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(Title)Intraspecific diet shifts of the sesarmid crab, *Sesarma dehaani*, in three wetlands in the Han River estuary, South Korea

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**ABSTRACT**

**Background:** Han River estuary is a national wetland reserve near the Demilitarized Zone (DMZ) between South Korea and North Korea. This trans-boundary estuary area has been well-preserved and shows distinctive plant communities along the salinity gradient. To elucidate energy flows and nutrient cycling in this area, we studied trophic relations between the dominant sesarmid crab, *Sesarma dehaani*, and food sources in three wetlands with different environments along the estuarine gradients.

**Results:** Stable isotope signatures (δ13C and δ15N) of the crabs were significantly different among the sites and body size classes. Seasonal changes in δ13C of small crabs were distinct from those of large individuals at all the sites. The isotopic values and fatty acid profiles of the crabs were more different among the sites in September than in May. In May, large sized crabs utilized more plant materials compared to other dietary sources in contrast to small sized crabs as revealed by a stable isotope mixing modeling, whereas contributions to diets of crabs were not dominated by a specific diet for different body size in September except at site 1. Based on PCA loadings, fatty acid content of 18:3ω3, known as a biomarker of plant materials, was main factor to separate size groups of crabs in May and September. The δ13C value of sediment had high correlation with those of small sized crabs at site 1 and 2 when one month time lag was applied to the value for crabs during the surveyed period.

**Conclusions:** Based on the stable isotope and fatty acid results, the consumption habits of *S. dehaani* appear to be distinguished by sites and their size. In particular, smaller size of *S. dehaani* appears to be more dependent on fewer food sources and is influenced more by the diet sources from the sediments in Han River estuary.

**Background**

 To elucidate ecosystem functions, such as energy flows and nutrient cycling, it is essential to understand the trophic relations of dominant organisms in food webs (Vander Zanden et al. 2006). The food webs in estuaries are complex; they incorporate different environments, such as terrestrial and brackish environments, and provide diverse habitats as well as different food sources for various organisms based on hydrological fluctuation (Peterson 1999; Nagelkerken et al. 2008; Kristensen et al. 2008). Numerous studies on estuarine food webs have been conducted for comparisons with habitats with a salinity gradient from riverine mouth to offshore, possessing different vegetation types (Lee 2000; Doi et al. 2005; Choy et al. 2008; Antonio et al. 2012; Bergamino and Richoux 2015). Several studies have focused on food webs in mangrove systems in subtropical and tropical regions (Kristensen et al. 2008; Lee et al. 2014); they have especially focused on the interactions between mangroves and crabs, such as grapsid and sesarmid crabs, which have been reported to be major players in carbon cycling in these systems and primarily consume mangrove litters and propagules (Lee 1998).

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In this study, we extended our previous investigation wherein we elucidated trophic relations between the sesarmid crab, *S. dehaani*, and food sources, particularly plant material and sediment, with respect to the size of crabs in the upper brackish areas, to three wetlands dominated with different plant species along the estuarine gradients in the Han River. In this regard, we estimated the diet contributions of the crabs, grouped by size, based on carapace width and seasonal change using stable isotope and fatty acid analyses with a stable isotope mixing model in three different sites with different vegetation and marine influence.

**Materials and Methods**

**(Sub-title may be different for each manuscript!)Study area**

The Han River runs through the middle part of the Korean peninsula, penetrating through Seoul to reach the Yellow Sea. The Han River estuary lies inside the Demilitarized Zone (DMZ) between South and North Korea (37° 36′ 56″ N, 126° 47′ 38″ E–37° 46′ 36″ N, 126° 31′ 34″ E). We studied three different wetlands encompassing a spatial gradient of environmental conditions. The Janghang wetland, containing site 1, is located 31.7 km upstream of the river mouth whereas the Gongreung wetland, containing site 2, and Seongdong wetland, containing site 3, are 18.3 and 14.4 km upstream, respectively (Fig. 1).

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**(Sub-title may be different for each manuscript!)Sample collection and preprocessing**

The sesarmid crab, the characteristic marsh plants (*S. subfragilis* for site 1, *P. communis* for site 2, and *B. planiculmis* and *P. communis* for site 3), and sediment were collected from the three sites every month between August, 2006 and November, 2007 except during the winter season (November, 2007 for site 2 and October and November, 2007 for site 3) when the crabs hibernated in their burrows and access to them was restricted. The crabs caught by hand from each study area were classified into four size groups, namely Group I (~10–20 mm), Group II (~20–30 mm), Group III (~30–40 mm), and Group IV (larger than 40 mm), based on its carapace width.

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**(Sub-title may be different for each manuscript!)Stable Isotope analysis**

All the samples were dried using a freeze dryer (Model FD2.5; Heto, Waltham, USA). The freeze-dried samples were further dried at 60°C in an oven for 48 h and subsequently ground into powder using pestle and mortar. …

 The δ13C and δ15N values were expressed as differences (in parts per thousand, ‰) in 13C:12C and 15N:14N ratios (R), respectively, between the samples and the standards [Pee Dee Belemnite marine limestone for 13C and atmospheric nitrogen for 15N], as follows:

$$δ^{13}C or δ^{15}N \left(‰\right)= \left[\frac{R\_{sample} – R\_{standard}}{R\_{standard}}\right] × 10^{3}$$

$$R= ^{13}C/^{12}C or ^{15}N/^{14}N$$

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**(Sub-title may be different for each manuscript!)Data analysis**

The body size and spatial difference in the stable isotope signatures of *S. dehaani* captured in all the seasons were identified by performing a two-way analysis of variance (ANOVA). This analysis used “Size Group” and “Site” as the two factors. PCA was conducted to compare the fatty acid compositions of crabs among the size groups and habitats. Prior to PCA, log transformations were applied to ensure the homogeneity of variance; this was followed by Pareto scaling to improve the fatty acid information content (van den Berg et al. 2006). To compare the stable isotope signatures and FA profiles of the crabs between the size classes and among sites, multivariate analysis of variance (MANOVA) was conducted (Cherel et al. 2000; Delaporte et al. 2005). MANOVA can examine whether the averages of the combined values of δ13C and δ15N or PCA score of FA profiles are significantly different between the size groups or among sites.

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**Results**

**(Sub-title may be different for each manuscript!)Isotopic signatures of *Sesarma dehaani* during the entire period**

The results of the two-way ANOVA on stable isotope signatures of *S. dehaani* sampled during the entire period of study, revealed significant differences among the crab size classes and among the different sites. The percent of variance explained by the site factor was higher than that explained by the size factor on both δ13C and δ15N of *S. dehaani*, especially on δ15N (Table 1). The δ13C and δ15N values of the crabs were respectively within certain ranges for each site; -25.2‰ ± 1.2 and 8.3‰ ± 1.0, n = 174 at site 1, -24.6‰ ± 1.6 and 9.7‰ ± 0.9, n = 62 at site 2, -22. 0‰ ± 2.3 and 11.3‰ ± 1.0, n = 58 at site 3. (Fig. 2).

**(Sub-title may be different for each manuscript!)Diet source contributions by size groups in two seasons**

To compare the effect of the surrounding circumstances on the contributions of diet to *S. dehaani* in detail, we selected two seasons for the analyses. The season during May provides a short time for the utilization of diets after hibernation, and the season during September provides more time for feeding as well as a wide range of diets compared to the spring. In addition, the monsoon rainfall during the summer influences their feeding between the two seasons.

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**(Sub-title may be different for each manuscript!)Trophic relations between sediment and *Sesarma dehaani***

Comparisons of the δ13C values of both the sediment and *S. dehaani* revealed that the values for individuals in group I and II were closer to those of the sediment compared to those of group III and IV (Fig. 6).

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**Discussion**

 The results of this study suggest that the consumption of food by the sesarmid crab, *S. dehaani*, was influenced by the landscape heterogeneity. Moreover, shifts in the feeding habits of *S. dehaani* between the size classes in the lower brackish area (site 3), which was less vegetated, were unclear compared to those in the upper area (site 1), which was densely forested by the *Salix* community. *Sesarma dehaani* had spatial differences in the isotopic signature, showing patterns similar to those of the benthic consumers, including sesarmid crabs, along the salinity gradient in estuarine ecosystems (Table 1 and Fig. 2, Bouillon et al. 2004; Doi et al. 2005; Bergamino and Richoux 2015).

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**Conclusions**

In conclusion, we determined the contributions of major food sources for *S. dehaani*, which is a dominant consumer species in the Han River estuary, where human influences are low. Food sources of *S. dehaani* were mainly influenced by the habitats, especially for small sized individuals. *Sesarma dehaani* at site 1 and site 2 in the upper estuary region were influenced more by the food sources of terrestrial origin whereas the crabs at site 3, which was more open and closer to the marine environment, were more influenced by the marine phytobenthos.

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**ABBREVIATIONS**

ANOVA: Analysis of Variance, DMZ: Demilitarized Zone, FA: Fatty Acid, FAMEs: Fatty Acid Methyl Esters, FID: Flame Ionization Detector, IRMS: Isotope Ration Mass Spectrometer, MANOVA: Multivariate Analysis of Variance, MPB: Microphytobenthos, PCA: Principal Component Analysis, PTV: Programmable Temperature Vaporizer, SIAR: Stable Isotope Analysis in R

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**AVAILABILITY OF DATA AND MATERIALS**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**AUTHORS’ CONTRIBUTION**

DY carried out the field study and performed the analysis, and wrote the manuscript. DH participated in the design of the study, conducted field study, reviewed the manuscript. SP conceived the study and reviewed/edited the manuscript. DY and DH made equal contributions to this study. All authors read and approved the final manuscript.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**CONSENT FOR PUBLICATION**

Not applicable.

**COMPETING INTEREST**

The authors declare that they have no competing interests.

**Table 1. Results of two-way ANOVA comparing the impacts of size groups (Group) and habitats (Site) of *Sesarma dehaani* on the values of δ15N and δ13C for the entire period of study**

|  |  |  |
| --- | --- | --- |
| **δ15N value** | 　 | **δ13C value** |
| **Source** | **df** | ***F*** | ***p*** | **% of variance explained** |  | **Source** | **df** | ***F*** | ***p*** | **% of variance explained** |
| Group | 3 | 10.87 | < 0.001 | 3.70% |  | Group | 3 | 5.40  | 0.001  | 3.00% |
| Site | 2 | 270.01 | < 0.001 | 60.60% |  | Site | 2 | 108.41  | < 0.001 | 40.70% |
| Group × Site | 6 | 5.88 | < 0.001 | 4.00% |  | Group × Site | 6 | 2.68  | 0.015  | 3.00% |
| Residual | 283 | 　 | 　 | 31.80% | 　 | Residual | 283 | 　 | 　 | 53.20% |

**Table 2. Results of MANOVA based on the values of δ13C and δ15N of *Sesarma dehaani* grouped by carapace width among the different sites in May and September, 2007**

|  |  |  |  |
| --- | --- | --- | --- |
| **Size****Group** | **May 2007** |  | **September 2007** |
| **Lambda** | ***p*** | **Sites** | ***T*2** | ***p*** |  | **Lambda** | ***p*** | **Sites** | ***T*2** | ***p*** |
| GroupI – II | 0.067  | < 0.001 | St.1- St.2 | 14.424  | 0.001  |  | 0.022  | < 0.001 | St.1- St.2 | 40.744  | < 0.001 |
|  |  |  | St.2- St.3 | 14.384  | 0.001  |  |  |  | St.2- St.3 | 27.125  | < 0.001 |
|  |  |  | St.1- St.3 | 59.395  | < 0.001 |  |  |  | St.1- St.3 | 136.352  | < 0.001 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| GroupIII - IV | 0.174  | 0.002  | St.1- St.2 | 5.154  | 0.032  |  | 0.279  | 0.003  | St.1- St.2 | 3.311  | 0.072  |
|  |  |  | St.2- St.3 | 1.654  | 0.245  |  |  |  | St.2- St.3 | 3.234  | 0.075  |
| 　 | 　 | 　 | St.1- St.3 | 16.634  | 0.001  | 　 | 　 | 　 | St.1- St.3 | 15.176  | 0.001  |

**Figure legends**

**Fig. 1** Location of sampling sites in the Han River estuary, South Korea: site 1 (Janghang wetland), site 2 (Gongreung wetland), and site 3 (Sungdong wetland)

**Fig. 2** Dual isotope plots of δ13C and δ15N mean values (± SD) for all sized groups of *Sesarma dehaani* at the site 1, site 2 and site 3 in the Han River estuary in whole studied period

**Fig. 3** δ13C and δ15N diagram of the Han River estuary wetland in the (a) early season after hibernation (May 2007) and (b) in the active season of foraging (September, 2007)

**Fig. 4** Mean percentage contributions (95% credibility interval) of food sources (plant materials, sediment, and microphytobenthos; MPB) to *Sesarma dehaani* at the three studied sites of the Han River estuary in May and September, 2007

**Fig. 5** Scores of principal component analysis based on the fatty acid profiles of *Sesarma dehaani* of each size class at site 1, 2, 3 in May, 2007 (a) and September, 2007 (b). Symbols indicate sites, circle: site 1, triangle: site 2 and square: site 3. Closed symbols indicate Group I-II. Open symbols indicate Group III-IV

**Fig. 6** Relationship between the δ13C values of detrital sediment and *Sesarma dehaani* in the three wetlands for the entire period of study. Symbols indicate size classes, circle: Group I-II, triangle: Group III-IV. Open symbols indicate that one month time lag was applied to the values for crabs

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**Fig. 1**

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**Fig. 2**

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