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Diet of the Mud-Flat Crab *Helice tientsinensis* in a Korean Salt Marsh

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Abstract

Crabs live at high densities in intertidal zones with various halophytes in salt marshes. However, the ecological relationships between crabs and halophytes as well as the impacts of herbivorous crabs on the plant distribution are not fully understood. In this study, we identified halophytic plant species consumed by crabs and determined the relative contributions of halophytes and other food sources to the diet. A DNA analysis of stomach contents was used to determine plant food sources for crabs. We found that the dominant crab species *Helice tientsinensis* consumed *Suaeda japonica*, even though several halophytes inhabited the study site. These results indicated that *H. tientsinensis* is a selective feeder. Based on a stable isotope analysis, we observed a dietary shift during crab development. Immature individuals mainly ate soil organic matter, whereas mature individuals showed more diverse food sources. We observed greater plant (*S. japonica*) consumption by middle-sized crabs than by crabs of other sizes. Our results for the feeding relationship between crabs and plants extend our understanding of the benthic food web in salt marshes.

Keywords Feeding habits · Food source · *Helice tientsinensis* · Korea · Siheung tidal flat · *Suaeda japonica*

Introduction

Crabs are abundant and generally dominant species in salt marsh ecosystems (Golley et al. 1962; Jones 1984). The feeding behavior of crabs can control the abundance and distribution of prey in salt marshes (Hoffman et al. 1984). Crabs also affect nutrient dynamics (Alberti et al. 2015; Martinetto et al. 2016) and can directly or indirectly affect community dynamics and food web interactions in salt marshes (Trussell et al. 2002).

The shore crab *Helice tientsinensis* lives in the upper part of the mud flat of the southwestern coast of Korea with salt marsh plants, and it is a very economically and commercially useful species for fishermen and local residents (Baek 2014). The crabs use halophytes as food sources and can affect their distribution (He et al. 2015). Extensive studies of mangroves have evaluated the effects of herbivorous crabs on plant communities. For example, crabs generally feed directly on mangrove leaves or store the leaves in burrows for later ingestion (Martinetto et al. 2016). Feeding by these crabs can result in the removal of 30–90% of annual litter in a mangrove habitat (Robertson 1986; Micheli 1993; Slim et al. 1997). Recent studies report that small herbivorous animals, such as crabs, can limit the marsh seaward boundaries (Alberti et al. 2010; He et al. 2015). In addition, crabs play important roles in salt marsh ecosystems, e.g., as herbivores, predators, and sediment consumers, and utilize diverse food types, depending on the species (Bertness 1999; Hsieh et al. 2002; Qin et al. 2010). Differences in food sources are also related to the size and sex of crabs (Divine et al. 2015; Hübner et al. 2015). Despite of previous studies on crabs in salt marshes, little is known about

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the feeding relationships between the crab *H. tientsinensis* and Korean native marsh plant species. Understanding the food sources of dominant crabs may provide insights into their roles in the food web and their impacts on the salt marsh plant community.

Stable isotope analyses are commonly used in feeding ecology to identify predator diet compositions and trophic levels (Peterson and Fry 1987; Post 2002; Layman et al. 2007). The isotopic signatures of consumers are often similar to those of plants, and food resources of consumers can be characterized by comparisons between the isotopic signatures of consumers and food resources (Fry et al. 1978; DeNiro and Epstein 1981). However, it is difficult to distinguish plant species from the particular diet of a consumer based on isotope analyses alone. Recently, increasing ecological studies have included DNA analyses to evaluate feeding habits (Valentini et al. 2009). A DNA analysis of food residues in the stomach can provide substantial and accurate prey information. Stable isotope analysis can provide relatively long-term diet information by analyzing the muscle tissue of the crab (Fry 2006; Mazumder et al. 2018). Therefore, DNA analyses of stomach contents and stable isotope analyses can provide complementary information about food sources (Peterson 1999).

In this study, we used a stable isotope analysis and a DNA analysis of stomach contents to identify the main food sources of the dominant crab *Helice tientsinensis* in the Siheung Tidal Flat, which is a Coastal Wetland Protected Area with a high tidal range (from 4 to 9 m) in Korea (Wells et al. 1990). *Helice tientsinensis* and salt marsh plants are widely distributed in Korean salt marshes, but their feeding relationships have not been studied. We determined the types of halophytes that are eaten by crabs and their importance relative to other food sources. We also used a stable isotope mixing model (SIAR) to evaluate the impact of the herbivorous crabs on salt marsh plant communities in the Siheung Tidal Flat. We hypothesized that (1) *H. tientsinensis* feeds on a variety of salt marsh plants at the study site and (2) the main food sources vary depending on the sex and carapace width (CW) of the crab.

Materials and Methods

Study Site

Field surveys were conducted in the Siheung Tidal Flat (37°23′52″N, 126°45′33″E), which is one of the large macrotidal salt marshes in Korea. Halophytes and tidal flats are widely distributed among the deep tidal channels formed by the high tidal range from 4 to 9 m (Wells et al. 1990), which has been designated a Coastal Wetland Protected Area (ca. 0.71 km^2) since 2012 owing to its ecological importance. The study area is geographically located in the mid-latitude temperate climatic zone characterized by a mean annual temperature of 12.1 °C, and a mean annual precipitation of 1234 mm, with 70% of annual precipitation falling in the rainy season from June to September (Korea Meteorological Administration, http://www.kma.go.kr).

The dominant salt marsh plants in this area are *Suaeda japonica* Makino and *Phacelurus latifolius* Ohwi; other species include *Carex scabrifolia* Steudel, *Artemisia fukudo* Makino, *Phragmites australis* Trin ex Steud and *Zoysia sinica* and hance are potential food sources for crabs (Bang et al. 2018). *Helice tientsinensis* Rathbun is the most abundant crab species at this study site (Bang and Lee 2019), with estimated densities of 27–47 individuals per m^2.

Sampling and Sample Processing

All samples were collected in August 2016 at three points; these locations were dominated by *S. japonica* and *P. latifolius* in the Siheung Tidal Flat. Sampling points were separated by at least 50 m (Fig. 1). Crab samples collected at the site using standard commercial crab traps (33 × 33 × 58 cm [width × height × length], 1-cm mesh) were immediately placed in an ice box to minimize digestion. After collection, the samples were transferred to the laboratory and the sex and CW, was determined using calipers, were recorded. Samples were then frozen at −20 °C until analysis.

Based on the literature and observations (Qin et al. 2010; Han et al. 2012; Mao et al. 2016), the potential food sources include halophytes, polychaetes, soil organic matter (SOM), and benthic microalgae (BMI). The leaves of marsh plants (*S. japonica*, *P. latifolius*, *C. scabrifolia*, *A. fukudo*, *P. australis* and *Z. sinica*) were collected by hand. Polychaetes were collected from the soil. Samples for SOM analyses were collected from depths of 0–1 cm in the surface soil and then stored in an ice box. Sediment samples were not acid washed because previous studies showed that there is no change in δ13C (SOM) value after acid treatment (Midwood and Boutton 1998; Kennedy et al. 2005). Reference samples for BMI were collected in the same season (summer) near the study site (Kang et al. 2016). To estimate the crab density in the study area, the number of burrows at each sampling point was counted in August 2016.

For the DNA analysis of stomach contents, the stomachs of 18 crabs were incised and stored in 70% ethanol. Muscle tissue of the leg of the crab was dissected for the stable isotope analysis because these tissues in decapods have less variation in ^{13}C and ^{15}N than that in other tissue types (Yokoyama et al. 2005). In addition, debris from the exoskeleton or other inorganic carbonates was not included in the samples and muscle samples were not oxidized. Since stable isotope ratios of crab muscle tissues apprears not to be affected by lipids (Bodin et al. 2007), normalisation adjustments were not made for lipid. In total, 30 crabs were used for the stable isotope analysis. All crab samples were classified into the following

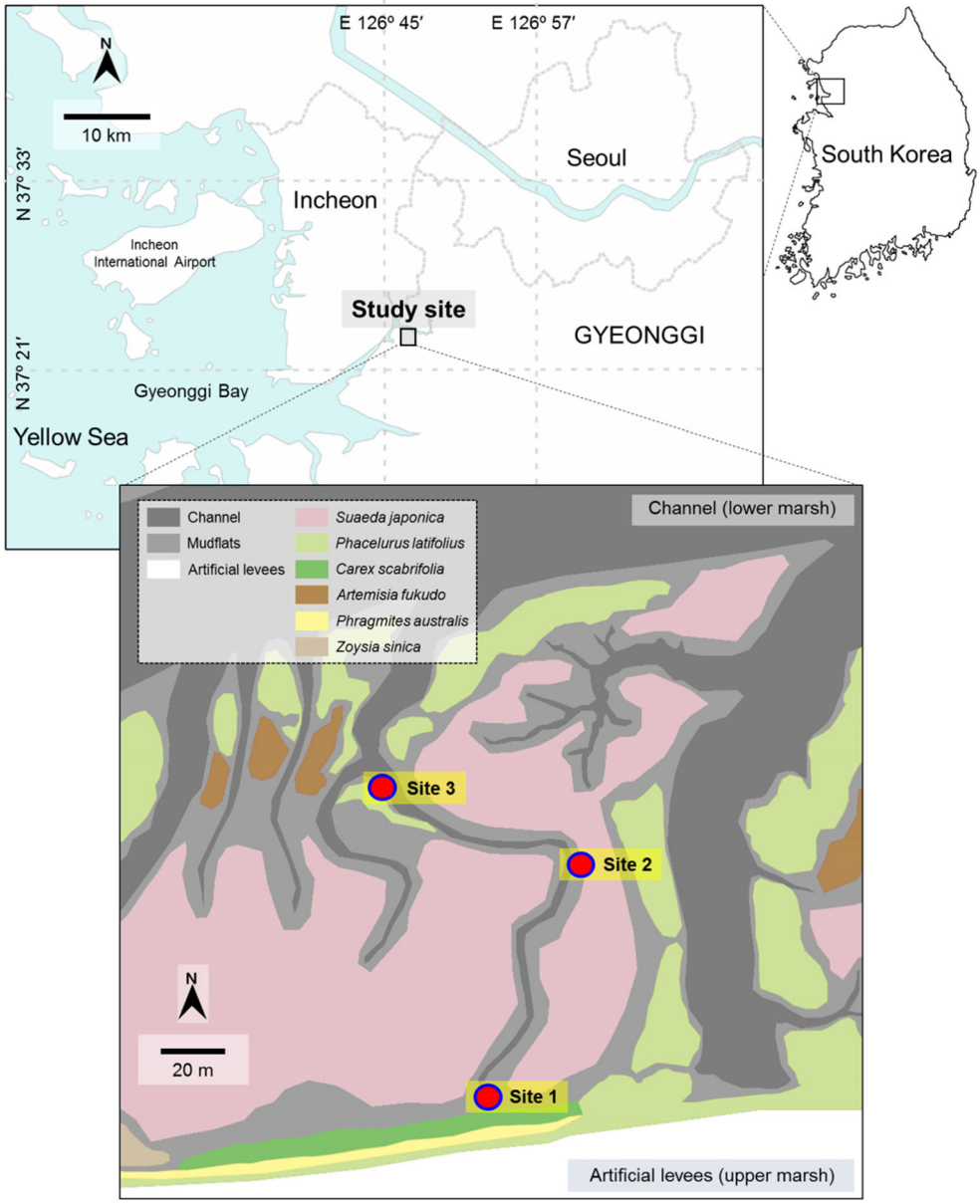

Fig. 1 Map showing the location of the sampling sites ($n = 3$), Siheung Tidal Flat

categories according to the CW or sex: G1 (2.0–2.5 cm, $n = 10$; Female/Male = 5/5), G2 (2.5–3.0 cm, $n = 10$; Female/Male = 5/5), and G3 (3.0–3.5 cm, $n = 10$; Female/Male = 5/5). All samples were freeze-dried (Kim et al. 2016) to remove moisture and then milled to a uniform particle size using a ball mill (Retsch MM301; Dusseldorf, Germany). The pulverized samples were added to a tin disc (Perkin Elmer, Waltham, MA, USA), made into compact spheres, and stored in plates, and then a stable isotope analysis was performed at the Stable Isotope Biogeochemistry Center at UC Berkeley.

Stable Isotope Analyses

The carbon and nitrogen isotope ratios were analyzed using a CHNOS Elemental Analyzer (vario ISOTOPE cube; Elementar, Hanau, Germany) coupled with a mass spectrometer (IsoPrime 100; Isoprime Ltd., Manchester, UK). The stable isotope ratio expressed as δX (‰) was determined according to the following formula:

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

where X is ^{15}N or ^{13}C and R is the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. R standards were the Vienna Pee Dee Belemnite (VPDB) standard for carbon and atmospheric N_2 for nitrogen. The analytical precision of these measurements was 0.20‰ for $\delta^{13}C$ and 0.30‰ for $\delta^{15}N$.

The relative contribution of each food source to the consumers (three groups of crabs) was assessed by the isotope

mixing model using the SIAR package in R. Potential food sources were chosen as plant (*S. japonica*) based on analyses of stomach content DNA (Table 2). Also, animal (polychaetes), SOM, and BMI were selected for potential food sources based on previous studies (Qin et al. 2010; Han et al. 2012; Mao et al. 2016). The following fractionation factors used the decapods model ($0.4 \pm 1.2‰$ for $\delta^{13}C$ and $2.2 \pm 1.6‰$ for $\delta^{15}N$) from previous studies (McCraith et al. 2003; Vanderklift and Ponsard 2003).

DNA Analysis of Stomach Contents

DNA was extracted from the stomach content samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, except for the lysis step. One or two 5-mm stainless steel beads (Qiagen) were added for sufficient homogenization in the lysis step and the samples were mixed by shaking on a Mixer Mill (Retsch) at 20 Hz for 1 min. The extracted DNA was finally eluted in 200 μl of AE buffer and stored at −20 °C until further analysis.

The ITS2 region was used because it could be a powerful marker for authenticating plant taxa (Chen et al. 2010). The ITS2 region of the ribosomal RNA (rRNA) gene was amplified using a universal ITS primer set, i.e., ITS2_S2F (5′-ATGCGATACTTGGTGTGAAT-3′) and ITS2_S2R (5′-GACGCTTCTCCAGACTACAAT-3′) (Chen et al. 2010). For PCR amplification, 2 μl of extracted DNA was added to amplification mixture, Intron FastMix/Frenche™ Premix (iNtRON Biotechnology, Seoul, South Korea), and forward and reverse primers (5 pmol) in a final reaction volume of 20 μl. PCR conditions were as follows: an initial denaturation at 94 °C for 5 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and elongation at 72 °C for 45 s, and a final extension step at 72 °C for 10 min. All PCR products were purified using the MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology). Purified PCR products were ligated into the pGEM-T Easy Vector according to the manufacturer's protocols (Promega, Madison, WI, USA) and transformed into DH5a chemically competent cells. Cells were plated in Luria–Bertani agar + ampicillin medium with 40 μl of X-gal solution (2% *w*/*v*) for antibiotic selection and blue-white screening. After the cloning step, five white colonies were selected and used for colony PCR with M13F and M13R primers. PCR conditions were as follows: an initial denaturation at 95 °C for 10 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min, and a final extension step at 72 °C for 7 min. After amplification, PCR products were purified and delivered to a commercial sequencing service (Genotech, Daejeon, South Korea). All DNA sequences were identified by BLASTN searches against the GenBank database and aligned using MEGA6 (Tamura et al. 2013). In addition, all BLASTN results were confirmed by comparisons with sequences of salt marsh plants collected in the field (Joo et al. 2014).

Data Analyses

Statistical analyses were performed using R (R Core Team 2017). Before the analysis, all data were checked for normality and homogeneity of variances. Correlations between variables were evaluated using Pearson correlation coefficients. Two-way ANOVA was used to explain variation in the $\delta^{13}C$ and $\delta^{15}N$ signatures of consumer groups using the CW and sex of crabs as the main fixed factors. Tukey's multiple comparison tests were used only if significant differences were detected by ANOVA ($P < 0.05$).

Results

Stable Isotope Analysis

The $\delta^{13}C$ and $\delta^{15}N$ values for consumer crabs (*H. tientsinensis*) differed according to the CW and sex of crabs (Table S1). There were no significant effects of Sex or the CW:Sex interaction, whereas the carbon and nitrogen isotope values were significantly different according to the CW of crabs (ANOVA, $P < 0.001$) (Table 1). As the CW of the crab increased, $\delta^{13}C$ increased (G1 < G2 < G3). The $\delta^{15}N$ values were higher in G2 than in G1 and G3. In particular, the difference in $\delta^{15}N$ between G2 and G3 was approximately 2–3‰.

The $\delta^{13}C$ and $\delta^{15}N$ values differed among the potential food sources collected from the Siheung Tidal Flat (Table S2). *Suaeda japonica* was the most ^{13}C-depleted food source in the Siheung Tidal Flat ($-27.99 \pm 0.67‰$), while *P. latifolius* was the most ^{13}C-enriched ($-14.52 \pm 0.26‰$). The $\delta^{13}C$ values for SOM ($-24.54 \pm 0.35‰$) and polychaetes ($-20.88 \pm 0.31‰$) were intermediate between those for *S. japonica* and *P. latifolius*. The $\delta^{15}N$ values for potential food sources ranged from *P. latifolius* ($2.36 \pm 1.35‰$) to *C. scabrifolia* ($7.15 \pm 1.95‰$).

Potential food sources and consumer crabs were well-differentiated with respect to carbon and nitrogen stable isotopes except for *S. japonica* and *C. scabrifolia* (Fig. 2). The $\delta^{13}C$ values for all crabs were distributed within the range of $\delta^{13}C$ values for potential food sources.

DNA Analysis of Stomach Contents

DNA was extracted from 24 samples, including six salt marsh plants and the stomach contents of 18 crab individuals (*H. tientsinensis*) in the Siheung Tidal Flat. Thirty unique sequences were detected from genes amplified from the

Table 1 Summary of two-way ANOVA results for consumer crabs in the Siheung Tidal Flat. (df: degrees of freedom; Sum Sq: sum of squares; Mean Sq: mean square; TSS: total sum of squares; CW: carapace width (CW) class)

Factor	df	Sum Sq	Mean Sq	*F*-value	Pr(>*F*)	Variance explained (%)
$\delta^{13}C$						
CW	2	23.29	11.65	11.86	<0.001	46.5
Sex	1	2.04	2.04	2.08	0.1620	4.1
CW × Sex	2	1.15	0.58	0.59	0.5633	2.3
Residuals	24	23.56	0.98			47.1
TSS		50.05				
$\delta^{15}N$						
CW	2	32.6	16.3	15.1	<0.001	52.5
Sex	1	0.55	0.55	0.51	0.4814	0.9
CW × Sex	2	3.04	1.52	1.41	0.2642	4.9
Residuals	24	25.91	1.08			41.7
TSS		62.1				

stomach content samples and only *S. japonica* was detected in all samples (Table 2). PCR amplification was successful for 10 out of 18 stomach content samples, and the amplification success rate was 55.6% (Table S3). Additionally, the amplification success rate differed depending on the CW class and sex of crabs and was higher in G3 (77.8%) than in G2 (33.3%) and in Females (77.8%) than in Males (33.3%).

Stable Isotope Mixing Model

A stable isotope mixing model (SIAR) was used to estimate the relative contributions of the four main categories of food sources to crabs assigned to the three CW classes (Fig. 3, Fig. S1). The ratios of SOM, BMI, animal, and plant materials varied according to the CW class. SOM was the most substantial food source in G1 (0.58–0.79) but contributed less to the food compositions of G2 (0.25–0.45) and G3 (0.25–0.42) (low–high 95% credibility intervals). BMI showed the inverse trend to that observed for SOM: G1 (0.11–0.19) < G2 (0.18–0.29) < G3 (0.3–0.38). The contribution of *S. japonica* was lower in G1 and G3 (0.0031–0.11 and 0.054–0.18, respectively) than in G2 (0.13–0.3). In the case of animal food sources, the ratio was higher in G3 (0.072–0.29) than in G1 and G2 (0.0036–0.1 and 0.016–0.2, respectively).

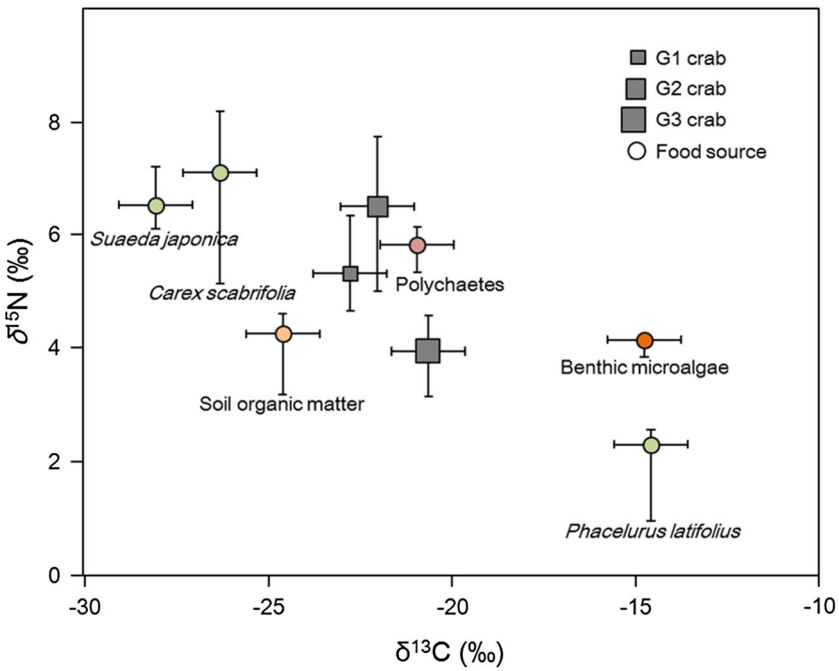

Fig. 2 Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$, mean ± SD, n = 5) for crabs (gray symbols) and food sources (other colors) collected in the Siheung Tidal Flat. G1: CW (2.0–2.5 cm); G2: CW (2.5–3.0 cm); G3: CW (3.0–3.5 cm); CW: carapace width

Table 2 Plant food sources detected in the stomach contents of crabs. Only one plant (*Suaeda japonica*) was detected as the results of pairwise alignment. G2: CW (2.5–3.0 cm); G3: CW (3.0–3.5 cm); CW: carapace width

No.	ID	Sex	Class	% identity
1	Sh_1	F	G2	100
2				99.71
3	Sh_2	F	G2	100
4	Sh_3	F	G2	98.73
5	Sh_4	F	G3	100
6				100
7				99.71
8				100
9				100
10	Sh_5	F	G3	99.71
11				97.56
12				100
13	Sh_6	F	G3	99.71
14				100
15				100
16	Sh_7	F	G3	99.72
17				99.71
18				100
19				100
20	Sh_8	M	G3	100
21				100
22				99.71
23	Sh_9	M	G3	100
24				100
25				99.71
26	Sh_10	M	G3	100
27				100
28				100
29				100
30				100

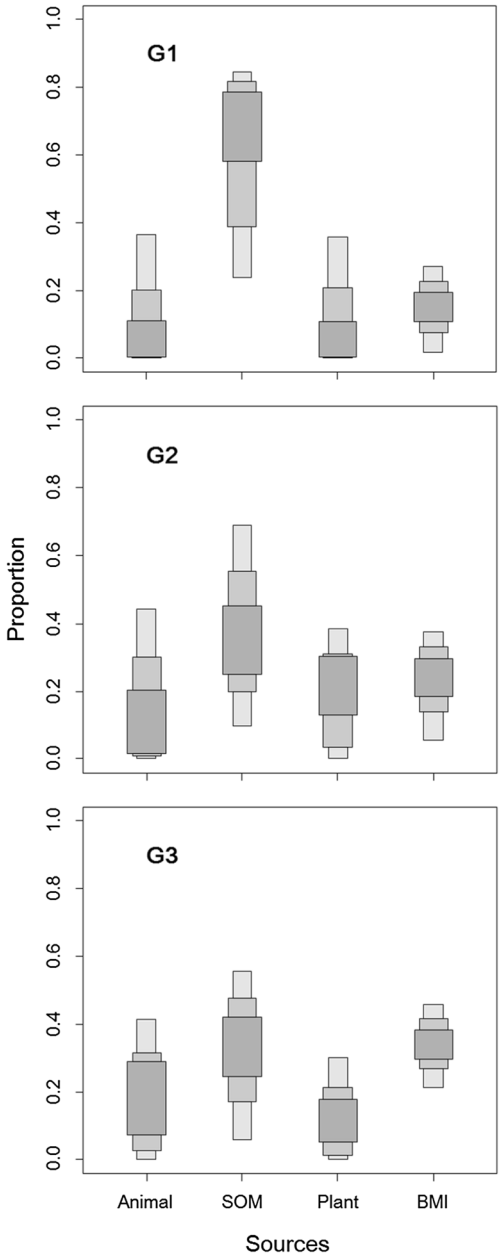

Fig. 3 Box plots showing the differential proportionate contribution of sources in modeled diet solutions for 3 class of crabs (G1, G2, G3). G1: CW 2.0–2.5 cm; G2: CW 2.5–3.0 cm; G3: CW 3.0–3.5 cm; CW: carapace width of crabs. Credibility intervals are presented as dark (95%), intermediate (75%), and light (50%) boxes

Discussion

This study is the first report to show the feeding relationship between the dominant crab *Helice tientsinensis* and salt marsh plants, which have received limited attention, in a Korean macrotidal salt marsh. In contrast to previous studies (Bodin et al. 2007; Han et al. 2012; Mao et al. 2016), we provided direct evidence for the preferred plant sources and determined their importance relative to other food sources. Data on these food sources of herbivorous crabs are essential to understanding plant-animal interactions and energy flow of the salt marsh ecosystem.

DNA analyses of stomach contents can provide direct evidence for preferred food sources based on comparisons between sequences in the GenBank database and sequences of salt march plants collected in the field (Table 2). Generally, DNA diet analysis shows recently consumed food. A stable isotope analysis of muscle tissues reflects the mean composition of the assimilated diet and provides relatively long-term information (Divine et al. 2015). These two methods are complementary and suitable for analyses of decapod food sources. Also, the ITS gene is an appropriate marker for food source analyses of herbivorous crabs. It contains considerable variation compared to that in other conserved regions (Baldwin

et al. 1995). In previous studies, the ITS2 region was used to classify medicinal plants, with an identification success rate of 92.7% at the species level (Chen et al. 2010).

In a previous study, *H. tientsinensis* consumed plants as its main food source in a salt marsh, particularly the leaves of *Phragmites australis* and *Spartina alterniflora* (Qin et al. 2010). At our study site, only *S. japonica* DNA was detected in the stomach contents of crabs, regardless of the CW and sex, even though several halophytes including *P. australis* inhabited the study site (Table 2). These results indicate that the dominant crabs (*H. tientsinensis*) are selective feeders in the Siheung Tidal Flat. *Helice tientsinensis* may have used *S. japonica* as its main food source for several reasons. First, *S. japonica* is the dominant plant in the Siheung Tidal Flat and is thus easily accessible. Second, *S. japonica* is shorter than other salt marsh plants, and energy expenditure may be lower for *S. japonica* consumption than for the consumption of other plants. Third, leaves and plant tissues are soft in *S. japonica*, and these properties may be preferable for *H. tientsinensis* (He et al. 2015). Since our DNA analysis was based on one time sampling in the summer, *H. tientsinensis* might have fed on other food sources in other time or seasons. To improve our understanding of the prey preferences of crabs, food preference experiments or comparisons of food sources at various sites with different vegetation structures should be performed.

The range of $\delta^{13}C$ values for potential food sources in the Siheung Tidal Flat ranged from −27.99‰ (*S. japonica*) to −14.52‰ (*P. latifolius*) (Fig. 2 and Table S2). A wide range of $\delta^{13}C$ values for the food sources can allow for better source discrimination in mixing models (Mao et al. 2016). In addition, the $\delta^{13}C$ values for the consumers were distributed within the $\delta^{13}C$ range for potential food sources, indicating that the carbon sources were potential food sources for consumers. The $\delta^{13}C$ values for SOM and polychaetes were intermediate, suggesting that they are major food sources, and were similar to the $\delta^{13}C$ values for consumers. Additionally, the $\delta^{13}C$ values for crabs differed significantly according to the CW (Table 1). These results show that diet of crabs may vary with respect to the CW. The range of $\delta^{15}N$ values of potential food sources was 2.36‰ (*P. latifolius*) to 7.15‰ (*C. scabrifolia*) and *C. scabrifolia* showed the most highly $\delta^{15}N$-enriched signatures (Fig. 2 and Table S2). The $\delta^{15}N$ values for the crabs also varied considerably depending on the CW category (Table 1). Among the consumers, the most highly $\delta^{15}N$-enriched signatures were those of the G2 group. G2 group crabs probably consumed mostly *S. japonica* or *C. scabrifolia*. In contrast, G3 crabs showed the most $\delta^{15}N$-depleted signatures. Based on the similar $\delta^{15}N$ values, G3 crabs probably consumed substantial BMI, unlike the G2 crabs. A sharp decrease in the $\delta^{15}N$ value for G3 crabs may be related to the molting period after achieving sexual maturation (Bodin et al. 2007). There was no significant difference in the isotopic results between sexes, unlike the results for CW (Table 1). However, unlike the other groups, there was a sex differences in $\delta^{13}C$ and $\delta^{15}N$ isotopes between males and females in G2 (Table S1). This may be due to differences in the activity area in the G2 crabs, which may be the most vigorous group (Squires and Dawe 2003). There may also be a difference due to the timing of pregnancy. Therefore, understanding the life history of crabs can improve the interpretation of ontogenic dietary changes in food source analyses.

Our results provided evidence for a mixed diet consisting of polychaetes, SOM, *S. japonica*, and BMI in crabs. Using SIAR, we evaluated the relative contributions of the major food resources (Fig. 3, Fig. S1). We found that SOM was the most highly represented food source in G1 crabs. This result indicated that relatively immature, small crabs may not eat a wide variety of food and SOM may be readily available and digested (Han et al. 2012). As the CW class increased, the proportion of SOM in the diet gradually decreased and the proportions of other food sources increased. G2 and G3 crabs tended to consume a variety of foods. Similar trends were observed in a study of the food sources of an estuarine crab (*Sesarma dehaani*) in Korea, indicating that immature individuals mainly consumed detrital sediments, whereas mature individuals used various carbon sources (Han et al. 2012). The stomach contents and stable isotope analysis showed that the primary salt marsh plant consumed by the crab was *S. japonica*, with a relative contribution of 11–30% (high 95% credibility intervals). Considering the high density of crabs, grazing may regulate the distribution of *S. japonica* in Siheung Tidal Flat. Previous studies have shown that crabs have a potential role in determining the plant distribution in wetlands (Costa et al. 2003). An herbivorous crab consumed *S. alterniflora* leaves and damaged up to 75% of the leaves (Alberti et al. 2007), and damaged leaves can also cause biomass loss due to infection by fungi (Daleo and Iribarne 2009; Freitas et al. 2015). He et al. (2015) recently reported that grazing crabs can limit the distribution of halophytes, together with abiotic stress or competition. These findings suggest that consumer pressures need to be considered in studies of the halophyte distribution.

The PCR amplification success rates for the stomach contents differed according to CW class and sex (Table S3). The amplification success rate for G3 samples (CW: 3.0–3.5 cm) was greater than that for G2 samples (CW: 2.5–3.0 cm). These differences may be explained by differences in the food source composition and in the amount of sample. For example, a small individual crab contains a relatively small amount of food compared with that in a large crab. Small sample volumes may be associated with lower DNA contents and lower amplification success rates. Also the stomachs of G1 crabs were too small to be incised, and excluded from the sample. The amplification success rate of male samples was lower than that of female samples. Squires and Dawe (2003) showed that female crabs consume highly accessible food sources, and male crabs consume different foods.

Conclusions

DNA diet analysis suggest that the dominant crabs (*H. tientsinensis*) consumed only *S. japonica*, despite the presence of other halophytes in the Siheung Tidal Flat. This crab may be a selective feeder and its feeding habits may significantly affect the distribution of *S. japonica*. Modelling based on carbon and nitrogen stable isotope signatures showed an ontogenic dietary shift in crab feeding habits, suggesting that immature individuals consume SOM predominantly and mature individuals exhibited diverse food compositions. In addition, the continuous monitoring of life history and population dynamics, including crab food source studies, is necessary to understand the plant distribution and the structure and function of the salt marsh benthic food web.

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