Isotopic shifts with size, culture habitat, and enrichment between the diet and tissues of the Japanese scallop *Mizuhopecten yessoensis* (Jay, 1857)

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Abstract

Use of stable isotope signatures to trace diet patterns in cultured marine bivalves, particularly when changing culture habitat, requires knowledge of the isotopic shift and enrichment between diet and consumer's tissues. The aim of this study was to determine the patterns of isotope change and the variability of enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) in different tissues (muscle, gonad, digestive gland) of the Japanese scallop (*Mizuhopecten yessoensis*). It was hypothesized that the isotopic signatures of a consumer's tissues changed during settlement, and that the changes were related to variations in the isotopic signatures of food sources and gut contents. Particular attention was paid to the isotope enrichment between the diet and a consumer's tissues using isotope analysis of gut content. Muscle $\delta^{15}N$ values decreased significantly 3-5 months post-settlement in a nearshore seabed, concomitant with the ingestion of lower $\delta^{15}N$ food. For juvenile scallops, sinking particles (SP) were considered a more important dietary source than suspended particulate organic matter (SPOM), based on the correspondence between SP and gut contents $\delta^{13}C$. Enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) varied with tissue and season. $\Delta\delta^{15}N$ was 2.4‰ in muscle, 1.2‰ in gonad, and 0.7‰ in the digestive gland. $\Delta\delta^{13}C$ was 3.2‰ in muscle, 2.3‰ in gonad, and -0.5‰ in the digestive gland. $\Delta\delta^{15}N$ was the lowest in summer (0.3‰) and $\Delta\delta^{13}C$ was the highest in autumn (2.8‰). $\Delta\delta^{15}N$ values were significantly influenced by age, but not $\Delta\delta^{13}C$. Patterns of isotope ratios and enrichment values may be related to physiological attributes and differences in diet. This is the first study to demonstrate isotopic shift and enrichment encountered in different tissues of a cultured scallop when changing culture habitat.

Keywords: Enrichment values, *Mizuhopecten yessoensis*, shell height; sinking particles, stable carbon and nitrogen isotopes

Introduction

Abundances of heavier stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) are increasingly being used to reconstruct diet patterns in animal species (Raikow and Hamilton 2001; Lorrain et al. 2002; Page and Lastra 2003; Yokoyama and Ishihi 2006; Aya and Kudo 2007). Stable carbon isotopic ratios of an

animal tissue are generally considered to be a little larger (about 1‰) than that of the diet through fractionation of isotopes (DeNiro and Epstein 1978). $\delta^{15}N$ of a consumer is enriched by 3-4‰ and can be used to determine the trophic position in a food web and therefore to identify predators and prey (Minagawa and Wada 1984; Post et al. 2002). Here, $\delta^{13}C$ and $\delta^{15}N$ were used to determine the diet of the Japanese scallop (*Mizuhopecten yessoensis*) in a two dimensional analysis.

Mizuhopecten yessoensis (Jay, 1857) is a commercially important temperate scallop cultured in the coastal areas of northern Japan. It is reared using the hanging method in a shallow estuarine lagoon (Lake Saroma) and by sowing or bottom culture in a nearshore seabed (Tokoro, Sea of Okhotsk). Scallops consume mainly phytoplankton (Shumway et al. 1987, 1997; Lorrain et al. 2002) and detritus in the form of sinking particles (Cranford and Grant 1990). Lake Saroma and Tokoro, in the Sea of Okhotsk, are only 5 km apart, but the two culture sites differed in the food source composition (Aya and Kudo 2007). Scallops reared at Lake Saroma feed on both suspended particulate organic matter (SPOM) and plankton, whereas scallops cultured at Tokoro, Sea of Okhotsk, feed only on SPOM (Aya and Kudo 2007).

Juvenile scallops, originating from estuarine lagoons, are introduced to nearshore seabeds. This practice allows us to conduct isotope tracking of scallops according to season. Seasonal changes in the carbon and nitrogen isotope signatures have been reported for age-0 smallmouth bass (Vander Zanden et al. 1998) and in other bivalve species (Kang et al. 1999; Rossi et al. 2004; Howard et al. 2005). It is important to properly examine the time required for a particular consumer tissue to approach the isotopic signature of its recent diet for isotopic shift in a consumer's tissues (Marín Leal et al. 2008). This approach would describe whether the patterns of isotope change are related to a change in culture habitat, resource-use patterns, developmental stages, or seasons.

Estimating isotope enrichment (i.e., the difference between isotope values in the diet and those in a particular tissue or entire animal) is necessary to interpret stable isotope patterns and to estimate the contributions of food sources to a consumer's diet (Gannes et al. 1997; Roth and Hobson 2000; Dubois et al. 2007; Deudero et al. 2009). Enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) have been evaluated experimentally, in which the diet is fully controlled (in quantity, quality and isotopic values); the enrichment factor is calculated when steady state is achieved. Here, we used the isotopic analysis of gut contents, which has been used in other studies (Page and Lastra 2003; Jardine et al. 2005; Fukumori et al. 2008; Guelinckx et al. 2006, 2008) to estimate enrichment values based on two assumptions: (1) The gut content is representative of the diet for only a short period (a few hours), whereas tissues integrate the isotope value of the food sources for several days (digestive gland) to several months (muscle); and (2) The gut content is not digested by scallops. This approach can provide not only direct information on the diet of consumers (Grey et al. 2002), but also information on the mechanisms regulating isotope enrichment (Guelinckx et al. 2008). Several studies have reported enrichment values mostly from a single size or age class of a marine invertebrate species. No data are available on the variability of

enrichment values, particularly for different age classes and seasons. Such an approach is necessary to determine the effects of age and season on the carbon and nitrogen isotope enrichment.

The objectives of this study were (1) to determine the seasonal variations of isotope signatures in muscle tissues in relation to the isotope signatures of food sources and gut contents; and (2) to evaluate the variability of enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) between the diet (gut content) and consumer's tissues (i.e. muscle, gonad, digestive gland). We hypothesized that the isotopic signatures of muscle tissues changed after settlement, and that the changes were related to dietary isotopic signatures. Particular attention was paid to the isotope enrichment between the diet and a consumer's tissues using isotope analysis of gut content.

Materials and methods

Study area and culture description

Scallops are reared in Lake Saroma and Tokoro, two of the main culture areas in northern Japan, that varied distinctly in depth, method of practice, and physical variables. Lake Saroma (44°11´N; 143°49´E) is a semi-enclosed brackish water lagoon that measures 150 km^2 in area and has an average depth of 16 m (Fig. 1). The surface salinity is 33.0-33.8 (Katsuki et al. 2009). Seed production is done through spat collection and "intermediate" culture. In the "intermediate" culture stage, spats are placed in suspended nets and grown to a size of 30-50 mm shell height in the first year (Paturusi et al. 2002).

Tokoro (44°13´N; 143°55´E) is in the Sea of Okhotsk, which has a maximum depth of 40-65 m (Fig. 1). In the spring and autumn 2007, surface salinity ranged from 32.3 to 34.1. At the time of seeding (every 3rd week of May to the 1st week of June), suspended-net-cultured juveniles are introduced to the Tokoro seabed for 3 years of grow-out culture. Juveniles are reared using a system referred to as "rotational" harvesting, wherein the culture ground is divided into four zones. In this system, a particular culture zone is seeded after the commercial-sized individuals are harvested (Paturusi et al. 2002).

Sampling design

Scallops

To determine seasonal patterns of isotopic change in muscle tissues, we obtained individuals at various developmental stages including spats, suspended-net-cultured juveniles in Lake Saroma, and post-settlement individuals in the Tokoro seabed, Sea of Okhotsk. Spats (18.3 ± 1.9 mm shell height, n = 37) and suspended-net-cultured juveniles (47.6 ± 6.9 mm shell height, n = 10) were collected in May and September 2006. Five post-settlement individuals were obtained every month from June or July to November in three seasons (2006-2008). For each of the three seasons, the mean shell heights of scallops averaged 86.1 ± 6.1 mm, 71.4 ± 10.4 mm, and 66.4 ± 8.6 mm, respectively.

Five samples each from 1- to 4-year-old individuals was collected in May (spring), July (summer), and November (autumn) 2007 to estimate the enrichment values between the diet (gut contents) and tissues. The mean shell heights for four age groups ranged from 90.8-124.0 mm, 62.3-

124.5 mm, and 82.6-118.1 mm, respectively in each season. Three tissues (muscle, gonad, and digestive gland) from 2-3 individuals were obtained in each age group every season. Isotopic values of these tissues were compared to the diet (gut contents) gathered from the same age groups and obtained at the same time in 2008. Interpretation of data (gut contents in 2008, tissue-specific responses in 2007) is considered valid based on a similar pattern of differences between gut contents and muscle isotopic signatures in 2008 (unpubl data).

Scallops were packed individually on ice in a styropore box and processed within 24 hours after sampling according to Aya and Kudo (2007). Briefly, shell height was measured to the nearest 0.1 mm using a venier caliper. Muscle, gonad, and digestive gland tissues were removed from the shell, washed with Milli-Q water, weighed using an electronic balance (Shimadzu, BX 4200H), and frozen for later analysis. Small portions of each tissue was freeze-dried, ground, and homogenized with mortar and pestle. Lipids were extracted from the tissues using a modified "Folch" extraction method (Post et al. 2007). Gut contents were carefully removed from five individuals using a small spatula and pooled for analysis. The 24-hour delay between sampling of individuals and collection of gut contents was presumed not to confound isotope values (Guelinckx et al. 2008). Gut content samples for isotopic analysis were treated with 1 N HCl for 24 hours to remove inorganic carbon. These samples were rinsed with Milli-Q water to remove the acid, freeze-dried, and ground (Page and Lastra 2003).

Food sources

To determine causes for any observed shifts in muscle isotopic signatures, isotopic signatures were compared between potential food sources sampled at two stations and gut contents collected from post-settlement individuals. Suspended particulate organic matter (SPOM) was collected at Station S-0 (44°10´N; 143°58.9´E) from May-November 2007 and July 2008 onboard the Training Ship (T/S) *Ushio-Maru* of the Faculty of Fisheries Sciences, Hokkaido University. Additional SPOM samples were obtained at B and C culture zones in Tokoro from September-December 2008. SPOM was sampled from 0-50 m depth using a 10 L Niskin bottle attached to a Kevlar wire at Station S-0, whereas SPOM samples from 0-40 m depth were collected using a Van Dorn water sampler in Tokoro. Each time, seawater samples were dispensed into 1 to 2 L polyethylene (PE) bottles. SPOM (1 to 2 L) was filtered onto pre-combusted (450ºC, 5 h) Whatmann GF/F (25 mm) glass-fibre filters, immediately after collection.

Sinking particles (SP) were collected by sediment traps with six cylinders (mouth, diameter, and height were 0.018 m^2, 7.5 and 30 cm, respectively) moored at 35 m depth (7 m above the sea floor) in Tokoro from September-December 2008. The sediment trap was deployed for 24 hours. No preservatives were used to avoid contamination of the isotopic signatures of samples. SP was filtered through pre-combusted and pre-weighted filters. Four to five SPOM and two SP filters were freeze-dried and treated with concentrated HCl fumes for 4 hours to remove inorganic carbon before isotopic analyses.

Elemental and stable isotope analyses

Two to three muscles, two gonads, and digestive gland tissues of scallops were weighed (about 1 mg each) and put into separate tin capsules. One pooled gut content sample was weighed (approximately 1.0-2.9 mg), and two trap samples, weighing 5-6 mg each, were put in tin capsules. SPOM filters were placed in tin capsules and folded using a compressor. All samples were analyzed for elemental carbon and nitrogen contents and stable carbon and nitrogen isotope ratios using a Thermo Electron Delta V Plus Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS; Bremen, Germany), coupled with a Thermo Electron Flash Elemental Analyzer 1112 Series (Milan, Italy). The analytical precision was better than 0.2‰ for both carbon and nitrogen isotope ratios, estimated with alanine and tyrosine as internal standards (Kyoto University, Kyoto, Japan). Isotopic signatures are expressed in delta notation ($\delta^{13}C$ or $\delta^{15}N$) and are reported in per mil (‰) using the equation:

$$\delta^{13}C_{sample} \text{ or } \delta^{15}N_{sample}\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000,$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ with Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (Air-N_2) as international reference standards for stable carbon and nitrogen isotope ratio analyses.

Data analysis

We used a linear regression analysis to explore the relationship between shell height and $\delta^{13}C$ and $\delta^{15}N$ of muscle tissues, and the relationship between elemental carbon and nitrogen contents and muscle tissue isotopic signatures. We performed one-way analysis of variance (ANOVA) to test the significance of slope from zero.

We performed a multivariate analysis of variance (MANOVA) to test responses of the variation in the isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in relation to three factors: tissue (three levels: muscle, gonad, and digestive gland); age (four levels: 1-, 2-, 3-, and 4-y old); and season (three levels: spring, summer, and autumn). Enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) between the diet (gut contents in 2008; *X*) and tissues (analyzed in 2007; *Y*) were calculated in terms of the difference in delta ($\delta^{13}C$ and $\delta^{15}N$) values using the Δ notation, where $\Delta = \delta Y - \delta X$. MANOVA was used to determine variations in enrichment values to the three factors. A post-hoc Tukey-Kramer test was used to compare the means if the main effects (tissue, age, and season) were significant.

All statistical analyses were done using the Number Cruncher Statistical System (NCSS, 07.1.4 version) 2007 software (Hintze 2007).

Results

Isotopic signatures of food sources

The $\delta^{15}N$ values of SPOM varied seasonally in both stations (Table 1). SPOM $\delta^{15}N$ values were the lowest (4.4 ± 1.1‰) in spring and were the highest (7.4 ± 0.7‰) in autumn at Station S-0. On the other hand, the $\delta^{15}N$ isotopic signatures of SPOM were lower in summer (2.5 ± 1.9‰) than in autumn

(5.1 ± 0.4‰) for the Tokoro area. Although the $\delta^{15}N$ values increased for SPOM, they decreased seasonally for SP (from 5.7 ± 0.3‰ to 4.3 ± 0.1‰). The $\delta^{13}C$ isotopic signatures of SPOM were relatively stable for both stations. SPOM ranged from -25.4 ± 0.4‰ to -23.0 ± 0.5‰ in $\delta^{13}C$ for Station S-0, and varied from -25.0 ± 0.3‰ to -23.4 ± 0.9‰ for the Tokoro area. The carbon isotopic signatures of SP had higher values than SPOM (Table 1). C:N ratios in SPOM were between 5.9 and 7.4, except for spring, during which living phytoplankton possibly dominated in the suspended organic matter pool (Table 1). SP exhibited higher C:N molar ratios of 9.2 and 10.4 than SPOM, indicating a worse physiological state.

Isotopic signatures of *Mizuhopecten yessoensis*

Spats and suspended-net-cultured individuals had $\delta^{15}N$ values of 9.3‰ and 8.5‰, respectively (Fig. 2a). These values decreased significantly 3-5 months post-settlement in a nearshore seabed (Table 2). Scallop $\delta^{15}N$ values were inversely correlated with shell height in two seasons ($r = -0.81$, $P < 0.05$, $n = 6$ in 2007 and $r = -0.97$, $P < 0.01$, $n = 6$ in 2008; Fig. 2a), indicating a dietary shift of post-settlement individuals. Values of $\delta^{13}C$ varied from -16.7‰ for spats to -15.8‰ for suspended-net-cultured individuals (Fig. 2b). The $\delta^{13}C$ isotopic signatures changed drastically in one month after the settlement and stabilized during the succeeding months (Fig. 2b, Table 2). No correlation was found between muscle $\delta^{13}C$ values and shell height because of the small variability in the $\delta^{13}C$ isotopic signatures (Fig. 2b).

The $\delta^{15}N$ isotopic signatures of muscle tissues changed in response to small seasonal variations in the $\delta^{15}N$ of gut contents (Fig. 2c). The $\delta^{15}N$ of muscle was 0.8-3.8‰ higher than that of gut contents (Fig. 2c, Table 3). The enrichment in ^{15}N of gut contents compared to that of food sources was relatively similar across seasons (i.e., SPOM and SP on the basis of their $\delta^{15}N$ values; Fig. 2c). The $\delta^{13}C$ values of muscle tissues were 2.9-3.8‰ higher than those of gut contents, reflecting no seasonality in their isotopic signatures (Fig. 2d, Table 3). Values of $\delta^{13}C$ in gut contents were closer to those of SP than SPOM, which confirmed a significant contribution of aggregated fresh microalgae for gut contents (Fig. 2d). The elemental nitrogen content generally increased in the muscle tissue of post-settlement individuals in three seasons (Table 2). However, the muscle $\delta^{15}N$ isotopic signatures were inversely correlated only with the elemental nitrogen content in 2008 ($r = -0.82$, $P < 0.05$, $n = 6$; Fig. 3a). Values of $\delta^{13}C$, on the contrary, were not correlated to the elemental carbon content in muscle tissues (Fig. 3b, Table 2).

Effects of age and season on isotopic ratios and enrichment values

Pairwise comparison tests indicated significant differences among tissues in all of the parameters examined (MANOVA, $\delta^{13}C$, $\delta^{15}N$, $\Delta\delta^{13}C$, $\Delta\delta^{15}N$, $P < 0.001$; Tables 4, 5, and 6). $\delta^{15}N$ was 7.8‰ in muscle, 6.6‰ in gonad, and 6.1‰ in the digestive gland. $\delta^{13}C$ was -18.3‰ in muscle, -19.3‰ in gonad, and -22.0‰ in the digestive gland. Further, $\Delta\delta^{15}N$ was 2.4‰ in muscle, 1.2‰ in gonad, and

0.7‰ in the digestive gland. $\Delta\delta^{13}C$ was 3.2‰ in muscle, 2.3‰ in gonad, and -0.5‰ in the digestive gland.

Enrichment values also varied seasonally, where $\Delta\delta^{15}N$ was the lowest in summer (0.3‰), and $\Delta\delta^{13}C$ was the highest in autumn (2.8‰) (Fig. 4, Tables 5, 6). $\Delta\delta^{15}N$ decreased in spring and summer by 2.0‰ for muscle and 1.7‰ both for gonad and the digestive gland. On the other hand, the corresponding increases for $\Delta\delta^{13}C$ in spring and autumn were 2.6‰ for muscle, 1.5‰ for gonad, and 2.3‰ for the digestive gland. The interaction among season, age, and tissue was significant for $\Delta\delta^{13}C$, but not for $\Delta\delta^{15}N$.

The effect of age was highly significant for $\delta^{15}N$ and $\Delta\delta^{15}N$, being significantly variable among tissues (Fig. 4, Tables 4, 5, and 6). Isotope ratios and enrichment values ($\delta^{15}N$ and $\Delta\delta^{15}N$) were significantly higher for adult than juvenile scallops (Tables 4, 5 and 6). However, the interaction among season, age, and tissue was significant for $\delta^{13}C$, and the interaction between season and age was highly significant for $\delta^{15}N$.

Discussion

Dietary effect on isotope ratios of muscle tissues

Changes in the dietary $\delta^{15}N$ values were explained by the observed $\delta^{15}N$ depletion of muscle tissues in post-settlement individuals. The higher $\delta^{15}N$ values in muscle tissues of spats and suspended-net-cultured scallops (9.3‰ and 8.5‰, respectively) are nearly equivalent to published mean values of benthic microalgae (Takai et al. 2002; Doi et al. 2005). The nitrogen isotopic signatures decreased significantly within 3-5 months after the settlement in a nearshore seabed, concomitant with the ingestion of lower $\delta^{15}N$ food. Changes in the muscle $\delta^{15}N$ isotope signatures were also in response to dietary shift, which varied seasonally according to changes in gut contents $\delta^{15}N$. A time lag responding to the changes in diet during settlement seemed to be influenced by the assimilation rate for that life stage and tissue type. Although the decreases in muscle $\delta^{15}N$ values were concomitant with the ingestion of a diet with lower $\delta^{15}N$, the similarity in the nitrogen isotopic signatures among gut contents, SPOM, and SP suggests an important contribution of these food sources to scallops' diet. This pattern is supported by the inverse relationship between the muscle $\delta^{15}N$ isotopic values and elemental nitrogen content, suggesting increased nitrogen retention in post-settlement juveniles. However, sample acidification may affect the measurement of $\delta^{15}N$, which may explain some of the discrepancy among gut contents, SPOM, and SP $\delta^{15}N$ isotopic signatures.

The elevated $\delta^{13}C$ values for spats and suspended-net-cultured scallops (-16.7‰ and -15.8‰, respectively) overlapped mean values of benthic microalgae reported in the literature (i.e. -16‰, Reira and Richard 1996; Kang et al. 1999; Takai et al. 2002; Doi et al. 2005). The muscle $\delta^{13}C$ isotopic signatures showed a short response time (1 month post-settlement), and appeared to be independent of the changes in gut contents $\delta^{13}C$. Assimilation of low amount carbon from ingested material could explain this pattern, particularly when available food supply is low (unpubl data). The pattern of $\delta^{13}C$ isotopic

signatures presented here is similar to that of *Chlamys islandica* when placed in cages at various depths and distances from shore (Nadon and Himmelman 2006). For juvenile scallops, SP was considered a more important food source than SPOM, based on the correspondence between SP and gut contents $\delta^{13}C$. SP is composed mainly of aggregated fresh microalgae and considered in a worse physiological state, and is transported from the surface photic zone to the seabed. The photic zone at the study site was estimated until 30 m based on the vertical profiles of Photosynthetically Active Radiation (PAR) (data not shown). Based on this observation, SPOM near the bottom will not be considered fresher than SP. However, C:N molar ratios in SP were higher than SPOM, indicating that the latter was much fresher than the former. This discrepancy is explained by the collection of SP samples in summer and autumn, during which primary production in the photic zone was not high (unpubl data). Contribution from resuspension of sedimented organic matter to SP during these periods was higher than the productive spring (Sigleo and Schultz 1993; Heiskanen and Leppänen 1995; Lund-Hansen et al. 1999).

Enrichment values between the diet and tissues

It is always assumed that the $\delta^{15}N$ values in the diet are lower than those in consumer tissues by approximately 3-4‰ (Minagawa and Wada 1984). The $\delta^{13}C$, on the contrary, exhibits a small enrichment of 0-1‰ (DeNiro and Epstein 1978). Several studies have demonstrated that these enrichment processes are tissue-specific (Yokoyama et al. 2005; Logan et al. 2006), and influenced by diet and sample preparation (McCutchan et al. 2003). Our results showed that enrichment values depended on tissue, season, and age.

Isotopic variation occurred among the three tissues in the order: muscle>gonad>digestive gland. These results agree with the previous findings of Lorrain et al. (2002), who demonstrated different isotopic signatures among scallop tissues. Tissue-specific differences in isotopic values are well known, as a result of biochemical composition, metabolism, and isotopic routing of compounds through the body of an organism (Gannes et al. 1997; Raikow and Hamilton 2001; Lorrain et al. 2002; Paulet et al. 2006; Bodin et al. 2007; Deudero et al. 2009). Isotopic routing of elements from the diet to consumer tissues can contribute to variation in the estimated enrichment values (Dalerum and Angerbjorn 2005; Podlesak and McWilliams 2006).

Our results showed that season influenced the enrichment values in scallops. Across seasons, $\Delta\delta^{15}N$ was lower relative to previous estimates, where $\Delta\delta^{15}N$ was the lowest in summer (0.3‰). Low $\Delta\delta^{15}N$ values have been reported for bivalves, because of the scallop's physiological attributes associated with reproductive or growth stage (Raikow and Hamilton 2001; Post 2002; Dubois et al. 2007; Fukumori et al. 2008; Marín Leal et al. 2008). Sample preparation affects the measurement of $\delta^{13}C$ in a consumer's tissues (McCutchan et al. 2003). Lipids show lower $\delta^{13}C$ compared to other biochemical fractions such as protein and carbohydrates (DeNiro and Epstein 1978). Variations in $\delta^{13}C$ values are negatively correlated with the amount of lipids present in a consumer's tissues (Lorrain et al. 2002). Extraction of lipids from our samples would have raised the seasonal $\Delta\delta^{13}C$; the highest values

were observed during autumn (2.8‰) with respect to the published literature values (DeNiro and Epstein 1978; McCutchan et al. 2003; Dubois et al. 2007). In the summer and autumn of 2008, large differences in $\Delta\delta^{13}C$ ranged from 2.7-4.8‰ (Table 3). This high discrepancy in $\Delta\delta^{13}C$ could reflect the quantity of food ingested by scallops. The overlapping of gut content $\delta^{13}C$ values to that of SP was explained by the preference of juvenile scallops of SP than SPOM as a food source. During the summer, SP flux was low in quantity (unpubl data). Low amount of food would not affect the isotopic signatures of muscle tissues, with the magnitude of $\Delta\delta^{13}C$ increasing with low ingestion efficiency. The patterns of differences in $\Delta\delta^{13}C$ are related to seasonal changes of carbon incorporation rates in a consumer's tissues (Paulet et al. 2006). The value of enrichment in ^{13}C reflects decreased carbon use efficiency, resulting in a decrease of food material available to post-settlement individuals. Therefore, there is a need to determine species-specific enrichment between the diet and consumer's tissues (Lorrain et al. 2002; Dubois et al. 2007). In addition, enrichment values need to be investigated in a temporal scale because values may show seasonal differences as reported here.

Our results for *M. yessoensis* documented age effects on $\delta^{15}N$ and $\Delta\delta^{15}N$ values. Larger values of $\delta^{15}N$ and $\Delta\delta^{15}N$ were observed for adult than for juvenile scallops. Similar enrichment in ^{15}N isotopic signatures with age has been reported for *Stizostedion vitreum* (Overman and Parish 2001) and freshwater mussels (Howard et al. 2005). The ^{15}N enrichment has been attributed to fractionation during transamination reactions, with the lighter isotope reacting faster in each direction of the reaction, leaving the remaining nitrogen pool enriched in ^{15}N (Macko et al. 1986). Dietary differences can also affect $\Delta\delta^{15}N$ values, as lower $\Delta\delta^{15}N$ values (1.4 ± 0.2‰) are found in consumers raised on invertebrate diets (McCutchan et al. 2003). Age effect on $\Delta\delta^{15}N$ is reported for red foxes (Roth and Hobson 2000), walleyes (Overman and Parrish 2001), and oysters (Lefebvre et al. 2009), but all of these are independent of diet. Other reports find no age effects in mussels (Minagawa and Wada 1984) or rainbow smelt (Jardine and Curry 2006). The strong correlation between muscle $\delta^{15}N$ values and age in Japanese scallops is related to differences in diet, which may explain the effect of age on the low $\Delta\delta^{15}N$ estimates reported here.

Conclusion

The observed decline in the $\delta^{15}N$ isotopic signatures of scallop tissues was influenced by the seasonal variations in the isotopic signatures of food sources and gut contents. For juvenile scallops, SP was considered a more important dietary source than SPOM. Enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) depended on tissue and season, as explained by the differences in diet. To the best of our knowledge, this study is the first to document patterns of isotope change and enrichment encountered in different tissues of *M. yessoensis* when changing culture habitat. This study will contribute to an understanding of isotope enrichment and the ecology of the scallop, and will help to improve tools for ecological studies in marine invertebrate species.

Table 1 Mean stable $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values and C:N molar ratios of potential food sources

Station and month	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		C:N molar ratios	
	SPOM	SP	SPOM	SP	SPOM	SP
Station S-0						
May 07	-23.9±1.1 (5)	nd	4.4±1.1 (5)	nd	3.8±0.1	nd
Jul 07	-24.8±0.6 (6)	nd	4.5±1.2 (6)	nd	6.6±0.9	nd
Nov 07	-25.4±0.4 (6)	nd	7.4±0.7 (5)	nd	5.9±0.2	nd
Jul 08	-23.0±0.5 (4)	nd	5.3±0.2 (4)	nd	7.4±0.2	nd
B and C culture zones off Tokoro						
Sept 08	-23.4±0.9 (5)	-21.4±1.4 (2)	2.5±1.9 (4)	5.7±0.3 (2)	7.3±0.9	9.2±0.4
Oct 08	-24.5±0.7 (5)	-21.8±0.8 (2)	4.6±0.6 (5)	4.8±0.5 (2)	7.0±0.6	10.1±0.3
Dec 08	-25.0±0.3 (5)	-21.9±0.7 (2)	5.1±0.4 (5)	4.3±0.1 (2)	7.4±1.0	10.4±0.1

Mean ± SD. Sample sizes in parenthesis. SPOM, suspended particulate organic matter; SP, sinking particles; nd, No data.

Table 2 $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰), elemental C (mg g^{-1}) and N contents (mg g^{-1}) of muscle tissues in different post-settlement times

Month	2006				2007				2008			
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	N (mg g^{-1})	C (mg g^{-1})	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	N (mg g^{-1})	C (mg g^{-1})	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	N (mg g^{-1})	C (mg g^{-1})
Jun	nd	nd	nd	nd	-17.9±0.1 (3)	8.0 (3)	97.6±1.0	420.2±0.8	-18.5±0.2 (2)	9.1±0.1 (2)	126.4±3.2	418.5±0.3
Jul	-17.1±0.1 (2)	7.9±0.2 (2)	113.5±4.0	415.8±1.5	-17.9±0.4 (3)	7.2 (3)	117.4±3.3	417.6±1.4	-18.6±0.1 (2)	8.4±0.5 (2)	135.2±0.7	420.3±0.1
Aug	-17.8±0.2 (2)	7.5±0.1 (2)	127.8±8.4	415.2±8.3	-18.6±0.5 (3)	6.9±0.5 (3)	116.1±0.7	415.1±1.6	-18.7±0.1 (2)	8.1±0.2 (2)	137.6±7.4	418.5±7.8
Sep	-17.8±0.3 (2)	7.4±0.3 (2)	136.6±5.3	428.0±2.3	-18.4±0.1 (3)	6.5±0.3 (3)	123.9±4.1	407.9±4.5	-18.3 (2)	7.8±0.1 (2)	133.1±0.2	411.8±7.1
Oct	-17.6±0.2 (3)	6.9±0.3 (3)	137.5±2.4	414.2±12.8	-18.1±0.4 (3)	7.0±0.3 (3)	135.8±4.1	409.5±7.1	-18.3 (2)	7.2±0.1 (2)	137.1±4.4	412.2±1.7
Nov	-17.7 (3)	7.2±0.1 (3)	142.5±1.3	416.7±4.3	-17.9±0.1 (3)	6.9±0.2 (3)	143.9±2.7	413.5±4.2	-18.2 (2)	7.2±0.3 (2)	140.3±6.3	413.9±6.9

Mean ± 1 SD. Sample sizes in parenthesis. nd, No data.

Table 3 Mean difference in enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) between age 1 gut content and muscle tissue in 2008

Sampling Date	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)
	M-Gut	M-Gut
Jun 23	3.6	3.8
Jul 23	3.2	1.9
Aug 13	2.7	2.1
Sep 10	2.9	2.0
Oct 30	2.9	2.2
Nov 17	4.8	2.0
Dec 11	3.8	0.8

M, Muscle; Gut, Gut content

Table 4 Summary of multivariate analysis of variance (MANOVA) results for isotopic variations among tissues

Source of variations	$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)			
	df	SS	MS	*F*	df	SS	MS	*F*
Season	2	1.19	0.59	$2.51^{n.s.}$	2	0.03	0.02	$0.10^{n.s.}$
Age	3	1.70	0.57	$2.39^{n.s.}$	3	6.96	2.32	14.33^{***}
Tissue	2	202.17	101.09	427.18^{***}	2	43.74	21.87	135.16^{***}
Season × Age	6	6.57	1.10	4.63^{***}	6	4.42	0.74	4.56^{***}
Season × Tissue	4	3.45	0.86	3.64^{*}	4	0.64	0.16	$1.00^{n.s.}$
Age × Tissue	6	7.48	1.25	5.26^{***}	6	1.87	0.31	$1.93^{n.s.}$
Season × Age × Tissue	12	6.00	0.50	2.11^{*}	12	2.51	0.21	$1.29^{n.s.}$
Residual	48	11.36	0.24		48	7.77	0.16	
Total	84				84			
Pairwise within level of factor Tissue	Muscle ≠ Gonad ≠ Digestive Gland				Muscle ≠ Gonad ≠ Digestive Gland			
Pairwise within level of factor Age	1 ≈ 2 ≈ 3 ≈ 4				1 ≠ 4 ≈ 2 ≈ 3			

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{n.s.}$Not significant difference

Table 5 Enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) among tissues

Season	Age	$\Delta\delta^{13}C$ (‰)			$\Delta\delta^{15}N$ (‰)		
		M-Gut	G-Gut	DG-Gut	M-Gut	G-Gut	DG-Gut
SP	1	1.7 ± 0.1 (3)	1.2 ± 0.1 (2)	-1.5 ± 0.1 (2)	1.9 ± 0.2 (3)	1.3 ± 1.3 (2)	0.7 ± 0.1 (2)
SP	2	1.2 ± 0.2 (3)	0.9 ± 0.2 (2)	-2.5 ± 0.6 (2)	2.9 ± 0.2 (3)	1.7 ± 1.5 (2)	1.1 ± 0.5 (2)
SP	3	2.7 ± 1.1 (3)	2.0 ± 0.2 (2)	-0.7 ± 0.2 (2)	3.8 ± 0.3 (3)	1.4 ± 0.3 (2)	1.2 ± 0.2 (2)
SP	4	2.1 ± 0.2 (3)	1.8 ± 0.6 (2)	-1.5 ± 1.0 (2)	4.3 ± 0.2 (3)	3.1 ± 1.3 (2)	2.2 ± 0.1 (2)
SU	1	3.9 ± 0.4 (3)	1.6 ± 0.0 (2)	0.4 ± 0.2 (2)	0.6 ± 0.0 (3)	-0.8 ± 0.1 (2)	-1.3 ± 0.0 (2)
SU	2	2.9 ± 0.2 (3)	2.6 ± 0.2 (2)	-1.9 ± 0.9 (2)	0.9 ± 0.1 (3)	0.1 ± 0.1 (2)	-0.5 ± 0.7 (2)
SU	3	2.9 ± 0.3 (3)	2.4 ± 0.2 (2)	-0.5 ± 0.3 (2)	1.3 ± 0.2 (3)	0.1 ± 0.1 (2)	-0.1 ± 0.4 (2)
SU	4	3.2 ± 0.2 (3)	2.7 ± 0.0 (2)	-0.6 ± 0.3 (2)	2.1 ± 0.1 (3)	0.9 ± 0.0 (2)	0.4 ± 0.0 (2)
AU	1	5.0 ± 0.1 (3)	2.1 ± 0.1 (2)	1.5 ± 0.1 (2)	1.7 ± 0.2 (3)	1.0 ± 0.1 (2)	0.7 ± 0.2 (2)
AU	2	5.0 ± 0.1 (3)	3.7 ± 0.2 (2)	1.8 ± 0.3 (2)	2.5 ± 0.3 (3)	2.0 ± 0.0 (2)	1.4 ± 0.2 (2)
AU	3	4.1 ± 0.2 (3)	3.2 ± 0.1 (2)	0.5 ± 1.4 (2)	3.0 ± 0.1 (3)	1.5 ± 0.2 (2)	1.4 ± 0.3 (2)
AU	4	4.3 ± 0.1 (3)	3.0 ± 0.2 (2)	-0.7 ± 1.5 (2)	3.5 ± 0.1 (3)	1.9 ± 0.5 (2)	1.5 ± 0.1 (2)

M, Muscle; G, Gonad; DG, Digestive gland; Gut, Gut content. SP, Spring; SU, Summer; AU, Autumn. Mean ± SD. Tissue sample sizes in parenthesis.

Table 6 Summary of multivariate analysis of variance (MANOVA) results for enrichment values ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$)

Source of variations	$\Delta\delta^{13}C$ (‰)				$\Delta\delta^{15}N$ (‰)			
	df	SS	MS	*F*	df	SS	MS	*F*
Season	2	63.30	31.65	133.76^{***}	2	49.98	24.99	154.46^{***}
Age	3	1.55	0.52	$2.19^{n.s.}$	3	24.72	8.24	50.92^{***}
Tissue	2	202.17	101.09	427.18^{***}	2	43.74	21.87	135.16^{***}
Season × Age	6	14.62	2.44	10.30^{***}	6	2.06	0.34	$2.12^{n.s.}$
Season × Tissue	4	3.45	0.86	3.64^{*}	4	0.64	0.16	$1.00^{n.s.}$
Age × Tissue	6	7.48	1.25	5.26^{***}	6	1.87	0.31	$1.93^{n.s.}$
Season × Age × Tissue	12	6.00	0.50	2.11^{*}	12	2.51	0.21	$1.29^{n.s.}$
Residual	48	11.36	0.24		48	7.77	0.16	
Total	84				84			
Pairwise within level of factor Tissue	Muscle ≠ Gonad ≠ Digestive Gland				Muscle ≠ Gonad ≈ Digestive Gland			
Pairwise within level of factor Age	1 ≈ 2 ≈ 3 ≈ 4				1 ≠ 4 ≈ 2 ≈ 3			
Pairwise within level of factor Season	Spring ≈ Summer ≠ Autumn				Summer ≠ Autumn ≈ Spring			

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{n.s.}$Not significant difference

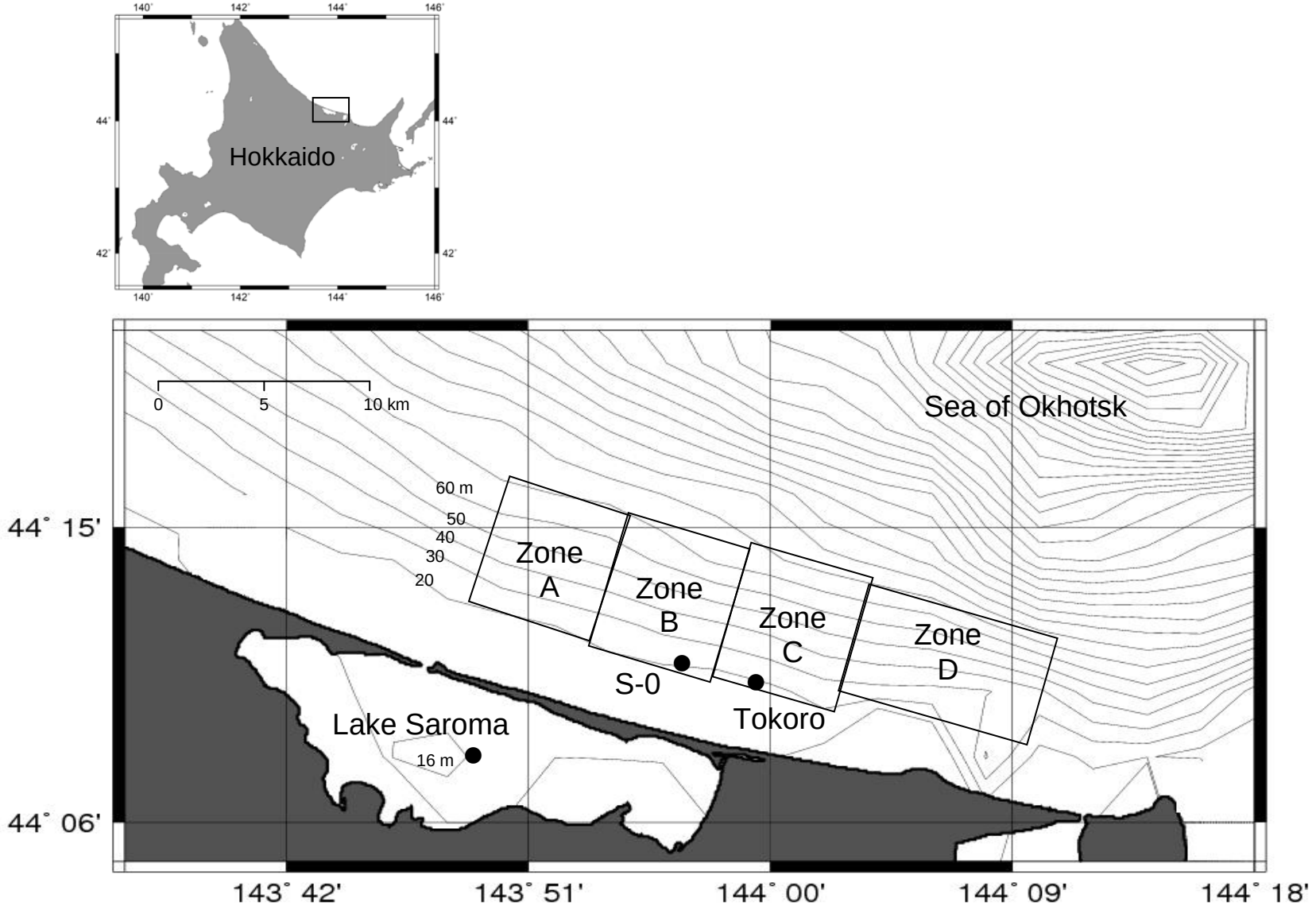
Figure legends:

Fig. 1 Location of sampling stations (represented by dots) and culture zones for scallops in the Sea of Okhotsk.

Fig. 2 Relationship between (a) $\delta^{15}N$ and (b) $\delta^{13}C$ of muscle tissues and shell height, and (c) $\delta^{15}N$ and (d) $\delta^{13}C$ of muscle (M) tissues, gut content,and food sources plotted against sampling date (day of year). Error bars indicate ± SD.

Fig. 3 Relationship between (a) muscle $\delta^{15}N$ values and elemental nitrogen content and (b) muscle $\delta^{13}C$ values and elemental carbon content. Error bars indicate ± SD.

Fig. 4 Dual isotope plots for gut content and scallop tissues (e.g., muscle (M), gonad (G), and digestive gland (DG)). Error bars indicate ± SD.



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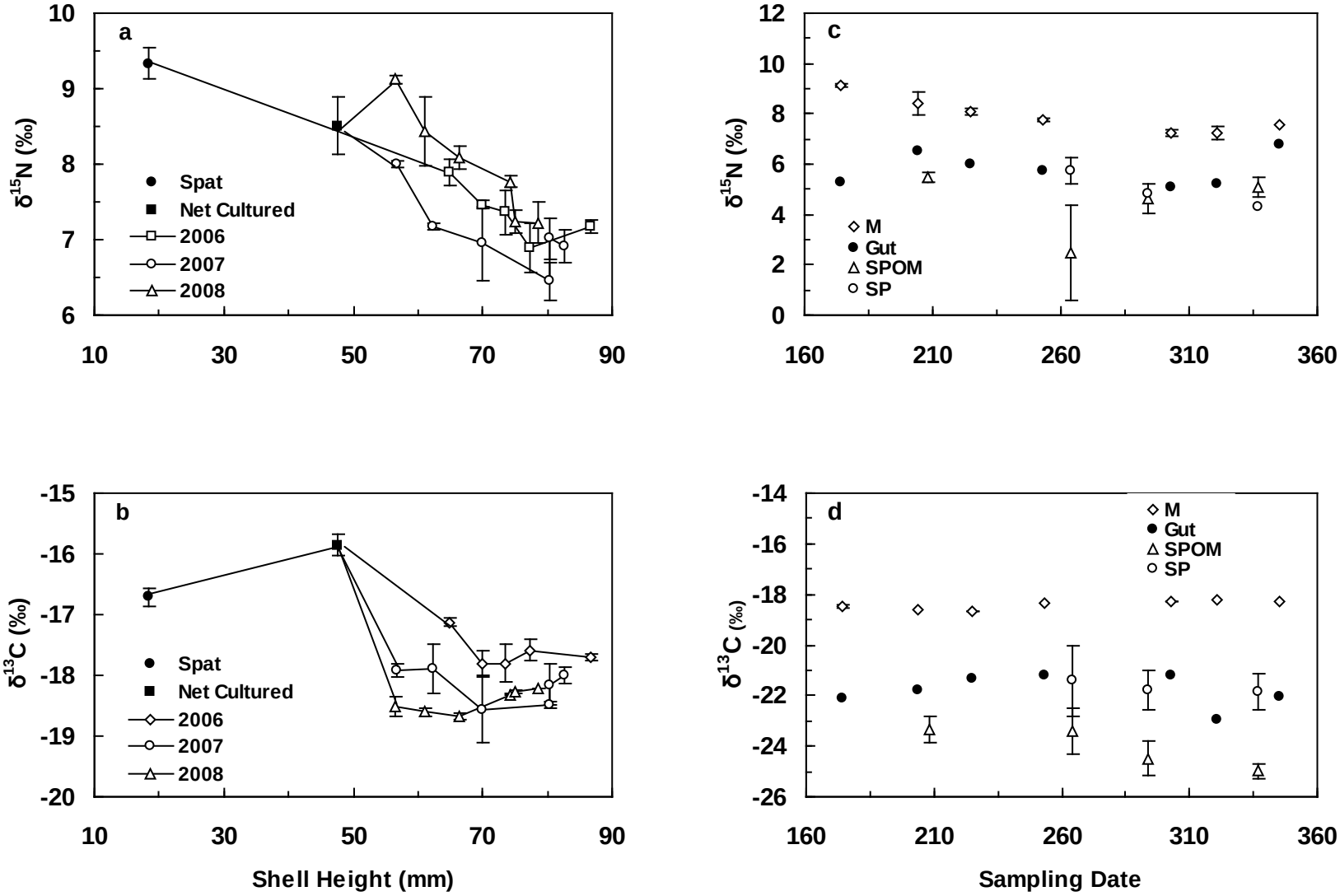

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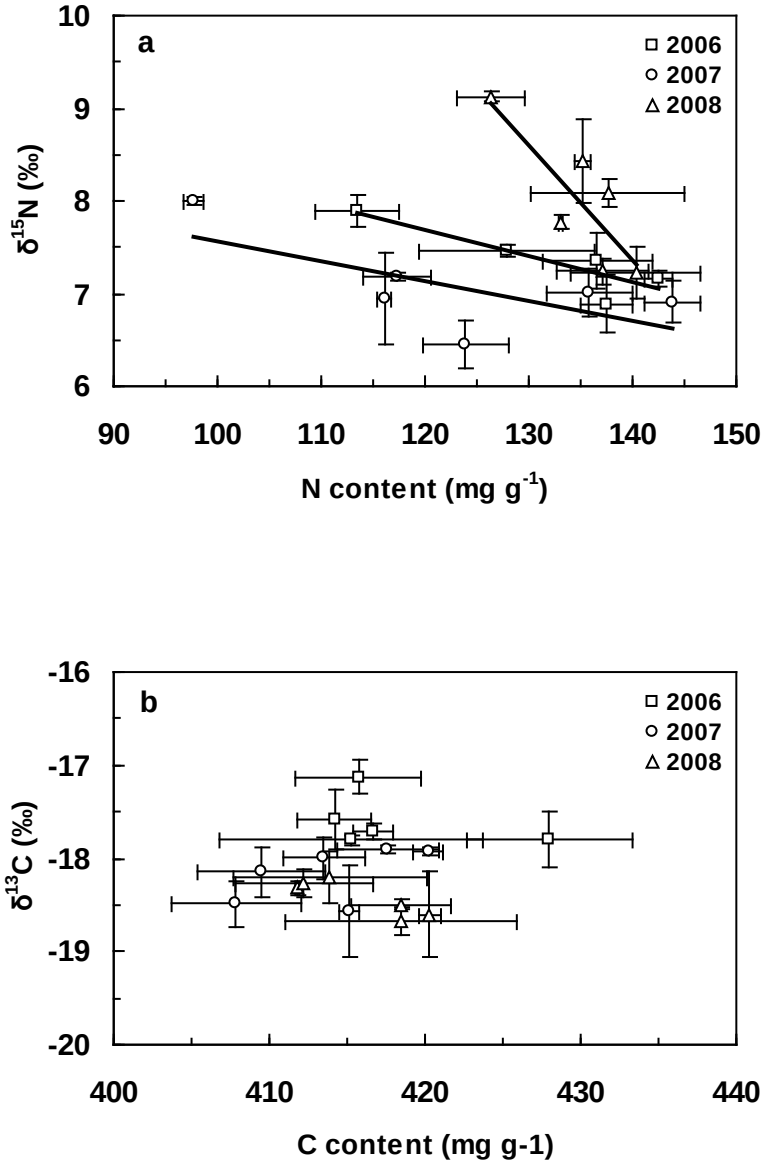

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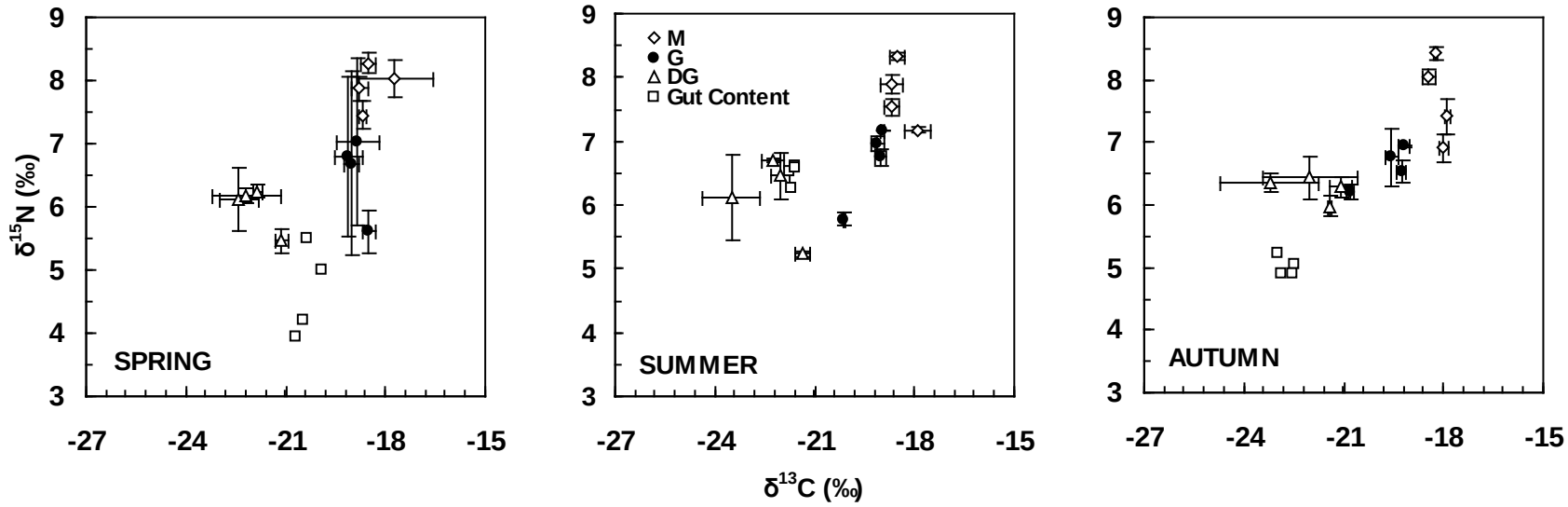

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