Environment International 131 (2019) 104981

Contents lists available at ScienceDirect

Environment International

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

Anthropogenic influences on benthic food web dynamics by interrupted freshwater discharge in a closed Geum River estuary, Korea

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ARTICLE INFO

Handling Editor: Tieyu Wang

Keywords:
Stable isotopes
Tidal flats
Sea dike
Dam
Yellow Sea
Marine invertebrates

ABSTRACT

Stable isotope analysis was used to investigate the benthic food web dynamics in the Geum River estuary where continuous river flow has been blocked by a sea dike over the past 25 years. In order to address the dike effect(s) on distribution of food sources (i.e., organic matters and microphytobenthos) and their utilization by marine predators, a three years monitoring survey (total of 30 surveys) was seasonally conducted at four fixed locations at both inside of dike and outer tidal flats. All the collected biota (total of 19 species; > 1100 individuals and microphytobenthos) and abiotic ($n = 118$) samples were analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes. In particular, two dominant marine bivalves inhabiting outer reach of tidal flats, *Mactra veneriformis* and *Cyclina sinensis*, were targeted to identify their feeding strategies that being related to a year-round population growth. In general, the stable isotopic signatures of samples indicated dissimilarity in distribution of organic matters between inside and outside of dike, supporting geographical and/or trophic isolation. The taxa-dependent trophic levels are also evidenced in consistent manner, with two to three levels being positioned over the years. Meantime, their dietary contributions varied in time, i.e., seasonal chances in compositions of major food sources (microphytobenthos and particulate organic matters) were observed for two target bivalves. Such temporal variations could be further linked to selective feedings that evidenced by age(size)-dependent and/or tissue specific distributions. Altogether, the present study suggested seasonality, diet preference, and growth dependent food web dynamics in the Geum River estuary. Overall, the present study suggested that the stable isotopic technique could be a powerful tool for characterizing the long-term anthropogenic influences of a sea dike on marine food-web dynamics.

1. Introduction

Estuarine ecosystem represents some of the most highly productive areas globally (Costanza et al., 1997; McLusky and Elliott, 2004). Such high productivity of estuaries is generally explained by a greatly complex environment, whereby the mixing of freshwater and seawater leads to habitats and biodiversity occupying dynamic environmental gradients. Accordingly, the benthic consumers in shallow intertidal zones utilize both autochthonous and allochthonous resources cross terrestrial and marine gradients (Nixon et al., 1986; Yokoyama and Ishihi, 2007; Thottathil et al., 2008; Antonio et al., 2012; McTigue et al., 2015; Dias et al., 2016). Several studies revealed that the relative contributions of these potential food sources would vary depending on the flooding frequency of freshwater (Delong et al., 2001), seasonal abundance of benthic primary producers (Kang et al., 2006), and feeding type or diet preferences of consumers (Davenport et al., 2011; Rossi et al., 2015). In particular, macrobenthos are relatively long-lived, lesser mobile, and locality-specific (Díaz-Castañeda and Reish,

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https://doi.org/10.1016/j.envint.2019.104981
Received 28 March 2019; Received in revised form 7 June 2019; Accepted 28 June 2019

2009), and thus could be regarded as ideal medium to elucidate the trophic history through the assimilation of varying food sources.

Natural food sources for consumers in the intertidal areas are diverse from phyto- and zooplankton, microphytobenthos (MPB), detritus, and to organic aggregations formed of exudates and colloidal materials. Those potential food sources are incorporated into the benthic food webs by deposit and/or filter feedings of macrobenthos (Kang et al., 2015). Several studies have reported the ecological significance of MPB in benthic food web, which regularly inhabit bare intertidal flats, as major food sources for many macrobenthos, because they exhibit higher productivity and biomass compared to planktonic organic matter (De Jonge and Van Beuselom, 1992; Lucas and Holligan, 1999; Kang et al., 2003). Further MPB contributes significant proportion, of pelagic organic matters, sometimes over 50% to total, by tide and/or wind driven mixing followed by prolonged resuspension in the shallow water system such as tidal flats (De Jonge and Van Beuselom, 1995; Koh et al., 2006). This periodic process allows deposit feeders, which prey on MPB in the sediment, and filter feeders to prey on resuspended MPB in the water column. Thus, intertidal flats are important because they provide constant sources of food for various benthic consumers, with locally produced MPB transporting organic carbon into the food web of nearshore marine habitats.

Stable isotope analysis of carbon and nitrogen is widely used to investigate the trophic structure of various ecosystems. It is also used to identify the contribution of potential food sources to upper level consumers. The stable nitrogen isotopic composition (δ^{15}N) is used to determine the trophic levels of organisms in food webs, while the stable carbon isotopic composition (δ^{13}C) is used to assess sources and pathways of organic matter from the dietary source to consumers (Fry and Sherr, 1984; Owens, 1988; Peterson and Fry, 1987). Earlier studies revealed that the relative isotope signatures of potential food sources vary across habitats and/or environments. For instance, vascular plants and mangroves (Lee, 1999; Rossi et al., 2010; Kristensen et al., 2017), terrestrial organic matters (Kasai and Nakata, 2005; Karlsson et al., 2012), macroalgal or seagrass detritus (Vizzini et al., 2002; Karlson et al., 2016), phyto- and zooplankton (Xu and Zhang, 2012; Golubkov et al., 2018), and anthropogenic effluent (McClelland and Valiela, 1998) exhibit distinct ranges in isotope signatures. Therefore, the stable isotopic approach is based on certain assumptions, namely, the consumers integrated in the sum of relative diet contributions and isotopic fractionation between dietary sources and consumers in one trophic level are predictable (Post, 2002).

For several decades, the hydrological modification of riverine and estuarine channels by sea dikes and weirs has been undertaken to control seasonal flooding and to secure agricultural and industrial water resources along the Yellow Sea in Korea and China (Koh and Khim, 2014; Yim et al., 2018). Such interruptions of the longitudinal connectivity of water flow isolate ecosystems situated between terrestrial and marine areas. This phenomenon leads to ecological degradation and the loss of habitats, decrease of biodiversity, and community changes of organisms inhabiting aquatic environment, elsewhere (Poff et al., 1997; Normile, 2010; Paalvast and van der Velde, 2014; Ko et al., 2017; Yoon et al., 2017; Hur et al., 2018; Table S1 and references therein).

Freshwater discharge into closed estuaries is artificially controlled by opening and closing of water-gates at low and high water, respectively. Thus, the input of terrestrial particulate organic matters (POM) and dissolved nutrients in discharged water might flow intensively during monsoon season in East Asia (Noh et al., 2018). A sea dike has closed off natural river flow from 1990 in the Geum River estuary, Korea; consequently, the dietary contribution of freshwater resources to estuarine benthic consumers might be strongly linked to freshwater discharge. Although the food sources available to the consumers may be limited in this anthropogenically altered environment, there are very few studies on the trophic contribution of potential food sources with terrestrial POM introduced by artificial freshwater discharge. Thus, study looking for marine benthic food web in the altered environment of closed estuary in varying perspectives, such as spatiotemporal, organ-specific, and age-related variations for assimilation of nutrition, would be timely necessary and important.

In the present study, we collected macrobenthos and their potential food sources from four locations along the Geum River estuary, encompassing eight seasons from 2015 to 2017. The clams *Mactra veneriformis* and *Cyclina sinensis* were specifically targeted because they are dominant in the study region and also commercially important along the intertidal flats of the Yellow Sea. The specific objectives were to: (1) evaluate the isotopic signatures of discharged-freshwater POM as potential food sources for intertidal macrobenthos in the closed Geum River estuary; (2) assess the trophic structure of the benthic ecosystem by comparing the stable isotope composition of potential food sources to benthic consumers; (3) determine seasonal variation in the contribution of potential food sources and identify the major food source (s); and (4) highlight organ- and size-specific isotope variations in the two target bivalves. Ultimately, this study supports to better understand benthic food web in the closed estuary and step towards enhanced management of estuarine ecosystem elsewhere.

2. Materials and methods

2.1. Study area and data collection

The study area, Geum River estuary, is located on the west coast of South Korea. Freshwater input occurs by irregular discharge through the sea dike. The sampling locations were selected based on the oceanographic settings, following the salinity gradient along the estuary, and included one freshwater area inside the sea dike (Geum River, GR) and three intertidal locations. Of the intertidal zones, two locations were in a coastal area near to the river mouth (Songlim-ri (SL) and Yubu Is. (YB) and one was located offshore (Dasa-ri, DS) far from river mouth (Fig. 1). The amount of freshwater discharged to the sea during study period was referenced from a record on freshwater discharge in Geumgang Project Office, Korea Rural Community Corporation (KRCC, 2019). Monthly precipitation rates of Gunsan City in the Geum River estuary was obtained from the open on-line source of the National Climate Data Service System (NCDSS, 2019) of Korea Meteorological Administration. Finally, we overlapped both the amount of daily discharged water and monthly precipitation rate (Fig. 2a). In general, 100 to 200 kt water was discharged on five to six occasions per month from the Geum River dike between 2015 and 2017, excluding summer (July to August). During summer, freshwater was discharged up to 30 times per day totaling > 800 kt water per month, because monthly precipitation is the highest by regular heavy monsoon and typhoon rains.

2.2. Sample collection and preparation

Sediment, sea/freshwater, and macrobenthos were sampled to analyze stable carbon and nitrogen isotopic compositions in sedimentary organic matter (SOM), particulate organic matter in freshwater (POM_{fw}) and seawater (POM_{sw}), and the soft tissue of biota in eight seasons over October 2015 to November 2017. Sampled macrobenthos were identified and separated into trophic groups followed by Yokoyama et al. (2005b), with diet and feeding type being taken into consideration. Discharged freshwater sample was randomly collected during irregular discharge times from June 2016 to November 2017, because the opening of the floodgate was determined from the amount of precipitation. In total, 22 samples (20 L per sample) were collected (Fig. 2a).

MPB samples were collected from two locations (DS and SL) in February and May, 2017, when microalgal mats were visible. The microalgal mats were scraped from the top of the sediment surface (~2 mm) and stored into a clean bottle on ice. The mats were transferred to the laboratory within 4 h. MPB was extracted from sediment

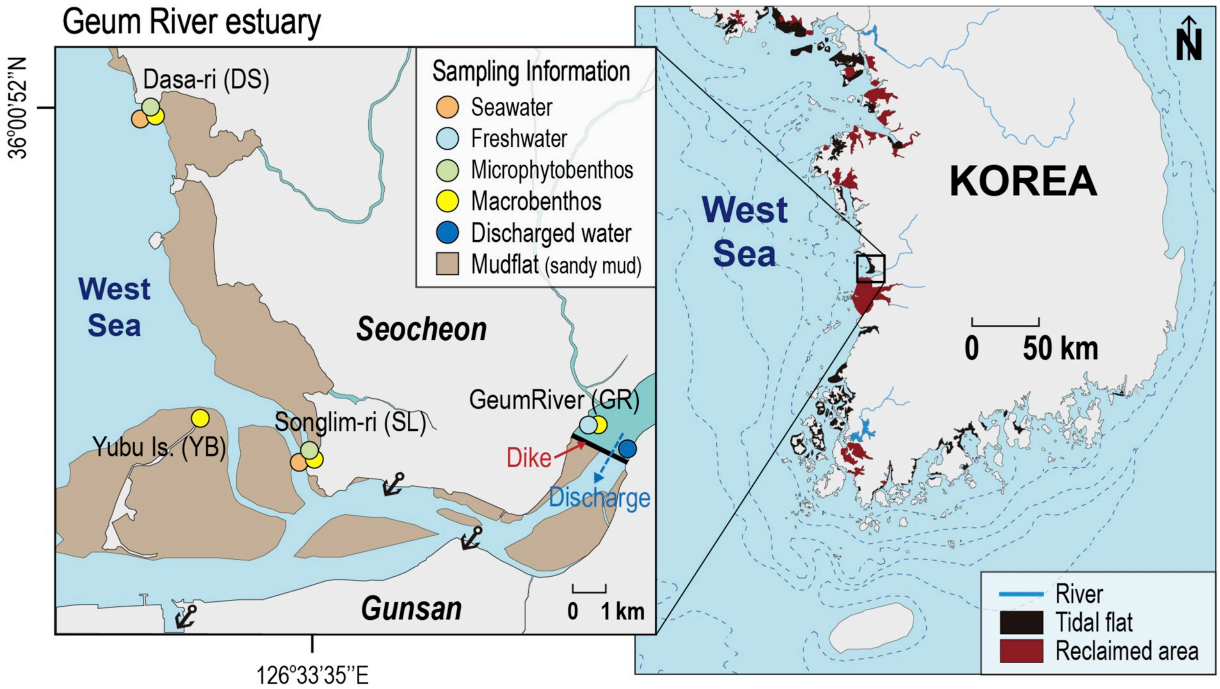

Fig. 1. Map of the study area along the Geum River estuary, Korea, showing the sediment types and sampling locations.

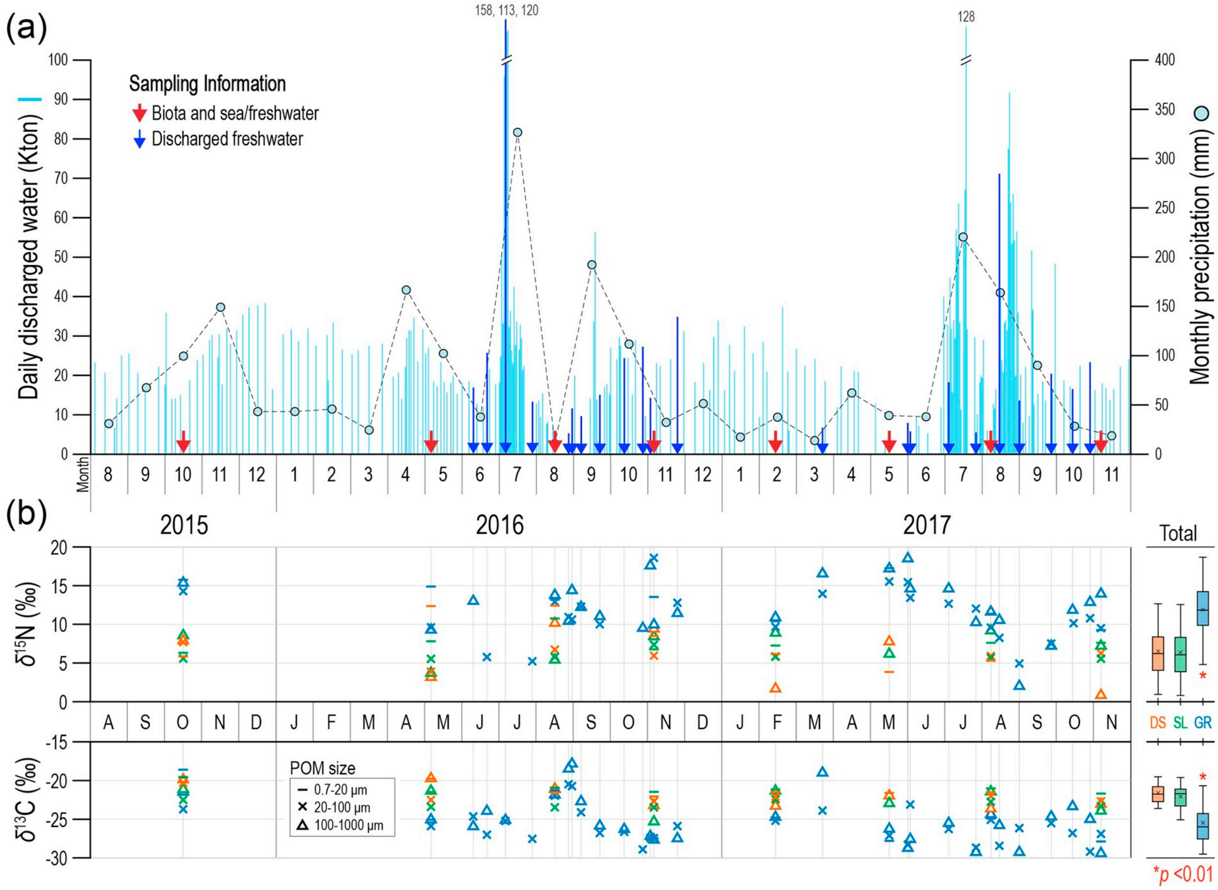

Fig. 2. (a) Daily discharge in water mass (kiloton, light blue bar), monthly precipitation rate (mm, light blue circle), and sampling information including seasonal biota and sea/freshwater samples (n = 8, red arrow) and discharge water samples (n = 22, blue arrow) from 2015 to 2017. (b) Stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of particulate organic matter (POM) in water samples. Water samples were collected from three locations including Dasa (DS, open coast), Yubu (YB, tidal flat on island), and Songlim (SL, river mouth). POM was separated into three size-classes: 0.7–20 µm (dash), 20–100 µm (cross), and 100–1000 µm (triangle). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Stable carbon and nitrogen isotopic compositions (δ^{13}C and δ^{15}N) of microphytobenthos (MPB), sediment organic matter (SOM), particulate organic matter (POM), and macrobenthos collected from four locations (Dasa-ri, DS; Songlim-ri, SL; Geum-River, GR; Yubu Is., YB) along the Geum River estuary in 2015 to 2017, with corresponding trophic level (TL). Isotopic values reported as means with standard deviations (s.d.), and *n* corresponds to the total number of samples.

Species	Location	*n*	δ^{15}N		TL	δ^{13}C	
			Mean	s.d.		Mean	s.d.
MPB	DS & SL	9	4.7	0.5	–	−17.6	0.7
SOM	DS & SL	8	5.9	1.1	–	−20.8	0.2
POM in seawater							
(0.7–20 μm)	DS	7	7.4	3.4	–	−21.5	0.4
(20–100 μm)		7	6.3	1.2	–	−22.0	0.9
(100–200 μm)		8	5.5	3.9	–	−22.0	1.6
Subtotal (DS)		22	6.5	3.2	–	−21.7	1.2
(0.7–20 μm)	SL	7	7.3	1.8	–	−21.1	0.7
(20–100 μm)		7	5.9	0.7	–	−22.9	0.5
(100–200 μm)		8	7.4	2.0	–	−22.1	1.6
Subtotal (SL)		22	6.9	1.7	–	−22.1	1.3
Total (DS & SL)		44	6.7	2.5	–	−21.9	1.2
POM in freshwater							
(0.7–20 μm)	GR	8	13.2	2.8	–	−24.7	3.3
(20–100 μm)		30	12.5	3.5	–	−25.4	1.9
(100–200 μm)		28	13.0	2.8	–	−24.9	2.7
Total (GR)		66	11.9	3.4	–	−25.4	2.8
Macrofauna							
Bivalvia							
Mactra veneriformis	DS	229	11.3	0.7	1.8	−17.9	0.8
	YB	193	11.7	0.5	2.0	−17.3	0.6
Cyclina sinensis	SL	37	12.0	0.6	2.1	−18.5	0.7
Dosinorbis japonicus	DS	5	13.0	0.1	2.4	−17.8	0.0
Anadara broughtonii	DS	1	12.0	0.0	2.0	−17.7	0.1
Solen strictus	DS	19	11.8	0.3	2.0	−18.7	0.8
Crassostrea gigas	SL	12	10.2	0.1	1.5	−19.2	0.1
Gastropoda							
Nassarius livescens	DS	147	13.7	0.3	2.6	−15.5	0.4
	SL	226	13.6	0.2	2.5	−15.5	0.1
Neverita didyma	DS	20	13.7	0.5	2.6	−16.1	0.4
Rapana venosa	DS	13	13.3	0.2	2.4	−17.0	1.0
Crustacea							
Palaemon paucidens[*]	GR	8	20.1	0.3	–	−22.8	0.1
Eriocheir sinensis[*]	GR	1	18.0	0.4	–	−22.5	0.0
Squilla oratoria	DS	3	14.6	0.1	2.8	−16.7	0.3
Hemigrapsus penicillatus	SL	26	13.4	0.1	2.5	−16.8	0.4
Callianassa japonica	DS	17	13.2	0.2	2.4	−16.8	0.1
	SL	23	13.2	0.2	2.4	−16.6	0.0
Upogebia major	DS	70	12.1	0.5	2.1	−18.2	0.2
Macrophthalmus japonicus	DS	18	11.9	0.2	2.0	−13.4	0.1
	SL	8	10.1	0.3	1.5	−15.4	0.5
Holothuroidea							
Apostichopus japonicus	DS	1	11.7	0.0	2.0	−16.7	0.1
Fish							
Synechogobius hasta	SL	2	15.9	1.0	3.2	−16.0	1.1
Tridentiger trigonocephalus	DS	3	15.1	1.0	3.0	−16.8	0.2
	SL	6	15.4	0.0	3.1	−19.4	0.2

[*] Trophic level was not calculated for freshwater crustaceans.

following the method of Couch (1989), as described by Riera and Richard (1996). We also collected SOM samples scraped from the top of the sediment surface. POM was size-fractionated in situ by use of nets (mesh sizes of 100–1000 μm and 20–100 μm). For collecting the 0.7–20 μm particle size, 20 L water was filetered through 20–100 μm net by eliminatiing the > 20 μm particle size. And then, the 20 L water sample was transported to the laboratory and concentrated by vacuum filtration onto a glass-fiber filter to obtain POM of 0.7–20 μm particle size. Finally, we separated POM into three of size classes: 0.7–20, 20–100, and 100–1000 μm. The size-fractionation reflects the plankton size classification described by Makoto & Tsutomu (1984): nanoplankton (2–20 μm), microplankton (20–100 μm), and macroplankton (100–1000 μm), respectively. MPB, SOM, and POM samples were stored at −20 °C until pretreatment.

Macrobenthos were collected by trap and capturing by hand. In total, > 1100 individuals of 19 species belonging to five taxa (Bivalvia, Gastropoda, Crustacea, Holothuroidea, and fishes) were collected in the intertidal area of the Geum River estuary during the study period (Table 1). Various size of dominant bivalves, *M. veneriformis* and *C. sinensis* (2.1–4.4 and 2.6–4.5 cm in shell length, respectively), were collected. In addition, two freshwater crustaceans, namely *Palaemon paucidens* and *Eriocheir sinensis* were sampled and analyzed which aids to link terrestrial isotopic signatures of dietary composition to freshwater organisms. All of the captured organisms were stored for 12–24 h in filtered sea/freshwater for evacuation. The hard shell of bivalves and gastropods was removed. The soft tissue was dissected into two sections: gut and remaining parts. For, *M. veneriformis* and *C. sinensis*, the shell length was recorded and individuals were grouped into size classes with a standard error of < 0.5 cm in shell length. The soft tissues were dissected into the three sections: adductor, gut, and remaining parts. For crustaceans and holothuroidea, the carapace and gut were removed, and only the remaining parts (in soft tissue) were used for the analysis. For fish, only the fillet was used. The same organs from the same species were pooled and rinsed with distilled water, homogenized, and stored at −20 °C until pretreatment. All of the frozen samples (e.g., macrobenthos, MPB, and POM) were lyophilized before stable isotope analysis. We referred to data of > 200 μm pelagic POM for the food web reported in same area of a previous study (Choi et al., 2017).

2.3. Stable isotope analysis

To determine the stable isotopic compositions of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in dried-samples, soft tissues of macrobenthos, MPB, SOM, and POM were used following methods slightly modified from that described previously (Schubert and Nielsen, 2000; Ferrari et al., 2003; Khodse et al., 2007; Svensson et al., 2014). The lipids were removed from a subsample of macrobenthos. In brief, the process involved adding approximately 10 mL dichloromethane (DCM)/methanol (2:1, v/v) to 10–20 mg freeze-dried soft tissues of macrobenthos. The mixture was then sonicated for 10 min and centrifuged at 4000*g* for 15 min. Then, organic solvents were pipetted off and discarded. This extraction procedure was repeated three to six times, depending on the lipid content of the sample. Lipid-free samples were evaporated under a gentle stream of N_2 gas at room temperature until fully dry. Before quantifying $\delta^{13}C$ in MPB, SOM, and POM (0.7–20 μm), the samples were de-carbonated overnight by fuming them with HCI in a desiccator. A portion of the POM (20–100 and 100–1000 μm) was acidified overnight with 1 N hydrochloric acid (HCl, Sigma Aldrich, St. Louis, MO) to eliminate inorganic carbon. It was then rinsed with distilled water. The acidified samples were repeatedly freeze-dried and subsequent the redried samples were thoroughly mixed, and these samples were then weighed in a tin capsule for isotopic analysis.

The values of $\delta^{13}C$ and $\delta^{15}N$ in MPB, SOM, and POM samples were pre-tested for each sample. Accordingly, the injection amounts of samples were then adjusted to determine the weight; approximately 3–10 mg for MPB and POM and 10–70 mg for SOM. Three replicates were analyzed for $\delta^{13}C$ and $\delta^{15}N$, and the mean of triplicates was used for the data analysis. Very small samples (particularly, POMs) were analyzed without replicates. $\delta^{15}N$ of some POM samples could not be measured due to the low concentration of N. $\delta^{13}C$ and $\delta^{15}N$ were measured with an Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) (Elementar, Gmbh, Hanau, Germany). High purity carbon dioxide and nitrogen gases were used as reference gases, while helium and oxygen gases were used as carrier and combustion gases, respectively. Stable carbon and nitrogen isotopic compositions were expressed as ‰ delta notation (Eq. 1):

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [R_{sam}/R_{ref} - 1] \times 1000 \tag{1}$$

where, R_{sam} and R_{ref} are the compositions ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample and reference, respectively. Isotopic compositions were reported relative to conventional reference materials, Vienna Peedee Belemnite (VPDB) for carbon, and atmospheric N_2 for nitrogen. International isotope standards, IAEA-N-2 and IAEA-CH-3, were used as reference materials to calculate analytical error of carbon and nitrogen, respectively. Measurement precision was approximately 0.04‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

2.4. Estimation of trophic position of macrobenthos

To estimate the trophic level, we used the trophic enrichment factor (TEF) of $\delta^{15}N$ 3.4‰ estimated in a previous study (Post, 2002). We used the mean $\delta^{15}N$ values of all collected bivalves to represent the second level of the food web, because filter feeders assimilate varying primary producers and organic particles, leading to the assumption that they occupy trophic level 2 (Ricciardelli et al., 2017). The trophic level of each consumer was determined using the equation proposed by Vander Zanden and Rasmussen (1999) (Eq. 2):

$$TL_i = (\delta^{15}N_i - \delta^{15}N_{base})/TEF + TL_{base} \tag{2}$$

where, TL_i is the trophic level of each species evaluated, $\delta^{15}N_i$ is the stable nitrogen isotopic composition of the species *i*, and $\delta^{15}N_{base}$ and TL_{base} are the mean stable nitrogen isotopic composition and the trophic level, respectively, of all bivalves.

2.5. Statistical analysis

SPSS 23.0 (SPSS INC., Chicago, IL) was used to perform the statistical analyses. The stable carbon and nitrogen compositions of target bivalves (*M. veneriformis* and *C. sinensis*) were analyzed to test for differences between the two species and for different locations (DS, YB, and SL) using a *t*-test and a one-way analysis of variance (ANOVA) with Bonferroni post-hoc test, respectively. Before analysis, the analysis of variance was determined to be homogeneous between groups by the Levene's homogeneity test, meeting the assumption of variance homogeneity ($p > 0.05$). The same statistical method was used to test differences in $\delta^{13}C$ and $\delta^{15}N$ of the organs (gut, adductor, and remaining parts) of the two bivalves.

Bayesian stable isotope mixing models in R (SIMMR) package (Parnell and Inger, 2016) were used to estimate the proportional contribution of potential food sources (POM and MPB) to the target bivalves as the primary consumers using the data analyzed in February, May, August, and October of 2017. The POM_{fw} and discharged freshwater were excluded for the SIMMR, because it showed that the high enrichment of POM $\delta^{15}N$ (mean 15.9‰) did not fit in model as a diet for primary consumers (e.g., mean 11.7‰ for $\delta^{15}N$ in bivalves) in the seawater food web of the Geum River estuary. The isotope values of the adductor and remaining parts (but not the gut) were used in the mixing model. For the contribution of POM and MPB, each value was pooled and evaluated as a total mean calculated without classifying the size of individuals below 100 μm. The $\delta^{13}C$ and $\delta^{15}N$ values in SIMMR were adjusted for trophic level using previous TEF estimates; 0.4 ± 1.3‰ for $\delta^{13}C$ and 3.4 ± 1.0‰ for $\delta^{15}N$ (Post, 2002).

The nonlinear regression function of SigmaPlot (v10.0, Systat Software, Inc.) was used to fit the curve for isotope signatures of *M. veneriformis* for part-specific variation and size (shell length), but not used for *C. sinensis* because sample size was limited ($n < 20$). The Kolmogorov-Smirnov test was performed, which resulted in data meeting the assumption of normality ($p > 0.05$) (Rosenthal, 1968). The age of *M. veneriformis* was estimated base on the growth curve for shell length by von Bertalanffy's equation following Kim and Ryou (1991). We then separated individuals into, six age-classes: below 1 yr (< 21.7 mm), 1–1.5 yr (21.7–29.0 mm), 1.5–2 yr (29.0–34.6 mm), 2–2.5 yr (34.6–38.8 mm), 2.5–3 yr (38.8–42.0 mm), and above 3 yr (> 42.0 mm) of age. The ages of *C. sinensis* were approximately assumed from a previous study (Lin et al., 2017).

3. Results

3.1. Variations of $\delta^{13}C$ and $\delta^{15}N$ of POM

$\delta^{13}C$ and $\delta^{15}N$ of the POM in seawater and freshwater (including discharged freshwater) varied both spatially and temporally (Fig. 2b and Table 1). Over most of the time series in GR, $\delta^{13}C$ and $\delta^{15}N$ of POM were typically between −30 to −25‰, and 10 to 20‰, respectively. The total mean $\delta^{15}N$ of POM in GR was 11.9‰, and it greatly fluctuated across seasons, ranging from 1.9 to 18.5‰. The smallest values for $\delta^{15}N$ of POM were recorded in June and July of 2016, and August and September of 2017 (mean 5.6, 5.2, 3.4, and 7.3‰, respectively), before massive and continuous freshwater discharges. In comparison, $\delta^{13}C$ of POM in GR was relatively consistent (mean: −25.4‰; range: −29.5 to −18.0‰). The highest values for $\delta^{13}C$ POM were observed in August, 2016 and March, 2017 (−19.5‰ and −19.1‰, respectively). While, both of $\delta^{13}C$ and $\delta^{15}N$ of POM_{sw} (DS and SL) were relatively stable

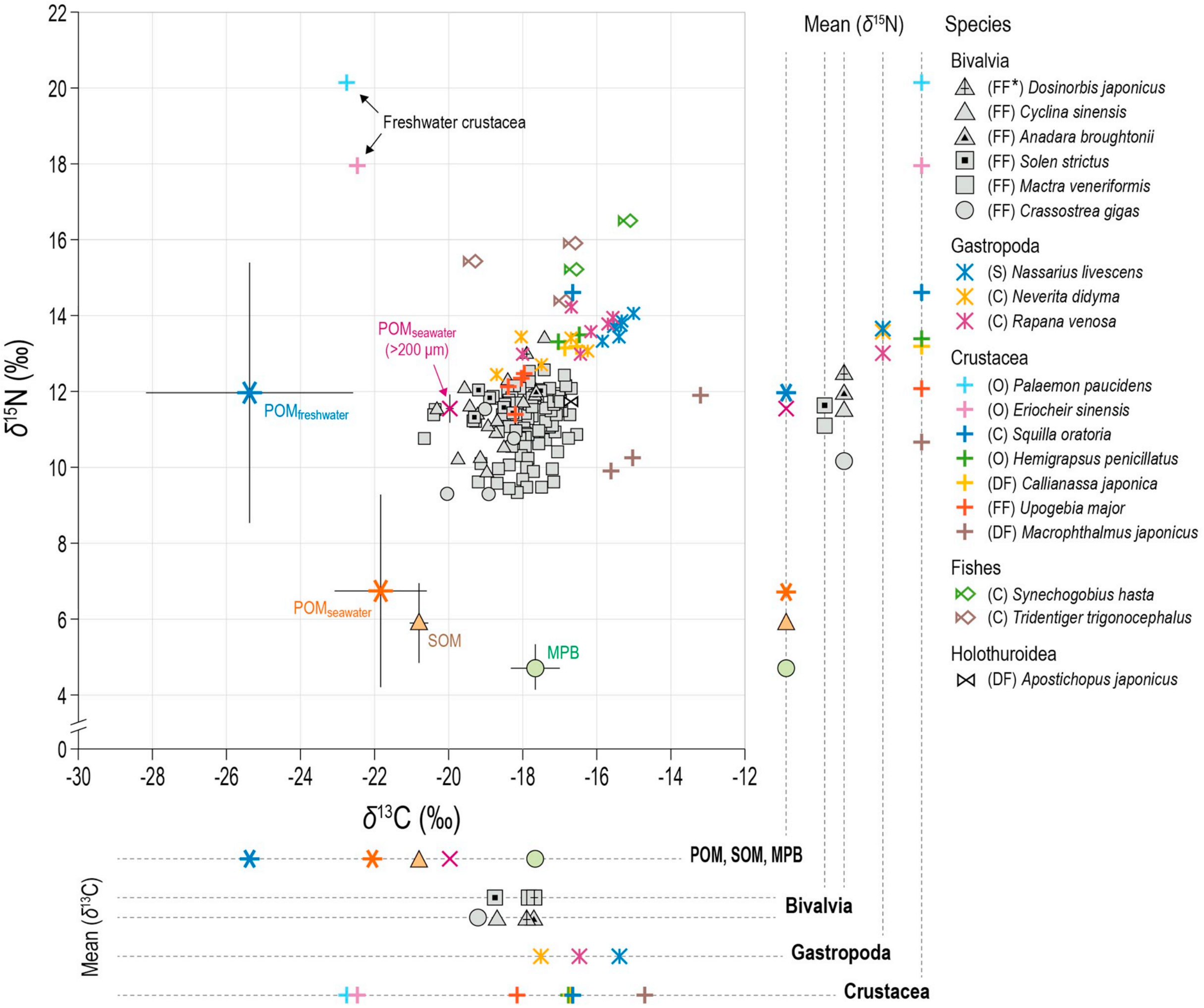

Fig. 3. Biplot of stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of macrobenthos and potential food sources in the Geum River estuary. The values of potential food sources [such as particulate organic matter (POM), sediment organic matter (SOM), and microphytobenthos (MPB)] represent the total means with standard deviations (black line). The values of macrobenthos represent the means of individuals collected at each sampling time. The value of over 200 μm POM in seawater was obtained from Choi et al. (2017) (*Acronyms in parenthesis: (FF) filter feeder, (S) scavenger, (C) carnivore, (O) omnivore, and (DF) deposit feeder).

between seasons (range: −25 to −20‰ for $\delta^{13}C$ and 5 to 10‰ for $\delta^{15}N$). The total mean for $\delta^{13}C$ and $\delta^{15}N$ of POM was −21.7‰ and 6.5‰ in DS, and −22.1‰ and 6.9‰ in SL, respectively. There was no significant difference in the stable isotopic compositions of POM between the two seawater locations (DS and SL); however, there was a significant difference ($p < 0.01$) between the freshwater location (GR and discharged freshwater samples) and the seawater locations (DS and SL) (Fig. 2b).

There was no isotopic difference in certain POM size-groups (0.7–20, 20–100, and 100–1000 μm) occupying the same site ($p > 0.05$, Table 1). However, for the > 200 μm POM size-group, the plausible size for zooplankton, POM values were greater for $\delta^{13}C$ (−19.9 ± 0.1‰) and $\delta^{15}N$ (11.6 ± 0.4‰). The values for $\delta^{13}C$ and $\delta^{15}N$ in > 200 μm POM seemed to be the result of feeding on pelagic POM and benthic (MPB) sources of zooplankton. Of note, depleted $\delta^{13}C$ (−22.6 ± 0.2‰) and enriched $\delta^{15}N$ (19.0 ± 1.2‰) isotopic signatures observed in two freshwater crustaceans indicated the separated trophic grouping from distinct intertidal food web. Altogether, we could successfully distinguish three groups of POM (POM_{fw}, POM_{sw}, and > 200 μm sized POM_{fw}), MPB, and macrobenthos in the biplot of the estuarine food web (Fig. 3).

3.2. Benthic food web

There were distinct differences in both $\delta^{13}C$ and $\delta^{15}N$ among potential food sources, including MPB, SOM, and POM for primary consumers (Table 1 and Fig. 3). As primary producers, MPB had the smallest mean $\delta^{15}N$ (4.7‰), but had relatively enriched mean $\delta^{13}C$ (−17.6‰) compared to those of SOM (−20.9‰), POM_{sw} (−21.9‰), and POM_{fw} (−25.4‰), respectively. Even though irregular freshwater discharge could supply the seawater area with POM, the very enriched-$\delta^{15}N$ and depleted-$\delta^{13}C$ values of freshwater POM were not considered to enter the trophic pathway of the seawater food web in the Geum River estuary. Therefore, three potential food sources for primary consumers were distinguished; POM_{sw} as pelagic sources and MPB and SOM from benthic sources.

Macrobenthos were assigned to its corresponding trophic group, such as filter feeder, deposit feeder, scavenger, carnivore, and omnivore (Fig. 3). Based on $\delta^{15}N$ values, the trophic levels of most invertebrate species were estimated to be between two to three. Crustaceans and gastropods occupied slightly higher trophic levels (+0.5 upper) compared to bivalves (TL = 2.0). However, *Upogebia major* and *Macrophthalmus japonicas* occupied similar positions. Carnivorous fish species (*Synechogobius hasta* and *Tridentiger trigonocephalus*) occupied the highest tropic level (3.0–3.2) in this food web. Macrobenthos exhibited

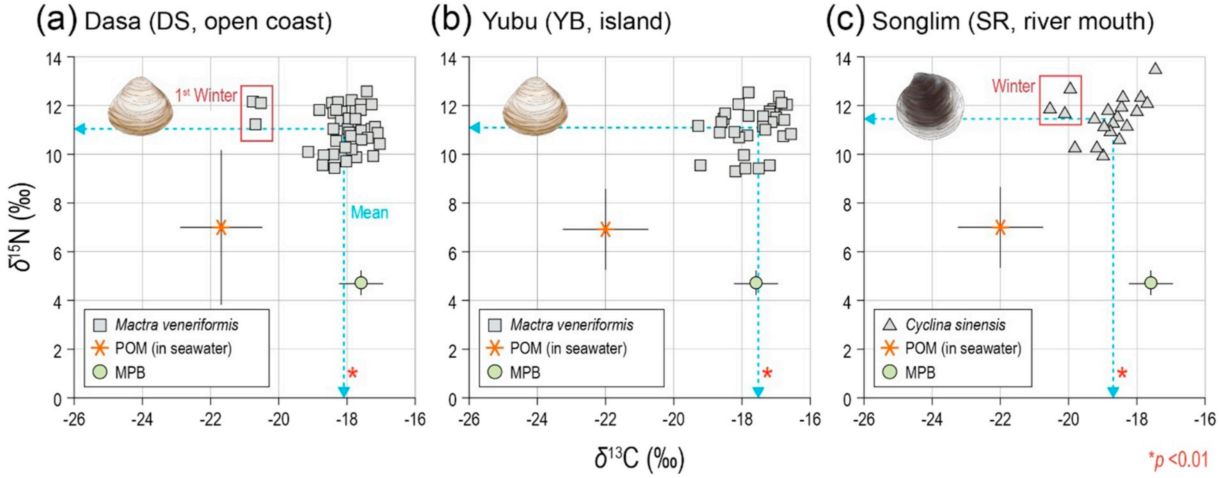

Fig. 4. Stable carbon and nitrogen isotopic compositions (δ^{13}C and δ^{15}N) of *Mactra veneriformis* and *Cyclina sinensis* at three locations: *M. veneriformis* collected from (a) DS, open coast and (b) YB, tidal flat on island; and *C. sinensis* collected from (c) SL, river mouth. The values of potential food sources [such as particulate organic matter (POM) and microphytobenthos (MPB)] represent total means with standard deviations (black line). The values of bivalves represent the means of individuals sampled at different times.

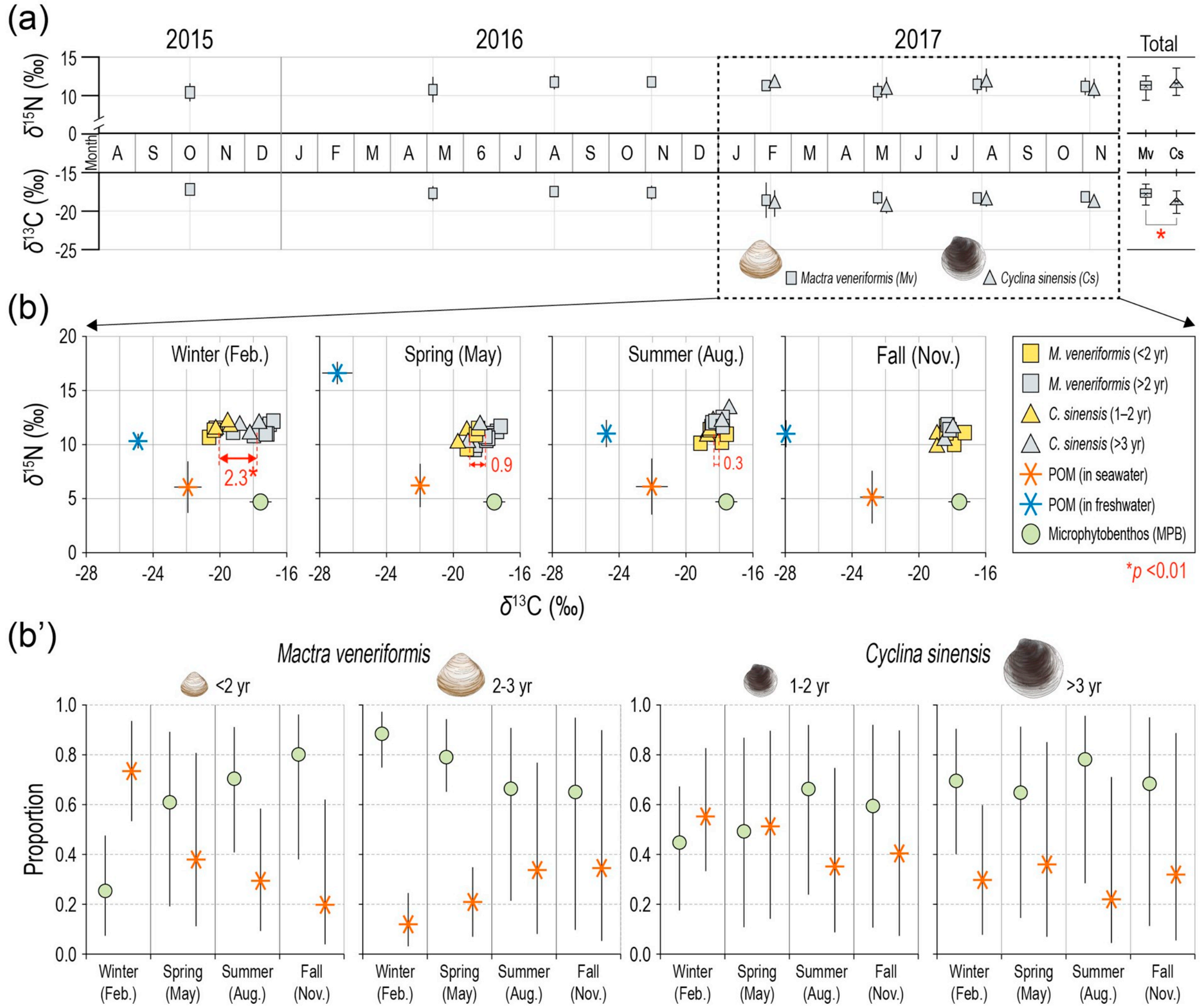

Fig. 5. (a) Seasonal values and total means of stable carbon and nitrogen isotopic compositions (δ^{13}C and δ^{15}N) in target bivalves *Mactra veneriformis* and *Cyclina sinensis* from 2015 to 2017, and (b) seasonal variation in stable isotopes between different size groups among the target bivalves, with potential food sources [such as particulate organic matter (POM) in seawater and freshwater, and microphytobenthos (MPB)] being collected in 2017. (b′) Seasonal proportions of each food source to *M. veneriformis* and *C. sinensis* collected in 2017. The two bivalves were separated into two size groups based on shell length. Each symbol for diet types shows the most likely value with 95% credibility intervals in Bayesian mixing model.

Table 2
Shell length and age of *Mactra veneriformis* and *Cyclina sinensis* collected from three locations (Dasa-ri, DS; Songlim-ri, SL; Yubu Is., YB) along Geum River estuary in 2015 to 2017. Shell length reported as mean with standard deviation (s.d.), and *n* corresponds to the number of individuals.

Species	Sampling date	Location	*n*	Shell length (cm)		Age (yr)
				Mean	s.d.	
Mactra veneriformis	Octs., 2015	DS	44	3.0	0.2	1.5–2.0
			15	4.3	0.2	3.0–3.5
		YB	10	3.3	0.3	1.5–2.0
			5	4.2	0.2	2.5–3.0
	May, 2016	DS	40	3.1	0.2	1.5–2.0
			20	4.4	0.2	3.0–3.5
		YB	18	3.1	0.2	1.5–2.0
			10	4.3	0.2	3.0–3.5
	Aug., 2016	DS	15	3.6	0.1	2.0–2.5
			11	4.3	0.2	3.0–3.5
		YB	22	3.4	0.1	1.5–2.0
			17	4.1	0.2	2.5–3.0
	Nov., 2016	DS	19	3.7	0.1	2.0–2.5
			12	4.4	0.2	3.0–3.5
		YB	35	3.0	0.4	1.5–2.0
			25	4.0	0.2	2.5–3.0
	Feb., 2017	DS	11	2.1	0.3	< 1.0
			9	4.2	0.2	2.5–3.0
		YB	6	4.3	0.1	3.0–3.5
	May, 2017	DS	7	3.5	0.2	2.0–2.5
			5	4.2	0.3	2.5–3.0
		YB	5	2.6	0.2	1.0–1.5
			7	3.9	0.1	2.5–3.0
	Aug., 2017	DS	6	2.7	0.2	1.0–1.5
			4	3.9	0.1	2.5–3.0
		YB	6	2.8	0.1	1.0–1.5
			3	3.9	0.2	2.5–3.0
	Nov., 2017	DS	6	3.8	0.2	2.0–2.5
			5	4.1	0.2	2.5–3.0
		YB	24	3.4	0.3	1.5–2.0
Cyclina sinensis	Feb., 2017	SL	7	2.6	0.3	1.0–2.0
			5	4.4	0.2	> 3.0
	May, 2017		9	2.8	0.2	1.0–2.0
			4	4.5	0.3	> 3.0
	Aug., 2017		4	2.9	0.1	1.0–2.0
			2	4.3	0.2	> 3.0
	Nov., 2017		3	2.7	0.5	1.0–2.0
			3	4.2	0.3	> 3.0

taxon-specific variation in δ^{13}C values (Fig. 3). The mean δ^{13}C values of bivalve species ranged from −19.2 to −17.7‰, and had more depleted-δ^{13}C compared to taxa in the upper trophic levels (Table 1). Gastropoda were represented by two carnivorous sea-sails (*Neverita didyma* and *Rapana venosa*) and one scavenger sea-snail (*Nassarius livescens*). In particular, *N. didyma* and *R. venosa* had enriched-δ^{13}C mean values (range: −17.0 to −16.1‰) with much greater δ^{15}N values (range: 13.3 to 13.7‰, TL: 2.4–2.6) than bivalves which are major prey. Crustacea and fishes had a relatively wide range of mean δ^{13}C values (range: −18.2 to −13.4‰ and −19.4 to −16.0‰, respectively). The total mean values of stable isotope signatures for *M. veneriformis* were −17.8‰ for δ^{13}C and 11.2‰ for δ^{15}N, respectively, while those of *C. sinensis* were −18.7‰ for δ^{13}C and 11.5‰ for δ^{15}N.

3.3. Spatiotemporal isotope variations in bivalves

The mean δ^{15}N values of target bivalves did not significantly differ among the three locations where they were collected (*M. veneriformis* in DS and YB, and *C. sinensis* in SL; $p > 0.05$), whereas differences in the mean δ^{13}C values were spatially significant (Fig. 4). Samples collected in winter (February, 2017) had more depleted-δ^{13}C values compared to other seasons for both *M. veneriformis* and *C. sinensis*. The stable isotope signatures between the target bivalves showed no significant difference in total mean δ^{15}N, but a significant difference for total mean δ^{13}C ($p < 0.01$) (Fig. 5a). *M. veneriformis* and *C. sinensis* of various sizes, as a proxy of age, were collected from the Geum River estuary, ranging from below one-year to above three-years in age (Table 2). Seasonal changes to stable isotopic compositions were detected for the two target bivalves. Both species were roughly separated into two groups according to their size: (1) small groups; < 2 yr for *M. veneriformis* and 1–2 yr for *C. sinensis* and (2) large groups; 2–3 yr for *M. veneriformis* and 2–3 yr for *C. sinensis*. The mean δ^{13}C value of target bivalves in smaller groups was significantly lower (i.e., ~2.3‰ depleted-δ^{13}C) compared to that of large groups in the winter of 2017 ($p < 0.01$). The difference in δ^{13}C between small and large groups gradually decreased as the season progressed (Fig. 5b).

The mixing model for the target bivalves, *M. veneriformis and C. sinensis*, indicated seasonal, species-, and size-specific variations in diet contributions (Fig. 5b′). Overall, the mean contributions of MPB to target bivalves exceeded that of POM$_{sw}$, except for the smallest size groups collected in February, 2017 (see Table 2). There was a greater contribution of POM$_{sw}$ in these groups (mean 74% and 56% in *M. veneriformis and C. sinensis*, respectively). MPB represented a relatively minor component of the diet in winter for small-sized groups of the two target bivalves; however, from spring to fall, MPB formed the main dietary component (mean 49–80%). In comparison, the mean dietary contributions of seawater POM tended to decline from winter to fall in the small-sized groups. MPB represented the main dietary component of large-sized bivalves for both species (mean: 65–88% and 64–78% for *M. veneriformis* and *C. sinensis*, respectively). In comparison, seawater POM was a relatively minor dietary component in large-sized bivalves, with its mean contribution being about 15% greater in small-sized groups.

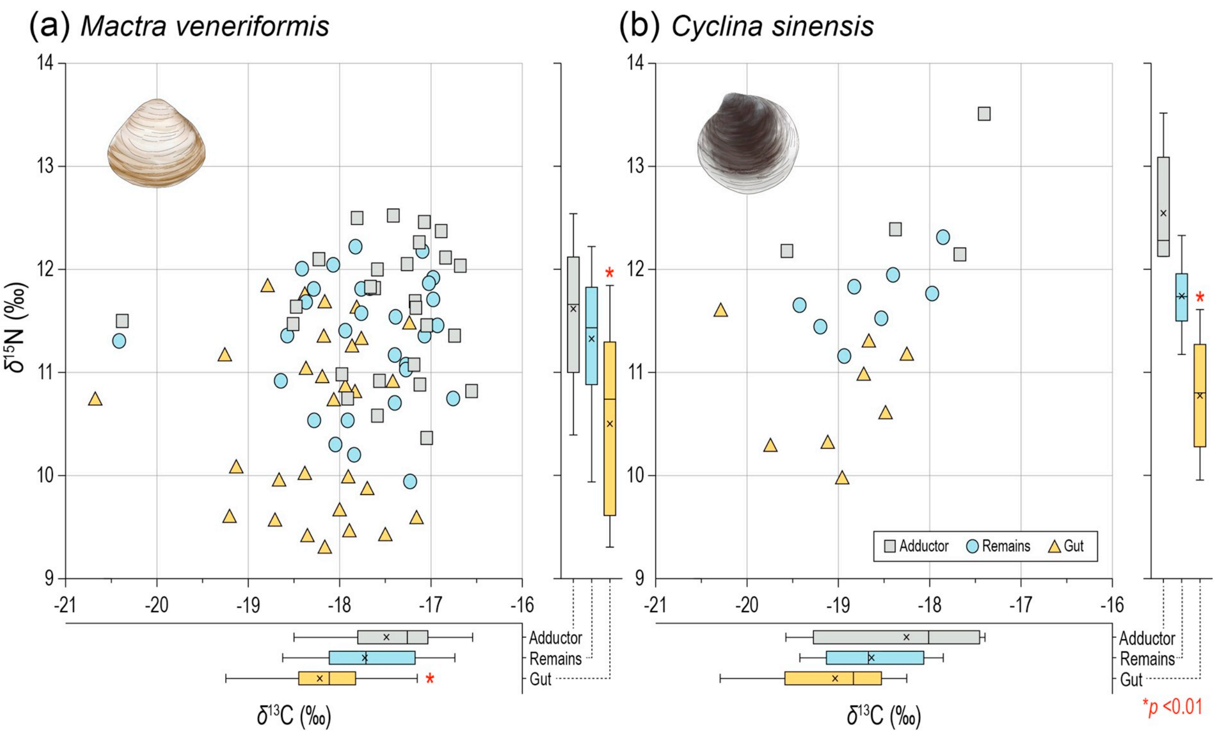

Fig. 6. Organ-specific values of stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) in *Mactra veneriformis* and *Cyclina sinensis*. Soft tissues of the bivalves were separated into three organs: adductor (grey square), gut (yellow triangle), and remaining parts (light blue circle). Each value represents the means of individuals sampled at different times. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The mixing model indicated that the diet was the most complex in fall (November, 2017), based on a wide range of credibility intervals between dietary contributions (i.e., 4–7% at the lowest limits and 62–96% at highest limits) (Fig. 5b′).

3.4. Organ- and size-specific isotope variations in bivalves

There was a significant difference in organ-specific isotopic enrichment in the target bivalves (Fig. 6). Gut tissue had distinctively depleted-$\delta^{15}N$ values compared to those in the adductor and remaining

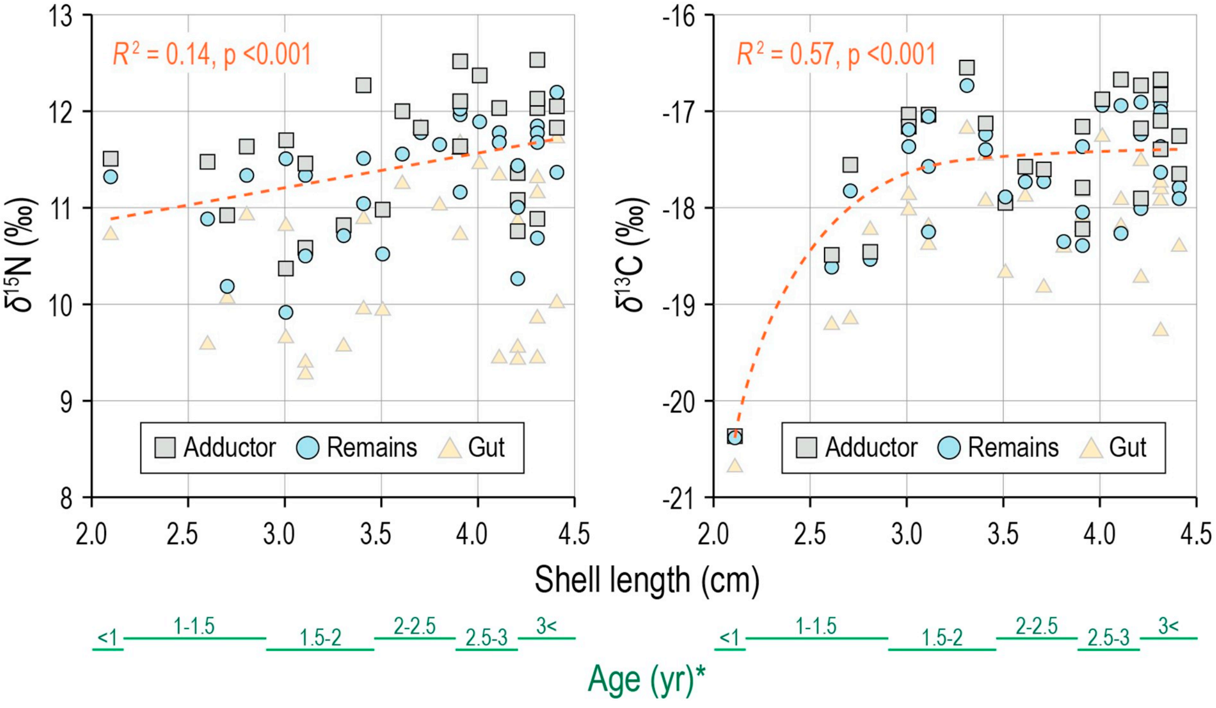

Fig. 7. Stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of *Mactra veneriformis* in relation to shell length as a function of adductor and remaining parts (i.e., organ-specific relationship). Value of gut tissues was excluded for fitting the curve. Each value represents the mean of individuals sampled at different times (*The age of *M. veneriformis* was estimated based on the growth curve for shell length by von Bertalanffy's equation following Kim and Ryou (1991)).

parts for both bivalve species; however, δ^{13}C was only significantly different in the gut tissue of *M. veneriformis* ($p < 0.01$). The mean δ^{15}N value of the gut was smaller about 1.0‰ in *M. veneriformis* and 1.4‰ in *C. sinensis*, compared to those in the other tissues (adductor and remaining parts). The mean δ^{13}C value of *M. veneriformis* was most depleted in gut (−18.2‰), and was significantly different to those in the adductor (−17.5‰) and remaining parts (−17.3‰) (Fig. 6a). The mean δ^{13}C value in the gut (−19.0‰) of *C. sinensis* was also smaller than those in the adductor (−18.6‰) and remaining parts (−18.3‰), but these differences were not significant ($p > 0.05$) (Fig. 6b).

The stable isotope values changed with size class (shell length) for *M. veneriformis* (Fig. 7). δ^{15}N values in total soft tissues showed no relationship with shell length; however, δ^{15}N of adductor and remaining tissues (excluding the gut) increased linearly with shell length ($\delta^{15}N = 10.1 + 0.36 \times$ shell length, $R^2 = 0.14$, $p < 0.001$, Fig. 7). On average, the δ^{15}N value of the largest size class (> 3 yr, $n = 74$) was slightly higher (+0.44‰) than that of the smallest class (< 1 yr, $n = 11$). δ^{13}C values of *M. veneriformis* (excluding gut) increased as a function of shell length, rising exponentially to a maximum, with this relationship being highly correlated ($\delta^{13}C = -1001 + 983.6 \times (1–0.064^{\text{shell length}})$, $R^2 = 0.14$, $p < 0.001$, Fig. 7). The growth of *M. veneriformis* noticeably changed in relation to δ^{13}C values during 1–1.5 yr cycle (age) in soft tissues. The mean δ^{13}C value of the smallest class (< 1 yr, $n = 11$) was −20.4‰, and noticeably increased until a peak value of −17.2‰ reached (1.5–2 yr, $n = 193$) when the shell length exceeded 3 cm.

4. Discussion

Many studies have reported ecological responses of marine organisms against habitat alteration, such as reclamation and/or artificial constructions. While those studies vary in locality, say from local to regional scales, survey or sampling strategies, and target taxa and/or response endpoints (mostly focused on community structure), thus direct comparison between studies would be limited (Tables S1). Of note, relatively few studies have examined spatiotemporal changes of trophic connectivity in a closed estuarine system. The present study revealed that freshwater POM in the closed Geum River estuary, where freshwater input is artificially controlled by a sea dike, is barely linked to the trophic status of benthic consumers on the outer tidal flats.

Large seasonal variation in the isotopic signature of freshwater POM was detected in the Geum River estuary. Thus, different process likely influenced δ^{13}C and δ^{15}N values of POM in the freshwater area compared to the seawater area (Fig. 2b). The dike has geographically isolated the Geum River from the seawater ecosystem, resulting in the freshwater area being more strongly influenced by terrestrial nutrient input from upstream. High δ^{15}N enrichment of freshwater POM is mainly influenced by the denitrification process of isotopic fractionation during the microbial transformation and phytoplanktonic assimilation of nitrogen under eutrophic conditions (Owens, 1988; Hadas et al., 2009; Gu, 2009). δ^{15}N was depleted in freshwater POM during June and July of 2016 and August of 2017, possibly a result of N_2-fixing cyanobacteria blooms under hypereutrophic conditions (Gu et al., 1996). Indeed, during the summer (July to September) of 2016 and 2017, relatively great concentration of Chl-*a* (57.7 ± 26.1 mg/L) and cyanobacteria blooms (up to 50 K cells/mL) were observed in the Geum River upstream (WEIS, 2019). Increased POM δ^{13}C in August, 2016 might reflect lower isotopic fractionation during carbon fixation when dissolved CO_2 decreased, because of increased temperature and highly photosynthetic conditions (Berman-Frank et al., 1998). The isotopic signatures of marine macrobenthos (especially bivalves as filter feeders) in the Geum River estuary provided no evidence for freshwater POM being a major food source for upper trophic levels, even when intermittent discharges occurred. Ultimately, the discrepancy in the isotopic signature of POM between the areas inside and outside the sea dike indicated that the “closed freshwater area” in the Geum River estuary is now more close to an isolated lake ecosystem, rather than free-flowing river.

The feeding pathway of filter feeders derives from various potential food sources, including SOM, phytogenic detritus, riverine and pelagic suspended matter, and resuspended particulate matter etc. Consequently, this variety could lead to highly complicated dietary contributions as demonstrated by the stable isotopic compositions. However, the flow of freshwater in the Geum River estuary is almost negligible, with extensive tidal flats being developed without salt-marshes, mangroves, or other vegetation and macroalgal communities. Therefore, the main food sources of filter feeders seemed to be limited with respect to pelagic and benthic sources, resulting two distinct groups existing, i.e., seawater POM and MPB (Table 1). The mean δ^{13}C values of seawater POM and MPB were similar to those obtained by previous studies, ranging from −24 to −18‰ in seawater POM and −20 to −13‰ in MPB (see Fig. S1 in Supplementary material). Although seawater POM and MPB had overlapping δ^{15}N values, δ^{13}C values were distinct; thus, the isotope biplot of δ^{13}C and δ^{15}N distinguished these primary producers (Fig. 3).

TEF is generally required to determine the food sources of consumers; however, ideally species-specific values must be applied, even though few studies are available (Post, 2002; Dubois et al., 2007). In the same manner, our results showed that the δ^{15}N of bivalves, as primary consumers, was ~5‰ greater compared to those of primary producers (seawater POM and MPB). This value exceeded the documented magnitude (3.4 ± 1.0‰, Post, 2002) of isotopic fractionation between consumers and their prey. The isotope signatures of POM of > 200 μm in size might be the result of zooplankton preying on seawater POM and/or MPB. The scavenger sea-snail, *N. livescens*, had a relatively enriched isotopic signature, possibly because it consumes the carcasses of benthic animals. Consequently, this species obtained a highly enriched source of carbon and nitrogen (Heinrich, 1988). The carnivorous sea-snails, *N. didyma* and *R. venosa*, had TLs of 2.6 and 2.4, respectively. These values indicated that they generally preyed on *M. veneriformis* (TL = 1.8–2.0); thus a TEF of < 3‰ was assumed. Crustacea had a wide range of isotope signatures due to a great diversity of feeding types. The carnivorous mantis-shrimp, *Squilla oratoria*, occupied the highest position (14.6‰ in mean δ^{15}N, TL = 2.8) for crustaceans. In comparison, *U. major* was a filter feeder that mainly fed on seawater POM and MPB, and had a similar isotopic signature to the bivalves.

Hemigrapsus penicillatus (an omnivore that incorporates ^{13}C-enriched materials) and *Callianassa japonica* (deposit feeder) had similar isotope signatures, suggesting that both were likely to utilize similar food sources although they employ different feeding strategies (Kang et al., 2015). *M. japonicus* is a consumer that incorporates ^{13}C-enriched materials and exhibited the widest range of isotope signatures because it used a variety of food sources from the sediment surface (Yokoyama et al., 2005a). The carnivorous Osteichthyes, *S. hasta* and *T. trigonocephalus*, had the greatest δ^{15}N values among the marine species, representing the top predators in the outside of the dike. While the most enriched δ^{15}N values were observed for two freshwater crustaceans, *P. paucidens* and *E. sinensis*, in the inside of the dike. Overall, this trophic pathway from primary producers to primary and secondary consumers was well-characterized as a trophic cascade in the benthic food web structure in the Geum River estuary (Fig. 3).

Several studies on food webs in tidal flat ecosystems have demonstrated spatiotemporal variations in the δ^{13}C and δ^{15}N values of macrobenthos because the pelagic and benthic contributions of potential food sources vary among species and/or habitat (Galván et al., 2008). Therefore, we initially hypothesized that if the oceanographic setting of the sampling locations followed a salinity gradient (i.e., along the estuary, including locations at a far distance (DS) and near to the river mouth (YB and SL)), the contribution of potential food sources of the target bivalves (*M. veneriformis* and *C. sinensis*) close to river mouth would be more influenced by riverine organic matter. However, the riverine input did not supply enough dietary items under the closed conditions. Despite this, we detected significant spatial differences in δ^{13}C values when comparing the three intertidal sites: *M. veneriformis* collected in DS and YB, and *C. sinensis* collected in SL (Fig. 4). Of note, intraspecific variation associated to the feeding sources could be directly influenced by pelagic and/or benthic production, whereas interspecific variation was the result of selective feeding behavior (Kiørboe and Møhlenberg, 1981; Prins et al., 1991; Kang et al., 1999; Cognie et al., 2001; Rossi et al., 2004; Nadon and Himmelman, 2006). Therefore, *M. veneriformis* individuals that were collected near the river mouth (YB) fed on more MPB compared to those collected from more distant coastal location (DS). Furthermore, *C. sinensis* selectively fed on more seawater POM compared to *M. veneriformis* in YB.

The dietary components of *M. veneriformis* and *C. sinensis* collected from the Geum River estuary showed clear seasonal variation over a 1-year period in 2017 (Fig. 5). MPB was the most important primary producer on the extensive bare intertidal flat of the Geum River estuary. In particular, the diurnal production and resuspension of MPB provided a continuous food supply for non-(or lesser) motile invertebrates (De Jonge and Van Beuselom, 1995; Lucas et al., 2000; Page and Lastra, 2003; Koh et al., 2006). Our results showed a seasonal dietary shift in the small-sized groups of both bivalves, associated with selective feeding on seawater POM and/or MPB. Although the smallest group of *M. veneriformis* (< 1 yr individuals collected in 1st winter, Fig. 4a) preferentially fed on seawater POM compared to MPB before reaching the next life stage (juvenile), the dietary contribution of MPB continuously accumulated over time (Kang et al., 2003, 2006). Interestingly, the large-sized group of *M. veneriformis* had the highest contribution of MPB during winter, which apparently corresponded to annual blooms of MPB in the intertidal flats from winter to early spring (Kwon et al., 2018). Over 50% to nearly 100% of carbon sources incorporated in the tissues of *M. veneriformis* and *C. sinensis* were derived from MPB. This active utilization was more prominent in the large-sized groups compared to the small-sized groups. The selective feeding strategies for growth (expressed by shell length) across seasons by the filter feeders studied here support previous studies, which demonstrated their ability to utilize nutritionally rich particles in quality or size during critical periods of growth and gamete production (Le Loc'h et al., 2008; Kang et al., 2009; Pernet et al., 2012; Kang et al., 2015).

Further, isotopic variation was detected among organs and size classes, indicating that isotopic shifts between the tissues of consumers and their diets (i.e., TEF) differ between organ types, with temporal variation (Lorrain et al., 2002). The isotope values of *M. veneriformis* in lipid-free tissues exhibited near parity between the adductor and remaining parts (averagely 0.2‰ and 0.3‰ shifts in δ^{13}C and δ^{15}N, respectively). In comparison, the gut tissues had significantly smaller values of both δ^{13}C and δ^{15}N; thus, an organ-specific allocation strategy for nutrient storage might exist (Paulet et al., 2006). Available nutritional sources exhibited seasonal variation in pelagic and benthic dietary contributions (Fig. 5); thus, variation in δ^{13}C and δ^{15}N values observed in the tissues of each organ (Fig. 6) was probably induced from seasonal growth patterns in somatic and reproductive tissues in the Geum River estuary (Strohmeier et al., 2000; Kang et al., 2019).

The positive correlation between the δ^{15}N values of soft tissues and the shell length (i.e., age) of *M. veneriformis* (Fig. 7) indicated shifts in diet through feeding of enriched-δ^{15}N sources, possibly due to differences in foraging ability such as selective feeding, metabolic process, and discrimination factor. Nevertheless, for better understanding of the enriched-δ^{15}N in bivalves, the δ^{15}N of bulk tissue was corrected using δ^{15}N of the source amino acid based trophic level removing variations of nitrogen trophic baseline, which is able to provide more accurate trophic information (Won et al., 2018). Juvenile bivalves have a small inhalant siphon that restricts their ability to consume fine particles, whereas adult individuals have larger siphons and can feed on a wider range of prey (Aya and Kudo, 2017). Our data supported this assumption, in that large-sized groups had a wider range of dietary contributions (for *M. veneriformis* and *C. sinensis*) compared to small-sized groups of *M. veneriformis* (Fig. 5b).

In contrast to the linear relationship for δ^{15}N enrichment, the relationship between the δ^{13}C values of soft tissues and the shell length of *M. veneriformis* showed an exponential increase that peaked at 1.5 years old (Fig. 7). Ryou and Chung (1995) reported that, after recruitment in June to July, spats (mean size: 250–350 μm) of *M. veneriformis* could reach to ~2.4 cm shell length after 160 d; however, there was no growth during winter (December to March). Therefore, the relatively depleted-δ^{13}C values of the < 1 yr group (mean 2.1 cm shell length) of *M. veneriformis* reflected rapid growth from summer to winter and the dietary contributions of the items that they fed on at that time. This phenomenon was followed by the subsequent enrichment of δ^{13}C values during the growing season. This finding clearly showed an age-related dietary shift from seawater POM to MPB, further supporting previous studies in that the seasonality of MPB determines the growth and reproduction of intertidal invertebrates (Fry and Sherr, 1984; Herman et al., 2000; Page and Lasta 2003; Kang et al., 2006; Grippo et al., 2011; Kang et al., 2015; Christianen et al., 2017).

5. Conclusions

The present study investigated the structure of benthic food web in the Geum River estuary and identified the main food sources for two dominant bivalves of *M. veneriformis* and *C. sinensis*, which are major fishery resources in the local area. In general, our results confirmed that prolonged geographical isolation of the Geum River estuary by a sea dike might have hindered natural distributions of dietary organic matters to intertidal marine organisms inhabiting outer open coastal tidal flats. The results showed that MPB was found to be the most important food source for primary consumers, with the riverine input of organic sources to the seawater ecosystem being interrupted by the dike. Thus, the irregular (or lagged) terrestrial input of riverine POM was found to be lesser associated with trophic status of benthic consumers on tidal flats. Further our results provided isotopic evidence for the organ-specific allocation of nutrients, as well as growth-related dietary shifts by bivalves. Overall, the stable isotopic analysis was powerful to address natural and/or anthropogenic influences of altered environmental condition towards understanding trophic structure and pathways.

Acknowledgments

This work was supported by National Research Foundation of Korea (NRF) grants funded by the South Korean government [grant number NRF-2017R1E1A1A01075067], and also was supported by projects entitled “Development of Integrated Estuarine Management System [grant number 20140431]”, “Integrated Management of Marine Environment and Ecosystems Around Saemangeum [grant number 20140257]”, “Development of Blue Carbon Information System and its Assessment for Management [grant number 20170318]”, and “Marine Ecosystem-Based Analysis and Decision-Making Support System Development for Marine Spatial Planning [grant number 20170325]”, funded by the Ministry of Oceans and Fisheries of Korea (MOF), South Korea granted to J.S.K.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.104981.

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Appendix A Supplementary Data

Anthropogenic influences on benthic food web dynamics by interrupted freshwater discharge in a closed Geum River estuary, Korea

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Seongjin Hong, Jaeseong Kim, Jongseong Ryu, and Jong Seong Khim

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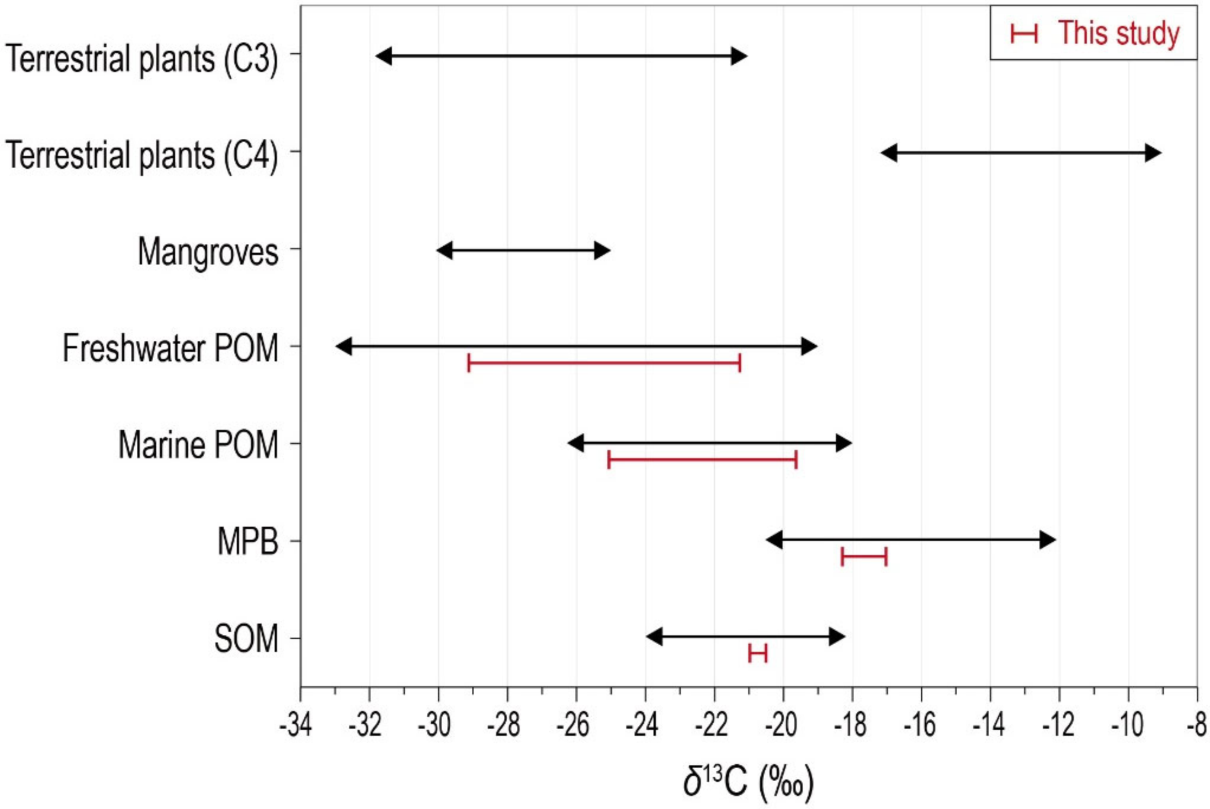

Fig. S1. Range of stable carbon isotopic compositions ($\delta^{13}C$) for organic inputs to coastal environments; terrestrial plants, mangroves, particulate organic matter (POM), microphytobenthos (MPB), and sediment organic matter (SOM) (data compiled from this study; Page 1997; Kang et al. 2003; Lamb et al. 2006; Finlay and Kendall 2007; Kristensen et al. 2017; and references therein).

Table S1. Mini-review for studies on the spatiotemporal changes in ecological responses of marine organisms to habitat alteration by artificial structures or reclamation (Abbreviations are corresponding; Sp: spring, Su: summer, F: fall, W: winter, and Re: region).

Study area		Type	Target		Sampling information								Remark	Reference
Country	Region		Organism	End-point	Period (yr)	Season				Number of				
						Sp	Su	F	W	Re*	Station	Survey		
China	Chongming Island	Reclamation	Nematode	Density, richness, diversity	<1				v	1	12	1	Reclamation altered nematode community structure.	Wu et al., 2005
China	Yancheng	Reclamation	Macrobenthos	Abundance, richness	<1	v				1	4	1	Biodiversity and functional composition of macrofauna was significantly affected by dike age in the reclaimed coast.	Ge et al., 2016
China	Yellow River delta	Tidal barrier	Macrobenthos	Density, biomass	<1	v		v		1	18	2	The macrobenthos communities differed greatly on opposite sides of tidal barriers.	Yang et al., 2017
Bahrain	Sitra island	Reclamation	Macrobenthos	Survival, burrowing	<1	v				1	10	1	Reclamation diminished abundance and distribution of macrobenthos	Naser, 2011
Netherland	Oosterschelde	Storm-surge barrier	Macrobenthos	Abundance, density, biomass	1-3		v			1	305	2	The impact of barrier construction on the macrofauna was relatively smaller than climate effects.	Meire et al., 1994
France	Bay of Biscay	Reclamation	Macrobenthos	Abundance, richness	1-3		v			1	6	3	After restoration (return land to the estuary), rapid recovery of benthic communities was observed.	Marquiegui & Aguirrezabalaga, 2009
Austarlia	Eastern Hunter River	Weir, dam	Fish community	Abundance, density	1-3		v			4	3	2	The barriers within river networks resulted in varying connectivity loss.	Rolls, 2011
India	Mumbai	Reclamation	Polychaete	Abundance, biomass	1-3			v		1	12	15	Reclamation caused the decrease of polychaete community.	Quadros et al., 2009
China	Tianjin	Reclamation	Phyto- and zooplankton, benthos	Density, biomass	1-3	v	v			1	8	3	Reclamation decreased the biodiversity and changed the structure of marine communities.	Li et al., 2010
China	North to south China coasts	Coastal infrastructures	Macrobenthos	Abundance, density, biomass	1-3		v		v	26	1	3	Artificial structures provided habitats for rocky shore species.	Dong et al., 2016
Korea	South coasts	Dike	Macrobenthos	Stable isotopes (food web)	1-3	v		v		2	4	2	Dike (local scale) induces changes in basal resource availability and faunal composition.	Park et al., 2017
Korea	Geum River estuary	Dike	Macrobenthos	Stable isotopes (food web)	1-3	v	v	v	v	1	4	30	Geographical isolation by a sea dike (regional scale) hindered terrestrial organic matters to macrobenthos.	***This study***

UK	Sedgeunkedunk Stream	Dam	Fish community	Density, biomass	>3	v	v			1	8	9	Dam removal has enhanced the fish assemblage by undisrupted stream gradient.	Hogg et al., 2015
Netherland	Oosterschelde	Storm-surge barrier	Macrobenthos	Abundance, density, biomass	>3		v	v		1	6-305[a]	2-18[a]	Climate caused more disturbance than barrier construction in the macrofaunal population.	Coosen et al., 1994
Korea	Saemangeum	Reclamation	Macrobenthos	Density, richness	>3	v	v	v	v	1	11-38[b]	12	Dike construction caused the change of benthic communities.	An et al., 2006
Korea	Seamangeum	Reclamation	Macrobenthos	Abundance, density, richness	>3	v	v	v	v	1	7	15	Since the closure of the dike, the number of species of macrobenthos	Koo et al., 2008

*The number of regions depending on geographical grouping by river-cathments, estuaries, or coasts.

[a] The number of stations, study period, and field survey differed depending on the dataset (total three datasets used).

[b] The number of stations differed depending on sampling date.

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