Possibility of direct utilization of seagrass and algae as main food resources by small gastropod, *Lacuna decorata*, in a subarctic lagoon, Hichirippu, eastern Hokkaido, Japan with stable isotope evidences of carbon and nitrogen

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Abstract: The small gastropod, *Lacuna decorata* Adams, living on macrophytobenthos or surface sediment, is one of the most dominant species of macrozoobenthos in Hichirippu lagoon covered with seagrass and macroalgae, eastern Hokkaido, Japan. We measured the standing stocks of primary producers and macrozoobenthos, and determined the stable carbon and nitrogen isotope ratios of the primary producers and *L. decorata*. With these results, we identify the main food items for *L. decorata* and discuss the feeding strategy of the small gastropod. This gastropod occupied about 64% in density and about 25% in biomass of the macrozoobenthos at all six sampling stations in the lagoon. It occurred densely on the surface of the sediment with dense patches of benthic microalgae (BMA), which contained extremely high levels of Chl-a between 84 to 226 mg m⁻² throughout the period of this study. Nevertheless, the stable isotope signatures of carbon and nitrogen of this gastropod clearly show the direct utilization of organic matter derived from seagrass, *Zostera japonica*, in the areas where the seagrass luxuriated. However, it shows also a flexible feeding strategy in food preference. It fed green algae such as *Ulva pertusa* and *Urospora wormskioldii* in the areas where the seagrass grew scarcely.

Key words: food resource, gastropod, green algae, *Lacuna decorata*, lagoon, seagrass, stable isotope, *Zostera japonica*

Introduction

There are many lagoons in the coastal areas of Hokkaido, subarctic areas in northern Japan, which are characterized by thickly grown seagrass (*Zostera japonica* Aschers & Graebn and *Zostera marina* Linnaeus) and macroalgae (*Ulva pertusa* Kjellman etc.) (Aioi 2005). In general, the seagrass meadows are extremely productive areas (McRoy & McMillan 1977, Duarte 1989), and provide habitats, feeding sites, refuges, etc., for various marine organisms (Adams 1976, Heck & Orth 1980). However, leaves of the vascular plants themselves are not easily exploitable for food by the animals, since few animals can digest the fibers of the vascular plants. The epiphytes and detritus attached on the leaves have been, therefore, the focus of ecological studies on the seagrass meadows as the foods for secondary production (Mann 1972, Fenchel 1977, Kikuchi 1980).

A small gastropod, *Lacuna decorata* Adams, is one of the most dominant species in the macrobenthic communities in the coastal areas of Hokkaido, but it also occurs densely on the leaves of seagrass and the thalli of macroalgae that grow thickly (Tomita & Mizushima 1984, Mizushima & Tomita 1984, Kanamori et al. 2004, Yamada et al. 2007). It is generally supposed that it utilizes the leaves of seagrass and the thalli of macroalgae as the sites to feed the epiphytes attached on them as a major part of its diet.
The description of feeding activities of the dominant species in the macrobenthic communities is important to follow the material or energy transportation from the primary producers to the primary consumers (the secondary producers) in the benthic ecosystem. However, it is often not easy to observe the feeding activities of small macrobenthic animals such as L. decorata and to identify their main food items by direct observation of feeding behaviors. For the description of food chain in the marine ecosystem, the techniques of stable isotope analysis have become popular recently, since the accumulation of empirical results on the carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) has revealed that each primary producer, such as phytoplankton, benthic microalgae, seagrass and macroalgae, has a unique range of the stable carbon isotope ratios (Fry & Sherr 1984, France 1995), animals have a slightly enriched carbon stable isotope ratio (0.8‰, $\delta^{13}C$) and $\delta^{15}N$: 3.4±1.1‰, $\delta^{15}N$) to those of their main food items, and the enrichment of nitrogen stable isotope ratio between two different trophic levels in the food chain is relatively constant ($\delta^{15}N$: 3.4±1.1‰) (DeNiro & Epstein 1978, Minagawa & Wada 1984). The determination of carbon and nitrogen isotope ratios of the small gastropod, L. decorata, also seems to be helpful for identification of main food items, particularly because it occurs in the two different habitats on the sediment of the sea floor and on the leaves of seagrass or the thalli of macroalgae.

We have studied the structure of lagoon ecosystems in eastern Hokkaido, Japan through field surveys and analysis of stable isotope signatures of carbon and nitrogen of the primary producers and macrobenthic animals. One of the primary areas, Hichirippu, is a lagoon, which is thickly covered by seagrass (mainly Z. japonica and Z. marina) and macroalgae. Here, we find typical macrobenthic communities as the lagoon ecosystem that a small gastropod, L. decorata, dominates both on the bottom sediment and on the leaves of seagrass or the thalli of macroalgae.

In this study, we carried out field surveys to describe the physico-chemical conditions of the water column and the bottom sediment, the distribution of the seagrass and macroalgae, and the faunal compositions of macrobenthic communities in Hichirippu, and determine the stable isotope signature of carbon and nitrogen of the primary producers and the macrobenthic animals in the lagoon ecosystem. The purposes of this study are to identify the main food items for L. decorata as one of the most dominant species in the macrobenthic communities in a subarctic lagoon, Hichirippu, in eastern Hokkaido, using the results of field surveys and stable isotope analysis of carbon and nitrogen, and to discuss the feeding strategy of the small gastropod.

### Materials and Methods

#### Study area

The study area, Hichirippu, is a lagoon located in the eastern part of Hokkaido, Japan (43°2’31”N, 145°1’5”E) (Fig. 1). This lagoon is semi-enclosed, and connected to the Pacific Ocean via a narrow channel. It is approximately 3.56 km² in area and shallow (mean water depth: approximately 1 m). In winter (November to March), the center to inner part of the lagoon is covered by ice. The catchment areas of this lagoon are covered mainly by reed and forest. We established six sampling stations in the subtidal zones from the mouth to the innermost part of this lagoon (Stn A~Stn F).

#### Sampling

We carried out sampling of water and sediment and quantitative sampling of macrobenthos from a boat at the six sampling stations in the lagoon bimonthly from June 2005 until April 2006 (June 19, August 15, October 17, 2005, and April 26, 2006) except in mid-winter, due to the formation of ice cover. At each station, a water sample for determination of chlorophyll $a$ (Chl.-$a$) concentration was collected from the surface and from 10 cm above the sea floor, respectively, using a motor pump, and kept in a 2 L plastic bottle. Water temperature and salinity were measured using a conductivity, temperature, and depth profiler (YSI 556, YSI Nanotech Inc.). A grab sample of the sediment was collected with an Ekman-Berge grab sampler (20 cm×20 cm). Ten core samples of the sediment were sub-sampled from a grab sample using an acrylic core tube (3 cm in diameter). The surface layers of the sediment up to 0.5 cm in depth were sliced from the core samples for determination of Chl.-$a$ content and the stable isotope signatures of carbon and nitrogen, and kept in a plastic bag. Three core samples of the sediment were also sub-sampled from a grab sample using the acrylic core tube. The surface layers of the sediment up to 5 cm in depth were sliced from the core samples for grain size analysis, and kept in a plastic bag. Grab samples for quantitative surveys of macrobenthic
animals were collected with the same Ekman-Berge grab sampler. Five sediment samples were sub-sampled from them with a handy core sampler (10 cm×10 cm×10 cm), and sieved with a 1 mm opening mesh screen. The residues of each sample on the sieve were kept in a plastic bag.

At a narrow channel near Stn F, 4 L of surface water was collected with a sampling bucket during flood tide to determine the stable isotope signatures of carbon and nitrogen of the suspended particulate organic matters (POM) in the water, and kept in a plastic bottle. At the tidal flat near Stn F, benthic microalgae (BMA) were also collected from the sediment surface following a modified Couch (1989) method for determination of the stable isotope signatures of carbon and nitrogen. The acryl boxes (30 cm×10 cm×3 cm) with precombusted glass beads (450°C, 5 h) on a 100 and 50 µm mesh screen were set in the field for one day. The motile microalgae migrated to the glass beads through the mesh screens. The BMA samples were kept in glass bottles with glass beads. We could not collect the epiphyte samples from the leaves of the seagrass, since it is too scarce on them.

Macrophytobenthos (seagrass and macroalgae) for quantitative surveys were collected with bottom sediment five times at each station, using a handy core sampler (25 cm×25 cm×10 cm), on August 15, 2005. They were sieved with a 1 mm opening mesh bag and the residues of each sample in the mesh bag were kept in a plastic bag. For stable isotope analysis, seagrass (Zostera japonica) and benthic macroalgae (Ulva pertusa Kjellman and Urospora wormskiioldii Mertens) were collected with the grab sampler on August 15, October 17, 2005, and April 26, 2006.

**Sample treatment**

For determination of Chl.-a concentration of the water, 0.4 L of the water sample was filtered with GF/F filter. Chl.-a on the filter was extracted in 90% acetone in a test tube kept in a freezer (dark conditions at −20°C) for a day. After ultrasonication treatment for five minutes, the concentration of Chl.-a in the supernatant of the test tube was determined with a fluorophotometer (Tuner 10-AU-5, Tuner designs), according to Lorenzen’s (1967) method as described by Parsons et al. (1984).

For determination of Chl.-a content of the sediment, about 0.1 g of the sediment sample was put in a test tube with 90% acetone, and the test tube was kept in the freezer for a day. The supernatant of the test tube was treated in the same manner with the extract of Chl.-a from the water sample. The water content of the sediment sample was obtained after drying at 60°C for 24 h. The Chl.-a content of the sediment was calculated from the data on the amount of Chl.-a extracted from the sediment sample and the water content. For grain size analysis of the sediment, the sediment sample was treated by the wet sieving method. For stable isotope analysis of carbon and nitrogen, the sediment sample was freeze-dried and ground into a powder with a mortar. Prior to the analysis, the samples were treated with 1 N HCl to remove inorganic carbon, rinsed with deionized and distilled water to remove the acid, and freeze-dried.

All of the macrozoobenthos were sorted from the residues of the quantitative sediment samples within three days after sampling. They were identified to species. The number of each species was counted, and the wet weight was measured after evacuation of the gut contents for 24 hours. The specimens of macrophytobenthos were sorted from the samples, identified to species, and the wet weight of each species was weighed.

For stable isotope analysis of carbon and nitrogen, all specimens of L. decorata, collected at the same station on the same sampling occasion were combined in each species to treat as a single sample, freeze-dried, and ground into a powder with a mortar. Prior to the analysis, the samples were treated with a chloroform-methanol mixture solution (2:1, v/v) for 24 h to remove lipids, filtered with a precombusted GF/F filter (450°C, 5 h), treated with 1 N HCl to remove inorganic carbon, rinsed with deionized and distilled water to remove the acid, and freeze-dried. The tissue of macroalgae and seagrass used for stable isotope analysis were sorted from the samples, rinsed with distilled water, freeze-dried, and ground into a powder with a mortar. For the analysis of POM, 4 L of the water sample was filtered with a precombusted GF/F filter (450°C, 5 h), and the residues on the filter were used for the analysis. For the analysis of BMA, the BMA adhering to glass beads was rinsed with filtered seawater and sieved with a 125 µm opening mesh screen to remove glass beads which were then filtered onto a precombusted GF/F filter (450°C, 5 h). Prior to the analysis, the filter of POM or BMA was treated with 1 N HCl to remove inorganic carbonates, rinsed with deionized and distilled water to remove the acid, and freeze-dried.

**Analysis of stable isotope ratios of carbon and nitrogen**

The stable isotope ratios of carbon and nitrogen of the samples treated for the analysis were determined with a mass spectrometer (Delta V plus, Thermo Electron) directly connected to an elemental analyzer (Flash Elemental Analyzer 1112 Series, Thermo Electron). All of the isotopic data are reported in the conventional delta notation as follows:

$$\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ (‰)}$$

where X is $^{13}$C or $^{15}$N, and R is $^{12}$C/$^{13}$C for carbon or $^{14}$N/$^{15}$N for nitrogen. Pee Dee Belemnite (PDB) for carbon and air N$_2$ for nitrogen were used as standard, respectively. The overall analytical error was within ±0.2‰.

A one-way ANOVA was performed to evaluate the differences of the Chl.-a and $\delta^{13}$C values among stations.
Results

Environmental conditions of water and sediment

Figure 2 shows the salinity and temperature of the water at Stn A, D, and F, which were represented by those of the surface water since there were no differences in the water column. The salinity of the water varied in a narrow range between 28.0 and 33.5 psu at all three stations. This indicates that there are limited sources of freshwater discharge to this lagoon. The water temperature was highest, 24.2°C, at Stn A (the innermost part of the lagoon) in June 2005, while it was more than 13°C lower at Stn D (11.1°C, the central part) and at Stn F (9.3°C, the mouth). In October, the water temperature was 14.8 to 15.6°C at all three stations. In April 2006, the lowest water temperature was recorded, but that at Stn A was 5°C higher than those at Stn D and F (4.3°C and 3.1°C, respectively). Thus, except during the cold season, the water temperature tended to be rather warmer in the innermost areas of the lagoon than in the central part and the mouth.

Figure 3 shows the grain size composition of the sediment at all six stations on June 19, 2005. The sediment in the lagoon was muddy sand with the mud content of less than 28.1% in the whole area, but the mud fraction of the sediment tended to increase toward the inner parts.

Standing stocks of Chl.-a in the water column and surface sediment

Figure 4 shows mean standing stocks of Chl.-a in the water column and surface sediment at the four sampling occasions at the six stations in the lagoon. The mean standing stock of Chl.-a of the water column varied in a narrow range between 1.8 and 2.6 mg m^{-2} at all of the six stations. Since the Chl.-a of the water column derived from the phytoplankton, there seemed to be few changes in the standing stock among these stations due to water exchange by the tidal current in the lagoon.

The mean standing stock of Chl.-a in the surface sediment at the four sampling occasions ranged between 84.2 and 225.9 mg m^{-2}, which was approximately 80 times larger than that of the water column in the same area. This high standing stock indicated that benthic microalgae luxuriated on the sediment surface widely in the lagoon.

Standing stocks of macrophytobenthos

Figure 5 shows the mean standing stock of the macrophytobenthos at the six stations in the lagoon on August 15, 2005, when the macrophytobenthos grew thickly in the year. Seagrass \((Zostera japonica)\) predominated among the macrophytobenthos in biomass at the four stations except Stn B and Stn C. The biomass of the seagrass reached 0.79 to 1.07 kgWW m^{-2} at these stations, while it did not grow at Stn B and Stn C, but green algae, \(Ulva pertusa\), was most popular in biomass at these stations (0.12 kgWW m^{-2} and 0.40 kgWW m^{-2}, respectively). Only at Stn C, filamentous green algae, \(Urospora wormskiioidii\), grew thickly (0.26 kgWW m^{-2} in biomass).
Standing stock of macrobenthic animals

Table 1 shows mean density and biomass of the dominant species of macrobenthic communities at four sampling occasions at six stations. A small gastropod, *Lacuna decorata*, was the most dominant species in the macrobenthic communities not only in density (63.7%), but also in biomass (25.1%) in spite of its small body size (approximately 2 to 3 mm in shell length). The highest density and biomass of this gastropod were recorded at Stn B, 14,065 indiv. m\(^{-2}\) and 40.5 gWW m\(^{-2}\), where only green algae, *U. pertusa*, grew thickly (Fig. 5). The second highest density and biomass, 6,245 indiv. m\(^{-2}\) and 25.2 gWW m\(^{-2}\), of this species were also noted at Stn C where the green algae grew most thickly with filamentous green algae, *U. wormskioldii*. At the other four stations (Stn A, Stn D to F) where seagrass, *Z. japonica*, luxuriated, relatively low densities, 285 and 3,495 indiv. m\(^{-2}\), were noted.

This gastropod has a unique behavior as it occurred densely not only in the sediment but also on the leaves of seagrass and the thalli of green algae (Fig. 6). In the case of the seagrass, it was hard to collect all of the individuals of the gastropod on the narrow leaves by the grab sampling. It is very likely that *L. decorata* occurred furthermore densely at the four stations.

In density, two small crustaceans, *Maera* sp. and *Nebalia* sp., were also dominant species in the macrobenthic com-
Stable isotope ratios of carbon and nitrogen

The mean $\delta^{13}C$ and $\delta^{15}N$ values of primary producers including macrophytobenthos, BMA, and POM containing phytoplankton, the bottom sediment, and the most dominant of the macrobenthic communities, L. decorata, at four sampling occasions (the values of macrophytobenthos are the mean of three sampling occasions excluding June, 2005) at the six stations.

The mean $\delta^{13}C$ values of L. decorata were divided into two groups, LA and LB. Group LA consisted of the results at the four stations, Stn A, D, E, and F, and their values were $-10.0\pm1.4\%$ (mean$\pm$S.D., n=11). Group LB has significantly lower values, $-12.2\pm0.8\%$ (n=8) (one-way ANOVA, $p<0.05$). The distributions of these two groups were corresponding to those of the different macrophytobenthos. Only seagrass, Z. japonica, luxuriated at the four stations of Group LA, while green algae grew thickly at the two stations of Group LB (Fig. 5). Because green algae, U. wormskiioidii, grew only October 2005 at Stn B, they did not appear on Fig. 5.

Furthermore, the ranges of $\delta^{13}C$ values of L. decorata of these two groups were overlapped with those of the dominant macrophytobenthos at the same stations, but were much higher than those of BMA ($-16.0\pm1.6\%$, n=4), the bottom sediment ($-18.1\pm0.6\%$, n=24), and POM ($-19.2\pm1.0\%$, n=4). Since the macrobenthic animals have a slightly enriched carbon stable isotope ratio to their main food items (DeNiro & Epstein 1978), these results indicate that L. decorata utilized mainly seagrass, Z. japonica, at the four stations in Group LA, and green algal, U. pertusa, at the two stations in Group LB as main food resources.

The mean $\delta^{15}N$ values of L. decorata, macrophytobenthos, and the bottom sediment tended to increase towards the mouth of the lagoon. However, the difference of $\delta^{13}C$ values between L. decorata and the seagrass in Group LA (Stn A, D, E and F) was $3.2\pm0.5\%$ (mean$\pm$S.D., n=4). Since the empirical results in the previous studies indicate that the enrichment of nitrogen stable isotope ratio between two different trophic levels in the food chain is $3.4\pm1.1\%$ in $\delta^{15}N$ value (Minagawa & Wada 1984), the relationship of $\delta^{15}N$ values between L. decorata and the seagrass in Group LA supports the utilization of the seagrass by L. decorata as main food resource.

The mean $\delta^{15}N$ values of the green algae, U. pertusa, were $1.0\%$ and $0.9\%$ lower than those of L. decorata in Group LB (Stn B and C, respectively). These relatively small differences in the mean $\delta^{15}N$ values indicate that L. decorata also utilized substitute food resources with rather lower $\delta^{15}N$ values than U. pertusa, which seem to be another species of green algae, U. wormskiioidii, and BMA on the surface sediment judging from their mean $\delta^{13}C$ and $\delta^{15}N$ values. However we took BMA samples at only Stn F, because of difficult to set and take the acrylic boxes at the surface sediment. In the feature, it is necessary to take the BMA samples at other stations and search the acrylic boxes at the surface sediment. In the feature, it is necessary to take the BMA samples at other stations and search the acrylic boxes at the surface sediment. In the feature, it is necessary to take the BMA samples at other stations and search the acrylic boxes at the surface sediment.
Discussion

In the study area, Hichirippu, a small gastropod, *Lacuna decorata*, was the most dominant species in the benthic communities both in density and biomass (Table 1). It occurred densely in the sediment, and the extremely high Chl.-*a* content of the surface sediment over 100 mg m\(^{-2}\) (Fig. 4 (b)) indicates the presence of dense patches of benthic microalgae (BMA) on the surface sediment. However, the stable isotope signatures of carbon and nitrogen of this gastropod clearly show the direct utilization of organic matter derived from seagrass, *Zostera japonica*, or green algae, *Ulva pertusa* and *Urospora wormskiioldii*, in the areas where they luxuriated on the sediment (Fig. 7).

The occurrence of this species on the surfgrass, *Phyllospadix iwatensis* Makino, and brown alga, *Cystoseira hakodatensis* (Yendo) Fensholt, was reported on the subtidal rocky shore of Akkeshi Bay, in eastern Hokkaido, Japan (Kanamori et al. 2004). However, these macrophytebenthos were recognized simply as settling sites and habitats. The results of this study indicate that the seagrass and the algae themselves are not only the feeding sites but also ones of the most favorable food resources.

The main food resource of the other species classified in the same genus of the gastropod (*Lacuna variegata* Capen-ter, *Lacuna vincta* Montagu) were also studied in each seagrass or macroalgae community based on stable carbon isotope ratio or combined with fatty acid analysis (McConnaughey & McRoy 1979, Frenfriksen 2003, Jaschinski et al. 2008). Their main food resource was reported to be surfgrass (McConnaughey & McRoy 1979) or epiphyte (Jaschinski et al. 2008) in the kelp forest. Especially, *L. vincta* can be seen grazing on holes and scars in the kelp lamina and is consider to graze directly on the living tissue of macrophytes (Frenfriksen 2003).

To enable *L. decorata* to digest the seagrass, it must have a digestive enzyme to decompose cellulose such as cellulase. Although the presence of such a digestive enzyme has not been found from *L. decorata* yet, it is not extremely rare. However, some invertebrates have been reported to have a cellulase. In particularly, endogenous cellulase genes have been cloned from a common Japanese brackish water clam, *Corbicula japonica* (Sakamoto et al. 2007), abalone (Suzuki et al. 2003), and mussel (Xu et al. 2001), proving direct digestion of cellulose by those aquatic invertebrates. Three species of estuarine gastropods were also reported to have cellulase activity (Antonio et al. 2010). It is very likely that the presence of a digestive enzyme will be confirmed from *L. decorata* in the near future.

Another noteworthy characteristic of the feeding strategy of *L. decorata* is its flexibility in food preference. The results of stable isotope signatures of carbon and nitrogen in this study revealed that this gastropod could utilize variety of primary producers in the lagoon including seagrass, green algae, brown algae, and BMA (Fig. 5, 7). Kanamori et al. (2004) reported that co-occurring *Lacuna uchidai* showed significant preference for the surfgrass, *Phyllospadix iwatensis*, whereas *L. decorata* had no preference for a particular substratum, and appeared in both *Phyllospadix* and *Cystoseira* beds. Such flexibility in food preference seems to enable *L. decorata* to have a wide distribution in the lagoon (eg. Hichirippi) with a variety of primary producers.

This study described the living conditions of *L. decorata* as the most dominant species in the macrobenthic communities in the subarctic lagoon, using the field surveys and analysis of stable isotope signatures of carbon and nitrogen. For further deeper understanding of how this species utilize the environment and ecosystem in the subarctic lagoon, the population dynamics of this species need to be followed not only on the sea floor but also on the seagrass and algae at the same time. An enzymological approach to this species will be also required to clarify the flexible feeding strategy.

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References


