Cellulase activity and stable isotope signature of benthic macroinvertebrates in estuarine habitats: potential assimilation of land-derived organic matter

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Abstract: We investigated inter-species dietary variation and potential cellulose digestion of 12 macroinvertebrate taxa collected from two locations in the estuarine Idoura Lagoon, Sendai Bay. All taxa exhibited cellulase activity (CA), which was higher among surface-deposit feeders (bivalve *Macoma contabulata*, polychaete *Tylorrhynchus osawai*, and ocypodid crabs) and obligatory suspension feeders (bivalves *Corbicula japonica* and *Nuttallia japonica*) (0.108 to 0.764 µmol min⁻¹ mg-protein⁻¹). In contrast, CA was lower among facultative suspension-feeding and deep-deposit feeding polychaetes (*Hediste* spp. and *Heteromastus* sp., respectively), and was lowest in the deep-deposit feeding polychaete *Notomastus* sp. The stable isotope ratios of the macroinvertebrates differed among feeding groups. A δ¹³C-based isotope mixing model revealed that the major dietary component of the surface-deposit feeders was microphytobenthos (34–50%), regardless of their high CAs. Although CAs of obligatory and facultative suspension feeders were comparable to or lower than those of surface-deposit feeders, they were highly dependent on river-derived materials at the station near the freshwater input (38–59%). These results indicate that CA is a common physiological characteristic of macroinvertebrates in estuarine soft-bottom habitats, but the dietary contribution of riverine detritus is not correlated with enzymatic activity. Our findings indicate that several factors affect the realized dietary components of macroinvertebrates, including feeding mode, the selectivity of ingestion, and digestive enzyme activity.

Key words: benthic invertebrate, δ¹³C and δ¹⁵N, detritus, digestive enzymes, feeding habit

Estuaries are major transition areas between riverine terrestrial and marine ecosystems and act as sinks for river-derived allochthonous organic matter (Levin et al. 2001, Kanaya et al. 2008a, b). Generally, river-derived detritus is rich in refractory carbohydrates, such as cellulose and lignin, which are barely assimilated by marine invertebrates (Kristensen 1972). However, recent studies have shown that some estuarine macroinvertebrates possess cellulases that can digest refractory plant materials (Kasai & Nakata 2005, Sakamoto et al. 2007, Niiyama & Toyohara 2011). They might play major roles in estuarine ecosystems by facilitating detritus processing and incorporating terrestrial primary production into the food web. The present study examined the stable isotope signatures (δ¹³C and δ¹⁵N) and cellulase activity (CA) of estuarine macrozoobenthos in a brackish estuarine lagoon to determine the relationship between cellulose digestion and realized dietary components.

Sampling was conducted on November 28, 2010 in the semi-enclosed brackish Idoura Lagoon (0.40 km², water depth <2.5 m), at the Natori River mouth, Sendai Bay, Japan (Fig. 1). Macroinvertebrates were collected from Station (Sta.) A (muddy sand flats in the central lagoon) and Sta. B (near the freshwater input, the Idoura River). In the innermost part of the lagoon (around Sta. B), salinity was much lower, and the sediment contained large amounts of river-derived detritus (δ¹³C < −25‰) than in the central lagoon (Kanaya et al. 2008ab, 2018). It was mainly due to the freshwater discharge from the Idoura River (Kanaya 2017). Twelve taxa were classified as surface-deposit, deep-deposit, and facultative and obligatory suspension feeders. Table 1 summarizes the samples, body sizes, and feeding habits. For polychaetes, the posterior part of the body was used for stable isotope analysis (SIA), and the remaining, including foregut and midgut, was used for CA analysis. For bivalves and crabs, adductor and leg muscles were used for SIA, respectively, and the remaining for CA analysis. Because of the limited time and difficulty in sampling, we were unable to collect sufficient number of specimens for statistical analysis (n > 3 for all taxa). A previous study showed that cellulase gene expression of a nereidid
polychaete differed among different parts of the body, for example, higher in the anterior part (Ito et al. 2011). Accordingly, differences in the selection of body parts used for CA analysis may lead to potential biases in the interpretation of CA values.

Cellulase activity was examined using the reducing sugar assay, as previously described (Kanaya et al. 2018). Each specimen was homogenized with 5 mL of phosphate-buffered saline and centrifuged at 9100×g for 10 min. After the measurement of protein concentration, the protein sample was adjusted to 1 mg mL⁻¹ and 5 μL of the enzyme solution, 5 μL of 1 M AcNa buffer (pH 5.5), and 40 μL of 1% carboxymethyl cellulose solution were mixed and incubated at 37°C for 20 min. The amount of reducing sugar was quantified using

Table 1. Stable isotope ratios and cellulase activity (CA) of macrozoobenthos in Idoura Lagoon. Data are presented as the mean and (SD) or (minimum/maximum). Size of specimens and reported feeding habits are also shown.

| Taxa                  | Sta. | Stable isotope ratio | CA  | Size (mm)† | Feeding habit          |
|-----------------------|------|----------------------|-----|------------|------------------------|
|                       |      | δ¹³C (‰)  | δ¹⁵N (‰) | µmol min⁻¹ mg-protein⁻¹ |                         |
| Polychaete            |      |           |           |                        |                         |
| *Hediste atoka*       | B    | 25.0 (0.3) | 13.0 (0.4) | 0.016 (0.015/0.016) | 2 0.88 to 1.30 facultative SFa |
| *Hediste diadroma*    | A    | 20.4 (0.3) | 10.6 (0.2) | 0.017 (0.010) | 4 0.88 to 1.45 facultative SFa |
|                       | B    | 26.5 (0.6) | 12.0 (0.4) | 0.052 (0.021) | 3 0.70 to 0.83           |
| *Tylorrhynchus osawai* | A   | 17.4 (0.4) | 12.6 (0.5) | 0.341 (0.418) | 4 0.45 to 1.13 SDFb |
| *Heteromastus* sp.    | A    | 19.9 (0.3) | 12.5 (0.7) | 0.076 (0.062) | 3 approx. 10 to 20 DDFc |
| *Notomastus* sp.      | B    | 20.8 (0.7) | 16.7 (0.3) | 0.001 (0.001) | 3 approx. 10 to 20 DDFb |
| Bivalve               |      |           |           |                        |                         |
| *Macoma contabulata*  | A    | 14.9 (0.6) | 11.3 (0.6) | 0.142 (0.050) | 3 19.5 to 35.6 SDFc |
| *Laternula marilina*  | A    | 21.2      | 8.6       | 0.165 (0.109/0.220) | 2 15.8 to 17.9 obligatory SFd |
| *Corbicula japonica*  | B    | 29.0      | 10.4      | 0.108 (0.101/0.114) | 2 6.5 to 30.0 obligatory SFe |
|                       | B    | 27.3 (0.5) | 10.5 (0.2) | 0.211 (0.114) | 4 19.5 to 32.5 obligatory SFe |
| Crab                  |      |           |           |                        |                         |
| *Scopimera globosa*   | A    | 13.6 (0.7) | 10.9 (0.6) | 0.103 (0.036) | 3 7.3 to 11.0 selective SDFb |
| *Ilyoplax pusilla*    | A    | 14.9      | 10.3      | 0.050 (0.000/0.100) | 2 8.5 to 11.3 selective SDFb |
|                       | B    | 19.2 (0.6) | 12.9 (0.4) | 0.320 (0.289) | 4 5.4 to 7.7              |
| *Macrophthalmus*      | A    | 16.5      | 11.7      | 0.764 (0.064/1.464) | 2 8.7 to 11.0 selective SDFb |

† The measurements are the width between eyespots (*Hediste* spp. and *Tylorrhynchus*), thorax length (*Heteromastus* and *Notomastus*), shell length (bivalves), and carapace width (crabs). † Reported in Kanaya et al. (2018). SF, suspension feeder; SDF, surface-deposit feeder; DDF, deep-deposit feeder. ‡ Toba & Sato (2013), † Doi et al. (2005), ‡ Kanaya et al. (2008a), † Kanaya et al. (2008b). Spatial changes in stable isotope ratios and CAs of *H. diadroma* were tested by a t-test.
the tetrazolium blue method, and CA was expressed as the glucose content per min per mg of protein. We have previously reported some of the CA data (Kanaya et al. 2018).

Samples for SIA were freeze-dried for 24 h, powdered, and treated with chloroform-methanol (2:1 by vol.) to remove lipids. Then, δ13C and δ15N were determined using an element analyzer interfaced with an isotope ratio mass spectrometer (EA-IRMS; NC2500/Delta plus systems, Finnigan MAT). The isotope ratios are expressed in δ notation as follows:

$$\delta^{13}C \text{ or } \delta^{15}N (\%o) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) \times 1000$$

where $$R$$ is the 13C/12C or 15N/14N ratio for δ13C or δ15N, respectively.

The dietary contributions of potential food sources were estimated using a δ13C-based Bayesian isotope mixing model (SIAR; Parnell et al. 2010), using reported δ13C values of microphytobenthos (MPB; −16.7±2.2‰, n=7), marine particulate organic matter (MPOM; −20.3±0.8‰, n=6), and riverine particulate organic matter (RPOM) and marsh plant litter (−27.0±1.6‰, n=13) from Japanese estuaries (see Kanaya et al. 2018 for the source datasets). In the procedure, δ13C values of RPOM and marsh plant litter were averaged as “RPOM/detritus” because their δ13C values were nearly identical. Trophic enrichment in δ13C was assumed to be +0.4±1.3‰ per trophic step (Post 2002). A t-test was used to test spatial changes in the stable isotope ratios and CAs.

CA was detected in all the 12 taxa collected from Sta. A and Sta. B, although there was marked inter-specific variation (Fig. 2 and Table 1). The activity was high in surface-deposit feeders, including the nereidid polychaete Tylorrhynchus osawai, bivalve Macoma contabulata, and ocypodid crabs Ilyoplax pusilla (specimens at Sta. B), Scopimera globosa, and Macrophthalmus japonicus (0.103–0.764 µmol min$^{-1}$ mg-protein$^{-1}$). Notably, M. japonicus and T. osawai showed the highest CA values with distinctive intraspecific variations even within the same sampling location (0.101–1.464 µmol min$^{-1}$ mg-protein$^{-1}$ for M. japonicus and 0.009 to 0.898 µmol min$^{-1}$ mg-protein$^{-1}$ for T. osawai). The variation in CA values was probably due to their intestinal fullness (Kawaida et al. 2013). Suspension-feeding bivalves, including Notatallia japonica, C. japonica, and L. marilina, also had high CAs (0.108–0.211 µmol min$^{-1}$ mg-protein$^{-1}$). In contrast, CA was low in the facultative suspension feeders (nereidid polychaetes Hediste diadroma and H. atoka) and deep-deposit feeders (capitellid polychaete Heteromastus sp.) (0.016–0.076 µmol min$^{-1}$ mg-protein$^{-1}$). The deep-deposit feeder Notomastus sp. (capitellid polychaete) showed the lowest CA among the taxa examined in this study (0.001 µmol min$^{-1}$ mg-protein$^{-1}$).

Spatial differences in CA were detected between the two stations. H. diadroma at Sta. B had a significantly higher CA than at Sta. A, which were 0.052 µmol min$^{-1}$ mg-protein$^{-1}$ at Sta. B and 0.017 µmol min$^{-1}$ mg-protein$^{-1}$ at Sta. A, t-test, p < 0.05. The CA of I. pusilla was six-fold higher at Sta. B than at Sta. A (0.320 µmol min$^{-1}$ mg-protein$^{-1}$ for Sta. B and 0.050 µmol min$^{-1}$ mg-protein$^{-1}$ for Sta. A), although a statistical test could not be performed because of small sample size. Habitat-specific changes in CAs have also been reported for I. pusilla in the Tanakagawa tidal flats (Kawaida et al. 2013). The high CAs could be attributed to the high demand for cellulose digestion at Sta. B, where the soil organic matter (SOM) was rich in refractory detritus (Kanaya et al. 2008a). It is assumed that such a spatial gradient in SOM composition is formed by the deposition of riverine suspended materials from the Idoura River into the northern part of the lagoon (Kanaya 2017).

The stable isotope ratios of the macroinvertebrates differed among feeding groups (Fig. 3). Surface-deposit feeders had the highest δ13C values, which was close to those of MPB (−16.7‰). The δ13C was the highest in S. globosa (−13.6‰), followed by M. contabulata and I. pusilla at Sta. A, and M. japonicus, T. osawai, and I. pusilla at Sta. B (−13.6 to −17.4‰). The 13C-based isotope mixing model estimated that MPB contributed to 34–50% of the assimilated diet of the surface-deposit feeders (Fig. 4). In comparison, suspension feeders were much more 13C-depleted (<−20.4‰) than the surface-deposit feeders, indicating a high dietary contribution of RPOM/detritus. They seemed to have more access to RPOM in the water column, a mixture of land-derived detritus and freshwater phytoplankton, discharged from inflowing rivers during low tides (Kamaya et al. 2008a, b).

The deep-deposit feeders Heteromastus sp. and Notomastus sp. had δ13C values (−19.9 to −20.8‰), which were inter-
mediate between the other two feeding groups. These species may assimilate degrading organic particles associated with microbes in the deeper sediment layer (Kikuchi & Wada 1996). The high \(\delta^{15}N\) value in Notomastus at Sta. B was also notable and was more than 4\(\bar{\ }\) higher than those of sympatric macroinvertebrates. Although the underlying mechanism remains unclear, one possible explanation is that it selectively assimilated \(15\) N-enriched fractions, such as microbially altered materials in the SOM pool (Macko & Estep 1984).

Previously, we inferred that estuarine deposit-feeders such as capitellid polychaetes, M. contabulata, and M. japonicus lacked CA and could use detritus-derived carbon only after it is transferred to the microbial biomass (Kanaya et al. 2008a). However, our results revealed that all of the macroinvertebrates examined exhibited CA. Niiyama and Toyohara (2011) found CA in 17 taxa of estuarine and mangrove macrobenthos, including nine gastropods, three bivalves, two polychaetes, two decapods, and one amphipod, although the activity varied among the taxa. These findings indicate that the dietary components of benthic invertebrates are altered by several eco-physiological characteristics of each taxon, including feeding habits, selectivity during ingestion, and activity of digestive enzymes. Future study is needed to examine the relationships between cellulose digestion and realized dietary components, to clarify the function of estuarine macrozoobenthos in the food web.

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