic discrimination (2.3‰–8.7‰) when using different tissue types in an oyster feeding experiment. Reich et al. (2008) also found different TDFs among tissue types in turtles, ranging from –0.6‰ to 1.6‰, due to the different isotopic turnover rate in different tissues. Meanwhile, various studies have discussed the relationship between nitrogen isotopic fractionation and diet in consumers. For example, a variation in TDF was observed in consumers with a high protein content in their diet (McCutchan et al., 2003). Moreover, another study established that the TDF of nitrogen isotope value is strongly affected by the quality of dietary protein, such as C/N ratio (Robbins et al., 2005). Accordingly, the factors affecting variability of TDF should be carefully considered when estimating TL using the nitrogen isotope ratio in bulk tissue.

Another problem with estimating TL using the nitrogen isotope ratio of bulk tissue is the different isotopic turnover rates between primary producers (nitrogen isotopic baseline) and consumers. Consumers reflect the time-integrated nitrogen isotope ratio from their diet, but they do not show the small fluctuations in nitrogen isotope ratio visible in primary producers. The different isotopic turnover rates between producers and consumers have often been discussed as a weak point of the stable isotope analysis approach to TL estimation (Hannides et al., 2009; Rolff, 2000). In particular, using the nitrogen isotope ratio for TL estimation can easily lead to misestimations in areas where the nitrogen source changes dramatically, such as coastal and stream environments (Kellman and Hillaire-Marcel, 2003; Watanabe et al., 2009). As an alternative, Post (2002) suggested applying the nitrogen isotope ratio for primary consumers as a nitrogen isotopic baseline because their isotope turnover time is longer than that of primary producers. Doing so would reduce the difference in isotopic turnover rate between producers and consumers (Eq. (1)).

$$\text{TL} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{primary consumer}}) / \text{TDF} + 2 \quad (1)$$

In fact, the $\delta^{15}\text{N}$ value of zooplankton is generally applied as the $\delta^{15}\text{N}$ for the primary consumer when applying Eq. (1). However, it is difficult to define a primary consumer in aquatic food webs because the variation in TL among zooplankton taxa has been well documented (McClelland and Montoya, 2002). Filter feeders, also generally known as primary consumers, often receive higher TLs, with values of more than 2 in various food webs, due to their consumption of particulate organic matter, including heterotrophic organisms (Chikaraishi et al., 2014; Choi et al., 2017). Accordingly,

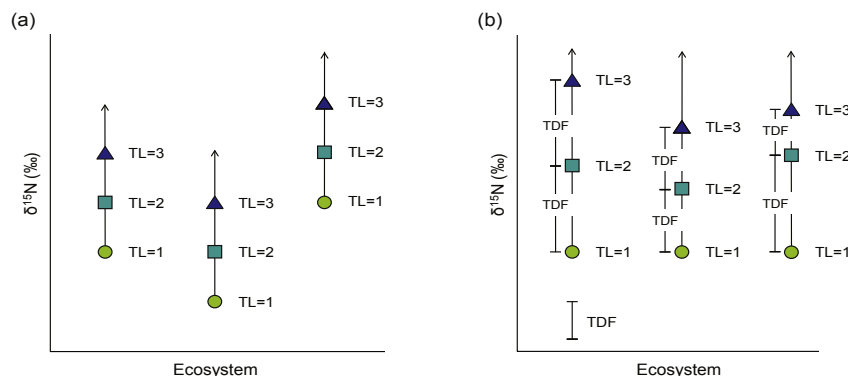


Fig. 3. Estimations of trophic level (TL) using nitrogen stable isotope ratio. Cases for applying (a) consistent TDF in food webs with different nitrogen isotopic baseline and (b) species specific TDF in food web with consistent nitrogen isotopic baseline.

Eq. (1) cannot be a fundamental solution to the current limitations of TL estimation in aquatic ecosystems.

3.2. Using the CSIA of amino acids for accurate TLs calculation in TMF studies

The gas chromatography/combustion/isotope ratio mass spectrometry (GC-C-IRMS) technique enables researchers to conduct a compound specific isotope analysis (CSIA) of nitrogen in individual amino acids (CSIA-AA). After the first report about the nitrogen isotope ratio in individual amino acids by Macko et al. (1997), McClelland and Montoya (2002) found the enrichment of ^{15}N with TLs in some amino acids (e.g., alanine, valine, isoleucine, and glutamic acid) but little or no change in other amino acids (e.g., methionin and phenylalanine). According to those isotopic characteristics, McCarthy et al. (2007) defined amino acids as “trophic” and “source”, respectively. Those characteristics are determined by different isotopic fractionation along the metabolic pathway in the organisms. The trophic amino acids experience large nitrogen isotopic fractionation by deamination, whereas the metabolic route for source amino acids has no nitrogen-associated reaction (Chikaraishi et al., 2007). The difference is simply explained by the deamination of amino acids. For example, the stable isotope ratio of amino acid classified into trophic amino acid (e.g., glutamic acid) has enriched ^{15}N in organisms due to the loss of amine group (NH_3) in deamination in metabolisms. In the case of phenylalanine (one of source amino acid), however, a little fractionation occurs as it converted into tyrosine via phenylalanine hydroxylase without losing amine group in the reaction. These different nitrogen isotopic properties were discussed for use in estimating TL (Fig. 4). Several studies attempted to determine the optimal pair of trophic and source amino acids to estimate TL in both marine and terrestrial food webs and settled on glutamic acid and phenylalanine, respectively (Hannides et al., 2009; McCarthy et al., 2007; Popp et al., 2007; Chikaraishi et al., 2010). As a result, Chikaraishi et al.

(2009) suggested a new equation to estimate TLs in aquatic food webs based on the nitrogen isotope ratio of glutamic acid and phenylalanine in a single consumer with a practical TDF (e.g., 7.6) and the isotopic offset between glutamic acid and phenylalanine in a primary producer (β , e.g., 3.4). This approach is also available for terrestrial food webs with different β value (-8.4) that associated with nitrogen isotopic fractionation of phenylalanine during lignin synthesis in vascular plant (Chikaraishi et al., 2010). Because the information on the nitrogen isotope ratios of both the resources (nitrogen isotopic baseline) and trophic enrichment information are retained in a single organism, the estimation of TL by CSIA-AA can be independent from variations in the nitrogen isotopic baseline. In particular, the larger trophic enrichment of the trophic amino acid decreases the error compared to that from applying the nitrogen isotope ratio of bulk tissue. For instance, Bowes and Thorp (2015) documented the significantly reduced error in estimated TL from applying this equation (less than 0.2) compared to that from using the bulk isotope equation (0.5). Moreover, a homogenate TDF for amino acids between different tissue types (Chikaraishi et al., 2014) enhances the availability of the nitrogen isotope in amino acids.

Accordingly, the nitrogen isotope ratio of amino acids is now regarded as an approach for estimating more accurate TLs for marine organisms and enhancing the accuracy of the TMF. For example, the nitrogen isotope ratio of amino acids has been suggested to estimate the TMF of Hg in Hawaiian bottom fish species (Sackett et al., 2015). In that study, the nitrogen isotope ratio of bulk tissue in bottom fish showed weak correlation with TL, indicating that different nitrogen sources were accumulating in the different species. The nitrogen isotope ratio of bulk tissue was corrected using the nitrogen isotope ratio of the source amino acid, which remove the variations of nitrogen isotopic baseline. The corrected nitrogen isotope ratio thus provided more accurate trophic information for each species, except for source information, and showed a relationship with the concentration of Hg in the body tissues of various species. Thus, Sackett et al. (2015) demonstrated the applicability of nitrogen isotope ratio in amino acids and suggested the importance of food web structure to TMF study. For several decades, TMF studies have simply applied the stable nitrogen isotope ratio in bulk tissue for TL estimation. However, in the future CSIA of amino acids is going to be very useful to determine accurate TL in trophic magnification studies for the better understanding of the food web transfers for various pollutants.

4. Conclusions

This review has summarized the recent studies relating to the environmental pollutants including metal and POPs in aquatic environments in the context of trophic magnification, highlighting the implications of TLs in trophic magnification study. Studies on the pollutants and their transference through food chains should continue in association with environmental management. Many of the reported interpretations of pollutants and ecosystem structures provide clear evidence of trophic magnification. Nonetheless, those results have also demonstrated the difficulties of accurately analyzing TLs and showed some weaknesses in interpreting the results. CSIA-AA, a recently suggested sensitive approach shows great promise as a useful tool for predicting TLs in food web structures and providing reliable information on the trophodynamics of pollutants when accompanied by a complete analysis of pollutants in organisms. However, successful differentiation of TLs using CSIA-AA will require a concerted effort throughout the scientific community for environmental chemistry and stable isotope ecology.

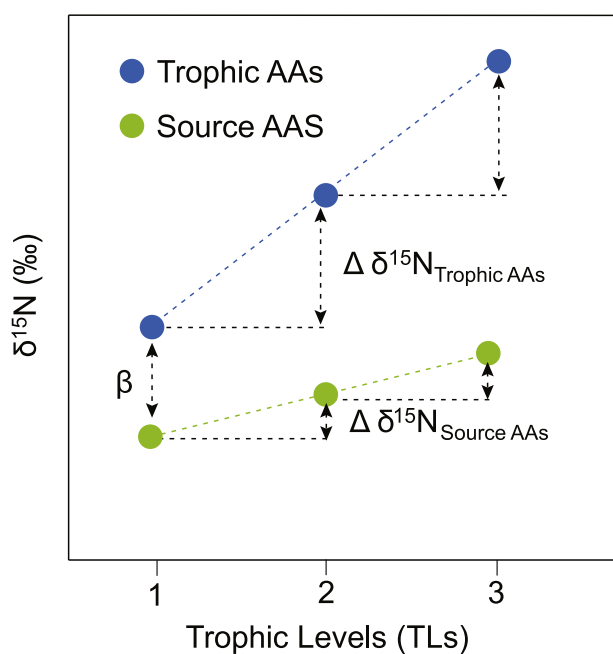


Fig. 4. A scheme for the relationship between the nitrogen isotope ratio of source and trophic amino acids and trophic level (TL) in a food web (altered from Chikaraishi et al., 2014). β indicates the offset between the nitrogen isotope ratio of the trophic amino acid and the source amino acid in a primary producer.

Acknowledgments

This research was a part of the project titled 'Development of techniques for assessment and management of hazardous chemicals in the marine environment – Establishment of ecological risk assessment and diagnosis system', funded by the Ministry of Oceans and Fisheries, Korea. This work was also supported by the National Research Foundation of Korea grant funded by the Korea government (MSIP) (NRF-2016R1E1A1A01943004).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2018.03.045>.

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Supplementary Materials

Table S1. Acronyms of target organic toxic chemicals presented in Table 1.

Target chemicals	Abbreviation
Polychlorinated biphenyls	PCBs
Organochlorine pesticides	OCPs
Hexachlorocyclohexane	HCH
Hexachlorobenzene	HCB
Dichloro diphenyl trichloroethane	DDT
Dichloro diphenyl dichloroethylene	DDE
Dichloro diphenyl dichloroethane	DDD
Non-PBDE halogenated flame retardants	NPHFRs
Pentabromo- <i>p</i> -xylene	pTBX
Hexabromocyclododecane	HBCDD
4-Bromobiphenyl	BB-101
2,2',4,4',5,5'-Hexabromobiphenyl	BB-153
Polybrominated diphenyl ethers	PBDEs
Polychlorinated naphthalenes	PCNs
Polycyclic aromatic hydrocarbons	PAHs
Perfluorinated compounds	PFCs
Perfluorooctanoic acid	PFOA
Perfluorononanoic acid	PFNA
Perfluorodecanoic acid	PFDA
Perfluoroundecanoic acid	PFUA
Perfluorododecanoic acid	PFDoA
Perfluorohexane sulfonic acid	PFH _x S
Perfluorooctane sulfonamide	PFOSA
Perfluorooctane sulfonic acid	PFOS
Perfluorotridecanoic acid	PFT _r DA
Perfluorotetradecanoic acid	PFT _e DA
Branched-PFOS	Br-PFOS
Linear-PFOS	L-PFOS
N-methylperfluoro-1-octanesulfonamidoacetic acid	MeFOSAA
Short chain chlorinated paraffins	SCCPs
Medium chain chlorinated paraffins	MCCPs
Volatile methylsiloxanes	VMSs
Octamethylcyclotetrasiloxane	D4
Decamethylcyclopentasiloxane	D5
Dodecamethylcyclohexasiloxane	D6
Alkylphenols	APs
4-octylphenol	4-OP
4- <i>n</i> -nonylphenol	4- <i>n</i> -NP
4- <i>t</i> -octylphenol	4- <i>t</i> -OP
4-nonylphenol	4-NP
Bisphenols	BPs
Bisphenol AF	BPAF
Bisphenol Z	BPZ
Bisphenol C	BPC

Table S2. Partition coefficients and TMF values of organic toxic chemicals presented in Table 1 and Fig. 2.

Chemicals	Log K _{ow}	References	Log K _{OA}	References	TMF value	References
<i>PCBs (polychlorinated biphenyls)</i>						
PCB 9	5.06	Walters et al., 2011	7.04	ChemSpider, 2017	1.95	Walters et al., 2011
PCB 17	5.25		7.92		1.94	
PCB 18	5.24		7.54		1.71	
PCB 19	5.02		7.51		1.46	
PCB 22	5.58		7.66		2.29	
PCB 25	5.67		7.85		2.23	
PCB 26	5.66		7.85		2.39	
PCB 27	5.44		7.85		1.82	
PCB 28	5.67		7.71		2.68	
PCB 31	5.67		7.71		2.65	
PCB 32	5.44		7.84		2.25	
PCB 37	5.83		8.29		2.01	
PCB 44	5.75		8.05		2.83	
PCB 45	5.53		8.63		2.98	
PCB 47	5.85		8.14		3.57	
PCB 48	5.78		8.63		2.65	
PCB 49	5.85		8.29		3.10	
PCB 52	5.84		8.18		3.21	
PCB 53	5.62		8.47		1.91	
PCB 56	6.11		8.63		2.96	
PCB 60	6.11		8.13		3.32	
PCB 64	5.95		8.58		3.06	
PCB 66	6.20		8.62		3.46	
PCB 70	6.20		8.62		3.10	
PCB 74	6.20		9.06		3.47	
PCB 77	6.36		10.05		2.54	
PCB 82	6.20		9.40		3.96	
PCB 84	6.04		9.40		3.25	
PCB 87	6.29		9.37		3.52	
PCB 91	6.13	9.40	3.60			
PCB 92	6.35	9.21	4.30			
PCB 95	6.13	8.86	3.10			
PCB 97	6.29	9.19	3.72			
PCB 99	6.39	9.71	4.06			

PCB 100	6.23		9.40		5.07	
PCB 101	6.38		9.23		3.48	
PCB 105	6.65		8.26		4.01	
PCB 110	6.48		8.64		3.26	
PCB 118	6.74		9.05		4.10	
PCB 124	6.73		9.40		4.87	
PCB 128	6.74		10.59		6.00	
PCB 130	6.80		10.21		4.99	
PCB 134	6.55		9.95		3.90	
PCB 135	6.64		9.79		4.65	
PCB 136	6.22		9.56		3.96	
PCB 137	6.83		9.99		5.89	
PCB 138	6.83		10.51		4.20	
PCB 141	6.82		10.22		6.18	
PCB 144	6.67		10.17		4.28	
PCB 146	6.89		10.11		5.01	
PCB 149	6.67		8.53		4.18	
PCB 151	6.64		10.24		5.78	
PCB 156	7.18		9.83		6.24	
PCB 157	7.18		10.17		4.83	
PCB 163	6.99		10.41		4.39	
PCB 164	7.02		10.17		4.63	
PCB 167	7.27		10.05		5.47	
PCB 170	7.27		11.70		6.63	
PCB 171	7.11		10.13		5.14	
PCB 174	7.11		11.51		4.38	
PCB 177	7.08		10.95		4.90	
PCB 178	7.14		11.30		4.96	
PCB 183	7.20		10.95		5.25	
PCB 187	7.17		10.95		5.60	
<i>OCPs (organochlorine pesticides)</i>						
α -HCH	4.14	ChemSpider, 2017	8.84	ChemSpider, 2017	1	Wang et al., 2012
β -HCH	4.14		8.84		1.25	
<i>p,p'</i> -DDE	6.51		9.68		1.96	
<i>p,p'</i> -DDD	6.02		10.10		1.68	
<i>p,p'</i> -DDT	6.91		9.82		1.62	
α -endosulfan	4.74		10.29		1.8	
β -endosulfan	3.83		8.64		1.04	

Endosulfan sulfate	3.66		8.54		1.76	
α -chlordane	6.22		8.92		0.84	
Aldrin	6.5		8.08		1.91	
Dieldrin	5.2		8.13		2.49	
Endrin aldehyde	4.12		7.89		0.83	
<i>NPHFRs (non-PBDE halogenated flame retardants)</i>						
<i>p</i> TBX	6.65	ChemSpider, 2017	8.82	ChemSpider, 2017	1.45	Su et al., 2017
HBCDD	7.74		10.47		5.14	
<i>PBDEs (polybrominated diphenyl ethers)</i>						
BDE 28	5.88	Puzyn et al., 2008	9.50	Yue and Li, 2013	2.34	Shao et al., 2016
BDE 47	6.77		10.53		3.31	
BDE 66	6.73		10.82		2.40	
BDE 85	7.03		11.66		2.75	
BDE 99	7.27		11.31		2.57	
BDE 100	7.49		11.13		3.09	
BDE 154	7.89		11.92		1.74	
BDE 153	7.58		12.32		0.88	Kelly et al., 2008
<i>PCNs (polychlorinated naphthalenes)</i>						
PCN 19	5.46	Puzyn and Falandysz, 2007	7.42	Puzyn and Falandysz, 2007	0.95	Helm et al., 2008
PCN 24	5.29		7.37		0.9	
PCN 42	5.9		7.96		1.23	
PCN 44	5.97		8.15		1.01	
PCN 47	5.85		7.95		1.08	
PCN 39	5.75		8.20		0.85	
PCN 35	5.63		8.17		0.97	
PCN 52	6.36		8.95		1.25	
PCN 60	6.2		8.90		1.25	
PCN 58	6.24		8.97		1.26	
PCN 61	6.21		8.95		1.3	
PCN 50	6.13		9.00		1.03	
PCN 51	6.17		9.02		1.02	
PCN 54	6.18		8.91		1.16	
PCN 57	6.18		8.93		0.86	
PCN 62	5.98		8.97		0.81	
PCN 53	6.19		8.94		1.04	
PCN 59	5.85		8.88		0.98	
PCN 67	6.43		9.73		1.42	
PCN 64	6.45		9.89		1.15	

PCN 68	6.43		9.87		1.17	
PCN 69	6.37		9.88		1.29	
PCN 65	6.55		9.73		1.12	
PCN 73	6.55		10.61		1.28	
PCN 74	6.49		10.62		1.04	
PCN 75	6.45		11.52		0.9	
PAHs (polycyclic aromatic hydrocarbons)						
Acenaphthylene	3.80	ChemSpider, 2017	6.52	ChemSpider, 2017	1	Wang et al., 2012
Acenaphthene	3.97		6.42		1.35	
Fluorene	4.14		6.81		1.28	
Phenanthrene	4.49		7.61		1.27	
Anthracene	4.63		7.63		1.35	
Fluoranthene	4.98		8.80		1.2	
Pyrene	5.06		8.79		1.12	
Benzo[<i>a</i>]anthracene	5.83		10.28		1.09	
Chrysene	5.67		10.30		1.11	
Benzo[<i>b</i>]fluoranthene	5.83		11.34		1.2	
Benzo[<i>k</i>]fluoranthene	5.85		11.37		0.91	
Benzo[<i>a</i>]pyrene	5.99		11.56		1.47	
Indeno[1,2,3- <i>c,d</i>]pyrene	6.57		12.43		1.9	
Dibenzo[<i>a,h</i>]anthracene	6.50		11.24		1.42	
Benzo[<i>g,h,i</i>]perylene	6.60		12.55		1.1	
PFCs (perfluorinated compounds)						
PFOA	6.30	ChemSpider, 2017	5.73	ChemSpider, 2017	6.3	Houde et al., 2006
PFNA	7.27		5.98		2.4	
PFDA	8.23	Arp et al., 2006	6.22 ^a		2.2	
PFUA	7.15	ChemSpider, 2017	8.08		2.3	
PFDoA	7.77		8.36		0.6	
PFHxS	4.34		6.13		0.1	
PFOSA	7.58		5.70		5	
PFOS	6.28		6.63 ^a		1.8	
PFTTrDA	8.25	Wang et al., 2011	8.63	Wang et al., 2011	0.66	Munoz et al., 2017
PFTeDA	8.9		8.87		0.33	
FOSA	5.62		7.58		2.3	
SCCPs (short-chain chlorinated paraffins)						
C ₁₀ H ₁₆ Cl ₆	5.22	Sun et al., 2017	6.81	Krogseth et al., 2013	0.151	Sun et al., 2017
C ₁₀ H ₁₅ Cl ₇	5.42		7.71		0.13	
C ₁₀ H ₁₄ Cl ₈	5.65		8.72		0.148	

C ₁₀ H ₁₃ Cl ₉	5.74		9.86		0.197	
C ₁₁ H ₁₉ Cl ₅	5.24		6.51		0.163	
C ₁₁ H ₁₈ Cl ₆	5.43		7.18		0.158	
C ₁₁ H ₁₇ Cl ₇	5.56		7.99		0.183	
C ₁₁ H ₁₆ Cl ₈	5.68		8.93		0.236	
C ₁₁ H ₁₅ Cl ₉	6.04		9.99		0.274	
C ₁₁ H ₁₄ Cl ₁₀	6.36		11.13		0.264	
C ₁₂ H ₂₀ Cl ₆	5.66		7.58		0.129	
C ₁₂ H ₁₉ Cl ₇	5.76		8.33		0.198	
C ₁₂ H ₁₈ Cl ₈	5.97		9.19		0.299	
C ₁₃ H ₂₃ Cl ₅	5.98		6.05		0.376	
C ₁₃ H ₂₂ Cl ₆	5.76		5.95		0.254	
C ₁₃ H ₂₁ Cl ₇	5.89		5.93		0.257	
C ₁₃ H ₂₀ Cl ₈	6.04		5.98		0.341	
C ₁₃ H ₁₈ Cl ₁₀	6.43		6.31		0.313	
VMSs (volatile methylsiloxanes)						
D4	6.98	ChemSpider, 2017	4.42	ChemSpider, 2017	0.6	Powell et al., 2017
D5	8.09		3.99		0.6	
D6	8.87		5.50		0.7	
APs (alkylphenols)						
4-OP	4.12	Gu et al., 2016	7.86	ChemSpider, 2017	2.93	Gu et al., 2016
4- <i>t</i> -OP	4.48		8.22		0.9	
4-NP	4.48		7.34		0.72	
BPs (bisphenols)						
BPAF	4.47	Wang et al., 2017	12.10	ChemSpider, 2017	2.52	Wang et al., 2017
BPC	4.74		14.08		2.69	
BPZ	5.48		14.41		1.71	

^aLog K_{OA} = Log K_{OW} – Log K_{AW}.

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