Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of epilithic microalgae in Gokasho Bay

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Abstract: Glass plates were immersed in the surface layer (1 m) in Gokasho Bay during a 7 day period every month over an annual cycle to allow periodic collection of epilithic microalgae and to analyze their year-round stable carbon and nitrogen isotope ratios. The bulk of the attached material was diatoms throughout the year. The $\delta^{13}C$ and $\delta^{15}N$ of epilithic microalgae varied seasonally, ranging from $-22.0$ to $-14.6\%$ and from $3.6$ to $10.2\%$, respectively. The $\delta^{13}C$ increased during a period from August to November when large microagal biomasses were found, while the $\delta^{15}N$ decreased after November through January in the next year. Such seasonal variations are discussed from the viewpoints of the algal growth and ambient nutrient concentrations. Comparison of the $\delta^{13}C$ (yearly mean $= -19.8\%$) and $\delta^{15}N$ (7.7%) for epilithic microalgae with the previously reported values for primary producers and organic matter in Gokasho Bay shows that the overlap of the $\delta^{13}C$ with marine phytoplankton ($-20.4\%$), which will result in poor resolution of sources.

Key words: attached diatom, benthic microalgae, epilithon, seasonal cycle, stable isotope

Introduction

Benthic microalgae are an important component in the estuarine and coastal waters as one of major primary producers to fuel ecosystems. The term benthic microalgae includes epiphyton growing attached to other plants, epipelon growing on sediments, epilithon growing attached to rock surfaces, etc. (Round 1971). Among them, epilithon (epilithic microalgae) have been shown to be a food source for many herbivores inhabiting intertidal rock shores and subtidal stony seabeds (e.g. Steneck & Watling 1982).

Stable carbon and nitrogen analyses have been successfully used to determine trophic links from primary producers to higher trophic levels in various ecosystems (reviewed by Peterson & Fry 1987). The analyses, however, are possible only when a few primary producers are important and those sources have distinct isotopic signatures. In order to trace the flow of epilithic microagal organic matter, it is necessary to know the isotopic compositions of these algae.

It has been reported that there are large seasonal or temporal variations in the $\delta^{13}C$ and $\delta^{15}N$ for marine and estuarine phytoplankton (Gearing et al. 1984, Cifuentes et al. 1988, Goering et al. 1990, Fogel et al. 1992, Nakatsuka et al. 1992, Rolff 2000, Vizzini & Mazzola 2003). The variability of $\delta^{13}C$ in phytoplankton has been discussed in relation to the dissolved CO$_2$ concentration (Rau et al. 1989, Fry 1996), the kind of dissolved inorganic carbon (DIC) substrate (i.e. CO$_2$, HCO$_3^-$) used by phytoplankton for photosynthesis (Fogel et al. 1992), the taxon and size of phytoplankton (e.g. diatoms, flagellates) (Gearing et al. 1984, Fogel et al. 1992) and the growth rate of each phytoplankton species (Cifuentes et al. 1988, Fry and Wainright 1991, Nakatsuka et al. 1992). On the other hand, it has been suggested that algal $\delta^{15}N$ depends on the isotopic fractionation during the assimilation of ambient inorganic nitrogen as well as on the isotopic composition of the inorganic nitrogen (Mariotti et al. 1984, Macko et al. 1987, Goering et al. 1990, Wada & Hattori 1991). That is, under limited nutrient conditions, isotopic fractionation during assimilation of dissolved inorganic nitrogen by algae is small resulting in enriched $\delta^{15}N$ for phytoplankton; on the contrary, when the nutrients are in excess of algal need, large isotopic fractionation occur resulting in reduced $\delta^{15}N$.

Extensive data exist on isotopic compositions of coastal phytoplankton, seaweeds, seagrasses, epipelagic microalgae, salt marsh plants and terrestrial organic matter (e.g. Maksymowska et al. 2000), however, there is much less information about the isotopic compositions of epilithic microalgae. The present paper offers a year-round data set on stable carbon and nitrogen isotope ratios ($\delta^{13}C$, $\delta^{15}N$) for epilithic
microalgae and the observed variability is briefly discussed from viewpoints of the algal growth and environments.

**Materials and Methods**

Ground glass plates (18×18×1 cm) were used as artificial substrata to collect epilithic microalgae throughout the year, because it is difficult to sample microalgae consistently from intertidal rock shores or subtidal stony bottoms due to the large grazing pressures by herbivores inhabiting there. Glass plates were maintained at a depth of 1 m from the sea surface for a period of 7 days each month using an anchor buoy (Fig. 1). Based on Tanaka (1987) who described that the almost exclusive occurrence of attached diatoms on an artificial substratum was attained after 3 to 7 days immersion, the duration was determined from the viewpoint that diatoms could be collected exclusively as much as possible. Samples were collected monthly from two stations, Stn 1 (water depth = 16 m) and Stn 2 (23 m), which were located at the inner and seaward parts in Gokasho Bay (Fig. 2), from May 2001 to April 2002. The two stations were located ≥250 m from the shore and ≥15 m above the seabed, of which distances are supposed to prevent the migration of grazers to the glass plates. Surface mean water temperature during the immersion period measured at the central part of Gokasho Bay ranged from 11.8°C in February 2002 to 28.4°C in August 2001 (National Research Institute of Aquaculture, in press).

In the laboratory, attached substances were scraped from the glass surface by hand with rubber gloves on and washed into a beaker with filtered seawater. The suspended mixture was passed through a 0.1-mm-mesh net to remove any animals and debris, and then divided into two subsamples (20 and 80% in volume), which served for the microscopic analysis of the species composition and cell density and for the measurements of the total organic carbon and total nitrogen contents and stable isotope compositions. The latter subsamples (80%) were filtered on a precombusted Whatman GF/F glass fiber filter, washed with 1.2 N HCl to remove carbonates, rinsed with distilled water, and dried at 60°C for 24 hrs. The 13C and 15N compositions of the samples were determined using a mass spectrometer (MAT 252, Finnigan MAT) coupled online, via a Finnigan ConFlo II interface, with an elemental analyzer (EA 1110, ThermoQuest Italia). Carbon and nitrogen contents were also determined with the elemental analyzer.

**Results**

Microscopic observation showed that the substances consisted exclusively of attached diatoms. *Nitzschia* spp., *Cylindrotheca closterium*, *Navicula* spp., *Licmophora* spp. including *L. abbreviata* and *L. flabellata*, and *Bacillaria paradoxa*, which have been reported to appear at early stages in the algal succession (e.g. Suzuki et al. 1987), occurred as dominant or subdominant species on the glass plates (Fig. 3). Diatoms including *Amphora* spp. *Fragilaria fasciculata*, *Thalassionema nitzschioides*, *Entomoneis* sp., *Cocconeis* spp. *Grammatophora* sp. *Thalassiosira* sp. *Pleurosira* spp. *Achnanthes brevipes v. intermedia*, *Bleakeleya notata* occurred as subordinate species. *Nitzschia* sp. and
Cy. closterium predominated generally during spring and summer, while Licmophora spp. predominated during autumn and winter. Seasonal trends in the species composition at the two stations were similar excluding for the periods during June and July when Nitzschia spp. predominated at Stn 2 whereas at Stn 1 other diatoms such as Cy. closterium occurred at high composition rates and for the periods during February and March when Navicula spp. dominated at Stn 1 whereas at Stn 2 Licmophora abbreviata dominated.

Density of algal cells, total organic carbon (TOC) and total nitrogen (TN) of microalgae attached on the glass plates are shown in Fig. 4. From May to August, these parameters remained at low levels, ranging from 89 to 460 cells cm$^{-2}$, from 0.06 to 0.25 mg C cm$^{-2}$ and from 0.01 to 0.05 mg N cm$^{-2}$, respectively. After August, values of these parameters increased rapidly and a large amount of organic matter (700–8,040 cells cm$^{-2}$, 0.81–2.45 mg C cm$^{-2}$, 0.16–0.43 mg N cm$^{-2}$) was found from September through November. Thereafter, the algal production decreased to the level before August.

The $\delta^{13}$C of epilithic microalgae varied seasonally (Fig. 5a). At both stations, the $\delta^{13}$C remained at low levels during a period from May to July (−22.0 to −20.7‰). After July, the $\delta^{13}$C increased rapidly and attained a maximum of −14.6‰ at Stn 2 in September and a maximum of −17.7‰ at Stn 1 in November. The microalgal $\delta^{13}$C at both stations decreased after November. From December to April the values were within the range of −21.3 to −18.2‰. The yearly mean (±SD) of all samples of epilithic microalgae was −19.8±1.7‰ (n=24).

The $\delta^{15}$N of epilithic microalgae also varied seasonally (Fig. 5b). From May through September epilithic microal-
gae had relatively constant $\delta^{15}$N values, ranging from 8.2 to 9.2% at Stn 1 and from 7.3 to 8.2% at Stn 2. After November, the algal $\delta^{15}$N at both stations decreased, and in January in the next year, minimum values, 5.8% at Stn 1 and 3.6% at Stn 2 were found. Thereafter, the $\delta^{15}$N increased and reached a maximum of 10.2% at Stn 1 in April and 9.1% at Stn 2 in March. The yearly mean ($\pm$SD) of all samples of epilithic microalgae was $7.7 \pm 1.4\%$ ($n=24$).

**Discussion**

During 7 day periods, substances containing a maximum of 2.5 mg C/cm$^2$ were found to attach on the glass plates. Taking into account that there was a significant relationship between the density of algal cells and the carbon content in the attached substances ($r^2=0.496$, $P<0.001$, $n=24$), it is probable that the major part of the substances was derived from microalgae, although we could not show the ratio between algae and other possible organic matter such as bacteria, mucus and detritus.

In the present study, we found a significant positive relationship between the carbon content of substances attached on the glass plates during 7 day periods and the DIC concentration. The observed relationship seems to be similar to the findings that enriched $\delta^{13}$C values for phytoplankton are usually attained during the bloom season (e.g. Goering et al. 1990). Theoretical models and experimental evidence have suggested that photosynthetic activity for a given growth period and the DIC concentration are the most important determinants of the $\delta^{13}$C of marine and freshwater phytoplankton (Takahashi et al. 1991, Nakatsuka et al. 1992, Gervais & Riebesell 2001). That is, when the carbon demand from the algae is large, discrimination against $^{13}$C will reduce, resulting in having enriched $\delta^{13}$C values. It is possible that the similar kinetic isotopic effects which have been found in phytoplankton also control the $\delta^{13}$C values of epilithic microalgae. In addition to this, the thickness of the algal layer on the glass plate might also explain the $\delta^{13}$C of the epilithic microalgae. As the thickness of the algal layer increases, diffusive transport of DIC into algal cells might decrease, resulting in the reduction of isotopic fractionation, which leads to enriched $\delta^{13}$C signatures.

In the present study, we found a decrease in the $\delta^{15}$N for epilithic microalgae in late autumn and early winter. There was no significant relationship between the algal biomass and the $\delta^{15}$N ($r^2=0.001$, $P=0.90$), suggesting that the microalgal $\delta^{15}$N was not affected by the growth rate of microalgae. In Gokasho Bay, inorganic nitrogen concentrations in the surface layer remained at low levels from spring through early autumn due to active primary production and the formation of thermal stratification, whereas after mid autumn the levels of nutrients recover (T. Sakami et al. unpubl. data). In a microalgal culture, in which the concentration of inorganic nitrogen was in excess of microalgal need, isotopic fractionation occurred during the nitrogen assimilation (Macko et al. 1987). A similar result was found in phytoplankton from the North Atlantic (Altabete et al. 1991) and from the Delaware estuary (Cifuentes et al. 1988). It is possible that the observed low $\delta^{15}$N values of the epilithic microalgae in Gokasho Bay resulted from large isotopic fractionation that occurred in nutrient-rich waters during the period of vertical mixing of water and low biomass of phytoplankton. On the other hand, there is a possibility that the variability of the $\delta^{15}$N for epilithic microalgae depends on the isotopic composition of ambient inorganic nitrogen (e.g. Goering et al. 1990), however, we cannot confirm this due to the lack of information on the $\delta^{15}$N of dissolved nitrogen.

The major organic matter sources for consumers in Gokasho Bay seems to include terrestrial plant material that flows into the bay in the form of the riverine particulate organic matter (POM), marine phytoplankton that were sampled as POM in seawater, seaweeds, epipelic microalgae on the mudflats and epilithic microalgae. The isotopic compositions of the former four kinds of organic matter were noted in Yokoyama & Ishihi (2003) (Table 1). The riverine POM has the most reduced $\delta^{13}$C and $\delta^{15}$N values (mean $=-26.5\%$ and $0.5\%$, respectively), which allow dis-
Table 1. Isotopic compositions of the organic matter source in Gokasho Bay.

<table>
<thead>
<tr>
<th>Organic matter</th>
<th>$\delta^{13}$C (%)</th>
<th>$\delta^{15}$N (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riverine POM$^a$</td>
<td>$-27.3$ to $-24.3$</td>
<td>$-26.5 \pm 0.8$</td>
<td>12</td>
</tr>
<tr>
<td>Marine phytoplankton$^a$</td>
<td>$-21.9$ to $-18.5$</td>
<td>$-20.4 \pm 0.9$</td>
<td>12</td>
</tr>
<tr>
<td>Seaweeds$^b$</td>
<td>$-22.6$ to $-8.8$</td>
<td>$-15.0 \pm 2.9$</td>
<td>129</td>
</tr>
<tr>
<td>Epipelic microalgae$^c$</td>
<td>$-19.5$ to $-10.3$</td>
<td>$-14.7 \pm 2.5$</td>
<td>13</td>
</tr>
<tr>
<td>Epilithic microalgae$^c$</td>
<td>$-22.0$ to $-14.6$</td>
<td>$-19.8 \pm 1.7$</td>
<td>24</td>
</tr>
</tbody>
</table>

$^a$ Based on Yokoyama & Ishihi 2003
$^b$ Data based on 7 Sargassum species (Ishihi et al. unpubl.); noted in Yokoyama & Ishihi (2003).
$^c$ This study

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