Environment International 133 (2019) 105194

Contents lists available at ScienceDirect

Environment International

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

Full length article

Multimedia distributions, bioaccumulation, and trophic transfer of microcystins in the Geum River Estuary, Korea: Application of compound-specific isotope analysis of amino acids

Dokyun Kim[a], Seongjin Hong[b,*], Hyuntae Choi[a], Bohyung Choi[a,c], Jaeseong Kim[d], Jong Seong Khim[e], Hodong Park[f], Kyung-Hoon Shin[a,*]

[a] Department of Marine Science and Convergence Engineering, Hanyang University, Ansan, Republic of Korea
[b] Department of Ocean Environmental Sciences, Chungnam National University, Daejeon, Republic of Korea
[c] Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan
[d] Water & Eco-Bio Co., Ltd., Jungboo Building, Miryong-dong, Kunsan, Republic of Korea
[e] School of Earth and Environmental Sciences & Research Institute of Oceanography, Seoul National University, Seoul, Republic of Korea
[f] Department of Environmental Science, Faculty of Science, Shinshu University, Matsumoto, Japan

A R T I C L E I N F O

Keywords:
Cyanobacterial toxin
Estuary
Coastal organisms
Stable isotope
Trophic position
Biodilution

A B S T R A C T

To determine distributions, bioaccumulation, and trophic transfer of freshwater cyanobacterial toxins such as microcystins (MCs), surface water, suspended solids, sediments, and coastal organisms were collected from seven stations in inner and outer regions of the estuary dam in the Geum River Estuary in June and July 2017. Concentrations of MC variants (MC-LR, -RR, and -YR) in the multimedia samples were analyzed using a HPLC-MS/MS. Trophic position (TP) of organisms (fish, bivalve, gastropod, decapod, and polychaete) was determined by nitrogen stable isotope analyses of both bulk tissues and amino acids. From July to August 2017, great concentrations of MCs were detected in discharged freshwater ranging from 0.4 to 75 $\mu g\ L^{-1}$. Considerable amounts of MCs are delivered to the Geum River Estuary in summer season. MCs spread far away as dissolved phases (18.7–49.5 $ng\ L^{-1}$) in July when large amount of freshwater was discharged during the rainy season. Concentrations of MCs in marine organisms varied among species, ranging from 40 to 870 $ng\ g^{-1}$ dw. Bioaccumulated MCs tend to decrease with increasing TP of organisms, suggesting that MCs are biodiluted through the marine food web. Compound-specific isotope analysis (nitrogen of amino acids) provides more reliable TPs compared with those by bulk isotope analysis in a closed estuary (such as the Geum River Estuary) with large fluctuations in the isotope ratio of primary producers.

1. Introduction

Cyanobacterial blooms in freshwater lakes are mainly caused by *Microcystis*, *Anabaena*, and *Planktothrix*, all of which produce microcystins (MCs) and are hepatotoxic to aquatic organisms (Vaitomaa et al., 2003). MCs have been reported in approximately 80 congeners according to differences in two amino acids (Welker et al., 2006), with the MC-LR, -RR, -YR and -LA variants occurring most frequently (De Figueiredo et al., 2004). MCs decompose poorly in aquatic environments and have an acute toxic effects on living organisms (Ibelings and Chorus, 2007) and a tendency to accumulate in freshwater organisms (Kozlowsky-Suzuki et al., 2012), such as fish, shrimp (Jia et al., 2016), daphnia (Sotton et al., 2014), gastropods (Lance et al., 2010), bivalves (Kim et al., 2017), and aquatic plants (Jia et al., 2016).

When the cyanobacterial cell membrane is damaged, intracellular toxins such as MCs could be released into the water column (Park et al., 1998). MCs entering organisms through drinking water show toxic effects on the liver (Harada et al., 1988). In cases of acute toxicity, an excess of phosphorylated protein damages keratin and plectin, causing breakdown and apoptosis of the cytoskeleton (MacKintosh et al., 1990). In addition, excess phosphorylated protein affects the cancer suppressor gene p53 and acts as a cancer-promoting factor. In cases of chronic toxicity, which can be caused by long-term exposure of MCs (Magalhães et al., 2001), MCs affect intracellular mitochondria and induce reactive oxygen species production and lipid peroxidation (Bouaïcha and Maatouk, 2004). Toxic effects of cyanobacteria have been reported in lakes and rivers around the world, including Lake Alexandrina in Australia (Francis, 1878), Tabocas Reservoir in Brazil (Pouria et al.,

* Corresponding authors.
E-mail addresses: hongseongjin@cnu.ac.kr (S. Hong), shinkh@hanyang.ac.kr (K.-H. Shin).

https://doi.org/10.1016/j.envint.2019.105194
Received 1 April 2019; Received in revised form 26 July 2019; Accepted 16 September 2019
0160-4120/ © 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).

1998), Monterey Bay in United States (Miller et al., 2010), and southern Adriatic Sea in Italy (De Pace et al., 2014). Although MCs can eventually enter marine ecosystems, most studies of MC dynamics have focused on freshwater ecosystems (Takahashi et al., 2014; Umehara et al., 2017). Thus, fate, multimedia distribution, and bioaccumulation of MCs in estuarine and coastal ecosystems are not yet fully understood.

The Geum River in South Korea is approximately 412 km long and drains an area of ~9800 km^2. Approximately 6.4×10^9 ton of freshwater flows into the Yellow Sea every year through the estuary dam (via water gate) of the Geum River. In the Geum River Estuary, an estuary dam constructed in 1994, has a length of 1.84 km, a height of 16.6 m, and 20 gates 30 m wide and 10 m high (Jeong et al., 2014). After construction of estuary dam, the Geum River Estuary acquired characteristics of a partially mixed or well-mixed estuary (Lee et al., 1999), and harmful algal blooms dominated by *Microcystis* began to occur frequently in summer (Noh et al., 2014; Oh et al., 2007). The number of phytoplankton cells ranged from 39.5 to 288.6×10^3 cells mL^{-1}, increasing dramatically from the middle reaches of the river to downstream portions by more than seven times (Lee and Boo, 2000), indicating algal blooms (Yeo, 2009). Thus, there is a possibility that toxic substances from great densities of cyanobacteria may have been introduced into the marine environments when they are released through the estuaries during river water discharge, potentially affecting the inhabitants of coastal environments, including tidal flats (Miller et al., 2010; De Pace et al., 2014). MCs have been reported that those were generally bioaccumulative in freshwater organisms. Several studies have discussed on the concentrations of MCs in organisms to figure out their fate in the prey-predator relations in a food web (Ibelings and Chorus, 2007; Kozlowsky-Suzuki et al., 2012).

To evaluate biomagnification of toxic substances, the trophic position (TP) of an organism should be determined. Previous study identified the TP of organisms using the nitrogen (N) stable isotope ratio of bulk tissues of living organisms (Vander Zanden et al., 1999). A trophic discrimination factor (TDF) with a $\delta^{15}N$ value of 3.4 ± 1.1‰, proposed by DeNiro and Epstein (1981), is frequently used to calculate the TP. However, it has been reported that the TDF would vary depending on the many factors such as species, diet quality, consumer's nutritional status, size, age, dietary ontogeny, and difference in metabolism of organism (Caut et al., 2009; Post, 2002). The $\delta^{15}N$ of a primary producers changes rapidly and irregularly, while consumers show a relatively slow turnover rate (Chikaraishi et al., 2009). This diversity in TDF and different isotopic turnover rate between diet and consumer may increase the error range of a TP (Vizzini et al., 2002). In order to overcome those problems, studies on TPs of marine organisms applying compound-specific isotope analysis (CSIA) have been conducted to understand marine ecosystem structure (Won et al., 2018). N stable isotope analysis of amino acids (AAs) provides more accurate and detailed information compared with bulk tissue analysis, as reported previously (Chikaraishi et al., 2009).

The objectives of the present study were to: (i) investigate MCs concentrations in discharged freshwater via an estuary dam; (ii) determine the fate and environmental multimedia distributions of MCs in an estuarine area; (iii) clarify the bioaccumulation characteristics of MCs in various coastal organisms; and (iv) elucidate the trophic transfer of MCs through a marine food chain.

2. Materials and methods

2.1. Sampling and sample preparation

The Geum River Estuary is a closed estuary where freshwater and seawater are completely separated by the estuary dam (Fig. 1). The discharge of freshwater is highly dependent on the amount of rainfall. In Korea, the majority of the annual precipitation falls between late June and the middle of September. Cyanobacterial bloom also occurs in summer upon increased high temperature in water column. Accordingly, the MCs originating from the freshwater lake could be introduced to the estuarine and coastal areas through the discharged freshwater during the summer. Thus, in order to determine the distributions and fate of MCs accurately, environmental multimedia samples were collected from inner and outer regions of the estuary dam in the Geum River Estuary before heavy rainfall (June) and after freshwater discharge (July) in 2017.

Surface water, suspended solids (SS), sediments, and marine biological samples were collected from seven stations (inner and outer regions of the estuary dam) (Fig. 1). During the June survey period, it was the lowest precipitation and there was little or no discharge of freshwater. On the other hand, there was the largest rainfall in July, leading to the largest volumes of freshwater discharge through the estuary dam (Fig. S1 of the Supplementary Materials (S)). Water quality parameters such as salinity, temperature, and pH were measured using a Hydrolab DS5X Multiparameter sonde (OTT Hydromet, Loveland, CO) in the field. A total of 10 L of surface water (0–0.5 m depth) was collected using a Van Dorn sampler. For analysis of particulate MCs, 0.5–1 L of water samples was filtered using a pre-combusted (450 °C, 5 h) 47-mm glass fiber filter (GF/F, Whatman, Maidstone, England) and immediately frozen at –80 °C until analysis. MCs dissolved in water were obtained from a filtrate (using GF/F) and immediately purified. Approximately 100 g of sediments was collected at 0–0.5 cm of depth using a Van Veen grab sampler, placed in a glass jar, and frozen at –20 °C until analysis.

In June, biological samples such as the bivalve *Mactra veneriformis* (n = 5, pooling), the fish *Mugil cephalus* (n = 2) and *Larimichthys* sp. (n = 1), the gastropod *Rapana venosa* (n = 2), the decapod *Portunus trituberculatus* (n = 1), and the polychaete *Neanthes* sp. (n = 5, pooling) were collected from the estuarine areas (Table S1). Fish, gastropod, and crab were caught with a net, and bivalve and polychaete were collected from surface sediments with a 1-mm sieve. To remove the gut contents, the biota samples were depurated in seawater (filtered with GF/F) for more than 6 h. Because MCs accumulate mainly in the liver of biota samples, dissection were performed immediately where possible, and the liver or midgut gland was isolated and frozen at –80 °C until analysis. Samples that could not be dissected were frozen as a whole body at –80 °C and stored in a glass jar until analysis.

2.2. Extraction and cleanup for MCs analysis

The pretreatment methods used in the present study were modified from Park et al. (1998), Xie et al. (2007), and Sedda et al. (2016), as summarized in Fig. S2. Briefly, for the extraction of particulate MCs, 10 mL of butanol:methanol:water (1:4:15, v/v/v) were added to a conical tube with lyophilized filter samples and 100 ng of enkephalin (Sigma-Aldrich, Saint Louis, MO) was added as a surrogate standard. The samples were then stirred for 24 h and sonicated for 5 min. Supernatant was collected by centrifugation at 3000 × g for 15 min. The supernatant obtained by repeating twice more was diluted two times with distilled water. For the extraction of MCs in sediments (1–2 g) and biota (100–200 mg), the lyophilized and homogenized samples were produced by the same process as the particle phase.

The extracts were injected into an Oasis HLB cartridge (500 mg, 6 cc, Waters, Milford, MA) pre-conditioned with 10 mL of methanol (Merck, Darmstadt, Germany) followed by 10 mL of deionized water (Aquapuri 5 series, Young In Scientific, Korea). After the sample loading, the cartridge was washed with 10 mL of deionized water, followed by 10 mL of 20% methanol. The remaining solution in the cartridge was air-dried with a mortar pump for 30 min and eluted with 10 mL of methanol. The flow rate of the solution was controlled so as not to exceed 10 mL min^{-1}. The eluate was concentrated with an N_2 purge at 40 °C and finally reconstructed with 1 mL of methanol. Finally, 10 ng of monolinuron (Sigma-Aldrich) in 100 μL of methanol was added as an internal standard (Fig. S2).

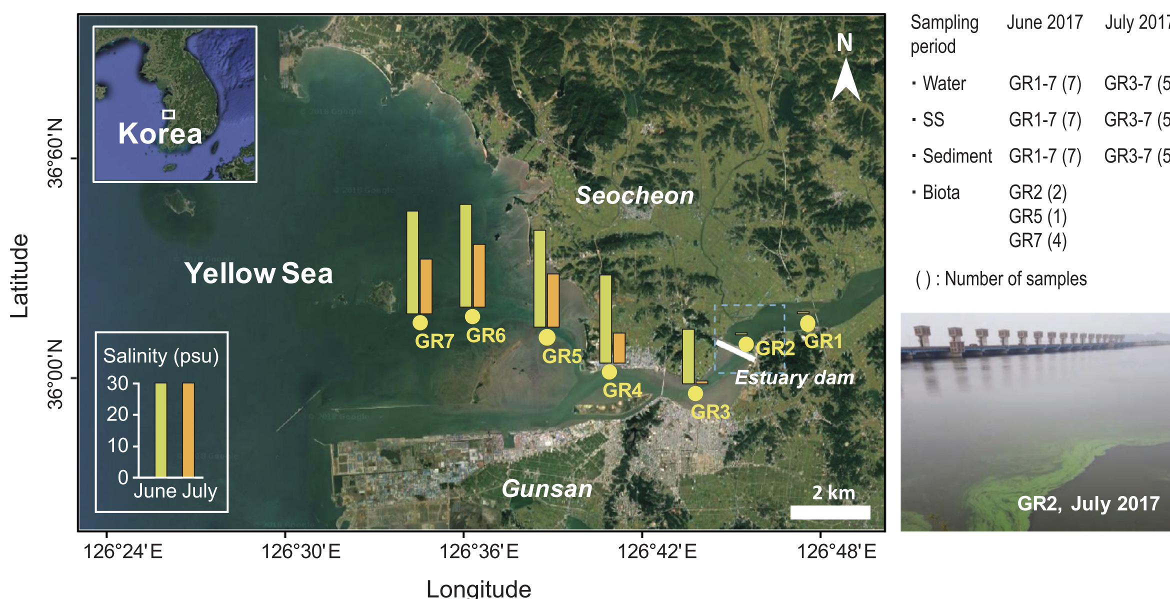

Fig. 1. Sampling sites along the Geum River Estuary in South Korea. Water, SS, sediments, and biological samples were collected from both inner and outer regions of the estuary dam in June and July 2017.

2.3. LC-MS/MS analysis

The sample was analyzed using a 1290 infinity II series HPLC (Agilent Technologies, Santa Clara, CA) combined with QTRAP 6500 series electrospray ionization tandem mass spectrometer (AB Sciex, CA). A UPLC column (Poroshell 120 EC-C18 4.6 × 50 mm, 2.7 µm, Agilent Technologies) was used with an injection volume of 10 µL. The mobile phase was (A) 0.1% formic acid in water and (B) 100% methanol. The gradient condition was maintained at 30% for 1 min and then increased slowly to 100% for 6 min, maintained for 1 min, and decreased to 30% for 2 min. The flow rate of the mobile phase was 0.3 mL min^{-1} (details in Table S2).

The ion mode was positive, ion spray voltage was 5.5 kV, source temperature was 600 °C, curtain gas was nitrogen (N_2) (35 psi), and the ion source gas 1 and 2 was nitrogen (N_2) (50 psi). As a result of multiple reaction monitoring (MRM), Q1/Q3 pairs for quantification were MC-LR $[M + H]^+$ (Q1/Q3 = m/z 995.4/103.1), MC-RR $[M + 2H]^{2+}$ (Q1/Q3 = m/z 519.9/135.2), MC-YR $[M + H]^+$ (Q1/Q3 = m/z 1045.4/103.0), enkephalin $[M + H]^+$ (Q1/Q3 = m/z 556.2/120.1), and monolinuron $[M + H]^+$ (Q1/Q3 = m/z 215.0/126.2) (optimized MS/MS parameters in Table S3).

2.4. Quality assurance and quality control for MCs analysis

Calibration standards of 1, 10, 50, 100, 300, 500, and 1000 ng L^{-1} of three homologues of MCs were used (R^2 > 0.997 for all MCs). The three MC standards (MC-LR, MC-RR, and MC-YR) were purchased from Alexis Biochemicals (Enzo Life Sciences, Lausanne, Switzerland). The limit of detection (LOD) and limit of quantitation (LOQ) for three MCs were calculated following the guidelines of the United States Environmental Protection Agency (USEPA, 1979). LODs were calculated as: 3.143 × SD (standard deviation, n = 7) for a one-sided 98% confidence interval. LOQs were calculated as: 10 × SD. Calibration standards and instrumental blanks were analyzed every 10 injections of samples to check for instrumental accuracy and repeatability. The LODs and LOQs for MC-LR were 0.5 and 1.5 ng L^{-1}, for MC-RR they were 1.8 and 5.8 ng L^{-1}, and for MC-YR they were 0.9 and 2.9 ng L^{-1}, respectively (Table S4). To confirm analytical recovery, 500 ng of three MCs were added to 500 mL of deionized water and quantitated according to an optimized analysis protocol. The analytical recovery of MC-LR, MC-RR, and MC-YR was 87 ± 1%, 96 ± 3%, and 92 ± 11%, respectively (Table S4).

2.5. Analyses of $\delta^{13}C$ and $\delta^{15}N$ in bulk tissue

The pre-treatments for inorganic carbon and lipids removal for bulk carbon isotope analysis were performed using the methods by Lorrain et al. (2003) and Logan et al. (2008) with slight modifications. In brief, filter samples were acidified with HCl fume in a desiccator for 12 h and placed with NaOH and silica gel to remove the residual HCl and moisture for 12 h. Biota and sediment samples were homogenized and placed in 10 mL of 1 M HCl for 12 h followed by neutralization with distilled water. Additionally, biota samples were defatted using chloroform/methanol (2:1, v/v). After sonicated for 15 min, the supernatant was collected by centrifugation at 2500 × g for 10 min and removed at three times. Residual solvents in samples were oven dried completely at 60 °C. For bulk nitrogen isotope analysis all samples were used without any treatment of acidification and defat.

Samples for the C and N stable isotope analyses were measured with an elemental analyzer (EA, Vario PYROcube, Elementar, Germany) coupled with isotope ratio mass spectrometer (IRMS, Isoprime 100, Isoprime, UK). The value of the stable isotope ratio was expressed as per mil (‰), which is defined as the δ value of the isotope ratio difference between the standard material and the sample.

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

where R represent the ratios of heavy isotopes to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and Vienna Pee-Dee Belemnite (VPDB) and atmospheric N_2 were used as standards for carbon and nitrogen isotope ratios ($R_{standard}$), respectively. IAEA reference materials, such as CH-3 and N-1 were analyzed every 10 injections of samples as a running standard to check the accuracy of $\delta^{13}C$ and $\delta^{15}N$, respectively. Standard deviations of CH-3 and N-1 were within ± 0.2‰ and ± 0.1‰, respectively, for all analysis.

2.6. Analysis of $\delta^{15}N$ in amino acids of organisms

Applying CSIA, we used an analytical protocol of AAs followed that of Chikaraishi et al. (2007). Approximately 5 mg of freeze-dried and homogenized biota samples were hydrolyzed at 110 °C for 12 h with 12 M HCl. After the hydrolysis, the samples were cooled at room temperature, and impurities were removed using GHP nanosep

(hydrophilic polypropylene, 0.45 μm, Pall corp., Tokyo, Japan). Lipids were separated and removed using n-hexane/dichloromethane (3:2, v/v). The remaining HCl in the samples was removed with an N_2 purge. Isopropyl esterification and pivaloylation were used for derivatization of AAs. The derivatization procedure was performed with thionyl chloride/2-propanol (TC/iPr, 1:4, v/v) and subsequently pivaloyl chloride/dichloromethane (PC/DCM, 1:4, v/v). The $\delta^{15}N$ of AAs in the samples were extracted with n-hexane/DCM (3:2, v/v) and then were measured using a gas chromatograph (GC, Agilent HP6890N, Agilent Technologies) coupled with an IRMS (Isoprime) (Table S5).

2.7. Estimations of trophic positions

TP based on the $\delta^{15}N$ of bulk tissue was estimated using a conventional equation as follows (Eq. (2)):

$$TP_{Bulk} = [(\delta^{15}N_{consumer} - \delta^{15}N_{base})/TDF] + \lambda \quad (2)$$

where TDF was set to 3.4‰ which is established by DeNiro and Epstein (1981), and λ represents the TP of the baseline organism. In this study, we used $\delta^{15}N$ values of particulate organic matter (POM) sampled at each site and that of the bivalve *M. veneriformis* sampled in GR5 as baseline organisms with λ = 1 and λ = 2, respectively.

TP was calculated using the $\delta^{15}N$ of AAs as proposed by Chikaraishi et al. (2009) (Eq. (3)).

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

Briefly, the $\delta^{15}N$ of glutamic acid ($\delta^{15}N_{Glu}$)provides trophic information because it largely increases (8.0‰) at a certain rate along the trophic transfer. The $\delta^{15}N$ of phenylalanine ($\delta^{15}N_{Phe}$) provides source information because it negligibly increases (0.4‰) along the trophic transfer. The empirical difference (β) between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ of primary producers in aquatic ecosystems is 3.4‰, and the TDF between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ is 7.6‰.

3. Results and discussion

3.1. Concentrations of MCs in discharged freshwater via estuary dam

In July and August of 2017, the water samples of river discharge were collected from the Geum River to measure MCs and water quality parameters (temperature, salinity, pH, and chlorophyll *a*) (Table S6). In summer 2017, the sum of MCs concentrations at the inner dam of the Geum River Estuary varied from 0.4 to 75 μg L^{-1} (Fig. 2a). MC-LR concentrations ranged from 0.2 to 40 μg L^{-1}, which were greater than the values for MC-RR and MC-YR. MC-LR concentrations are similar to or greater than the World Health Organization (WHO) guideline for drinking water of 1 μg L^{-1} (Poste and Ozersky, 2013). In particular, on 4 July, the concentrations of MCs were about 40 times greater than the WHO guideline. The water temperature of the Geum River discharge ranged from 25.9 to 31.2 °C, which was the most suitable temperature range for cyanobacteria blooms (Bui et al., 2018), and the chlorophyll *a* concentration was also great.

There is a strong correlation between MCs and chlorophyll *a* concentrations in discharged water samples (Fig. 2b). The MCs concentrations increase exponentially with increasing chlorophyll *a* concentrations. This suggests that cyanobacteria containing MCs dominate phytoplankton communities in the Geum River during sampling periods (Hollister and Kreakie, 2016; Noh et al., 2014). Duarte et al. (1992) reported that when cyanobacteria account for approximately 90% of an algal biomass community, the total algal biomass reached about 100 mg L^{-1} in 165 lakes in the Florida. At the biomass, chlorophyll *a* and MCs concentrations have also reportedly reached or exceeded 50 μg L^{-1} and 1 μg L^{-1}, simultaneously (Bigham et al., 2009; Bukaveckas et al., 2017; Yu et al., 2014). The MCs quota was calculated as MCs content per unit chlorophyll *a*. The MCs quota ranged from 0.02 to 0.46 μg MCs μg chlorophyll a^{-1} in the summer season. In particular, when the concentrations of MCs was 75 μg L^{-1} (in 4 July) and 29 μg L^{-1} (in 5 July), the MCs quota was 0.46 and 0.26 μg MCs μg chlorophyll a^{-1}, respectively. This value was similar to the 0.4 μg MCs μg chlorophyll a^{-1} recorded when the concentrations of MCs were 20 μg L^{-1} (Bui et al., 2018; Ha et al., 2011).

Using the daily amount of discharge water, the standing stock of MCs released into the estuary in July and August 2017 was calculated. Approximately 4.4 ton of MCs were discharged into the Geum River Estuary for two months. In Isahaya Bay, Japan, approximately 64.5 kg of MCs were released a year through a sea dike in 2009 (Umehara et al., 2012). The amount of MCs discharged through the estuary dam of the Geum River Estuary seems to be relatively great, thus the impact of the MCs on estuarine and coastal ecosystems is concerned.

3.2. Distributions of MCs in environmental multimedia samples

MCs concentrations in multimedia samples (water, SS, and sediments) collected from the inner and outer seven stations in the Geum River Estuary in June and July 2017 were investigated (Fig. 3). During the sampling periods, the concentrations of MCs in water samples ranged from 0.5 to 154 ng L^{-1}, SS samples ranged from < LOD to 1060 ng L^{-1}, and sediment samples ranged from < LOD to 17.7 ng g^{-1} dw. These results indicate that the distribution trends in MCs are different between before and after rainfall season. In June, the relatively great concentrations of MCs (water: 154–157 ng L^{-1}; SS: 790–1060 ng L^{-1}; sediments: 16.1–17.7 ng g^{-1} dw) at the two stations of the inner dam (GR1 and GR2) decreased gradually from the outer dam (GR3, water: 132 ng L^{-1}; SS: 41.5 ng L^{-1}; sediment: 2.2 ng g^{-1}

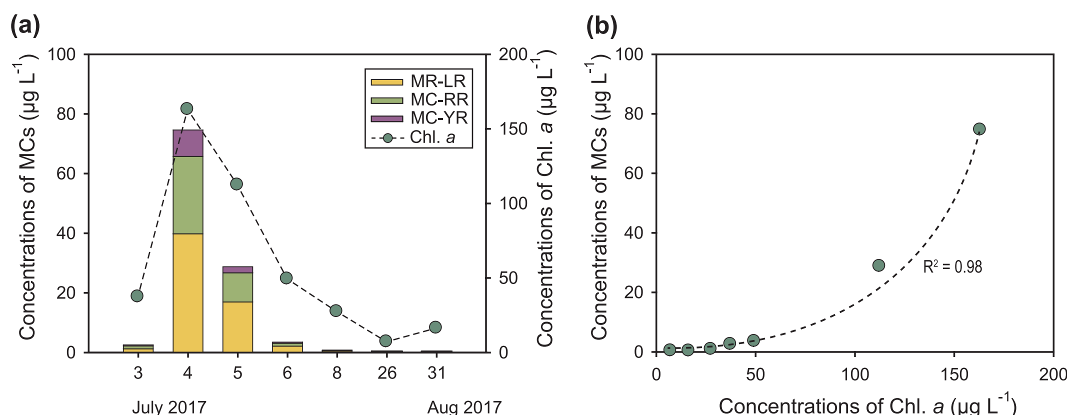

Fig. 2. (a) Daily MCs concentrations (μg L^{-1}), chlorophyll *a* concentrations (μg L^{-1}) and (b) the relationship between MCs and chlorophyll *a* concentrations collected from river discharge in the Geum River Estuary.

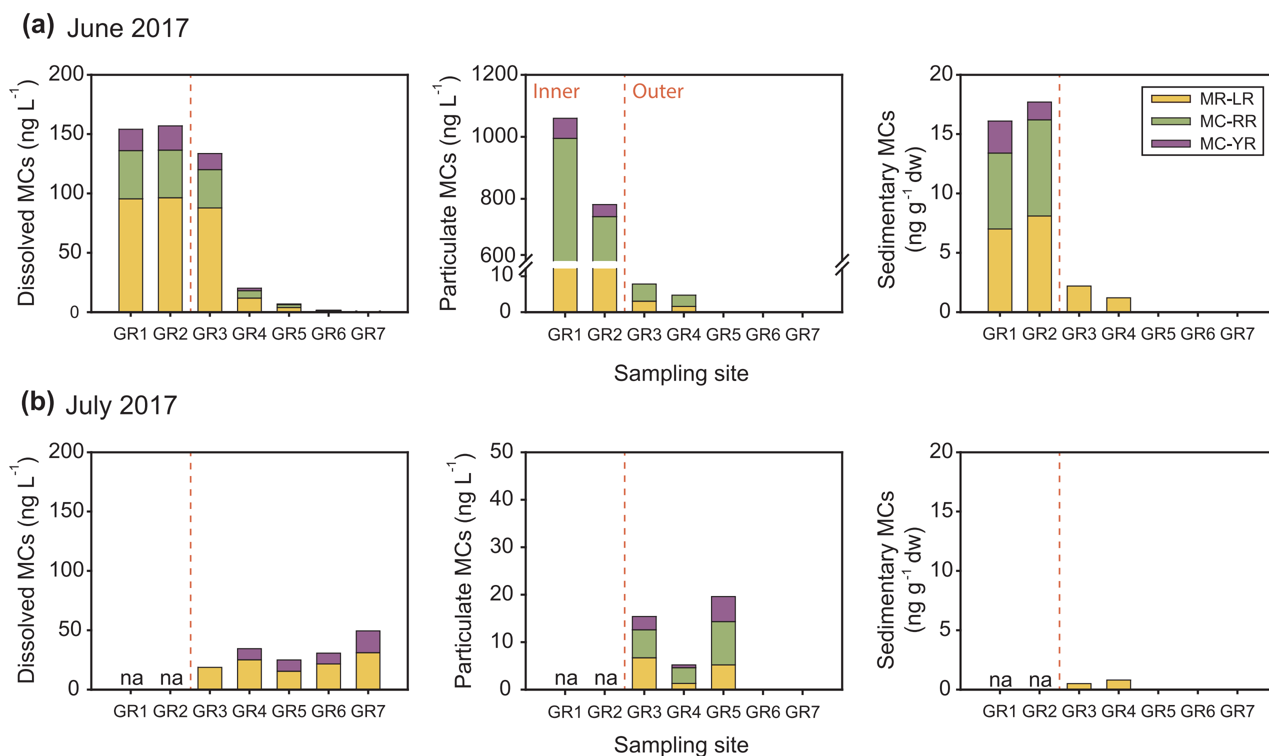

Fig. 3. Distributions of MCs concentrations on environmental multimedia samples in the Geum River Estuary in (a) June and (b) July 2017.

Table 1
Concentrations of microcystins in biological samples and trophic positions in the Geum River Estuary.

Site	Common name	Scientific name	Tissue	MC-LR	MC-RR	MC-YR	ΣMCs	$\delta^{13}C_{Bulk}$ (‰)	$\delta^{15}N_{Bulk}$ (‰)	TP_{Bulk_POM}	$TP_{Bulk_bivalve}$	TP_{AAs}
				(μg g^{-1} dw)								
GR 2	Striped mullet	*M. cephalus*	Liver	0.42	0.010	0.37	0.81	−25.8	12.1	0.4	2.4	2.2
			Liver	0.38	-	0.25	0.63	−25.8	12.1	0.4	2.4	2.2
GR 5	Surf clam	*M. veneriformis*	Liver	0.40	-	0.50	0.87	−19.1	10.2	2.1	2.0	1.8
GR 7	Yellow croakers	*Larimichthys* sp.	Midgut gland	0.010	-	0.03	0.04	−17.5	13.4	2.1	2.9	3.3
	Clam worm	*Neanthes* sp.	Midgut gland	0.020	-	0.09	0.11	−16.7	12.6	1.9	2.7	2.4
	Blue crab	*P. trituberculatus*	Midgut gland	0.13	-	0.03	0.16	−16.9	13.3	2.1	2.9	2.6
	Veined rapa whelk	*R. venosa*	Midgut gland	0.18	-	0.25	0.43	−15.5	12.4	1.9	2.6	2.2
			Whole body	0.25	-	0.26	0.51	−15.5	12.4	1.9	2.6	2.2

-: below the detection limit.

dw) to the farthest station (GR7, water: 0.5 ng L^{-1}; SS: < LOD; sediments: < LOD). The concentrations of MCs in sediments at the outer dam were also detected in the range of < LOD to 2 ng g^{-1} dw (Fig. 3 and Table S7). In July, peak rainfall during the year, concentrations of MCs in SS increased at the stations of the outer dam (GR3-GR5) compared with those in June, although they were < LOD at GR6 and GR7. The concentrations of MCs in water gradually decreased from GR3 (132 ng L^{-1}) to GR7 (0.5 ng L^{-1}) in June, while they increased (18.7–49.5 ng L^{-1}) at all stations except GR3 in July. This is because the cyanobacteria that flowed out through the estuary dam for a few days before sampling appears to persist for several days and the toxin also remain for an extended period in the coastal environment (Ibelings and Chorus, 2007).

The MCs can occur in sediments through several routes including direct deposition of cyanobacterial cells in discharged freshwater and redistributions of MCs through tide-induced sediment re-suspension (Umehara et al., 2017). At greatest discharge in July, it was assumed that cyanobacteria that have been cultivated due to lengthy drought and temperature rise before the strong rainfall (see Fig. S1) flowed into the coastal area following mass discharge after strong precipitation (Fig. 3). Cyanobacteria that flowed out through the estuary dam with mass discharge after heavy rainfalls have spread to the coastal area, dissolved in water along the low-salinity layer (Table S8) (Umehara et al., 2015). When cyanobacteria flow into the marine environment, their cell membranes are partly destroyed by osmotic pressure depending on salinity (Preece et al., 2017). The MCs in the cells are then released into the water column and extracellular MCs can persist for days to months (Lahti et al., 1997; Tonk et al., 2007; Zastepa et al., 2017) because it is chemically and physically stable in the aquatic environments (Fetscher et al., 2015; Graham et al., 2012).

3.3. Species-specific bioaccumulation of MCs in coastal organisms

The sum of MCs concentrations of the organisms collected from the Geum River Estuary varied from 40 to 868 ng g^{-1} dw. The greatest concentrations of MCs were 868 ng g^{-1} dw (MC-LR: 420 ng g^{-1} dw; MC-RR: < LOD; and MC-YR: 450 ng g^{-1} dw) in the *M. veneriformis* (Table 1 and Fig. 4a). Concentrations of MC-RR are lower than those of other congeners in all organisms. Ni et al. (2017) also showed a similar result to our study. MC-LR concentrations were the highest and MC-RR concentrations were the lowest in the bighead carp *Aristichthys nobilis* in Hangzhou. MC-RR, which has two arginine amino acids, is relatively

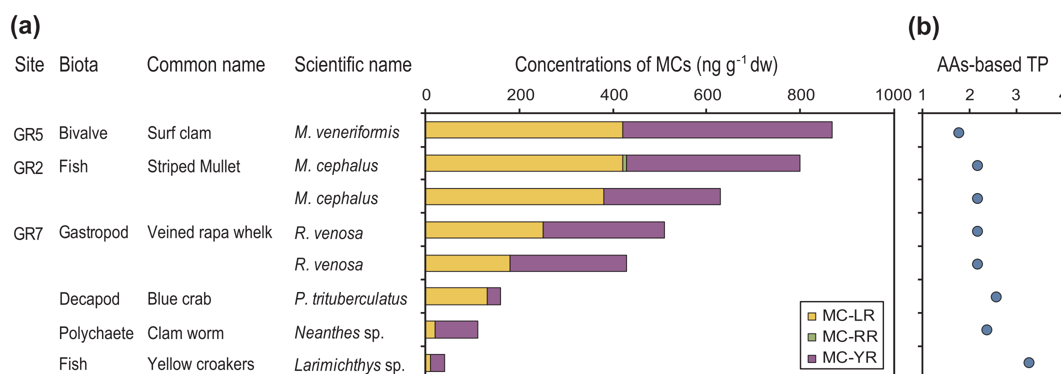

Fig. 4. (a) MCs concentrations (ng g^{-1} dw) and (b) TP_{AAs} of organisms in the Geum River Estuary.

hydrophilic among variants, and hydrophilic MCs can be excreted more easily than hydrophobic variants (Gupta et al., 2003). This difference could cause changes in organotropism, toxicokinetics, and bioaccumulation (Vesterkvist and Meriluoto, 2003). Therefore, in bioaccumulation studies, it is difficult to represent the total MCs amount with only the sum of MC-LR, MC-RR, and MC-YR in vivo because the composition of MC congeners may vary in vivo depending on the organ (Ni et al., 2017; Sotton et al., 2014).

The MCs concentrations in the organisms obtained from this study are relatively lower than those previously reported in freshwater species. However, they are similar or relatively greater than those from brackish and marine environments (Table S9). Coastal organisms are considerably influenced and contaminated by freshwater MCs in the Geum River Estuary. In this study and previous studies, freshwater organisms generally showed greater concentrations of MCs than did seawater species. Exceptionally, Miller et al. (2010) reported that the concentration of MCs in liver samples of sea otters who had consumed MCs-contaminated mollusks ranged from 1360 to 348,000 ng g^{-1} dw in Monterey Bay. This level is much greater than previously reported for freshwater organisms (Table S9) and may be attributable to "superblooms" involving 2,900,000 μg L^{-1} of MCs in freshwater flowing into coastal areas.

3.4. Determination for trophic positions of marine organisms

TPs of organisms were estimated to evaluate trophic transfer of MCs originating from the Geum River moved through the coastal food web. To obtain more accurate trophic information, we compared TP estimated by $\delta^{15}N$ of bulk tissue and that by $\delta^{15}N$ of AAs for each organisms (Table 1). The TP of organisms calculated with POM as a baseline (TP_{Bulk_POM}) is inaccurate compare to those of $\delta^{15}N$ of bulk in bivalve ($TP_{Bulk_bivalve}$) and $\delta^{15}N$ of AAs (TP_{AAs}). For instance, the TP_{Bulk_POM} of the *M. cephalus* sampled in GR2 was 0.4 that is even lower than typical TP of primary producer. A main diet for *M. cephalus* is known as POM (de Oliveira et al., 2014), which mainly consists with primary producer (i.e., phytoplankton). Thus, the $TP_{Bulk_bivalve}$ and TP_{AA} of this species (2.4 and 2.2, respectively) seem to be more reliable than TP_{Bulk}. The $\delta^{15}N$ of the primary producers changes rapidly and irregularly, while consumers show a relatively slow turnover rate (Chikaraishi et al., 2009). Because the freshwater from the Geum River has been occasionally discharged through the estuary dam, contributions of freshwater primary producer for POM in the estuarine area varied temporally and spatially. Our finding on the great ranges of $\delta^{15}N_{POM}$ values in the Geum River Estuary (7.4–14.6‰, Fig. S3) and the extremely small TP_{Bulk_POM} of the *M. cephalus* is well explained by this issue.

The $\delta^{15}N$ value of *M. veneriformis* sampled in GR5 was used as a represent baseline for $TP_{Bulk_bivalve}$ estimation, for the organisms sampled in GR7 due to the lack of representative primary consumer (typical TP = 2). However, based on the difference in $\delta^{15}N$ value of POM between GR5 (7.4‰) and GR7 (9.5‰), the $\delta^{15}N$ baseline (λ = 2) in the GR7 may not be identical with that in the GR5. Thus, the differences between $TP_{Bulk_bivalve}$ and TP_{AA} for the consumers sampled in GR7 can be caused by the uncertainty of $\delta^{15}N$ value of baseline in the GR7 (Table 1). Our results clearly demonstrate that potential error of TP estimations can be attributed from the determination of baseline in the TP_{Bulk} estimation. A more accurate TP can be calculated using an AAs analysis that does not need the considerations of the difference in isotope value between diet and consumer (Chikaraishi et al., 2009; Won et al., 2018). Thus, we suggest again that TP estimates using $\delta^{15}N$ of AAs provides more reliable results compared to those of using bulk $\delta^{15}N$ values, particularly in where dynamic change of environmental conditions is occurring such as estuaries.

3.5. Trophic transfer of MCs through the marine food chain

To investigate food web structure and the main food source of living organisms, the $\delta^{13}C$ and $\delta^{15}N$ of POM, sedimentary organic matter (SOM), and biota were analyzed at stations GR2, GR5, and GR7 in June 2017. Based on the differences in $\delta^{13}C$ and $\delta^{15}N$, the food sources were separated into freshwater and marine origins (Fig. S3). At station GR2, POM and SOM were considered to be food sources of freshwater origin with relatively low $\delta^{13}C$ values, while those from GR5 and GR7 showed relatively high $\delta^{13}C$. At station GR5, *M. veneriformis*, which is a primary consumer, has higher $\delta^{13}C$ (−19.1‰) than those of POM (−23.2 ± 0.5‰) indicating that they feed mainly on precipitating POM on surface sediments at their living site (Navarro et al., 2009). Meanwhile, *M. cephalus*, a primary consumer, has the lowest $\delta^{13}C$ among those of organisms at GR2, GR5, and GR7. *M. cephalus* is known to be strongly salinity tolerant and moves in a wide radius in the brackish zone (Yoon et al., 2015). The main food sources of *M. cephalus* appear to be POM of both freshwater and marine origins, with relatively low $\delta^{13}C$ at the inner dam and high $\delta^{13}C$ at the outer dam (de Oliveira et al., 2014). The others species, deposit feeders or predators (*R. venosa*, *P. trituberculatus*, *Neanthes* sp., and *Larimichthys* sp.), have relatively high $\delta^{13}C$ values, reflecting the carbon isotopes of SOM at their living sites.

The TP_{AAs} of organisms varied from 1.8 to 3.3. *M. veneriformis* (TP_{AAs} = 1.8) and *M. cephalus* (TP_{AAs} = 2.2), which are herbivores, showed relatively great concentrations of MCs (Table 1 and Fig. 4b). *Larimichthys* sp., with a TP_{AAs} of 3.3, showed the lowest concentrations of MCs among the collected organisms. *R. Venosa* (TP_{AAs} = 2.2), *Neanthes* sp. (TP_{AAs} = 2.4), and *P. trituberculatus* (TP_{AAs} = 2.6), which are omnivores and likely feed predominantly on organic matter deposited in surface sediments, showed MC concentrations between those of herbivores and carnivores. Benthic organisms including deposit feeders, are also exposed to precipitated toxic algae (Trainer et al., 1998) as well as bivalves that directly feed on toxic algae (FAO, 2004). Concentrations of MCs show a tendency for a negative relationship with TP_{AAs} (Fig. 5). Cyanobacteria appears to directly affect herbivorous organisms due to widespread blooming from the river to the coastal

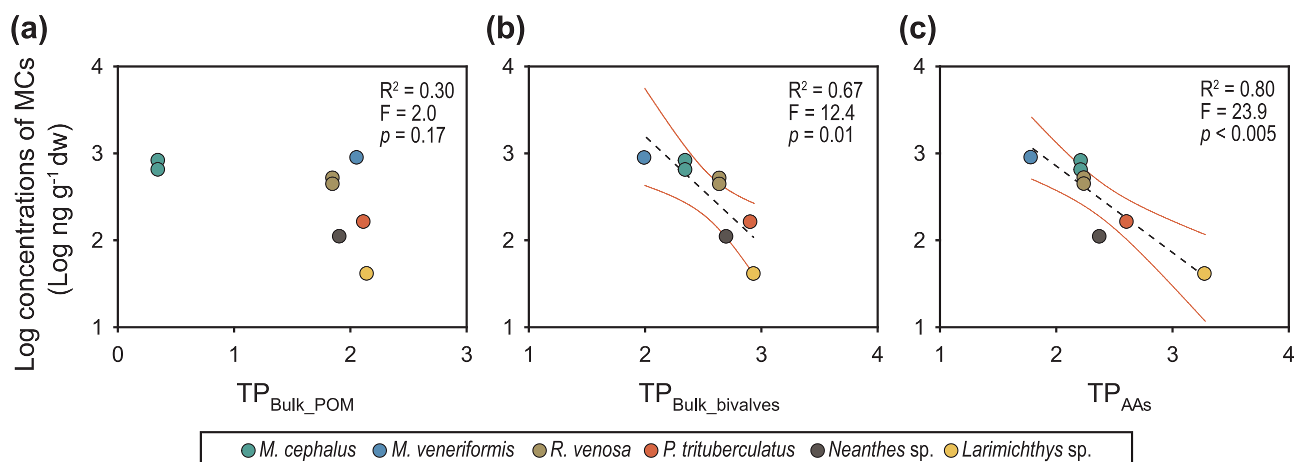

Fig. 5. Relationships between concentrations of MCs and TPs calculated based on (a) POM, (b) bivalve, and (c) amino acids of organisms in the Geum River Estuary.

estuarine area through the dam in summer. MCs, which are widely distributed in coastal ecosystems, likely precipitate on surface sediments and influence omnivorous organisms that mainly feed on benthic food sources. MC concentrations in the *Larimichthys* sp., with a TP_{AAs} higher than 3, are relatively low compared with levels in other organisms.

These results suggest that MCs in organisms do not biomagnify through the food chain of coastal ecosystem (Fig. 5). In addition, this tendency is more pronounced in TP_{AAs} than those of using TP_{Bulk_POM} and $TP_{Bulk_bivalve}$ (Fig. 5). This result could be explained by a biodilution effect through food web (Ibelings and Chorus, 2007; Kozlowsky-Suzuki et al., 2012) and/or metabolism processes such as biodegradation and biotransformation of MCs in the higher TP organisms (Poste and Ozersky, 2013). In addition, MCs concentrations can vary depending on the feeding behavior of organisms. This is because filter feeders and deposit feeders, which directly feed on suspended particles (including cyanobacteria), show greater MCs concentrations than those in carnivorous fish, which only feed on MCs indirectly (Bukaveckas et al., 2017). Overall, even though MCs do not biomagnify in predators who do not feed directly on cyanobacteria, they can be transferred to predators through feeding routes in coastal food webs (Sotton et al., 2014).

4. Conclusions

In this study, we investigated the fate of MCs produced by freshwater cyanobacteria and the ecological impacts of introducing them into a coastal ecosystem through an estuary. Several key findings on the fate of MCs in a coastal environment and bioaccumulation characteristics were obtained as summarized below:

- Great concentrations of MCs are detected in discharged freshwater via an estuary dam.
- When MCs enter the coastal ecosystem, they largely dissolve in surface seawater.
- Relatively great concentrations of MCs are found in herbivorous organisms.
- MC-LR is the most abundant among three MC variants in coastal organisms.
- MCs in coastal organisms do not biomagnify along the food chain in coastal ecosystem.

This study provides valuable information to understand the behavior, distribution, and biological effects of the cyanobacterial toxin (MCs) in coastal environments. In particular, CSIA can be a powerful tool to characterize the bioaccumulation properties and food web transfer potential of MCs. However, the mechanism of MCs biodegradation in living organisms has remained unclear, and future study should identify the detoxification mechanism to clarify the adverse effects of MCs on marine organisms.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (Ministry of Science and ICT) (No. NRF-2016R1E1A1A01943004 & No. 2017R1A4A1015393).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105194.

References

Bigham, D.L., Hoyer, M.V., Canfield, D.E., 2009. Survey of toxic algal (microcystin) distribution in Florida lakes. Lake Reserv. Manag. 25, 264–275.
Bouaïcha, N., Maatouk, I., 2004. Microcystin-LR and nodularin induce intracellular glutathione alteration, reactive oxygen species production and lipid peroxidation in primary cultured rat hepatocytes. Toxicol. Lett. 148 (1–2), 53–63.
Bui, T., Dao, T.S., Vo, T.G., Lürling, M., 2018. Warming affects growth rates and microcystin production in tropical bloom-forming microcystis strains. Toxins (Basel) 10 (3), 123.
Bukaveckas, P.A., Lesutienė, J., Gasiūnaitė, Z.R., Ložys, L., Olenina, I., Pilkaitytė, R., Pūtys, Ž., Tassone, S., Wood, J., 2017. Microcystin in aquatic food webs of the Baltic and Chesapeake Bay regions. Estuar. Coast. Shelf Sci. 191, 50–59.
Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443–453.
Chikaraishi, Y., Kashiyama, Y., Ogawa, N.O., Kitazato, H., Ohkouchi, N., 2007. Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: implications for aquatic food web studies. Mar. Ecol. Prog. Ser. 342, 85–90.
Chikaraishi, Y., Ogawa, N.O., Kashiyama, Y., Takano, Y., Suga, H., Tomitani, A., Miyashita, H., Kitazato, H., Ohkouchi, N., 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol. Oceanogr. Methods 7, 740–750.
De Figueiredo, D.R., Azeiteiro, U.M., Esteves, S.M., Gonçalves, F.J.M., Pereira, M.J., 2004. Microcystin-producing blooms - a serious global public health issue. Ecotoxicol. Environ. Saf. 59, 151–163.
de Oliveira, M.C.L.M., Bastos, R.F., Claudino, M.C., Assumpção, C.M., Garcia, A.M., 2014. Transport of marine-derived nutrients to subtropical freshwater food webs by juvenile mullets: a case study in southern Brazil. Aquat. Biol. 20 (1), 91–100.
De Pace, R., Vita, V., Bucci, M.S., Gallo, P., Bruno, M., 2014. Microcystin contamination in sea mussel farms from the Italian Southern Adriatic coast following cyanobacterial blooms in an artificial reservoir. J. Ecosyst. 2014, 1–11.
DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of carbon isotopes in

animals. Geochim. Cosmochim. Acta 42 (5), 495–506.
Duarte, C.M., Agustí, S., Canjield, D.E., 1992. Patterns in phytoplankton community structure in Florida lakes. Limnol. Oceanogr. 37, 155–161.
FAO, 2004. Marine biotoxins - Food and Nutrition Paper 80, Food and Agriculture Organization of the United Nations, Rome.
Fetscher, A.E., Howard, M.D.A., Stancheva, R., Kudela, R.M., Stein, E.D., Sutula, M.A., Busse, L.B., Sheath, R.G., 2015. Wadeable streams as widespread sources of benthic cyanotoxins in California, USA. Harmful Algae 49, 105–116.
Francis, G., 1878. Poisonous Australian lake. Nature 18 (444), 11–12.
Graham, J.L., Ziegler, A.C., Loving, B.L., Loftin, K., 2012. Fate and Transport of Cyanobacteria and Associated Toxins and Taste-and-Odor Compounds from Upstream Reservoir Releases in the Kansas River, Kansas, September and October 2011, US Department of the Interior, US Geological Survey, 2012.
Gupta, N., Pant, S.C., Vijayaraghavan, R., Rao, P.V.L., 2003. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology 188, 285–296.
Ha, J.H., Hidaka, T., Tsuno, H., 2011. Analysis of factors affecting the ratio of microcystin to chlorophyll-a in cyanobacterial blooms using real-time polymerase chain reaction. Environ. Toxicol. 26 (1), 21–28.
Harada, K.I., Matsuura, K., Suzuki, M., Oka, H., Watanabe, M.F., Oishi, S., Dahlem, A.M., Beasley, V.R., Carmichael, W.W., 1988. Analysis and purification of toxic peptides from cyanobacteria by reversed-phase high-performance liquid chromatography. J. Chromatogr. A 448, 275–283.
Hollister, J.W., Kreakie, B.J., 2016. Associations between chlorophyll a and various microcystin-LR health advisory concentrations. F1000Research 5, 151.
Ibelings, B.W., Chorus, I., 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: a review. Environ. Pollut. 150, 177–192.
Jeong, Y.H., Yang, J.S., Park, K., 2014. Changes in water quality after the construction of an Estuary dam in the Geum River Estuary Dam system. Korea. J. Coast. Res. 298, 1278–1286.
Jia, J., Chen, Q., Lauridsen, T.L., 2016. A systematic investigation into the environmental fate of microcystins and the potential risk: study in Lake Taihu. Toxins (Basel) 8 (6), 170.
Kim, M.S., Lee, Y.J., Ha, S.Y., Kim, B.H., Hwang, S.J., Kwon, J.T., Choi, J.W., Shin, K.H., 2017. Accumulation of microcystin (LR, RR and YR) in three freshwater bivalves in Microcystis aeruginosa bloom using dual isotope tracer. Mar. Drugs 15, 1–15.
Kozlowsky-Suzuki, B., Wilson, A.E., da Silva Ferrão-Filho, A., 2012. Biomagnification or biodilution of microcystins in aquatic foodwebs? meta-analyses of laboratory and field studies. Harmful Algae 18, 47–55.
Lahti, K., Rapala, J., Färdig, M., Niemelä, M., Sivonen, K., 1997. Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. Water Res. 31, 1005–1012.
Lance, E., Neffling, M.R., Gérard, C., Meriluoto, J., Bormans, M., 2010. Accumulation of free and covalently bound microcystins in tissues of *Lymnaea stagnalis* (Gastropoda) following toxic cyanobacteria or dissolved microcystin-LR exposure. Environ. Pollut. 158, 674–680.
Lee, S.-H., Kwon, H.-K., Choi, H.-Y., Yang, J.-S., Choi, J.-Y., 1999. Physical environment changes in the Keum River estuary by the dyke gate operation: II. Salinity structure and estuary type. Sea 4, 255–265.
Lee, S.K., Boo, S.M., 2000. Standing crops of freshwater phytoplankton in Kumgang river. Korea. Korean J. Environ. Biol 18, 347–353.
Logan, J.M., Lutcavage, M.E., 2008. A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. Rapid Commun. Mass Sp. 22 (7), 1081–1086.
Lorrain, A., Savoye, N., Chauvaud, L., Paulet, Y.M., Naulet, N., 2003. Decarbonation and preservation method for the analysis of organic C and N contents and stable isotope ratios of low-carbonated suspended particulate material. Anal. Chim. Acta 491 (2), 125–133.
MacKintosh, C., Beattie, K.A., Klumpp, S., Cohen, P., Codd, G.A., 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett. 264 (2), 187–192.
de Magalhães, V.F., Soares, R.M., Azevedo, S.M.F.O., 2001. Microcystin contamination in fish from the Jacarepaguaa Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon 39 (7), 1077–1085.
Miller, M.A., Kudela, R.M., Mekebri, A., Crane, D., Oates, S.C., Tinker, M.T., Staedler, M., Miller, W.A., Toy-Choutka, S., Dominik, C., Hardin, D., Langlois, G., Murray, M., Ward, K., Jessup, D.A., 2010a. Evidence for a novel marine harmful algal bloom: Cyanotoxin (microcystin) transfer from land to sea otters. PLoS ONE 5 (9), e12576.
Miller, T.W., Omori, K., Hamaoka, H., Shibata, J.Y., Hidejiro, O., 2010b. Tracing anthropogenic inputs to production in the Seto Inland Sea, Japan - a stable isotope approach. Mar. Pollut. Bull. 60 (10), 1803–1809.
Navarro, E., Méndez, S., Ibarrola, I., Urrutia, M.B., 2009. Comparative utilization of phytoplankton and vascular plant detritus by the cockle Cerastoderma edule: digestive responses during diet acclimation. Aquat. Biol. 6, 247–262.
Ni, W., Zhang, J., Luo, Y., 2017. Microcystin accumulation in bighead carp (*Aristichthys nobilis*) during a Microcystis-dominated bloom and risk assessment of the dietary intake in a fish pond in China. Environ. Sci. Pollut. Res. 24, 8894–8902.
Noh, S., Park, H., Choi, H., Lee, J., 2014. Effect of climate change for cyanobacteria growth pattern in Chudong station of Lake Daechung. J. Korean Soc. Water Environ. 30 (4), 377–385.
Oh, H.M., Ahn, C.Y., Lee, J.W., Chon, T.S., Choi, K.H., Park, Y.S., 2007. Community patterning and identification of predominant factors in algal bloom in Daechung reservoir (Korea) using artificial neural networks. Ecol. Modell. 203, 109–118.
Park, H.D., Iwami, C., Watanabe, M.F., Harada, K.I., Okino, T., Hayashi, H., 1998. Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic microcystis species in a hypertrophie lake, Lake Suwa, Japan (1991–1994). Environ. Toxicol. Water Qual. 13, 61–72.
Post, D.M., 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83 (3), 703–718.
Poste, A.E., Ozersky, T., 2013. Invasive dreissenid mussels and round gobies: a benthic pathway for the trophic transfer of microcystin. Environ. Toxicol. Chem. 32, 2159–2164.
Pouria, S., De Andrade, A., Barbosa, J., Cavalcanti, R.L., Barreto, V.T.S., Ward, C.J., Preiser, W., Poon, G.K., Neild, G.H., Codd, G.A., 1998. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. Lancet 352 (9121), 21–26.
Preece, E.P., Hardy, F.J., Moore, B.C., Bryan, M., 2017. A review of microcystin detections in Estuarine and Marine waters: environmental implications and human health risk. Harmful Algae 61, 31–45.
Sedda, T., Baralla, E., Varoni, M.V., Pasciu, V., Lorenzoni, G., Demontis, M.P., 2016. Determination of microcystin-LR in clams (*Tapes decussatus*) of two Sardinian coastal ponds (Italy). Mar. Pollut. Bull. 108, 317–320.
Sotton, B., Guillard, J., Anneville, O., Maréchal, M., Savichtcheva, O., Domaizon, I., 2014. Trophic transfer of microcystins through the lake pelagic food web: evidence for the role of zooplankton as a vector in fish contamination. Sci. Total Environ. 466–467, 152–163.
Takahashi, T., Umehara, A., Tsutsumi, H., 2014. Diffusion of microcystins (cyanobacteria hepatotoxins) from the reservoir of Isahaya Bay, Japan, into the marine and surrounding ecosystems as a result of large-scale drainage. Mar. Pollut. Bull. 89, 250–258.
Tonk, L., Bosch, K., Visser, P.M., Huisman, J., 2007. Salt tolerance of the harmful cyanobacterium Microcystis aeruginosa. Aquat. Microb. Ecol. 46, 117–123.
Trainer, V.L., Adams, N.G., Bill, B.D., F., A.B., Wekell, J.C., 1998. Concentration and dispersal of a Pseudo-nitzschia bloom in Penn Cove, Washington, USA. Nat. Toxins 6(3-4), 113–125.
U.S Environmental Protection Agency (USEPA), 1979. Handbook for analytical quality control in water and wastewater laboratories, Environmental Protection Agency. Washington, D.C.
Umehara, A., Komorita, T., Tai, A., Takahashi, T., Orita, R., Tsutsumi, H., 2015. Short-term dynamics of cyanobacterial toxins (microcystins) following a discharge from a coastal reservoir in Isahaya Bay, Japan. Mar. Pollut. Bull. 92, 73–79.
Umehara, A., Takahashi, T., Komorita, T., Orita, R., Choi, J.W., Takenaka, R., Mabuchi, R., Park, H.D., Tsutsumi, H., 2017. Widespread dispersal and bio-accumulation of toxic microcystins in benthic marine ecosystems. Chemosphere 167, 492–500.
Umehara, A., Tsutsumi, H., Takahashi, T., 2012. Blooming of Microcystis aeruginosa in the reservoir of the reclaimed land and discharge of microcystins to Isahaya Bay (Japan). Environ. Sci. Pollut. R. 19, 3257–3267.
Vaitomaa, J., Rantala, A., Halinen, K., Rouhiainen, L., Tallberg, P., Mokelke, L., Sivonen, K., 2003. Quantitative real-time PCR for determination of microcystin synthetase E copy numbers for microcystis and anabaena in lakes. Appl. Environ. Microbiol. 69 (12), 7289–7297.
Vander Zanden, M.J., Casselman, J.M., Rasmussen, J.B., 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401 (6752), 464.
Vesterkvist, P.S.M., Meriluoto, J.A.O., 2003. Interaction between microcystins of different hydrophobicities and lipid monolayers. Toxicon 41, 349–355.
Vizzini, S., Sarà, G., Michener, R.H., Mazzola, A., 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. Acta Oecol. 23 (4), 277–285.
Welker, M., Von Döhren, H., 2006. Cyanobacterial peptides - nature's own combinatorial biosynthesis. FEMS Microbiol. Rev. 30, 530–563.
Won, E.J., Choi, B., Hong, S., Khim, J.S., Shin, K.H., 2018. Importance of accurate trophic level determination by nitrogen isotope of amino acids for trophic magnification studies: a review. Environ. Pollut. 238, 677–690.
Xie, L., Yokoyama, A., Nakamura, K., Park, H., 2007. Accumulation of microcystins in various organs of the freshwater snail *Sinotaia histrica* and three fishes in a temperate lake, the eutrophic Lake Suwa, Japan. Toxicon 49 (5), 646–652.
Yeo, H., 2009. Phytoplankton community and phytohydrographic characteristics of the inside and outside of saemangeum reservoir. J. Korea Acad. Coop. Soc. 10, 2021–2025.
Yoon, J., Park, S., Chang, K., Choi, J., 2015. Characteristics of Fish Fauna in the Lower Geum river and identification of trophic guilds using stable isotopes. Analysis 9999, 34–44.
Yu, G., Jiang, Y., Song, G., Tan, W., Zhu, M., Li, R., 2014. Variation of Microcystis and microcystins coupling nitrogen and phosphorus nutrients in Lake Erhai, a drinking-water source in Southwest Plateau, China. Environ. Sci. Pollut. R. 21, 9887–9898.
Zastepa, A., Taranu, Z.E., Kimpe, L.E., Blais, J.M., Gregory-Eaves, I., Zurawell, R.W., Pick, F.R., 2017. Reconstructing a long-term record of microcystins from the analysis of lake sediments. Sci. Total Environ. 579, 893–901.

<Supplementary Materials>

Multimedia distributions, bioaccumulation, and trophic transfer of microcystins in the Geum River Estuary, Korea: Application of compound-specific isotope analysis of amino acids

Dokyun Kim, Seongjin Hong *, Hyuntae Choi, Bohyung Choi, Jaeseong Kim, Jong Seong Khim, Hodong Park, Kyung-Hoon Shin *

Supplementary Tables

Table S1. Information on the biological samples, for microcystins analysis, collected from the Geum River Estuary. S2
Table S2. Instrumental conditions of HPLC-MS/MS for microcystins analysis. S3
Table S3. Optimized HPLC-MS/MS parameters for microcystins analysis. S4
Table S4. Results of quality assurance and quality control for microcystins analysis. S5
Table S5. Instrumental conditions of GC-IRMS for analysis of nitrogen stable isotope of amino acids. S6
Table S6. Summary of microcystins concentrations and water quality parameters (temperature, salinity, pH, and chlorophyll *a*) of discharged water samples from Geum River during July to August, 2017. .... S7
Table S7. Summary of microcystins concentrations on multi-media samples in the Geum River Estuary. S8
Table S8. Locations and environmental parameters (temperature, salinity, and pH) of study area. S9
Table S9. Concentrations of microcystins in freshwater, brackish water, and marine organisms reported in the previous studies and from the present study. S10

Supplementary Figures

Fig. S1. Discharge ($\times 10^6$ ton d^{-1}) and precipitation (mm d^{-1}) in Geum River Estuary. The Sampling was conducted in 9 June and 18 July 2017. .... S11
Fig. S2. Scheme of analytical methods for determination of microcystins in the environmental multimedia samples (modified from Xie et al. (2007), Park et al. (1998), and Sedda et al. (2016)). S12
Fig. S3. δ^{13}C and δ^{15}N dual plot of POM, SOM, and biota in GR2, GR5, and GR7. .... S13

References S14

***Corresponding authors.**
E-mail addresses: hongseongjin@cnu.ac.kr (S. Hong); shinkh@hanyang.ac.kr (K.H. Shin).

Supplementary Tables

Table S1. Information on the biological samples, for microcystins analysis, collected from the Geum River Estuary.

Station	Common name	Scientific name	*n*	Size (mm)	Wet weight (g)	Tissue
GR2	Striped mullet	*M. cephalus*	2	500	720	Liver
				450	500	Liver
GR5	Surf clam	*M. veneriformis*	5	41 ± 19		Liver
GR7	Yellow croakers	*Larimichthys* sp.	1	200	127	Midgut gland
	Clam worm	*Neanthes* sp.	5	34 ± 15		Midgut gland
	Blue crab	*P. trituberculatus*	1	200	138	Midgut gland
	Veined rapa whelk	*R. venosa*	2	110	160	Midgut gland
				120	207	Whole body

Table S2. Instrumental conditions of HPLC-MS/MS for microcystins analysis.

HPLC system	1290 infinity II
Column	Poroshell 120 EC-C18 4.6 x 50mm 2.7-Micron
Separation scheme	Gradient: A 70% (0-1 min) → A 70 - 0% (1-7 min) → A 0% (7-8 min) → A 0 - 70% (8-10 min) B 30% (0-1 min) → B 30 - 100% (1-7 min) → B 100% (7-8 min) → B 100 - 30% (8-10 min)
Mobile phase A	0.1% Formic acid in water
Mobile phase B	MeOH
Flow rate	0.3 mL min^{-1}
Injection volume	10 μL
MS/MS system	**QTRAP 6500**
Source	ESI (electrospray ionization)
Ion mode	Positive
Ion Spray voltage	5.5 kV
Source Temp.	600°C
Curtain gas	N_2 (35 psi)
Ion Source gas 1	N_2 (50 psi)
Ion Source gas 2	N_2 (50 psi)
Detection mode	Multiple reaction monitoring (MRM)

Table S3. Optimized HPLC-MS/MS parameters for microcystins analysis.

Compound	RT	Molecule weight	Parent ion	Daughter ion	Dwell (s)	CE (eV)	DP (volts)	CXP (volts)
Microcystin-LR	6.99	995.2	995.4 $[M+1]^+$	103.1*	50	129	41	14
				135	50	121	41	16
Microcystin-RR	6.43	1038.2	519.9 $[M+2]^{2+}$	135.2*	50	37	146	6
				103	50	91	146	12
Microcystin-YR	6.83	1045.2	1045.4 $[M+1]^+$	103.0*	50	127	16	14
				213	50	73	16	12
Enkephalin	5.45	555.6	556.2 $[M+1]^+$	120.1*	50	71	141	8
				136	50	67	141	8
				397	50	29	141	12
Monolinuron	6.85	214.7	215.0 $[M+1]^+$	126.2*	150	25	111	6
				148	150	19	111	20

* Daughter ion used for quantification.

Table S4. Results of quality assurance and quality control for microcystins analysis.

Compounds	Linear (R^2)	LOD (μg L^{-1})	LOQ (μg L^{-1})	Recovery (%)
Microcystin-LR	0.9994	0.5	1.5	87 ± 1
Microcystin-RR	0.9973	2	6	96 ± 3
Microcystin-YR	0.9993	1	3	92 ± 11
Enkephalin (IS)	0.9991	1	4	93 ± 21

LOD: Limit of detection.

LOQ: Limit of quantitation.

Table S5. Instrumental conditions of GC-IRMS for analysis of nitrogen stable isotope of amino acids.

GC-IRMS system	Agilent HP6890N – Isoprime GV instruments
Column	HP Ultra-2 (50 m × 0.32 mm, 0.52 μm)
Oven program	40°C (2.5 min. hold) → 110°C (15°C/min.) → 150°C (3°C/min.) → 220°C (6°C/min., 17.3 min. hold)
Flow rate	1.4 mL min^{-1}
Injection volume	2 μL
Oxidation column	950°C, CuO, NiO, and Pt
Reduction column	200°C, Cu and Rh

Table S6. Summary of microcystins concentrations and water quality parameters (temperature, salinity, pH, and chl. *a*) of discharged water samples from Geum River during July to August, 2017.

Sampling date	Particulate + dissolved MCs (μg L^{-1})			Temperature (°C)	Salinity (psu)	pH	Chl. *a* (μg L^{-1})	Discharge amount (kton)
	MC-LR	MC-RR	MC-YR					
3 July	1.24	0.97	0.35	27.2	0.26	8.2	37	18,156
4 July	39.8	26.0	8.84	26.0	0.23	6.9	163	26,299
								18,395
5 July	17.0	9.77	2.02	31.2	0.22	7.1	112	18,366
								13,293
6 July	2.15	0.95	0.40	28	0.22	7.5	49	15,615
8 July	0.51	0.20	0.11	27.8	0.20	6.8	28	29,355
26 July	0.17	0.12	0.11	27.6	0.12	7.1	7	10,188
31 Aug	0.18	0.11	0.07	25.9	0.14	7.4	16	13,416

Table S7. Summary of microcystins concentrations on multi-media samples in the Geum River Estuary.

Sampling period	Estuary dam	Site	Dissolved MCs (ng L^{-1})			Particulate MCs (ng L^{-1})			Sedimentary MCs (ng g^{-1} dw)		
			MC-LR	MC-RR	MC-YR	MC-LR	MC-RR	MC-YR	MC-LR	MC-RR	MC-YR
June	Inner	GR1	95	41	18	420	570	65	7.0	6.4	2.7
		GR2	97	40	20	280	470	40	8.1	8.1	1.5
	Outer	GR3	88	30	14	2.5	39	-	2.2	-	-
		GR4	12	6.2	2.1	1.3	2.6	-	1.2	-	-
		GR5	3.9	2.4	0.60	-	-	-	-	-	-
		GR6	1.0	-	0.50	-	-	-	-	-	-
		GR7	0.50	-	-	-	-	-	-	-	-
July	Outer	GR3	-	-	-	-	-	-	-	-	-
		GR4	-	-	-	-	-	-	-	-	-
		GR5	19	-	-	6.7	5.9	2.8	0.5	-	-
		GR6	25	-	9.4	1.3	3.3	0.60	0.8	-	-
		GR7	15	-	9.6	5.2	9.1	5.3	-	-	-

- : below the detection limit.

Table S8. Locations and environmental parameters (temperature, salinity, and pH) of study area.

Station	Location		June 2017			July 2017		
	Latitude	Longitude	Temperature (°C)	Salinity (psu)	pH	Temperature (°C)	Salinity (psu)	pH
GR1	36°01'41"N	126°47'17"E	23.7	0.3	9.1			
GR2	36°01'07"N	126°45'13"E	24.1	0.3	9.0			
GR3	35°59'49"N	126°43'32"E	22.6	18.6	7.6	24.8	1.2	7.0
GR4	36°00'22"N	126°40'40"E	21.2	28.8	7.6	25.1	9.5	7.2
GR5	36°01'18"N	126°38'32"E	20.2	33.7	7.7	26.2	17.1	7.8
GR6	36°01'51"N	126°36'04"E	20.5	34.0	7.7	26.2	20.6	8.0
GR7	36°01'41"N	126°34'18"E	20.5	33.0	7.8	26.0	19.8	7.9

Table S9. Concentrations of microcystins in freshwater, brackish water, and marine organisms reported in the previous studies and from the present study.

Type	Salinity (psu)	Location	Biota	Scientific name	Tissue	MCs conc. (ng g^{-1} dw) Min.–Max.	Mean	References
Freshwater		Lake Suwa, Japan	Gastropod	*Semisulcospira reiniana*	Hepatopancreas		1,860	Xie et al., 2007
		Lake Biwa, Japan	Gastropod	*Sinotaia histrica*	Hepatopancreas	1,950–3,200		Ozawa et al., 2003
		Lake Pamvotis, Greece	Fish	*Carassius auratus*	Muscle	20–1,500	336	Gkelis et al., 2006
		Lake Suwa, Japan	Fish	*Carassius auratus*	Liver	1,080–8,790	5,380	Xie et al., 2007
		Lake Taihu, China	Bivalves	*Hyriopsis cumingii*	Hepatopancreas	76–12,500		Chen and Xie, 2005
				Cristaria plicata		16–38,480		
				Anodonta woodian		150–5,960		
				Lamprotula leai		380–13,230		
		Fish farm, Egypt	Fish	*Oreochromis niloticus*	Liver		532	Mohamed et al., 2003
		Lake Albufera, Spain	Fish	*Liza* sp.	Liver		2480	Romo et al., 2012
Brackish water (Estuary)	< 1	Barataria estuary, USA	Blue crab	*Callinectes sapidus*	Hepatopancreas	ND–820	80	Garcia et al., 2010
	< 0.2	James River Estuary, Virginia	Finfish	*Ictalurus furcatus*	Liver	26.0–93.0		Wood et al., 2014
		San Francisco Estuary, USA	Finfish	*Morone saxatilis*	Liver	1.0–3.4		Lehman et al., 2010
	0.3–34	Geum River Estuary, Korea	Striped mullet	*Mugil cephalus*	Liver		810	This study
					Liver		630	
			Surf clam	*Mactra veneriformis*	Liver		870	
			Yellow croakers	*Larimichthys* sp.	Midgut gland		40	
			Clam worm	*Neanthes* sp.	Midgut gland		110	
			Blue crab	*Portunus trituberculatus*	Midgut gland		160	
			Veined rapa whelk	*Rapana venosa*	Midgut gland		430	
					Whole body		510	
Seawater		Adriatic Sea, Italy	Mussels	*Mytilus galloprovincialis*	Muscle	1.7–256		De Pace et al., 2014
			Finfish	*Cyprinus carpio*		0.42–3.0		
	ca. 30	Isahaya & Ariake Bay, Japan	Polychaetes	*Polynoidae* sp.	Whole	1.4–1,400		Umehara et al., 2017
				Maldanidae sp.		260–450		
				Nectoneanthes oxypoda			290	
			Oysters	Mollusca		4.6–2,800		
				Crassostrea gigas		ND–190		
		Monterey Bay, USA	Sea otter	*Enhydra lutris nereis*	Liver	1,360–348,000		Miller et al., 2010
	30–34	Amvrakikos Gulf, Greece	Mollusca	*Mytilus galloprovincialis*	Whole	45–142		Vareli et al., 2012
		Sepetiba Bay, Brazil	Crab	Unidentified	Muscle	0.25–100		Magalhães et al., 2003
			Fish	Unidentified		ND–40		

Supplementary Figures

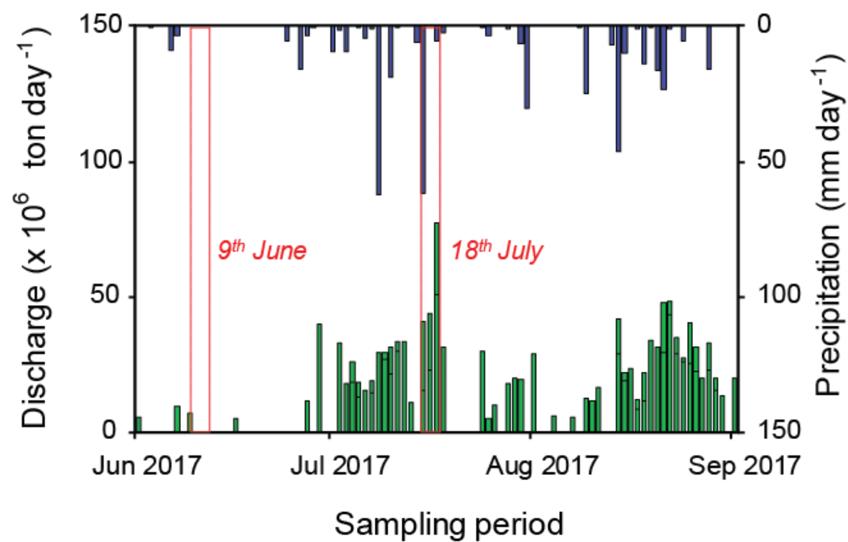

Fig. S1. Discharge ($\times 10^6$ ton d^{-1}) and precipitation (mm d^{-1}) in Geum River Estuary. The Sampling was conducted on 9 June and 18 July 2017.

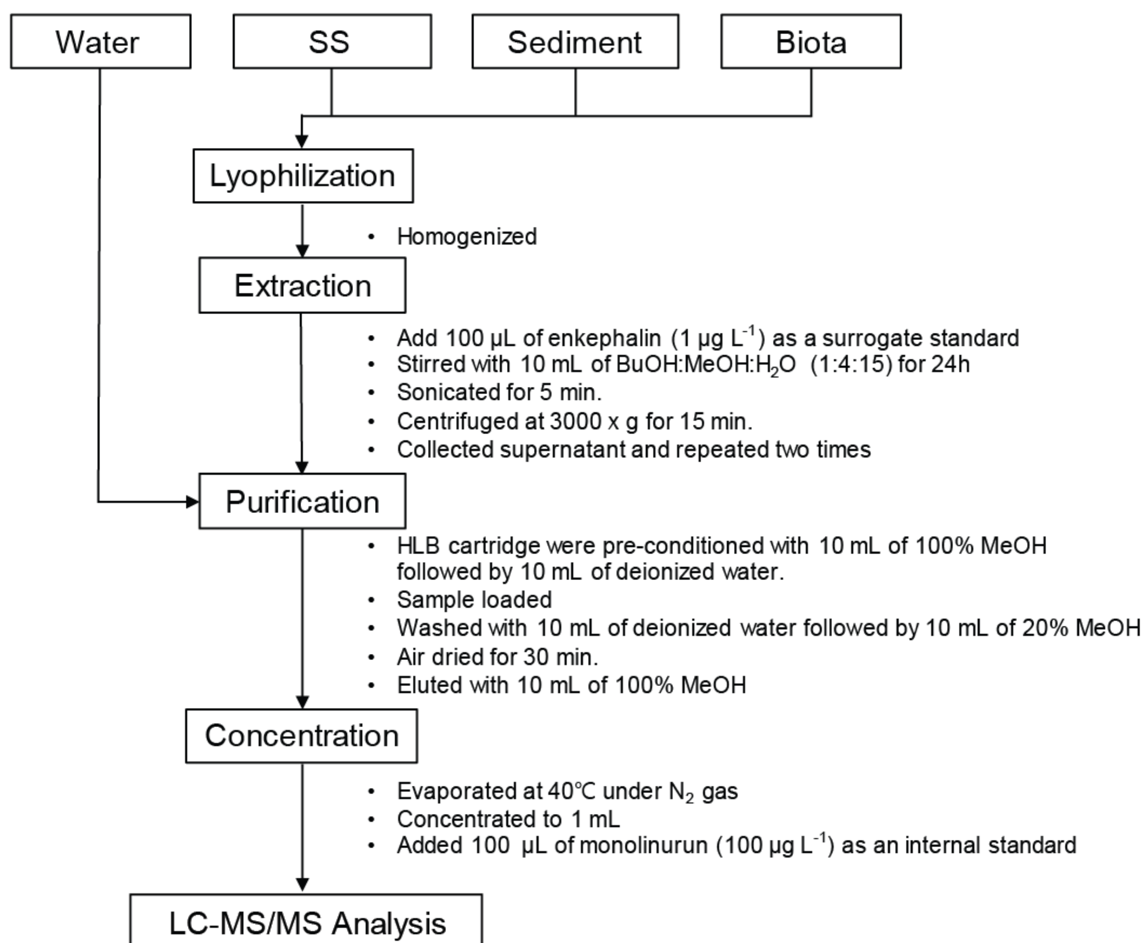

Fig. S2. Scheme of analytical methods for determination of microcystins in the environmental multimedia samples (modified from Xie et al. (2007), Park et al. (1998), and Sedda et al. (2016)).

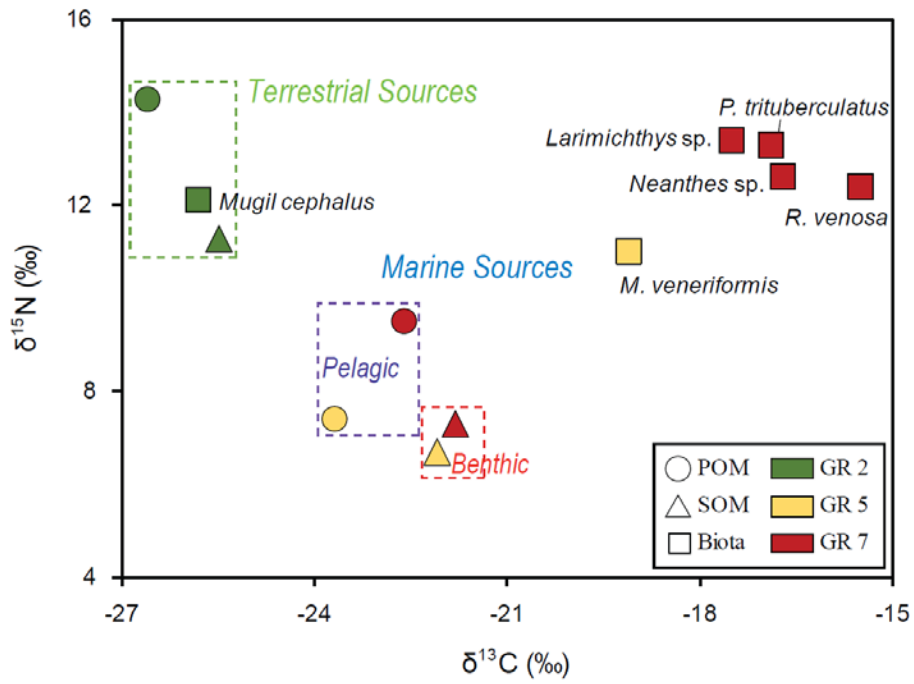

Fig. S3. $\delta^{13}C$ and $\delta^{15}N$ dual plot of POM, SOM, and biota in GR2, GR5, and GR7.

References

Chen, J., Xie, P., 2005. Seasonal dynamics of the hepatotoxic microcystins in various organs of four freshwater bivalves from the large eutrophic Lake Taihu of subtropical China and the risk to human consumption. Environ. Toxicol. 20(6), 572–584.

De Pace, R., Vita, V., Bucci, M.S., Gallo, P., Bruno, M., 2014. Microcystin Contamination in Sea Mussel Farms from the Italian Southern Adriatic Coast following Cyanobacterial Blooms in an Artificial Reservoir. J. Ecosyst. 2014, 1–11.

Garcia, A.C., Bargu, S., Dash, P., Rabalais, N.N., Sutor, M., Morrison, W., Walker, N.D., 2010. Evaluating the potential risk of microcystins to blue crab (Callinectes sapidus) fisheries and human health in a eutrophic estuary. Harmful Algae 9, 134–143.

Gkelis, S., Lanaras, T., Sivonen, K., 2006. The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. Aquat. Toxicol. 78(1), 32–41.

Lehman, P.W., Teh, S.J., Boyer, G.L., Nobriga, M.L., Bass, E., Hogle, C., 2010. Initial impacts of Microcystis aeruginosa blooms on the aquatic food web in the San Francisco Estuary. Hydrobiologia 637(1), 229–248.

Magalhães, V.F., Marinho, M.M., Domingos, P., Oliveira, A.C., Costa, S.M., Azevedo, L.O., Azevedo, S.M.F.O., 2003. Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). Toxicon 42, 289–295.

Miller, T.W., Omori, K., Hamaoka, H., Shibata, J.Y., Hidejiro, O., 2010. Tracing anthropogenic inputs to production in the Seto Inland Sea, Japan - A stable isotope approach. Mar. Pollut. Bull. 60(10), 1803–1809.

Mohamed, Z.A., Carmichael, W.W., Hussein, A.A., 2003. Estimation of microcystins in the freshwater fish Oreochromis niloticus in an Egyptian fish farm containing a Microcystis bloom. Environ. Toxicol. 18(2), 137–141.

Ozawa, K., Yokoyama, A., Ishikawa, K., Kumagai, M., Watanabe, M.F., Park, H.D., 2003. Accumulation and depuration of microcystin produced by the cyanobacterium Microcystis in a freshwater snail. Limnology 4(3), 131–138.

Park, H.D., Iwami, C., Watanabe, M.F., Harada, K.I., Okino, T., Hayashi, H., 1998. Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic microcystis species in a hypertrophie lake, Lake Suwa, Japan (1991-1994). Environ. Toxicol. Water Qual. 13, 61–72.

Romo, S., Fernández, F., Ouahid, Y., Barón-Sola, Á., 2012. Assessment of microcystins in lake water and fish (Mugilidae, Liza sp.) in the largest Spanish coastal lake. Environ. Monit. Assess. 184, 939–949.

Sedda, T., Baralla, E., Varoni, M.V., Pasciu, V., Lorenzoni, G., Demontis, M.P., 2016. Determination of microcystin-LR in clams (Tapes decussatus) of two Sardinian coastal ponds (Italy). Mar. Pollut. Bull. 108, 317-320.

Umehara, A., Takahashi, T., Komorita, T., Orita, R., Choi, J.W., Takenaka, R., Mabuchi, R., Park, H.D., Tsutsumi, H., 2017. Widespread dispersal and bio-accumulation of toxic microcystins in benthic marine ecosystems. Chemosphere 167, 492–500.

Vareli, K., Zarali, E., Zacharioudakis, G.S.A., Vagenas, G., Varelis, V., Pilidis, G., Briasoulis, E., Sainis, I., 2012. Microcystin producing cyanobacterial communities in Amvrakikos

Gulf (Mediterranean Sea, NW Greece) and toxin accumulation in mussels (Mytilus galloprovincialis). Harmful Algae 15, 109–118.
Wood, J.D., Franklin, R.B., Garman, G., McIninch, S., Porter, A.J., Bukaveckas, P.A., 2014. Exposure to the cyanotoxin microcystin arising from interspecific differences in feeding habits among fish and shellfish in the james river estuary, Virginia. Environ. Sci. Technol. 48, 5194–5202.
Xie, L., Yokoyama, A., Nakamura, K., Park, H., 2007. Accumulation of microcystins in various organs of the freshwater snail Sinotaia histrica and three fishes in a temperate lake, the eutrophic Lake Suwa, Japan. Toxicon 49, 646-652.