Identification of the Food Sources-Metabolism of the Pacific Oyster *Crassostrea gigas* using Carbon and Nitrogen Stable Isotopic Ratios

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Abstract - In order to understand food sources-metabolism for the pacific oyster (*Crassostrea gigas*), the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of its gut, gill, and muscle as well as potential food sources (particulate organic matter, sedimentary organic matter, benthic microalgae, seagrass detritus) were determined in Dongdae Bay. Average $\delta^{13}C$ and $\delta^{15}N$ values reflect that oysters primarily fed on sedimentary organic matter as opposed to suspended organic matter during summer and winter seasons. However, the relatively enriched ^{15}N values of particulate organic matter ($>$250 μm) and sedimentary organic matter in the summer may be due to the photosynthetic incorporation of ^{15}N-enriched nitrogen (DIN) or the spawning events of bivalves. Specific oyster tissues (gut, gill, and muscle) revealed different metabolic pathways, which were determined through analysis of $\delta^{13}C$ and $\delta^{15}N$ in each organ. The present results suggest the determination of carbon and nitrogen stable isotopes to be a useful approach in ecological research related to the food sources- metabolism of *Crassostrea gigas*.

Key words : Dongdae Bay, stable isotope ratios, *Crassostrea gigas*, food source

INTRODUCTION

Oysters (Mollusca: Bivalvia: Pteioida: Ostreidae) can be divided into three genus (*Crassostrea*, *Ostrea*, *Pycnodonta*) and more than 100 species, with a worldwide distribution ranging from the tropical regions to the cold latitudes (Ranson 1950; 1960). Pacific oysters (*Crassostrea gigas*), suminoe oysters (*Crassostrea ariakensis*), iwagaki oysters (*Crassostrea nippona*), and flat oysters (*Ostrea denselamellosa*) make up the dominant species among nine oyster species reported in Korea (Kwon *et al.* 1993).

Crassostrea gigas plays a large role in Korea as a principal oyster export (Rana 1998) and has become an important species for farming throughout the world (Ventilla 1984; Kusaki 1991; Kobayashi *et al.* 1997; Hyun *et al.* 2001). Oysters, invertebrates that filter-feed large volumes of ambient water, have been investigated as bioindicators of the water quality in their surrounding environment by numerous researchers (Boesch and Rosenberg 1981; Clark and Warwick 1994; Park and Yi 2002). However, energy flow patterns among primary producers and macrobenthos remain unclear in estuarine and coastal environments.

Stable isotope ratios of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) have traditionally been used to trace energy flow in the food web as well as the assimilation of nutrient and organic matter sources (Hobson and Welch 1992; Hansson *et al.* 1997). In general, the $\delta^{13}C$ value of an organism reflects the $\delta^{13}C$ value of its diet with little to no change (Fry and Sherr 1984), and the $\delta^{15}N$ value of an organism increases an average of 3～4‰ with each raise in trophic level (Minagawa and Wada 1984). This nitrogen enrichment is due to the excretion of ^{15}N-depleted nitrogen (Peterson and Fry, 1987).

Additionally, the effects of human-derived waste or efflu-

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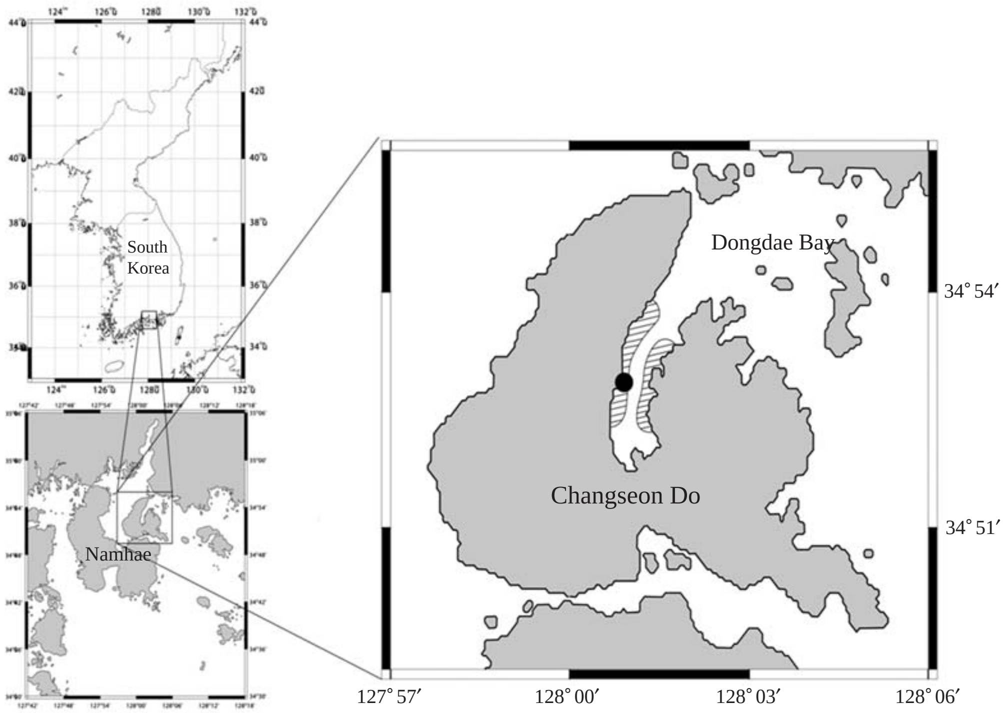

Fig. 1. Sampling location in Dongdae Bay (●).

ent were observed via anomalous increases in δ^{15}N value (McKinney *et al*. 2002) and variations of δ^{13}C were dependant on the turnover rates of metabolic tissue related to the health of the organism (Gannes *et al.* 1997).

The objective of this study was to identify potential energy sources and to assess seasonal variation among *C. gigas* food sources-metabolism using carbon and nitrogen stable isotopic ratios.

MATERIALS AND METHODS

1. Study area and sample collection

Particulate organic matter (POM), sedimentary organic matter (SOM), seagrass detritus, benthic microalgae (BM), and oysters (*C. gigas*) were sampled monthly during the winter and summer seasons (November 2004～March 2005 and June 2005～September 2005, respectively). The sampling site (34° 52′57′′N × 128° 1′2′′E) is located at Dongdae Bay on the southern coast of Korea (Fig. 2). The bay is semi-closed, 5 km in length, 1 km in width, and has extensive seagrass beds. It has a fluctuating water depth of 3～12 m at high tide, with some part of the seagrass bed exposed to the atmosphere at low tide. *C. gigas* is a dominant filter feeder in the bay.

A method modified by Couch (1989) was used to separate benthic microalgae from sediments, with a more detailed procedure described by Riera and Richard (1996). The sediment, which was collected by scraping the upper 2 mm of dense microalgal mats, was spread under a light and covered with combusted silica powder (60～120 μm) on a nylon screen (63-μm mesh).

Oysters were taken by hand, cleaned, and kept alive overnight at the laboratory in filtered seawater to allow the evacuation of gut contents. They were then removed from their shells, rinsed in distilled water, and dissected into three types of tissue (adductor muscle, gill, and gut) for tissue specific analysis.

Samples of POM were filtered onto pre-combusted glass fibre filter papers (GF/F) for 4 hours at 450°C. The summer season POM sample was divided into three size fractions (＜30 μm, ＞30 μm, and ＞250 μm) using zoo- and phytoplankton nets to verify the chemical specificity among the different sizes of POM.

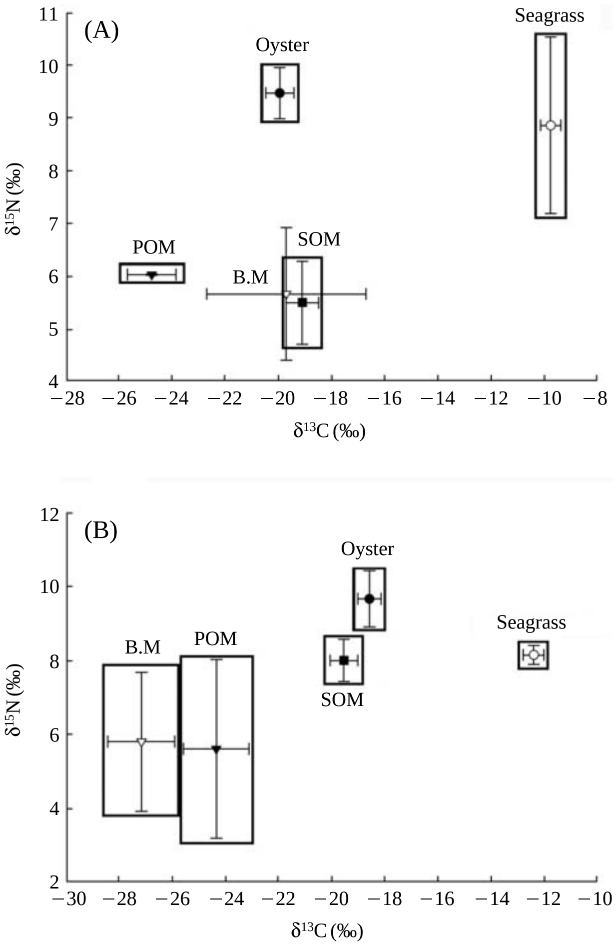

Fig. 2. Carbon and nitrogen stable isotope values for oysters and primary producers in Dongdae Bay during the winter (November 2004～March 2005, A) and summer seasons (June 2005～September 2005, B).

2. Organic carbon and total nitrogen stable isotope analysis

Freeze-dried and homogenized samples were treated with 1 N HCl to remove carbonates, and then placed into tin cups. Samples were not acidified for nitrogen isotope ratio analysis because acidification could significantly influence $\delta^{15}N$ values (Carabel *et al.* 2006). The samples were analyzed for organic carbon and nitrogen isotope ratios using an elemental analyzer (Costech ECS4010) combined with mass spectrometer (Delta plus, Finnigan MAT). All isotopic results are provided in conventional delta (δ) notation in units of parts per thousands (‰) and the standards for $\delta^{13}C$ and $\delta^{15}N$ are Pee Dee Belemnite and atmospheric nitrogen, respectively:

$$\delta^{13}C \text{ or } \delta^{15}N = \{R\,(\text{sample})/R\,(\text{standard}) - 1\} \times 1000\ (‰)$$

where

$$R = {}^{13}C/{}^{12}C,\ {}^{15}N/{}^{14}N.$$

RESULTS AND DISCUSSION

1. Seasonal isotopic variation of potential food sources and oysters

The average stable isotope values of oysters and potential food sources (POM, SOM, BM, seagrass) were plotted during the winter and summer seasons (Fig. 2A and 2B). There were distinctive differences between the stable isotope ratios of the winter and summer seasons, indicating significant seasonal variation of environmental conditions in the bay. The average $\delta^{13}C$ values of POM ranged from −26 to −23 ‰, reflecting the mixed contributions of isotopically lighter terrigenous organic matter and isotopically heavier phytoplanktonic organic matter to the estuarine POM pool (Fichez *et al.* 1993).

On the other hand, seagrass detritus demonstrated consistently heavier $\delta^{13}C$ values (−12 to −10‰ range) that are typically observed in C_4 plants due to the different photosynthetic pathways in C_3 plants (Ehleringer 1991). In contrast to the seagrass detritus $\delta^{13}C$ values, $\delta^{15}N$ variation in seagrass detritus during the winter season was larger than the summer season (Fig. 2). Variation in $\delta^{15}N$ values of seagrass detritus was caused by nitrogen isotopic fractionation during microbial decomposition, which included deamination once seagrass withered during the winter season (Danovaro 1996).

In the winter season, $\delta^{13}C$ and $\delta^{15}N$ values of SOM were similar to the isotopic range of benthic microalgae, demonstrating that benthic microalgae significantly contributed to surface sediment organic matter. Indeed, primary production of benthic microalgae greatly exceeded the production of phytoplankton in shallow aquatic environments including estuarine tidal flats (Knox 1987; Fielding *et al.* 1988; Pinckney and Zingmark 1993). Oysters demonstrated similar $\delta^{13}C$ values to SOM, but they experienced a 4‰ rise in $\delta^{15}N$ values (Fig. 2A). In the winter season, oysters preferentially consumed resuspended surface sedimentary organic matter, consisting mainly of benthic microalgae, over suspended particulate organic matter. Oysters as filter feeders could accelerate the utilization of sedimentary organic matter resuspended by wind and tide in estuarine tidal flats (Heral *et al.* 1983).

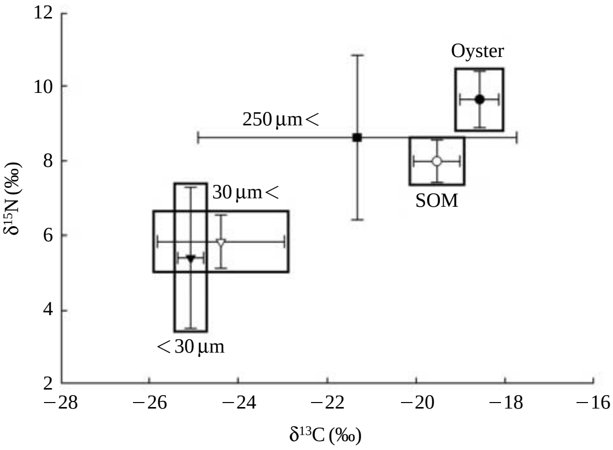

Fig. 3. Carbon and nitrogen stable isotope values for oysters, SOM, and size-fractionated POM during the summer season.

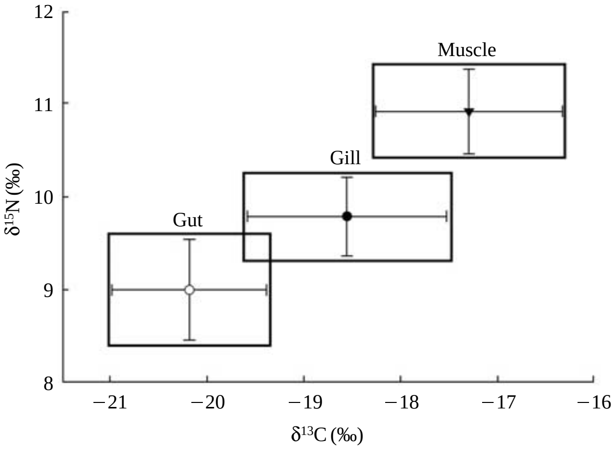

Fig. 4. Average (±SD, n=65) carbon and nitrogen stable isotope values for oyster tissues collected over 12 months.

Increased SOM $\delta^{15}N$ values in the summer may be due to the contributions of organic matter, which include enriched $\delta^{15}N$ values derived from ammonium and oxidized nitrogen in wastewater (Fig. 2B). McClelland *et al.* (1997) have reported that nutrients derived from wastewater could be reflected in phyto- and zooplankton, benthos, and sediments via photosynthesis, aggregation, and deposition in the estuarine food chain.

To determine factors contributing to surface sedimentary organic matter, the $\delta^{13}C$ and $\delta^{15}N$ values of three size-fractionated POM during the summer period are provided in Fig. 3. POM larger than 250 µm had distinctive $\delta^{13}C$ and $\delta^{15}N$ values when compared to POM smaller than 250 µm, which exhibited relatively similar values with SOM in the summer period. Although the isotopic distinction of SOM in the summer can be explained by various possibilities, the surface sediments can be significantly influenced by larvae originating from the spawning of bivalves such as oysters and scallops. Typically, the heavier isotope ratios (e.g. ^{13}C or ^{15}N) in the proteins of organisms are enriched through isotopic fractionation in the deamination and transamination processes, compared to those in lipids (DeNiro *et al.* 1981; Hobson *et al.* 1993). Considering that the eggs of bivalves consist primarily of protein (Marcelo *et al.* 2003), it is highly probable that larvae have anomalously heavier stable isotope values. Mature oysters spawn at a temperature ranging from 21 to 26°C and the fertilized eggs grow to a larvae size of 270～350 µm within a few weeks (Nelson *et al.*, 1928; Breese *et al.*, 1975). According to the National Oceanographic Research Institute of Korea database (http://www.nori.go.kr/), water temperature averages in the study area are 10.5±4.05 °C and 23.2±1.60°C for the winter and summer seasons, respectively. It is possible that bivalve larvae mainly affect POM larger than 250 µm. SOM in the summer appears to have heavier $\delta^{15}N$ values than in the winter because of potential influence by the supplement of ^{15}N-enriched organic matter via aggregation and sedimentation of large POM such as larvae.

In summer, oysters exhibited similar $\delta^{13}C$ values to SOM, whereas $\delta^{15}N$ values of oyster demonstrated a 2～4‰ difference between SOM in the summer season and the winter season. The difference in nitrogen isotopic values between oyster and SOM may be caused by the release of eggs containing relatively ^{15}N-enriched protein during spawning periods, as discussed previously.

2. Fractionation of internal specific tissues in oysters

Carbon and nitrogen stable isotope ratios of specific tissues in organisms have been applied to understand different energy metabolisms as well as to trace the incorporation of nutrients over various temporal and spatial scales (Riera 1998; Piola *et al.* 2006). Results for the specific tissues (gut, gill, and muscle) in oysters during the study period indicated different isotopic fractionation among the three organs (Fig. 4). The lighter isotopes (e.g. ^{12}C or ^{14}N) tend to react more than the heavier isotopes (e.g. ^{13}C or ^{15}N) as a result of weaker coherence (Hoefs, 1980). From analysis of $\delta^{13}C$ values (～−19‰) for SOM, which may have been consumed by oysters during this study period, it could be speculated

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that carbon and nitrogen became isotopically heavier through selective elimination of the lighter isotope (e.g. ^{12}C or ^{14}N) during metabolism via food digestion and the resorbed energy pathway. In this study, the heaviest isotopic values of the muscle demonstrated that the muscle may be the last organ to resorb food-derived carbon and nitrogen in the oyster. The same results have been reported for the Sydney rock oyster, *Saccostrea glomerata* (Piola *et al.* 2006), and several fish species (Gaston 2004).

The digestive pathway in oysters supports the idea that the stable isotope ratios of the gut and gill reflect the ingesta for a short period of time. However, the mature oyster gut is comprised of the gonad and digestive systems, and the oysters' biochemical state (physiological conditions and spawning events) may substantially affect the carbon and nitrogen stable isotope ratios of the gut (Beesley *et al.* 1998; Piola *et al.* 2006). As a result, oysters have demonstrated potential as bio-indicators for aquatic environments, with the gill and muscle in oysters as appropriate parts for the assessment of food ingestion over short- and long-term scales, respectively.

CONCLUSION

In this study, food sources for *C. gigas* were investigated using carbon and nitrogen isotope signatures, thus showing that the oysters selectively assimilated sedimentary organic matter. Also, this study suggests that specific tissues in oysters may be useful as bio-indicators in environmental monitoring research over diverse time scales.

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