Environment International 135 (2020) 105361

Contents lists available at ScienceDirect

Environment International

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

Variability of trophic magnification factors as an effect of estimated trophic position: Application of compound-specific nitrogen isotope analysis of amino acids

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ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Compound-specific isotope analysis (CSIA)
Food web
Trophic position
Trophic magnification factor (TMF)

ABSTRACT

The trophic magnification of persistent organic pollutants (POPs), which is the relationship between POP concentration and the trophic position (TPs) of an organism, is considered an important factor for prioritizing chemicals of concern in the environment. Organismal TPs are typically based on nitrogen isotope ratios of bulk tissue ($\delta^{15}N_{bulk}$). In this study, nitrogen isotope ratios of amino acids ($\delta^{15}N_{AAs}$), a more precise approach for TP estimation (TP_{AAs}), was applied and compared with estimations of TP based on $\delta^{15}N_{bulk}$ (TP_{bulk}) in marine organisms living in Masan Bay, South Korea. Compound-specific isotope analysis of the amino acids (CSIA-AAs) in fish samples allows us to calculate robust TPs by correcting the variation in baseline isotope values with use of the $\delta^{15}N_{bulk}$ technique. In a benthic food chain, this approach reveals more significant magnification trends for POPs [polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs)] than those revealed by analysis of the relationship between TP_{bulk} and POPs. The trophic magnification factors (TMF) associated with TP_{AAs} were significant for some POPs, especially *pp′*-DDD and chlordane. The results presented in this study suggest that TP calculations based on $\delta^{15}N_{AAs}$ are an effective tool for characterizing trophic magnification trends related to the fates of various pollutants.

1. Introduction

Previous studies designed to assess the environmental health of coastal ecosystems have used fish or invertebrates as biomonitoring species, as these species have integrated information on pollutants (Tanabe et al., 2000; Voorspoels et al., 2004). In particular, persistent organic pollutants (POPs), which accumulate at high levels due to high affinities in organisms and are easily transferred to high trophic levels, have received substantial attention. POP trends illustrate that pollutants can remain in an ecosystem despite a ban or reduction in use (Tanabe et al., 2000; Hong et al., 2003; Zheng et al., 2018). The transfer and biomagnification of pollutants in a food web shows that the biota itself represents a pollutant vector, highlighting the need for pollution guidelines (Borgå et al., 2012). Many studies on POPs have aimed to identify trends of contamination in organisms, based on the feeding relationships among them (Fan et al., 2017; Zheng et al., 2018). Estimation of the trophic magnification factor (TMF) is a good method to determine the fate of pollutants in a food web and has been investigated frequently in aquatic ecosystems (Walters et al., 2011; Wang et al., 2012). In a study on biomagnification, knowledge of trophic relations is a very important factor, as TMF is calculated from the slope of a regression between trophic positions (TPs) and their accumulated pollutants in the food web. In a recent study, Starrfelt et al. (2013) pointed out that the trophic level (e.g., TP) is not considered to be related to variability, although uncertainty in contaminant concentrations was appreciated in the TMF study. Won et al. (2018) also showed the possibility of mis-estimating TL in two consumers with similar $\delta^{15}N$ values but different baseline values or trophic discrimination factors (TDF) in a recent review on trophic magnification. In fact, the simple concept of trophic level as a unidirectional linear food chain rarely applies to

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https://doi.org/10.1016/j.envint.2019.105361
Received 6 August 2019; Received in revised form 21 November 2019; Accepted 22 November 2019

natural ecosystems.

Many studies on trophic relations (e.g., food webs) have lauded stable isotope analysis as an approach to determine the source of TPs in diverse environments (Hobson and Welch, 1992; Choy et al., 2010). The carbon stable isotope ratios ($\delta^{13}C$) of biological samples provide information on diets with limited discrimination among food sources (~0.4‰ for $\delta^{13}C$). The nitrogen stable isotope ratio is used to estimate trophic positions following stepwise increases in discrimination throughout the food chain (~3.4‰ for $\delta^{15}N$) (Minagawa and Wada, 1984; Post, 2002). However, there are limitations associated with the estimation of TPs (e.g., trophic levels) of aquatic organisms using $\delta^{15}N$ in bulk tissue ($\delta^{15}N$ bulk) (e.g., TP_{bulk}), as it requires knowledge of the baseline $\delta^{15}N$ (nitrogen source). Won et al. (2018) and Ishikawa et al. (2018) reported that TPs calculated using $\delta^{15}N_{bulk}$ can be confounded by variation in baseline values of $\delta^{15}N$ in primary producers, according to the environment inhabited. The use of long-lived consumers or a time-series baseline to reduce overlapping values of potential resources indicates the variables associated with bulk isotope ratios (baseline $\delta^{15}N$ values), due to the rapid response of local biogeochemical processes in low-level organisms (e.g., phytoplankton) and their isotope ratios. An ecologically relevant baseline and knowledge of spatial and temporal differences are now considered prerequisites of a food web study, although stable isotope analysis of bulk carbon and nitrogen has dramatically improved the understanding of the food web (Layman et al., 2012). The wide ranges of TDF in species and environments also demonstrate the inefficiency of bulk isotopes in food web studies. Many studies have demonstrated that the significantly large variability in $\Delta^{15}N$ (from 0.6 to 5.5‰) produces uncertainty in TP estimation using average TDF (Vander Zanden and Rasmussen, 2001; Post 2002; Dubois et al., 2007; Chikaraishi et al., 2009). Thus, TP calculation using $\delta^{15}N_{bulk}$ (TP_{bulk}) with a literature value for trophic discrimination factor (TDF, e.g., 3.4‰) can be misleading and should be used with caution when a complete understanding of the species-specific value of TDF is lacking (Layman et al. 2012).

Recent studies have emphasized the robust TPs of organisms to understand transfers of pollutants because the identification of a reliable food chain guild and its position are important factors in tracking the fate of pollutants in food webs (Fan et al., 2017; Won et al., 2018). The improved approach using stable isotope analysis, known as compound-specific isotope analysis of amino acids (CSIA-AAs), provides accurate information on TP (e.g., TP_{AAs}) based on isotopic fractionation patterns among individual amino acids in consumers (McClellend and Montoya, 2002; McCarthy et al., 2007; Popp et al., 2007; Chikaraishi et al., 2009; McMahon et al., 2017). As a novel tool for the analysis of TPs in the ecosystem, two different groups of amino acids (e.g., source and trophic amino acids) showed great potency as tracers for source (e.g., approximately 0.4‰ for phenylalanine $\delta^{15}N$, $\delta^{15}N_{Phe}$) and trophic levels (e.g., approximately 8.0‰ for glutamic acid $\delta^{15}N$, $\delta^{15}N_{Glu}$) to discriminate $\Delta^{15}N$ (offset between diet and consumer) (Chikaraishi et al., 2009). Many studies have shown that the strength of trophic and source amino acids is the ability to determine TP, as the isotopic offset between these two amino acid types provides trophic shift information and an integrated baseline value for single organisms (Sackett et al., 2015). This is based on the theory that source and trophic amino acids have different fractionations in metabolism; $\delta^{15}N_{Glu}$ contains enriched ^{15}N, rather than ^{14}N, due to deamination (loss of NH_3) during nitrogen metabolism, whereas the typical source amino acid (e.g., phenylalanine) does not (Chikaraishi et al., 2009). This means that TP can be estimated from nitrogen isotope values of two amino acids in a single sample, without the need for a baseline sample from producers or lower-level organisms. This is why the isotope values of amino acids are a promising approach for the estimation of TP. Many studies have difficulties in specifying the appropriate baseline values in environments (Post, 2002). A recent comparative study of CSIA and $\delta^{15}N_{bulk}$ in the calculation of TDF for teleosts supports the feasibility of CSIA-AAs ($\delta^{15}N_{AAs}$) in estimating reliable TPs in a food web, as TDF values are relatively constant (Blanke et al., 2017).

In this study, the trophic transfer of POPs in aquatic food webs was assessed, and robust trophic positions influencing the trophodynamics of POPs in the food web were clarified based on CSIA. Trophically magnified POPs such as polychlorinated biphenyls (PCBs) polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) that have already been reported to be magnified in food web (Kobayashi et al., 2015; Verhaert et al., 2017) were targeted and compared using $\delta^{15}N_{bulk}$-derived TP (TP_{bulk}) and $\delta^{15}N_{AAs}$-derived TP (TP_{AAs}) to characterize aquatic organisms from Masan Bay, Korea. Masan Bay is one of the most heavily polluted areas of Korea and has been identified as a special management area (1982) and monitored and remediated for several decades by the government (MOMAF, 2013). However, there remains scant knowledge on the trophic hierarchy, guilds, and the biomagnification of POPs in this area. PCBs, PBDEs, and OCPs are globally common POPs, and Masan is not an exception (Hong et al., 2003). Due to their hydrophobic and lipophilic characteristics, these compounds are persistent in the environment, accumulate in organisms, are transferred through the food chain, and pose a health hazard to the ecosystem (Tanabe et al., 2000; Voorspoels et al., 2004).

We hypothesized that comparative results would support the importance of accurate TP calculation for TMF estimation in the characterization of aquatic organisms. The results facilitate a better understanding of the trophodynamics of POPs driven by ecological niches in benthic food webs and robust TP estimation.

2. Materials and methods

2.1. Sampling and preparation

Biological samples were collected from Masan Bay in September 2016 (Fig. 1A). Benthic invertebrates and fish (n = 69, 14 species) were sampled (Table S1 of the Supplementary Materials) using a grab sampler and/or a fishing pole and were immediately cleaned with filtered seawater. For species classification, morphological analysis was conducted. Except for small invertebrates (e.g., polychaetes, mussels, crabs, and sea cucumbers) whose whole bodies were used for analysis, all fish samples were immediately dissected for additional procedures, and muscles were used for isotopic and chemical analyses. The samples were frozen with dry ice and stored at −80 °C until freeze-drying and homogenization (Planetary Mono Mill PULVERISETTE 6, Fritsch, Idar-Oberstein, Germany). The samples were analyzed for $\delta^{13}C_{bulk}$, $\delta^{15}N_{bulk}$, $\delta^{15}N_{AAs}$, and POPs (PCBs, PBDEs, and OCPs). In addition, particulate organic matter (POM) was sampled by filtering seawater for source discrimination (GF/F, Whatman Co., Middlesex, UK).

2.2. Stable isotope analysis of bulk samples

Levels of $\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$ were measured in all samples (n = 69) and POM. Briefly, homogenized samples underwent further processing to remove inorganic carbon and lipid fractions for carbon isotope analysis. In brief, each sample was sequentially treated with 1 M HCl and a mixture of chloroform and methanol (2:1, v/v), as described by Choi et al. (2017). For $\delta^{15}N_{bulk}$ analysis, the homogenized samples were used directly without any treatment. Carbon and nitrogen isotope ratios were measured using an elemental analyzer (EA-3000, Eurovector, Italy) with an isotope ratio mass spectrometer (Isoprime100, Isoprime Ltd., UK). The isotope ratios of carbon and nitrogen are expressed as parts per thousand, as follows:

$$\delta X\,(‰) = [(R_{sample}/R_{standard} - 1) \times 1000] \quad (1)$$

where X is ^{13}C or ^{15}N, and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for carbon or nitrogen, respectively. As standards for each value of carbon and nitrogen, Pee Dee Belemnite and air (N_2) were used, respectively. The degree of analytical precision was within 0.2‰ for both $\delta^{13}C_{bulk}$ and

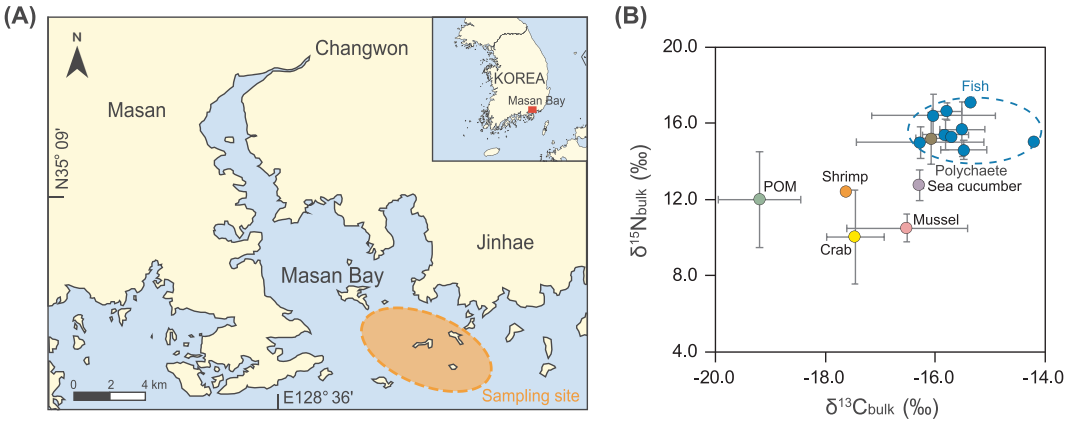

Fig. 1. (A) Study site at Masan Bay and (B) the dual plot of $\delta^{13}C$ and $\delta^{15}N$ values for biotic samples including POM (n = 69). POM was sampled to compare source differences.

$\delta^{15}N_{bulk}$. Average stable isotope ratios from each species were used for the discussion of food web structure.

2.3. Compound-specific isotope analysis: $\delta^{15}N_{AAs}$

TP was recalculated from $\delta^{15}N_{AAs}$ values. For the comparison, 10 fish and 5 benthos (14 species, Table 1) were selected for CSIA. Two flounders (*Pleuronichthys cornutus*) were selected, as these 2 samples had TP values derived from $\delta^{15}N$ bulk values that differed significantly. The $\delta^{15}N_{AAs}$ analysis was performed according to the procedure reported previously (Chikaraishi et al., 2009). Briefly, 3–5 mg of dried sample was hydrolyzed with 12 M HCl at 110 °C overnight (approximately 12 h) in a reacting vial, and lipids were removed with n-hexane/dichloromethane (3:2, v/v). The hydrolysate was derivatized with thionyl chloride/iso-propanol (1:4, v/v) for 2 h at 110 °C and sequentially treated with pivaloyl chloride/dichloromethane (DCM) (1:4, v/v) under the same conditions used for the next derivatization. The derivatives were then extracted with n-hexane/DCM (3:2, v/v). Gas chromatography (Agilent 6890N, Agilent Technologies, Santa Clara, CA) coupled with an isotope ratio mass spectrometer (GC-IRMS, Isoprime, GV Instruments) linked via a combustor (GV Instrument) was used to determine the nitrogen isotope ratios for individual amino acids. In the GC system, an ultra-2 capillary column (50 m long, 0.32 mm i.d., 0.52-μm film thickness, Agilent) was used with helium as carrier gas in constant flow mode (1.1 mL min^{-1}). The temperatures used for combustion and the reduction furnace were 980 °C and 650 °C, respectively. Standards were measured every 5 samples to assure precision in the isotope ratios. The analytical precision was within 0.5‰.

Table 1
Trophic positions (TPs) of organisms from Masan Bay, in Sept. 2016 (The measurements are for each individual, n = 15).

Organisms	Trophic position (TP)	
	TP_{bulk}[a]	TP_{AAS} (CSIA)[b]
Vertebrate (Fish)		
Bartail flathead *Platycephalus indicus*	4.1	2.9
Rock fish *Sebastes inermis*	3.7	3.1
Spotbelly rockfish *Sebastes pachycephalus*	3.9	3.1
Masked greenling *Hexagrammos otakii*	3.7	3.1
Ridged-eye flounder #1 *Pleuronichthys cornutus*	3.1	2.5
Ridged-eye flounder #2 *Pleuronichthys cornutus*	3.8	2.5
Crimson seabream *Evynnis japonica*	3.2	2.8
Multicolorfin rainbowfish Halichoeres sp.	3.4	2.6
Tidepool gunnel *Pholis nebulosa*	3.5	3.1
Japanese scad *Decapterus maruadsi*	3.3	3.0
Invertebrate		
Shrimp	2.5	2.4
Sea cucumber	2.4	2.4
Polychaete	3.5	3.1
Crab	2.3	2.7
Mussel *Mytilus sp.*	2.0	2.0

[a] bulk: TP calculated using $\delta^{15}N$ isotope value of bulk nitrogen (TDF: 3.4‰) (Minagawa and Wada, 1984).

[b] CSIA: TP calculated using $\delta^{15}N$ isotope value of amino acid (glutamic acid and phenylalanine) (TDF: 7.6‰) (Chikaraishi et al., 2009).

2.4. Analysis of POPs in biological samples

Ten fish (Table 1) used to analyze TP were selected based on a $\delta^{13}C$ values (heavier than −16.5‰) with a ranges of benthic food sources for measuring POPs and comparative studies. The quantification of PCBs, PBDEs, and OCPs was conducted using protocols consistent with U.S. Environmental Protection Agency methods (EPA 1618, US EPA 1989), with some modifications in selected samples (n = 10). In brief, 5 g wet weight of sample biota tissue was homogenized with anhydrous sodium sulfate (Na_2SO_4). Before extraction, surrogate standards were spiked into the samples (Table S2). These samples were extracted via ultrasonification in dichloromethane and hexane solution (1:1, v/v). Extracts were then centrifuged, concentrated under N_2 gas, and passed through a column containing silica gel, with elution via 200 mL of dichloromethane and hexane solution (1:1, v/v).

All targeted POPs were measured using gas chromatography coupled with high-resolution mass spectrometry (JMS-700D, JEOL Ltd., Tokyo, Japan). The 28 PCB congeners (CB 8, 18, 28, 29, 33, 44, 52, 66, 70, 87, 101, 105, 110, 118, 128, 138, 153, 168, 170, 180, 187, 194, 195, 199, 200, 205, 206, and 209) were quantified, and total PCBs (ΣPCBs) were derived as the sum of the measured congeners. The ΣDDTs, ΣHCHs, and ΣChlordane were reported as the sum of 6 metabolites (*o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT, and *p,p′*-DDT), 4 isomers (α-HCH, β-HCH, γ-HCH, and δ-HCH), and 8 isomers (α-chlordane, β-chlordane, γ-chlordane, oxy-chlordane, trans-chlordane, cis-chlordane, trans-nonachlordane, and cis-nonachlordane), respectively. The target compounds for PBDEs were from BDE-17, 28, 47, 66, 99, 100, 138, 153, 154, 183, and 209, which were present at levels over the detection limit of 0.12–1.9 pg g^{-1}. Finally, the concentrations of POPs in organisms were normalized using lipid-level measurements obtained with the gravimetric method. The recovery of surrogate standards for analysis of POPs in fish samples was generally acceptable, ranging from 40 to 145%, 43 to 92%, and 22 to 151% for PCBs, OCPs, and PBDEs, respectively (Table S2). In addition, the recovery of matrix-spiked samples ranged from 94 to 104% for OCPs and PBDEs, with % RSD < 10%. The reproducibility of the results ranged from 7.0 to 17% and from 5.0 to 18.5% for OCPs and PBDEs, respectively.

2.5. Estimation of TPs and TMFs

TP was calculated using Eqs. (2) and (3) for $\delta^{15}N_{bulk}$ and $\delta^{15}N_{AAs}$, respectively.

$$TP_{bulk} = [(\delta^{15}N_{sample} - \delta^{15}N_{filterfeeder})/TDF] + 2 \quad (2)$$

$$TP_{AAs} = [(\delta^{15}N_{glu} - \delta^{15}N_{phe} - \beta)/TDF] + 1 \quad (3)$$

where 3.4‰ (Minagawa and Wada, 1984) and 7.6‰ (Chikaraishi et al., 2009) were used as the TDF of $\delta^{15}N$ for TL calculations based on bulk and amino acids, respectively, and 3.4‰ was used as β-value (Chikaraishi et al., 2009). An isotope value from a filter feeder (mussel, $\delta^{15}N_{bulk}$ = 10.50‰) was assumed to represent that of a secondary consumer when calculating TL_{bulk}, and its TL_{AAs} value was correct, based on Eq. (3) (Table S1).

The relationships between concentrations of POPs and TP were compared in 10 fish selected as benthic organisms based on carbon isotope ratio: bartail flathead, rockfish, spotbelly rockfish, masked greenling, flounder (n = 2), seabream, rainbowfish, tidepool gunnel, and Japanese scad (Table 1). The relationships between accumulated concentrations of POPs and TP in a given organism are shown in Eq. (4), and TMFs were calculated using Eq. (5).

$$\log C_{POPs}(lipid\ \ normalized) = a + b\ \ TP \quad (4)$$

$$TMF = 10^{b} \quad (5)$$

2.6. Statistical analysis

Data are expressed as mean (± standard deviation) of bulk isotope values. Simple linear regression and correlation analysis based on Pearson correlations were used to assess relationships between variables. The homogeneity of variance was assessed with Levene's test. SPSS ver.18.0 (SPSS Inc., Chicago, IL) was used for all statistical analysis. A *p*-value below 0.05 was regarded as statistically significant.

3. Results and Discussion

3.1. Masan Bay food web structure

The $\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$ values for all samples ranged from −18.8 to −14.2‰ and from 8.3 to 18.03‰, respectively (Fig. 1B, Table S1). The ^{13}C values of consumers in the illustrated food web differed between pelagic and benthic trophic pathways. The $\delta^{13}C$ signatures of several fish were similar to those of benthic invertebrates but not to those of pelagic POM and shrimp (−19.0 ± 0.9‰ and −17.6‰, respectively, Fig. 1B). The wide range of $\delta^{13}C$ values in organisms from Masan Bay suggests that the organisms feed on different and/or diverse carbon sources. The similar ranges of carbon isotope ratios observed in fish and benthos suggest that a benthic diet (i.e., benthic microalgae) rather than POM (plankton, etc.) is likely a substantial component of the food web in Masan Bay. The ranges of $\delta^{13}C$ values in those consumers were similar to values reported previously for benthic algae (−17‰), whereas marine phytoplankton had values of approximately −22‰ (McConnaughey and McRoy, 1979; France, 1995; Kim et al., 2012). Furthermore, despite a slight shift and a wider range due to annual variation in the contribution of diverse sources, the consistency of our results with those reported previously in studies of carbon isotope values indicates that the food chain guilds of pelagic POM are not likely a major dietary resource for benthic invertebrates and fish in this region (Kim et al., 2012).

The wide range of bulk nitrogen isotope ratios indicates the existence of diverse trophic levels in the structured food web of this study area. In particular, the $\delta^{15}N$ values in fish were higher than those of POM and benthic invertebrates (Fig. 1B). This finding is similar to previous reports (Baker and Sheaves, 2006; Kim et al., 2012) and supports the general consensus that higher $\delta^{15}N$ values are found in organisms with higher TP. Among the tested samples, the top predators of the benthic ecosystem were flathead and rockfish with a carnivorous feeding strategy (Baker and Sheaves, 2006; Park et al., 2018). The high $\delta^{15}N_{bulk}$ of these benthic fishes demonstrates that both organisms have a high TP, and their benthic environment habitat may be a good window to recognize transfers of POPs in this area. The $\delta^{15}N_{bulk}$ of several organisms including mussel, benthic fish (flounder, rockfish, seabream, and greenling), and shrimp showed ranges similar to those observed previously for measurements of Masan Bay organisms (Kim et al., 2012), providing evidence that the feeding habitats and behaviors of these consumers are well defined.

3.2. TP as determined by $\delta^{15}N_{bulk}$ and $\delta^{15}N_{AAs}$

The $\delta^{15}N$ values of phenylalanine (the representative source amino acid) ranged from 6.8‰ to 18.5‰, while the $\delta^{15}N$ values for glutamic acids (the representative trophic amino acid) showed greater enrichment (from 10.7‰ to 27.7‰ in 10 fish) (Table S3). The $\delta^{15}N$ value for glutamic acid was about 8‰, which was greater than the bulk nitrogen isotope value. The variation in the isotope ratio of phenylalanine was smaller than that observed for other amino acids, indicating that this amino acid did not change significantly from source values. The patterns of $\delta^{15}N$ values for these source and trophic amino acids (e.g., phenylalanine and glutamic acid) are consistent with the general findings that these isotope values increased from source to consumer, as they traveled through a food web with different fractionation factors (Choi et al., 2017).

The TP_{bulk} values calculated from all samples are listed in Tables 1 and S1. These values ranged from about 1.9 (± 0.7, crab) to 4.0 (spotbelly rockfish, *Sebastes pachycephalus*) when the mussels were assigned a TP of 2.0. The TP_{bulk} of spotbelly rockfish was similar to that of bartail flathead (*Platycephalus indicus*) (3.7 ± 0.3) and masked greenling (*Hexagrammos otakii*) (3.8 ± 0.1), and greater than that observed for rockfish (*Sebastes* sp.) (3.5 ± 0.4) and Japanese scad (*Decapterus maruadsi*) (3.3 ± 0.2). The TP_{bulk} of benthic invertebrates ranged from 1.9 (± 0.7, crab) to 3.6 (± 0.01, polychaete). Meanwhile, estimated TP_{AAs} values ranged from 2.0 to 3.1 for selected invertebrates and from 2.6 to 3.1 for fish (n = 15). In particular, the TP_{AAs} of two ridged-eye flounder showed the same TP (2.5, n = 2), while those based on $\delta^{15}N_{bulk}$ (TP_{bulk}) showed significantly different values (3.1 and 3.8), even though these two individuals were similar in size (Tables 1 and S3).

The TPs of consumers in a food web provide information on connectedness and energy flow (Chikaraishi et al., 2014). The values obtained from samples in this study showed that most organisms, except crab, were higher when derived from TP_{bulk} than when derived from the corresponding TP_{AAs} (Table 1). In recent studies, TPs calculated from TP_{bulk} and TP_{AAs} yielded different values, but the patterns of these differences were not consistent (Chikaraishi et al., 2009; Kobayashi et al., 2019). In our study, for TP calculation using $\delta^{15}N_{bulk}$, mussels (*Mytilus* sp.) were used as the baseline organism, based on the hypothesis that filter feeders are annotated at trophic level 2 (Post, 2002). It was suggested that a primary consumer (TP = 2) would reduce the difference in isotopic turnover rate between producers and consumers, as they have relatively long isotopic turnover rates. However, the unexpected TP observed in crab (TP_{bulk} = 1.9 ± 0.7) disproved the inefficiency of nitrogen isotope ratios from bulk samples with different or overlapping sources. This is because TP_{bulk} was calculated based on the isotope value of the instant diet we measured at that time. In this study, we used mussel *Mytilus* sp. as a primary consumer. In general, crab holds a carnivorous position in the food chain and has the potential to accumulate high levels of pollutants from its prey (Voorspoels et al., 2004). In a study conducted in the same area in 2009, Kim et al. (2012) also mentioned that bulk isotope ratios of nitrogen in crab (13.7–14.7‰; mussels: 9.7–11.2‰) explained their role as a benthic

carnivore (trophic level = 3) in this ecosystem. The discrepancy in the TPs of these top predators suggests that their diets are derived from different sources (overlapping), and/or that species-specific and spatial variability (fish) in feeding may affect nitrogen values. The result observed in two ridged-eye flounders of similar size, which varied substantially in TP_{bulk} but not in TP_{AAs}, indicates that spatial variability and its effects on sources can be normalized with amino acids because the range is more stable than that measured in bulk nitrogen samples, thus providing more reliable TPs. Bowes and Thorp (2015) also demonstrated small errors in TP using new equations with CSIA-AAs, compared to the error obtained with the use of bulk isotope values. The large differences in TP between these two approaches (TP_{bulk} and TP_{AAs}) demonstrate that the bulk isotope approach cannot incorporate long-term integrated baseline information for fish, as they have diets with wide ranges of baseline values.

3.3. Pops in organisms

Despite the ban on use of these compounds in many countries, including South Korea, the properties of PCBs, PBDEs, and OCPs causes significant bodily burden in several aquatic organisms, such as invertebrates (e.g., clams) and mammals (finless porpoises) (Park et al., 2010; Choi et al., 2014). In particular, sedimentary POPs have received attention because of transfers to higher trophic organisms (including humans), as sediment-dwelling organisms such as benthic invertebrates and fish have great potential for taking up the pollutants and passing on large burdens of chemicals to their predators (Voorspoels et al., 2004; Hardell et al., 2010; Liu et al., 2010; Choi et al., 2014). Ten fish samples collected from Masan Bay contained measurable concentrations of PCBs, PBDEs, and OCPs (Fig. 2, Tables S4–S6). The targeted compounds were PCBs (22 congeners, which excludes 6 congeners below the detection limit), PBDEs (11 congeners), OCPs, three isomers of HCHs, chlordane, and DDT and its metabolite forms (DDD and DDE). Lipid levels in fish varied widely among species, with values ranging from 0.3% in rockfish to 24.4% in Japanese scad (Table S7). Total concentrations of PCBs, PBDEs, and OCPs in aquatic organisms ranged from 1.8 to 39 ng g^{-1} lipid weight, from 0.54 to 11 ng g^{-1} lipid weight, and 1.5 to 18 ng g^{-1} lipid weight, respectively. Although total PCB concentration in Japanese scad was the lowest among all fish samples, most congeners showed detectable concentrations, with only a few below limit of quantification (LOQ) (CB-33, −70, −168, −194, −199, and −205). The greatest concentrations of PCBs were found in rockfish, likely due to low lipid content (0.33%). Tetra-, hexa-, and hepta-CBs (CB-44, −52, −66, −87, −101, −105, −110, −118, −128, −138, and −153) accounted for about 77% of total PCBs. BDE-47 and 209 accounted for the largest proportions of total PBDEs, with BDE-47 accounting for an average of 35%. Three isomers of HCHs (α-HCH, β-HCH, and γ-HCH) and five forms of chlordane were detected. For OCPs, the greatest concentrations were found in crab. The concentration of ΣDDTs accounted for 85 ± 9% (65–94%) of total OCPs, indicating a substantial contribution of DDT metabolites DDE and DDD.

For several decades, many studies have reported that the sediments in Masan Bay have significant concentrations of pollutants including trace metals, mercury, and several organic toxic substances (Khim et al., 1999; Kim et al., 2012; Cho et al., 2015). For the PCBs investigated in this study, the ratio of lower chlorinated congeners (tri- and tetra-CBs, CBs-18, −28, −29, −44, −52, and −66) to total PCBs was not significant (16%) in organisms, indicating that these congeners are susceptible to biological metabolic processes. In fact, octanol-water distribution coefficient (log K_{ow}) values (Voorspoels et al., 2004; Walters et al., 2011) are key physicochemical factors of the bioaccumulation of organic pollutants. The proportions of penta-, hexa-, and hepta-CBs among total PCB levels were greater than 77% (45–93%), indicating that the accumulated features of PCBs in marine organisms from Masan Bay are governed by these chemical properties. In the accumulated patterns of organisms, the concentrations of ΣDDT, ΣChlordane, and ΣHCH correlated strongly (Pearson correlation, $R > 0.7$; $p < 0.01$, Table S8). This finding suggests that these POPs (PCBs, PBDEs, and OCPs) have similar sources and/or behavior in this study area and that the mode of action among individuals may be used to extrapolate accumulation trends in the marine food web.

3.4. Trophic magnification factors based on TP_{bulk} and TP_{AAs}

To understand the fate of pollutants and their accumulation in organisms, recognizing the TPs of organisms based on transfer through the food chain is quite helpful for trophic magnification studies (Azevedo-Silva et al., 2016; Won et al., 2018; Kobayashi et al., 2019). The POP concentrations in organisms included in this study were compared with TP_{bulk} and TP_{AAs} values, and positive correlations were found for several target compounds (Fig. 3). The increasing trends observed for TP show that some POPs can be biomagnified through the benthic food chain in Masan Bay. Previous studies in aquatic environments have shown evidence of POP magnification in some organisms (Wang et al., 2012; Kobayashi et al., 2015; Verhaert et al., 2017). In particular, the TMF values for PCB, OCPs, DDT, and DDT metabolites are > 1 in many studies, although values depend on trophic relations among various environments. The lower magnified patterns in this study, however, are consistent with the pattern observed in a comparative study on TMF in pelagic and benthic ecosystems (Fan et al., 2017). Benthic organisms have flattened biomagnification trends, as they take in extra contaminants via sediment, resulting in reduced interspecies differences in contaminant levels (Fan et al., 2017). The study of TMF in benthic environments is difficult, as the various feeding strategies and the degrees of source dependence on POM and sediments can govern the uptake of pollutants (Evenset et al., 2016). As TMF

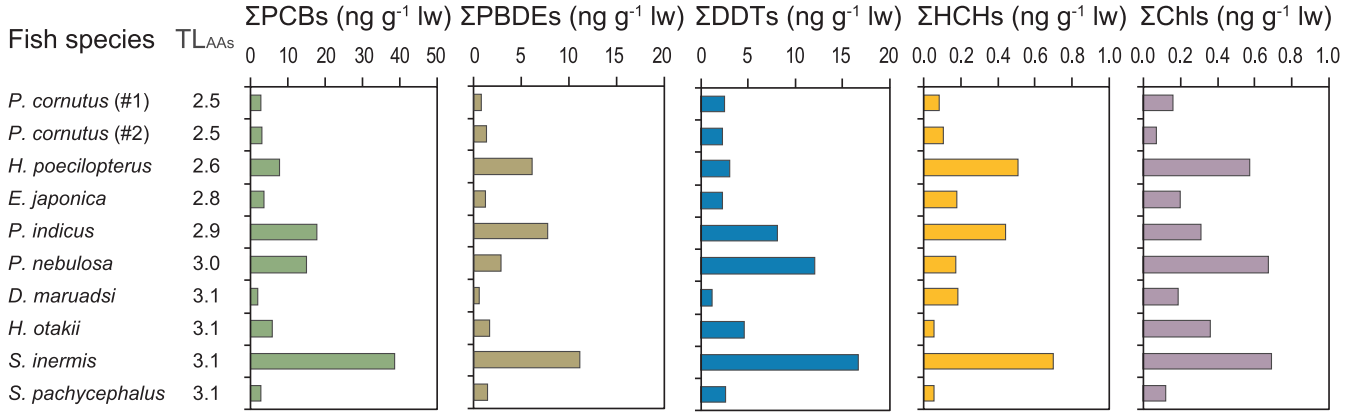

Fig. 2. PCB, PBDE, and OCP levels (expressed in ng/g lipid weight) for fish samples collected from Masan Bay.

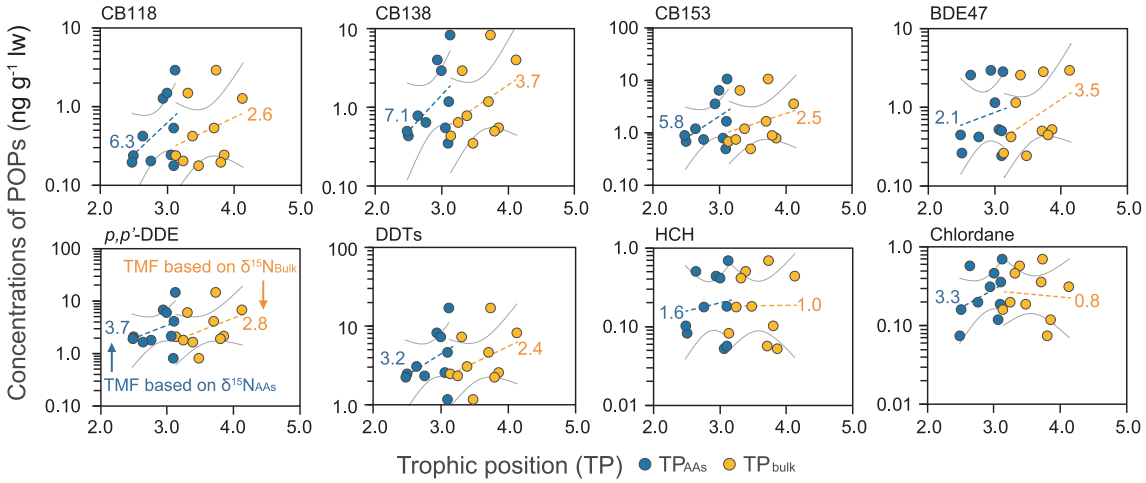

Fig. 3. Relationships between trophic position (TP, TP_{bulk}, and TP_{AAs}) and organic pollutants (PCB 118, PCB 138, PCB 153, BDE47, *p,p*-DDE, DDTs, HCH, and chlordane (log ng/g lipid weight) in fish collected from Masan Bay.

represents an average increase in contaminants per TP, our concern was that the narrow range of TP (3.1–4.1 for TP_{bulk} and 2.5–3.1 for TP_{AAs}) in this study might be insufficient to obtain a robust TMF. However, the positive trends and modulations observed in the relationships between logarithmic concentrations of POPs and TPs (TP_{bulk} vs. TP_{AAs}) demonstrate that TP is a key factor in determining TMF. Despite the difficulty of noting significance due to the small number of samples, statistical significance was noted for the effects of *pp'*-DDD and cis-nonachlordane on the relationships between TP_{AAs} and logarithmic concentrations of POPs ($p < 0.05$).

The TMFs calculated from the slopes of logarithmic concentrations of pollutants and TPs varied according to pollutant and isomer (Table S9). For several congeners and isomers, differences in slope (TMF) in the relationships of TP and pollutants (PCBs, PBDEs, and OCPs) were greater for TP_{AAs} than for TP_{bulk}. In particular, high TMF values were observed for high molecular (from penta- to deca-) weight PCBs (e.g., CB-118, −138, −158), some congeners of BDE, and total concentrations of OCP (ΣHCH, ΣDDT, and ΣChlordane) in this study (Fig. 3). In studies that compared TP_{bulk} and TP_{AAs}, the TP_{AAs} values were smaller than the TP_{bulk} values for some samples. This is why the expected TMF was larger for the TP_{AAs} than for the TP_{bulk} in this study (Fig. 4A). In particular, the difference between TP_{bulk} and TP_{AAs} increases with the isotope ratio of $\delta^{15}N_{bulk}$ (Figs. 4A and S1). This indicates that the information pertaining to different baseline values is involved in $\delta^{15}N_{bulk}$ and may affect TP, as arbitrary and similar values were adopted for all samples (10.8‰ for $\delta^{15}N_{bulk}$ for mussels in Eq. (2)). Nitrogen isotope values that were corrected for source amino acids ($\delta^{15}N_{bulk\text{-}phe}$) showed significant positive correlation with the differences between trophic and source ($\delta^{15}N_{glu\text{-}phe}$) isotope values, demonstrating the effects of baseline values in calculating TP for each sample (Fig. 4B–D).

The results of this study also show that calculating TP based on bulk nitrogen (TP_{bulk}) may be inaccurate in a region with variable sources, particularly when using fish, which are mobile organisms, as target samples. Although the small number of samples lowers the reliability of the calculated TMF values, the increase in TMF and the significance observed for some compounds were probably caused by the accuracy of the CSIA approach in calculating TP. In a biomagnification study conducted in Yellow Sea, Byun et al. (2013) reported that active migration of whale cause limitation to identify their TP using $\delta^{15}N$ (bulk nitrogen) approach. Recent studies using CSIA-AAs approach to figure out TP have also reported that TP_{bulk} have difficulty in calculating reliable TP for migratory organisms (bird) due to the spatial and/or temporal differences in baseline values and wide habitat (Dolgova et al., 2018; Hebert and Popp, 2018). Sackett et al. (2015) also suggest that $\Delta\delta^{15}N$, which normalizes bulk nitrogen values to source amino acid values ($\delta^{15}N_{bulk} - \delta^{15}N_{source\ amino\ acids}$), can be a proxy for more robust TP in a food web, because bulk isotope values include baseline isotopic variation. This was the reason recent studies have attempted to use CSIA-AAs to determine TP, in an attempt to overcome key issues associated with values measured from bulk tissue (Chikaraishi et al., 2009; Sackett et al., 2015; O'connell, 2017; Ek et al., 2018). The comparative results presented here (see Fig. 2) indicate a useful application of CSIA-AAs in characterizing the fate of and risks associated with pollutants in environments associated with trophic transfer within the food web.

4. Perspectives

Recent studies using the CSIA approach have broadened its applications in ecology, physiology, and environmental sciences. The results of this study suggest that nitrogen isotope ratios of amino acids are helpful to determine reliable positions (e.g., TP) of organisms in the aquatic food web in aquatic ecosystems. As TP estimation may reflect the uncertainties and complexities of the $\delta^{15}N$ of primary producers (baseline), CSIA-AA approaches have great potency in constructing reliable and robust TPs for fish that may have been affected by wide-ranging baseline values ($\delta^{15}N$-producers) due to their movement and feeding strategy. This study demonstrated that the TPs of organisms may be a key factor for clarifying the burden of organic pollutants in food webs.

For an improved understanding of trophic magnifications of pollutants in aquatic environments, the accuracy of the concentrations used for TMF calculation is important. In the current study, age, maturity, sex, and size of the fish were not taken into account, despite the fact that physiological condition and life cycle may lead to a wide range of lipid levels in fish. In addition, the comparative results and their application to TMF studies showed that a more extensive dataset is required to improve our understanding in this study area, and more intensive studies using a novel approach (e.g. CSIA-AAs) have to be conducted to fill the knowledge gap between pollutants and their fates in benthic ecosystems.

CRediT authorship contribution statement

Eun-Ji Won: Conceptualization, Visualization. **Bohyung Choi:** Conceptualization, Visualization, Supervision, Validation, Formal analysis. **Chang Hwa Lee:** Supervision, Validation, Visualization. **Seongjin Hong:** Conceptualization, Visualization, Formal analysis. **Jong-Hyeon Lee:** Supervision, Validation, Visualization. **Kyung-Hoon Shin:** Conceptualization, Visualization.

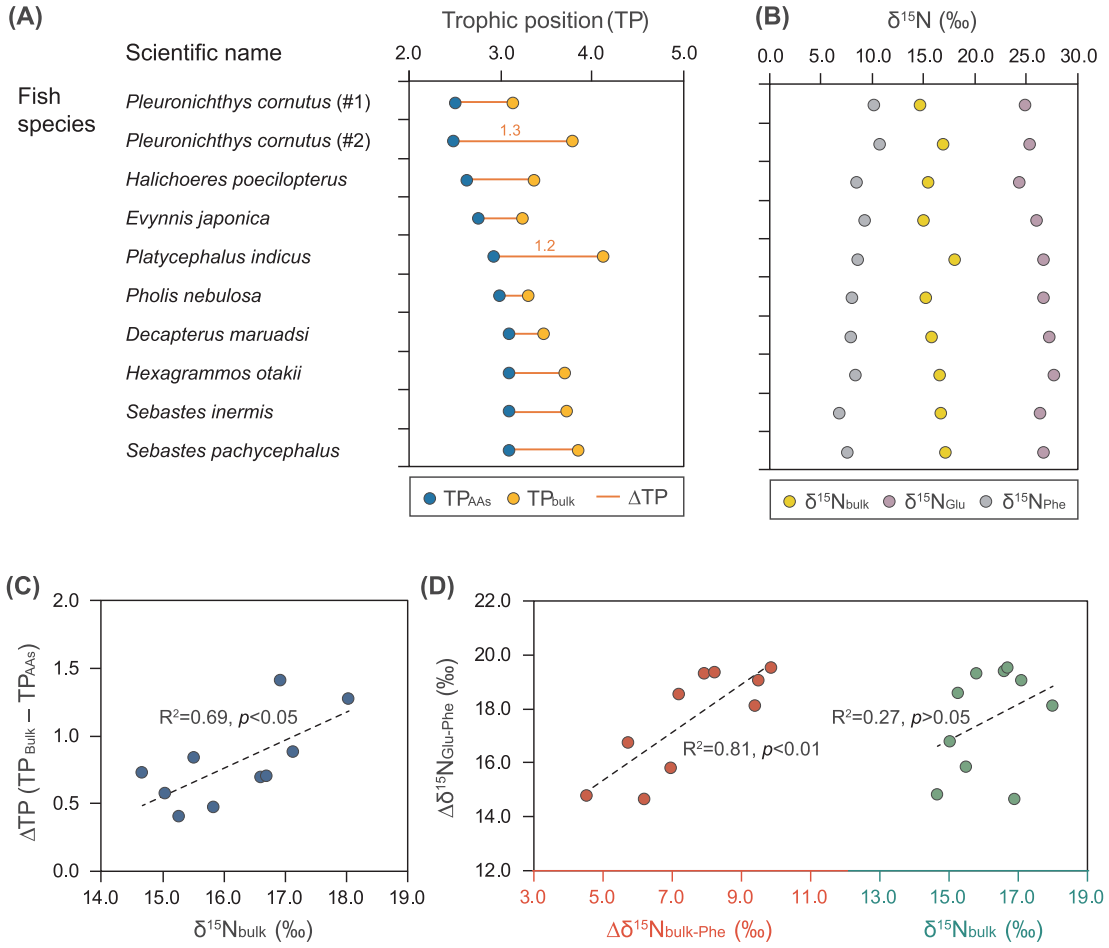

Fig. 4. (A) Differences between TPs for fish calculated based on nitrogen bulk isotope ratios and compound-specific isotope analysis of amino acids. (B) Nitrogen isotope values based on bulk tissue and amino acids (glutamic acid and phenylalanine) measurements from 10 fish collected from Masan Bay. (C) Correlations of stable isotope ratios for bulk nitrogen and differences between TL_{bulk} and TL_{AAs} (ΔTL). (D) Linear relationships between nitrogen stable isotopes in bulk samples and source-normalized values ($\Delta\delta^{15}$Nbulk-source, Bulk-Phe, x-axis), in comparison to trophic discrimination factors (mean TDF, $\Delta\delta^{15}N_{Trophic\text{-}source}$, Glu-Phe, y-axis) for fish.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgments

This study was supported by a project entitled “Development of techniques for assessment and management of hazardous chemicals in the marine environment (2014-0342)” funded by the Ministry of Oceans and Fisheries of Korea. This work was also supported by a National Research Foundation of Korea grant funded by the Korean government (NRF-2016R1E1A1A01943004 and NRF-2018R1A6A3A03012206).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105361.

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<Supplementary Materials>

Variability of Trophic Magnification Factors as an Effect of Estimated Trophic Position: Application of Compound-Specific Nitrogen Isotope Analysis of Amino Acids

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Supplementary Tables

Supplementary Figure

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Table S1. $\delta^{13}C$ and $\delta^{15}N$ values of all biotic samples measured in this study (Mean±S.D, n=69 (14 species), Sep. 2016, Masan Bay).

[*] n indicates number of sample used for analyzing bulk carbon and nitrogen isotope ratios/

[**] N/A indicates not available sample to measure the size.

Group	Organisms	Isotopic value (‰)			Remarks	
		$\delta^{13}C$	$\delta^{15}N_{bulk}$	TP_{bulk}	Size	n*
Fish	Bartail flathead *Platycephalus indicus*	-16.03 ± 1.12	16.40 ± 1.11	3.7	27 ± 2.5	5
	Rock fish *Sebastes inermis*	-15.21 ± 0.41	15.68 ± 1.44	3.5	15.6 ± 4.3	2
	Spotbelly rockfish *Sebastes pachycephalus*	-15.36	17.12	4.0	22.6	1
	Masked greenling *Hexagrammos otakii*	-15.79 ± 0.28	16.62 ± 0.47	3.8	18.7 ± 2.3	16
	Ridged-eye flounder *Pleuronichthys cornutus*	-15.82 ± 0.42	15.39 ± 0.80	3.4	15.5 ± 1.8	12
	Tidepool gunnel *Pholis nebulosa*	-15.69	15.26	3.4	N/A***	1
	Crimson seabream *Evynnis japonica Tanaka*	-14.20	15.04	3.3	11.5	1
	Multicolorfin rainbowfish *Halichoeres sp.*	-15.48 ± 0.42	14.61 ± 0.52	3.2	13.9 ± 0.68	8
	Japanese scad *Decapterus maruadsi*	-16.27 ± 1.16	15.01 ± 0.83	3.3	16.3± 3.2	12
Invertebrate	Shrimp	-17.61	12.43	2.6	N/A	1
	Crab	-17.46 ± 0.52	10.04 ± 2.47	1.9	N/A	2
	Sea cucumber *Protankyra bidentata*	-16.29 ± 0.06	12.75 ± 0.79	2.7	N/A	2
	Polychaeta	-16.07 ± 0.27	15.21 ± 1.34	3.6	N/A	3
	Mussel *Mytilus sp.*	-16.52 ± 1.10	10.50 ± 0.72	2.0	N/A	3

Table S2. Percent recoveries of surrogate standards for persistent organic pollutants measured in the present study.

Analytes			Recovery (%)		
			Min	Max	Median
PCBs	Tri	2,2',6 (#19L)	42.4	99.9	71.5
	Tri	2,4,4' (#28L)	85.3	114.4	93.5
	Tetra	3,4,4',5 (#81L)	56.0	84.8	67.2
	Tetra	3,3',4,4' (#77L)	57.8	79.1	68.7
	Tetra	2,2',5,5' (#52L)	53.3	86.9	68.8
	Tetra	2,3',4',5 (#70L)	83.6	94.7	89.0
	Penta	2,2',4,6,6' (#104L)	40.4	110.3	69.0
	Penta	2',3,4,4',5 (#123L)	47.7	80.2	67.0
	Penta	2,3',4,4',5 (#118L)	46.0	95.0	68.0
	Penta	2,3,4,4',5 (#114L)	44.0	92.8	72.8
	Penta	2,3,3',4,4' (#105L)	44.0	90.8	73.1
	Penta	3,3',4,4',5 (#126L)	47.8	83.2	62.3
	Penta	2,2',3,5',6 (#95L)	51.2	112.4	72.5
	Penta	2,2',4,5,5' (#101L)	55.6	112.4	65.6
	Hexa	2,3',4,4',5,5' (#167L)	41.5	88.6	64.4
	Hexa	2,2',4,4',5,5' (#153L)	56.1	125.2	93.1
	Hexa	2,2',3,4,4',5' (#138L)	46.3	101.1	75.9
	Hepta	2,2',3,4',5,6,6' (#188L)	71.8	124.4	109.7
	Hepta	2,3,3',4,4',5,5' (#189L)	68.9	91.5	74.1
	Hepta	2,2',3,4,4',5,5' (#180L)	49.4	145.4	85.6
	Hepta	2,2',3,3',4,4',5 (#170L)	39.7	145.4	67.8
	Octa	2,3,3',4,4',5,5',6 (#205L)	52.5	134.5	72.7
	Deca	2,2',3,3',4,4',5,5',6,6' (#209L)	66.3	82.7	70.3
HCHs	13C-α-HCH		52.7	81.9	69.2
	13C-β-HCH		57.1	90.8	75.3
	13C-γ-HCH		48.7	82.9	61.4
Chlordane	13C-γ-Chlordane		42.9	76.8	59.7
DDTs	13C-o,p'-DDE		59.1	75.8	60.7
	13C-p,p'-DDE		62.3	82.3	71.9
	13C-o,p'-DDD		52.7	72.9	59.7
	13C-o,p'-DDT		48.9	81.3	64.8
	13C-p,p'-DDD		52.9	88.1	68.4
	13C-p,p'-DDT		61.7	91.7	79.8
PBDEs	13C-TrBDE	2,4,4` (#28L)	27.1	136.3	62.0
	13C-TeBDE	2,2`,4,4` (#47L)	29.2	170	61.3
	13C-PeBDE	2,2`,4,4`,5 (#99L)	38.8	151.2	61.8
	13C-HeBDE	2,2`,4,4`,5,6` (#154L)	37.5	144	61.5
	13C-HeBDE	2,2`,4,4`,5,5` (#153L)	41.7	130.2	70.2
	13C-HpBDE	2,2`,3,4,4`,5`,6 (#183L)	41.9	142.6	69.0
	13C-OcBDE	2,2',3,3',4,4',6,6' (#197L)	35.2	116.0	59.2
	13C-NoBDE	2,2',3,3',4,4',5,6,6' (#207L)	29.9	101.5	46.3
	13C-DeBDE	2,2',3,3',4,4',5,5',6,6' (#209L)	21.6	51.8	36.8

Table S3. Nitrogen stable isotope ratios (‰) of trophic and source amino acids and the calculated trophic position (TP).

Organisms		Glutamic acid (Trophic)	Phenylalanine (Source)	TP_{AAs}*	Size (cm)
Fish	Bartail flathead *Platycephalus indicus*	26.8	8.6	2.9	30.6
	Rock fish *Sebastes inermis*	26.4	6.8	3.1	18.6
	Spotbelly rockfish *Sebastes pachycephalus*	26.7	7.6	3.1	22.6
	Masked greenling *Hexagrammos otakii*	27.7	8.4	3.1	20
	Ridged-eye flounder *Pleuronichthys cornutus* (#1)	25.0	10.2	2.5	15.5
	Ridged-eye flounder *Pleuronichthys cornutus* (#2)	25.4	10.7	2.5	14
	Tidepool gunnel *Pholis nebulosa*	26.7	8.09	3.1	N/A†
	Crimson seabream *Evynnis japonica Tanaka*	26.1	9.3	2.8	11.5
	Multicolorfin rainbowfish *Halichoeres poecilopterus*	24.4	8.5	2.6	14
	Japanese scad *Decapterus maruadsi*	27.2	7.9	3.1	15
Invert.	Shrimp	22.8	9.0	2.4	N/A
	Crab	25.5	9.6	2.7	N/A
	Sea cucumber *Protankyra bidentata*	23.6	9.2	2.4	N/A
	Polychaeta	26.5	7.1	3.1	N/A
	Mussel	18.5	7.4	2.0	N/A

* TP_{AAs} was calculated by following Chikaraishi et al. (2009).

† N/A indicates not available sample to measure the size (cm).

Table S4. Concentrations of all PCB congeners in all biotic samples (ng g^{-1} ww, Sep., 2016, Masan Bay).

Congener		Fish*									
		1	2	3	4	5	6	7	8	9	10
di	CB 8	0.0050	0.0030	0.0040	0.0050	0.0060	0.0050	0.0050	0.0050	0.0120	0.0080
tri	CB 18	0.0030	0.0020	0.0030	0.0030	0.0040	0.0030	0.0040	0.0040	0.0092	0.0060
	CB 28	0.0070	0.0050	0.0060	0.0070	0.0080	0.0070	0.0080	0.0080	0.0180	0.0120
	CB 29	0.0040	0.0030	0.0030	0.0030	0.0040	0.0040	0.0040	0.0040	0.0090	0.0060
tetra	CB 44	0.0040	0.0030	0.0040	0.0040	0.0050	0.0040	0.0040	0.0050	0.0130	0.0070
	CB 52	0.0030	0.0020	0.0030	0.0056	0.0040	0.0030	0.0040	0.0040	0.0452	0.0060
	CB 66	0.0050	0.0040	0.0107	0.0162	0.0154	0.0076	0.0060	0.0060	0.0535	0.0090
penta	CB 87	0.0040	0.0030	0.0030	0.0112	0.0040	0.0040	0.0040	0.0040	0.0207	0.0205
	CB 101	0.0070	0.0107	0.0173	0.0484	0.0246	0.0107	0.0059	0.0040	0.1292	0.0978
	CB 105	0.0058	0.0065	0.0084	0.0203	0.0066	0.0030	0.0030	0.0030	0.0172	0.0242
	CB 110	0.0093	0.0055	0.0103	0.0325	0.0146	0.0050	0.0060	0.0060	0.0558	0.0470
	CB 118	0.0202	0.0210	0.0280	0.0658	0.0262	0.0133	0.0094	0.0067	0.1243	0.0886
hexa	CB 128	0.0068	0.0069	0.0054	0.0170	0.0053	0.0030	0.0030	0.0030	0.0070	0.0226
	CB 138	0.0639	0.0593	0.0619	0.1444	0.0471	0.0339	0.0287	0.0123	0.2420	0.1728
	CB 153	0.0572	0.0773	0.0901	0.2052	0.0768	0.0607	0.0340	0.0188	0.5351	0.2484
hepta	CB 170	0.0153	0.0165	0.0085	0.0199	0.0020	0.0051	0.0020	0.0020	0.0713	0.0020
	CB 180	0.0357	0.0239	0.0207	0.0533	0.0111	0.0151	0.0123	0.0105	0.1430	0.0482
	CB 187	0.0234	0.0205	0.0250	0.0486	0.0169	0.0156	0.0118	0.0059	0.1841	0.0678
octa	CB 195	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010	0.0020	0.0020	0.0099	0.0020
	CB 200	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010	0.0020	0.0020	0.0151	0.0020
nona	CB 206	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010	0.0020	0.0020	0.0176	0.0020
deca	CB 209	0.0020	0.0010	0.0010	0.0020	0.0020	0.0020	0.0020	0.0020	0.0284	0.0030
ΣPCBs		0.2917	0.2834	0.3222	0.7277	0.3015	0.2192	0.1785	0.1327	1.7684	0.9193

*Fish: 1 Bartail flathead *Platycephalus indicus*, 2 Rock fish *Sebastes inermis*, 3 Spotbelly rockfish *Sebastes pachycephalus*, 4 Masked greenling *Hexagrammos otakii*, 5 Ridged-eye flounder #1 *Pleuronichthys cornutus*, 6 Ridged-eye flounder #2 *Pleuronichthys cornutus*, 7 Crimson seabream Evynnis japonica, 8 Multicolorfin rainbowfish *Halichoeres poecilopterus*, 9 Tidepool gunnel *Pholis nebulosi*, and 10 Japanese scad *Decapterus maruadsi*.

Table S5. Concentrations of polybrominated diphenyl ethers (PBDEs) in aquatic organisms collected from Masan bay (ng g^{-1} ww, Sep., 2016).

Congener	**Fish***									
	1	2	3	4	5	6	7	8	9	10
BDE17	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0030	0.0169
BDE28	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010	0.0020	0.0020	0.0068	0.0120
BDE 47	0.0470	0.0203	0.0591	0.0612	0.0285	0.0303	0.0190	0.0399	0.0939	0.1193
BDE 66	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0020	0.0072
BDE 99	0.0090	0.0157	0.0241	0.0226	0.0100	0.0090	0.0100	0.0200	0.0232	0.0381
BDE 100	0.0138	0.0127	0.0229	0.0213	0.0090	0.0080	0.0090	0.0138	0.0232	0.0188
BDE 138	0.0010	0.0010	0.0010	0.0202	0.0010	0.0010	0.0010	0.0010	0.0171	0.0072
BDE 153	0.0020	0.0010	0.0110	0.0054	0.0020	0.0020	0.0020	0.0020	0.0102	0.0030
BDE 154	0.0112	0.0139	0.0207	0.0229	0.0010	0.0070	0.0010	0.0063	0.0341	0.0169
BDE 183	0.0056	0.0010	0.0010	0.0121	0.0082	0.0056	0.0020	0.0020	0.0068	0.0072
BDE 209	0.0318	0.0116	0.0179	0.0332	0.0288	0.0235	0.0080	0.0080	0.0228	0.0209
Total BDEs	0.1245	0.0802	0.1608	0.2018	0.0925	0.0894	0.0560	0.0969	0.2432	0.2675

*Fish: 1 Bartail flathead *Platycephalus indicus*, 2 Rock fish *Sebastes inermis*, 3 Spotbelly rockfish *Sebastes pachycephalus*, 4 Masked greenling *Hexagrammos otakii*, 5 Ridged-eye flounder #1 *Pleuronichthys cornutus*, 6 Ridged-eye flounder #2 *Pleuronichthys cornutus*, 7 Crimson seabream Evynnis japonica, 8 Multicolorfin rainbowfish *Halichoeres poecilopterus*, 9 Tidepool gunnel *Pholis nebulosi*, and 10 Japanese scad *Decapterus maruadsi*.

Table S6. Concentrations of all OCPs in aquatic organisms collected from Masan Bay (ng/g ww, Sep., 2016).

OCPs	**Fish***									
	1	2	3	4	5	6	7	8	9	10
α-HCH	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010	0.0020	0.0020	0.0040	0.0030
β-HCH	0.0030	0.0020	0.0020	0.0030	0.0030	0.0030	0.0030	0.0030	0.0232	0.0815
γ-HCH	0.0030	0.0020	0.0030	0.0030	0.0040	0.0030	0.0030	0.0030	0.0070	0.0050
ΣHCH	0.0070	0.0050	0.0060	0.0070	0.0090	0.0070	0.0080	0.0080	0.0342	0.0895
o,p′-DDE	0.0040	0.0030	0.0040	0.0040	0.0050	0.0040	0.0050	0.0050	0.0110	0.0077
p,p′-DDE	0.1093	0.1074	0.2490	0.5099	0.2336	0.1324	0.0829	0.0262	0.5129	0.4047
o,p′-DDD	0.0020	0.0010	0.0020	0.0020	0.0054	0.0020	0.0020	0.0020	0.0050	0.0284
p,p′-DDD	0.0020	0.0010	0.0186	0.0359	0.0147	0.0020	0.0020	0.0020	0.0410	0.1037
o,p′-DDT	0.0020	0.0010	0.0020	0.0020	0.0020	0.0020	0.0020	0.0020	0.0050	0.0060
p,p′-DDT	0.0100	0.0070	0.0181	0.0107	0.0110	0.0100	0.0110	0.0110	0.0230	0.0299
ΣDDT	0.1293	0.1204	0.2937	0.5645	0.2717	0.1524	0.1049	0.0482	0.5979	0.5805
a-chlordane	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010	0.0020	0.0020	0.0030	0.0216
b-chlordane	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010	0.0020	0.0020	0.0030	0.0105
oxy-	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010	0.0020	0.0010
trans-nona	0.0010	0.0010	0.0010	0.0140	0.0052	0.0010	0.0020	0.0020	0.0132	0.0199
cis-nona	0.0010	0.0010	0.0097	0.0265	0.0074	0.0010	0.0020	0.0020	0.0177	0.0403
ΣChlordane	0.0050	0.0050	0.0137	0.0435	0.0176	0.0050	0.0090	0.0090	0.0389	0.0933
Total OCP	0.1413	0.1304	0.3134	0.6150	0.2983	0.1644	0.1219	0.0652	0.6710	0.7633

*Fish: 1 Bartail flathead *Platycephalus indicus*, 2 Rock fish *Sebastes inermis*, 3 Spotbelly rockfish *Sebastes pachycephalus*, 4 Masked greenling *Hexagrammos otakii*, 5 Ridged-eye flounder #1 *Pleuronichthys cornutus*, 6 Ridged-eye flounder #2 *Pleuronichthys cornutus*, 7 Crimson seabream Evynnis japonica, 8 Multicolorfin rainbowfish *Halichoeres poecilopterus*, 9 Tidepool gunnel *Pholis nebulosi*, and 10 Japanese scad *Decapterus maruadsi*.

Table S7. Total concentrations of organic pollutants (ng g^{-1} lipid weight) from fish samples collected from Masan Bay.

Organisms		Organic pollutants (ng/g lipid weight)					Remarks
		ΣPCBs[a]	ΣPBDEs[b]	ΣOCPs ΣHCHs[c]	ΣDDTs[d]	ΣChlordanes[e]	Lipid (%)
Vertebrate (Fish)	Bartail flathead *Platycephalus indicus*	17.8	7.77	0.437	8.07	0.31	0.79
	Rock fish *Sebastes inermis*	38.5	11.14	0.694	16.72	0.69	0.33
	Spotbelly rockfish *Sebastes pachycephalus*	2.8	1.41	0.053	2.58	0.12	5.68
	Masked greenling *Hexagrammos otakii*	5.8	1.65	0.057	4.61	0.36	5.84
	Ridged-eye flounder #1 *Pleuronichthys cornutus*	2.6	0.84	0.082	2.46	0.16	5.50
	Ridged-eye flounder #2 *Pleuronichthys cornutus*	3.1	1.32	0.103	2.24	0.07	3.33
	Crimson seabream *Evynnis japonica*	3.6	1.23	0.176	2.31	0.20	2.60
	Multicolorfin rainbowfish Halichoeres sp.	7.6	6.15	0.508	3.06	0.57	1.00
	Tidepool gunnel *Pholis nebulosa*	15.0	2.92	0.171	12.07	0.67	8.33
	Japanese scad *Decapterus maruadsi*	1.8	0.54	0.181	1.17	0.19	24.37

[a]Σ PCBs: total PCBs concentrations (sum of 22 congeners).

[b]Σ PBDEs: total PBDEs concentrations (sum of BDE 17, 28, 47, 66, 99, 100, 138, 153, 154, 183, and 209).

[c]Σ HCHs: sum of 3 isoforms (α-HCH, β-HCH, γ-HCH) of Hexachlorocyclohexane.

[d]Σ DDTs: sum of 6 metabolites including *o*,*p*′-DDE; *p*,*p*′-DDE; *o*,*p*′-DDD, *p*,*p*′-DDD; *o*,*p*′-DDT; *p*,*p*′-DDT.

[e]Σ Chlordanes: total chlordane concentrations ((α–Chlordane, β–Chlordane, γ–Chlordane, oxy-Chlordane, trans-Chlordane, cis-Chlordane, trans-nona Chlordane, cis-nona Chlordane.

Table S8. Correlations between different pollutants (ΣPCBs, ΣPBDEs, ΣHCHs, ΣDDTs and ΣChlordane ng g^{-1} lipid weight) (n=10).

Pollutants		ΣPCB	Total BDE	ΣHCH	ΣDDT	ΣChlordane
ΣPCB	r	1				
	p value					
PBDE	r	0.863	1			
	p value	<0.01				
ΣHCH	r	857	0.913	1		
	p value	<0.01	<0.01			
ΣDDT	r	0.979	0.869	0.787	1	
	p value	<0.01	<0.01	<0.01		
ΣChlordane	r	0.799	0.802	0.879	0.757	1
	p value	<0.01	<0.01	<0.01	<0.01	

r: Pearson correlation coefficient.

Table S9. Pearson correlation (R) and trophic magnification factors (TMF) between pollutants and trophic positions (TP) calculated using bulk and amino acid approaches in ten fish collected in Masan Bay (Sept, 2016, Masan Bay, *P* indicates significances).

Pollutant	TP_{bulk}			TP_{AAs}			**Pollutant**	TP_{bulk}			TP_{AAs}		
	R	TMF	*P*	R	TMF	*P*		R	TMF	*P*	R	TMF	*P*
PCB 8	0.15	1.7	0.67	-0.11	0.6	0.76	BDE 138	0.11	2.6	0.75	0.45	**13.9**	0.20
PCB 18	0.09	1.3	0.81	-0.11	0.6	0.77	BDE 153	0.33	5.3	0.35	0.18	3.5	0.62
PCB 28	0.14	1.6	0.70	-0.09	0.7	0.81	BDE 154	0.59	37.1	0.07	0.47	34.0	0.17
PCB 29	0.17	1.9	0.64	-0.09	0.7	0.80	BDE 183	0.20	2.8	0.59	-0.22	0.5	0.54
PCB 44	0.13	1.6	0.72	-0.07	0.7	0.85	BDE 209	0.46	7.5	0.18	-0.05	1.1	0.89
PCB 52	-0.02	0.9	0.95	0.03	**1.2**	0.93							
PCB 66	0.04	1.1	0.92	-0.04	0.9	0.91	Total BDE	0.40	5.0	0.26	0.20	2.9	0.59
PCB 87	0.14	1.5	0.70	0.20	**2.1**	0.58							
PCB 101	0.08	1.3	0.82	0.47	**5.3**	0.17	α-HCH	-0.05	0.6	0.88	-0.12	**0.8**	0.74
PCB 105	0.35	2.9	0.33	0.46	**6.0**	0.18	β-HCH	-0.03	0.9	0.94	0.24	**2.7**	0.51
PCB 110	0.13	1.5	0.72	0.36	**3.6**	0.30	γ-HCH	0.17	1.8	0.64	-0.10	0.7	0.80
PCB 118	0.30	2.6	0.40	0.47	**6.3**	0.17							
PCB 128	0.42	4.1	0.23	0.37	**4.6**	0.30	ΣHCH	0.09	1.0	0.99	0.13	**1.6**	0.73
PCB 138	0.39	3.7	0.27	0.46	**7.1**	0.18							
PCB 153	0.29	2.5	0.42	0.43	**5.8**	0.22	*o,p'*-DDE	0.12	1.5	0.74	-0.08	0.7	0.82
PCB 170	0.44	14.4	0.21	0.30	9.9	0.40	*p,p'*-DDE	0.39	2.8	0.27	0.37	**3.7**	0.29
PCB 180	0.37	4.4	0.30	0.36	**6.1**	0.31	*o,p'*-DDD	-0.04	0.9	0.92	-0.04	0.9	0.91
PCB 187	0.30	2.9	0.40	0.42	**6.4**	0.22	*p,p'*-DDD	-0.10	0.8	0.79	0.65	**8.2**	**<0.05**
PCB 195	-0.10	0.7	0.78	-0.10	0.6	0.78	*o,p'*-DDT	0.19	1.8	0.60	-0.03	0.9	0.94
PCB 200	-0.13	0.6	0.73	-0.08	**0.7**	0.83	*p,p'*-DDT	0.25	2.1	0.49	0.04	1.1	0.92
PCB 206	-0.14	0.6	0.71	-0.07	**0.7**	0.85							
PCB 209	-0.02	0.9	0.96	-0.04	0.8	0.91	ΣDDT	0.36	2.4	0.31	0.38	**3.2**	0.27
Total PCB	0.27	2.4	0.45	0.36	**4.3**	0.31	α-chlordane	-0.11	0.7	0.77	0.04	**1.2**	0.91
							β-chlordane	-0.08	0.8	0.83	-0.03	**0.9**	0.94
							oxy-chlordane	0.23	2.4	0.53	-0.06	0.8	0.88
BDE 17	0.14	1.6	0.71	0.24	**2.5**	0.50	trans-nona	-0.28	0.4	0.44	0.24	**2.5**	0.51
BDE 28	-0.16	0.8	0.66	0.03	**1.4**	0.94	cis-nona	-0.16	0.7	0.66	0.64	**7.6**	**<0.05**
BDE 47	0.40	3.5	0.26	0.20	2.1	0.56							
BDE 66	0.21	2.1	0.57	0.13	1.8	0.73	ΣChlordane	-0.08	0.8	0.82	0.43	**3.3**	0.22
BDE 99	0.26	3.2	0.47	0.21	3.0	0.57							
BDE 100	0.35	5.5	0.33	0.18	3.2	0.62	Total OCP	-0.20	2.2	0.60	0.51	**3.2**	0.13

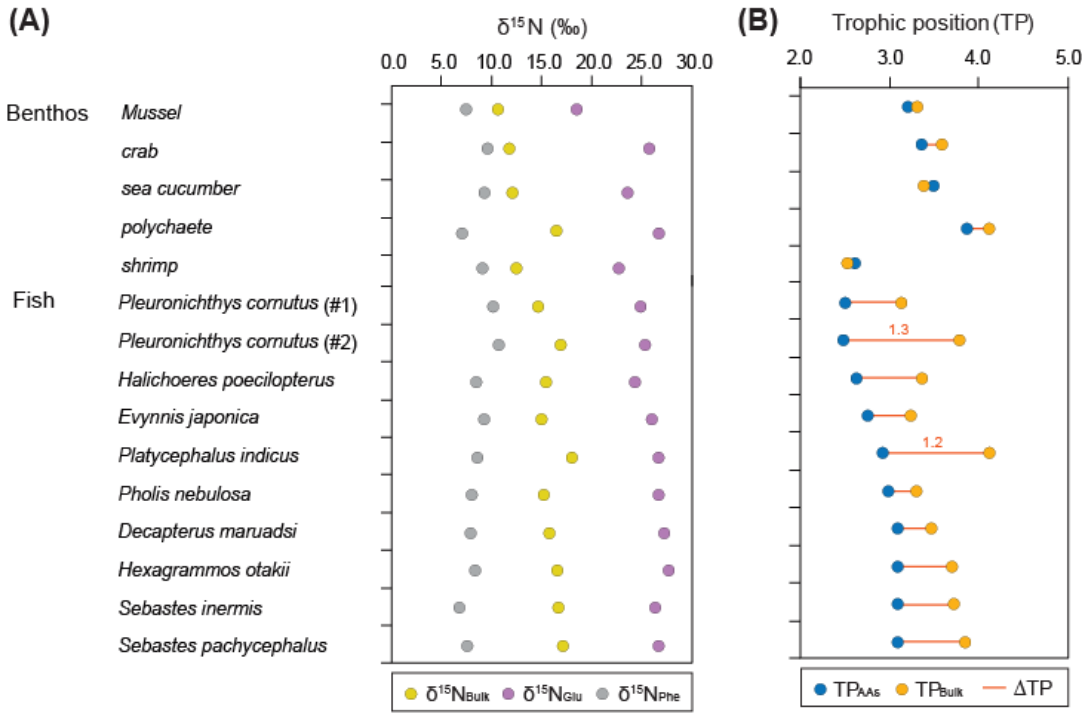

Fig. S1. (A) Nitrogen isotope values of bulk and amino acids, glutamic acid and phenylalanine measured from benthic organisms and fish collected from Masan Bay, (B) the differences between TP of fish calculated by nitrogen bulk isotope ratios and compound specific isotope analysis of amino acids.