Title	Nitrogen stable isotopes reveal age-dependent dietary shift in the Japanese scallop <i>Mizuhopecten yessoensis</i>
Author(s)	Aya, Frolan A.; Kudo, Isao
Citation	Isotopes in environmental and health studies, 53(1), 80-90 https://doi.org/10.1080/10256016.2016.1186024
Issue Date	2017-03
Doc URL	http://hdl.handle.net/2115/68401
Rights	This is an Accepted Manuscript of an article published by Taylor & Francis in Isotopes in Environmental and Health Studies on March 2017, available online: http://www.tandfonline.com/10.1080/10256016.2016.1186024 .
Type	article (author version)
File Information	Kudo.pdf

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Nitrogen stable isotopes reveal age-dependent dietary shift in the Japanese scallop

***Mizuhopecten yessoensis* (Jay, 1857)**

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Running Head: Dietary shift in Japanese scallop

Abstract

Ontogenetic niche shifts in diet are a consequence of changes in body size or resource partitioning between age classes. To better resolve the feeding patterns of the Japanese scallop *Mizuhopecten yessoensis*, we examined the relative importance of age and size in the diet of this species using stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) from 2006 to 2009. Contribution of food sources was quantified using an isotope mixing model by comparing the muscle tissue isotope ratios to those of suspended particulate organic matter (SPOM) and their zooplankton prey (e.g. micro- and meso-zooplankton). Unlike the $\delta^{13}\text{C}$ values which remained constant with age and size, muscle $\delta^{15}\text{N}$ values were more positively correlated with age accounting for 69% of variations than size with only 46%. Increasing ^{15}N values with age suggested that shifts in diet from SPOM to micro- and meso-zooplankton occurred during ontogeny in *M. yessoensis*. Results of the isotope mixing model indicated that SPOM contribution to scallop's diet decreased from 68% to 8% while those of zooplankton increased from 15 to 50% with increasing age. This study concludes that age-related dietary shift explains the enrichment of ^{15}N , as a result of predation on zooplankton by *M. yessoensis*.

Keywords: Age; ^{15}N enrichment; *Mizuhopecten yessoensis*; Diet shift; **Size**; Zooplankton; Isotope mixing

Introduction

Ontogenetic diet shifts are common among invertebrates, as a direct consequence of increase in body size [1] or resource partitioning between age classes. Body size is one of the most fundamental traits that affect individual characteristics determining food acquisition [2]. The age of the individuals however, is related with resource use capability [3]. Age-related shifts in diet composition lead to decreased potential intra-specific competition for resources [4,5]. As a predator ages, it will feed on larger and high quality food sources to meet the required energy for growth and reproductive functions [6] and an ontogenetic change in diet is expected to occur [7,8].

For a long time, scallops feed mainly on phytoplankton [9] and sinking particles [10,11], even though other larger zooplankton has been found in the guts of marine bivalves [9]. It has been shown that bivalves are not only herbivores, but omnivores that feed on bacteria, nano-zooplankton and micro-zooplankton [12–14]. **In fact, mussels are reported to have assimilated about 37 to 54% of meso-zooplankton, an evidence of a strong trophic link between mussels and zooplankton [14].** Scallops reared at Lake Saroma, Hokkaido, Japan feed not only on suspended particulate organic matter (SPOM) but also on zooplankton [15].

Stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are now increasingly used to estimate trophic position and the food sources of marine organisms [16–18]. The $\delta^{15}\text{N}$ of an animal tissue is enriched by 3–4‰ relative to its diet [19] while $\delta^{13}\text{C}$ is less, about 1‰ [20]. Several studies have shown that factors such as age or size are known to influence the isotopic composition of bivalve tissues. For instance, large differences in $\delta^{15}\text{N}$ occur with size, particularly in mussels and fishes than with age [19,21] but not in walleye [22]. Hence, it is necessary to re-examine the importance of these factors as determinants of ontogenetic niche shifts in diet of many bivalve species.

The Japanese scallop *Mizuhopecten yessoensis* (Jay, 1857) is a commercially important temperate scallop reared in the coastal waters of northern Japan. It is cultivated using the “rotational” harvesting system, where a particular culture zone is seeded with juveniles after the adult-sized individuals are harvested [23]. Previous studies have analyzed some aspects of *Mizuhopecten yessoensis* biology and ecology, including distribution and abundance [24], recruitment [23], and feeding rates [25,26]. However, information concerning the ontogenetic dietary changes of this species is not known.

Therefore, this study aimed (1) to examine age and size-specific shifts in the diet of Japanese scallop; and (2) to estimate the contribution of suspended particulate

organic matter (SPOM) and zooplankton prey in the scallop's diet using stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. We hypothesized that if the ingestion of ^{15}N -rich larger zooplankton is responsible for the heavy nitrogen found in adult scallops, then it follows that juvenile individuals should have a lighter nitrogen isotope ratio, similar to that of phytoplankton-derived organic matter.

Materials and Methods

Study area

The study was carried out in Tokoro, Sea of Okhotsk ($44^{\circ}13'\text{N}$, $143^{\circ}55'\text{E}$), where there is an extensive culture of Japanese scallops on the seabed (**Figure 1**). It measures a maximum depth of 40–65 m. The sediments are mainly coarse sand or pebbles [27]. In the spring and autumn 2007, surface salinity ranges from 32.3 to 34.1. The fishing ground is divided into four culture zones (Zone A to D), where each zone contains different age class at a given time [24].

Sampling and analyses

Suspended particulate organic matter (SPOM) was sampled at Station S-O ($44^{\circ}10'\text{N}$ and $143^{\circ}58.9'\text{E}$) between May and November 2007, July 2008, and July 2009. Depth-

integrated water samples for SPOM were collected with a 10-L Niskin bottle attached to a Kevlar wire from 0, 10, 20, 30 and 40 m depths. SPOM (1 to 2 L) samples were filtered onto pre-combusted (450°C for 5 h) Whatman GF/F (25 mm) glass fiber filters immediately after collection.

Zooplankton samples were collected with vertical net tows from bottom to surface at Station S-0 with NORPAC nets of **100 and 330 µm mesh sizes** during the same collection dates with SPOM and stored either in a 125 or 250 mL wide-mouth polyethylene (PE) bottle. Zooplankton fractions (100–330 µm and >330 µm) were filtered onto pre-weighed, pre-combusted (450°C for 5 h) Whatman GF/F glass fiber (25 mm) filters.

Scallop individuals in four age classes (**1⁺ to 4⁺-year-old**) from six **cohorts** were obtained monthly either from April or July to December in three seasons (**2006–2008**) and May 2009, except during the winter months. Shell height was measured to the nearest 0.1 mm using a vernier caliper. Soft tissues were dissected and muscle samples were removed, washed with Milli-Q water and weighed.

All samples were stored at -30°C until analysis. Small portion of muscle samples were freeze-dried and ground with mortar and pestle. Lipids were extracted from muscle tissues using the modified “Folch” extraction method [28]. SPOM and

108 zooplankton filters were freeze-dried and exposed to HCl fumes for 4 h to eliminate
 109 inorganic carbon.

110 All samples were measured for elemental and stable isotopes. About 1 mg of
 111 two to three muscle samples were weighed with a microbalance and packed in tin
 112 capsules for analysis. SPOM filters were put in tin capsules and fashioned into a tablet
 113 using a hand compressor. Zooplankton that was retained on the filters (1 to 1.5 mg for
 114 100–330 μm and 1.5 to 2 mg for $>330 \mu\text{m}$ fractions) were scraped carefully, prepared in
 115 duplicate and placed in tin capsules and folded. Stable isotope ratios of carbon and
 116 nitrogen were measured with a Thermo Electron Delta V Plus Continuous Flow IRMS
 117 (Bremen, Germany) attached to a Thermo Electron Flash Elemental Analyzer 1112
 118 Series (Milan, Italy). Precision was better than 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ estimated
 119 with alanine and tyrosine as internal standards (Kyoto University, Kyoto, Japan). Stable
 120 isotope ratios are expressed in delta notation ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) and are reported in per mil
 121 (‰) using the equation: $\delta^{13}\text{C}_{\text{sample}}$ or $\delta^{15}\text{N}_{\text{sample}} = (R_{\text{sample}}/R_{\text{standard}} - 1)$, where R
 122 $= {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$ with Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen
 123 as **international reference standards** for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. **Delta $\delta^{13}\text{C}$**
 124 **values are normalized using VPDB-LSVEC calibration curve.**

125

Data analyses

All statistical analyses were done using the Number Cruncher Statistical System (NCSS, 07.1.4 version) 2007 software [29]. We applied one-way analyses of variance (ANOVA) to test significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among scallop populations and post hoc Tukey's-Kramer test was used when significant differences were detected. Linear regression analysis was performed to examine relationships between muscle tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and their age and size. Prior to regression analysis, the actual age of scallops was recalculated from the difference between sampling dates and seeding time (3rd week of May until 1st week of June) and expressed as fractions of a year.

Scallop's trophic position (TP) was estimated based on a 3-year average $\delta^{15}\text{N}$ isotope ratio of SPOM ($5.3 \pm 0.8\text{‰}$; F. Aya, unpubl. data) and using the average trophic fractionation of 1.7‰ [16, F. Aya, unpubl. data] where the average trophic level of the Japanese scallop can be calculated as [30]:

$$\text{TP} = 1 + (\text{scallop } \delta^{15}\text{N} - \text{SPOM } \delta^{15}\text{N}) / 1.7 \text{ using SPOM as the basal level of the}$$

food web

To account for the trophic increases among the populations, the percentage $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ contributions of micro- (**PL100–330**), mesozooplankton (**PL>330**) and SPOM to scallops' diet were estimated with an isotope mixing model [31]. The isotopic values of SPOM during 2007 and 2008 at Station S-0 were reported elsewhere [11].

Application of the mixing model required correction of consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values using tissue-diet isotopic fractionation values. The difference between isotopic ratios of gut content and that of muscle tissue was used as representation of tissue-diet isotopic fractionation [11, F. Aya, unpubl. data]. Muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were corrected for 3.4‰ and 1.7‰ isotopic fractionation, respectively, prior to analysis [16, F. Aya, unpubl. data]. The contribution is calculated as follows:

$$\text{Mix } \delta^{13}\text{C} = f_{\text{SPOM}} * \delta^{13}\text{C}_{\text{SPOM}} + f_{\text{PL100-330}} * \delta^{13}\text{C}_{\text{PL100-330}} + f_{\text{PL>330}} * \delta^{13}\text{C}_{\text{PL>330}};$$

and

$$\text{Mix } \delta^{15}\text{N} = f_{\text{SPOM}} * \delta^{15}\text{N}_{\text{SPOM}} + f_{\text{PL100-330}} * \delta^{15}\text{N}_{\text{PL100-330}} + f_{\text{PL>330}} * \delta^{15}\text{N}_{\text{PL>330}};$$

with

$$f_{\text{SPOM}} + f_{\text{PL100-330}} + f_{\text{PL>330}} = 1$$

where the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of the consumer is the combination of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of potential food sources (SPOM, micro- and meso-zooplankton) and f is the fractional contribution of a source [31]. The last equation specifies that the fractions must equal to 1.

Results

Suspended particulate organic matter (SPOM) and zooplankton showed no significant differences in $\delta^{13}\text{C}$ ($P < 0.05$) (Table 1). **C/N molar ratios in SPOM and zooplankton were between 6.1 and 6.8**, indicating phytoplankton-derived organic material. However, **zooplankton, composed of micro- and meso-zooplankton, was 1.2 to 1.5‰** more enriched in ^{15}N than SPOM, suggesting different trophic position between primary producers and zooplankton.

Muscle $\delta^{15}\text{N}$ values significantly increased from 7.2 to 8.3‰ with age ($P < 0.01$; Table 2), indicating differences in food preferences and trophic position. The $\delta^{15}\text{N}$ values of age-3⁺ and 4⁺ scallops were similar and significantly higher than those of age-1⁺ and 2⁺ individuals ($P < 0.05$). Muscle $\delta^{15}\text{N}$ were independent of location or site with values ranging from 7.6 to 8.1‰ (mean = 7.9‰). Ontogenetic changes in stable isotope ratios were found for all the cohorts examined, showing significant positive trends of

increasing $\delta^{15}\text{N}$ values with age ($n = 83$, $r^2 = 0.69$, $P < 0.001$): $\delta^{15}\text{N} = 6.7 + 0.35 (\text{age})$ (in years) and size ($n = 83$, $r^2 = 0.46$, $P < 0.001$): $\delta^{15}\text{N} = 5.3 + 0.02 (S_H)$ (in mm) (**Figures 2a,b**). However, muscle $\delta^{15}\text{N}$ values were highly correlated with age than with size among cohorts, indicating greater influence of age on $\delta^{15}\text{N}$. On the contrary, the $\delta^{13}\text{C}$ values did not vary significantly among the four age classes (Table 2), although age-1⁺ individuals had less depleted ^{13}C . Moreover, $\delta^{13}\text{C}$ values remained constant on average among parameters age and size, in which the $\delta^{13}\text{C}$ values were neither correlated with age nor size (**Figures 2c,d**). On the average, *M. yessoensis* occupied a trophic position between 2.3 to 2.7 (Table 3).

Application of the mixing model to muscle $\delta^{15}\text{N}$ values indicated that **SPOM contribution to scallop's diet decreased from 68% to 8%, being the highest** in age-1⁺ and 2⁺, and lowest for age-3⁺ and 4⁺ individuals (**Figure 3, Table 4**). **However, consumption of zooplankton increased from 15 to 50% as scallop aged (Figure 3, Table 4)**. These results indicated that *M. yessoensis* to some degree shift their diet from SPOM to zooplankton, potentially influencing the $\delta^{15}\text{N}$ values of this species. **However, application of the mixing model to muscle $\delta^{13}\text{C}$ values did not agree well with the isotope values of their presumed potential food sources (Figure 3), indicating that**

scallops were preferentially assimilating highly enriched component (i.e. sinking particles [11]) in the suspended organic matter pool.

Discussion

Our study provides information on the relative importance of age as an indicative of dietary change in *Mizuhopecten yessoensis*. It also uncovers an ontogenetic shift in diet wherein juvenile scallops mainly depend on suspended particulate organic matter (SPOM), whereas adult individuals forage on zooplankton as the most important dietary component. We indeed observed with our isotopic dataset that juveniles and adult scallops, independent of age and size, exhibited similar $\delta^{13}\text{C}$ values indicative of similar quality of food sources. This is also evident from the lack of significant relationship between the $\delta^{13}\text{C}$ values for muscle and age or size because of the small variability in the $\delta^{13}\text{C}$ isotopic values. These results however are not supported by previous dietary studies using bivalves [31,32], where carbon isotope ratios showed greater differences than nitrogen isotope ratios. The large differences in $\delta^{15}\text{N}$ between young and adult scallops may suggest that they preferred different food sources but to some extent, scallops still occupied the same trophic position (2.3–2.7). Our estimates of scallop's trophic position supported the results of [33] confirming the herbivorous and

detrivorous nature of *M. yessoensis*. Bivalve species, scallops in particular, are feeding
 on a mixed diet sources but mainly on phytoplankton [9] or sinking particles in their
 natural habitats [10,11]. The increase in ^{15}N values with age may result from foraging
 on zooplankton, which supports the observed enrichment in ^{15}N . The $\delta^{15}\text{N}$ isotope ratios
 could be expected to increase with age if nitrogen undergoes fractionation during the
 transamination or deamination process where the lighter $\delta^{14}\text{N}$ is excreted at a faster rate
 than $\delta^{15}\text{N}$ from the body of some animals [19]. The isotope fractionation, with the
 lighter isotope reacting faster in each direction of the reversible reactions has been
 determined to occur during transamination reactions [34]. We may interpret that age is a
 stronger determinant of the diet of *M. yessoensis* and the increase of ^{15}N values with age
 as indicative of the ontogenetic foraging shift from SPOM to zooplankton.

Mizuhopecten yessoensis, as they age and grow, gradually shift their diet from
 SPOM to zooplankton, and through assimilation, the isotopic ratios of their food
 sources were reflected in the scallop muscle tissues. Our study indicated significant
 discrepancy in the isotopic composition between SPOM and zooplankton which may
 have a large impact on the $\delta^{15}\text{N}$ values observed in the different age classes of *M.*
yessoensis. In fact, a significant increase in $\delta^{15}\text{N}$ values with age in the present study
 suggests ontogenetic shifts similar to that of freshwater mussels [35] and walleye [22]

and shows that age is a stronger determinant of dietary change than size. However, previous studies on mussels [19] and rainbow smelts [21] failed to identify any significant change of $\delta^{15}\text{N}$ with age. The percentage of the total variation in $\delta^{15}\text{N}$ as explained by size (46%) was lower than those accounted for by age (69%), possibly due to constant growth among adult scallops. Indeed, *M. yessoensis* reached an asymptote size of 126 mm between age-3⁺ and 4⁺, the difference in growth rate between these age classes was not significant [F. Aya, unpubl. data].

Our analysis of the isotope mixing model supports the hypothesis that juveniles (age-1⁺ and 2⁺) exploit higher proportion of SPOM than adults (age-3⁺ and 4⁺), possibly due to difference in foraging capabilities. Juveniles have small mouth gape which limits their consumption to SPOM, whereas adult individuals, with larger mouth gape size, can feed on a wider range of prey including SPOM to large-sized food sources such as zooplankton. Previous work on the same species showed that for juveniles, sinking particles, composed of aggregated fresh microalgae, are considered more important energy source although they also consumed SPOM [11]. This explains the higher contributions of SPOM for the age-1⁺ and 2⁺ scallops, while zooplankton was significantly important for the age-3⁺ and 4⁺ individuals. Feeding studies analyzing stomach contents of other bivalve species have observed shifts from planktivorous to

piscivorous diets in different size classes [31]. In other studies, zooplankton is considered a potential food source for zebra mussels [14], while larger zebra mussels have been reported to suppress zooplankton abundance [36]. Also, [35] reported that the stomach contents of fan shell *Pinna nobilis* consist of water column calanoid copepods, an indication of exploitation on zooplankton by this species. Older and larger mussel individuals, with larger inhalant siphons, may eat drifting invertebrates or other organisms that feed higher on the food chain [35]. These age-related dietary resource partitioning is consistent with physiological differences arising with age [37]. **However, the large discrepancy between the diet $\delta^{13}\text{C}$ values and those of scallop muscle tissues could be attributed to the low assimilation of low amount of carbon from ingested material [11].**

In summary, the present study demonstrates the greater influence of age than size on $\delta^{15}\text{N}$ isotope ratios of *M. yessoensis*. Results of the isotope mixing model suggest unequal contributions of SPOM and zooplankton to scallops' diet, indicating gradual shift in prey consumption. These findings explain the potential accumulation of ^{15}N with age, as suggested by a strong nutritional dependence of *M. yessoensis* on zooplankton.

Acknowledgements

The authors thank Messrs. M. Fujiwara and M. Kimura of the Tokoro Fisheries Cooperative Association for providing scallop samples, the captain and crew of the *T/S Ushio-Maru* of the Hokkaido University and Y. Hidaka, Y. Yamada, E. Kakuta for their assistance during sampling. This work was supported by a research grant from the Ministry of Education, Science and Technology (MEXT) to F. A. Aya.

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Figure legends

Figure 1. Sampling stations and culture zones for scallops in the Sea of Okhotsk.

Figure 2. Relationship between muscle $\delta^{15}\text{N}$ and (a) age and (b) size, and between muscle $\delta^{13}\text{C}$ and (c) age and (d) size (as shell height, S_H) from different cohorts of *Mizuhopecten yessoensis*.

Figure 3. Dual isotope plots showing the natural $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values (mean $\pm$ SD) of scallop muscle tissues in different age classes and potential food sources (PL100–330, micro-zooplankton; PL>330, meso-zooplankton; SPOM, suspended particulate organic matter). **Scallop age classes are denoted by open symbols, with expected diet values after trophic correction of 3.4‰ and 1.7‰ for muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values, respectively, indicated by solid symbols.**

Figure 1

Figure 2

Fig. 3

Table 1

Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and carbon to nitrogen (C/N) molar ratios of potential food sources collected at Station S-0, Sea of Okhotsk

Food source	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C/N molar ratios
SPOM	-24.0 ± 1.1 (25) ^a	5.4 ± 1.2 (24) ^a	6.1 ± 1.4 ^a
Zooplankton			
100–330 μm	-23.4 ± 0.8 (8) ^a	6.6 ± 0.8 (8) ^b	6.7 ± 1.4 ^a
>330 μm	-23.5 ± 0.6 (8) ^a	6.9 ± 0.6 (8) ^b	6.8 ± 0.9 ^a

SPOM, suspended particulate organic matter. Values in parenthesis represent the number of samples analyzed. Column means followed by a different letter superscript are significantly different at $P < 0.05$

Table 2

Stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of muscle tissues in different age classes and sizes

Age class (in Years)	Shell height (mm)	<i>N</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
1 ⁺	65-95	13	-18.1 ± 0.3 ^a	7.2 ± 0.2 ^a
2 ⁺	95-116	24	-18.3 ± 0.4 ^a	7.6 ± 0.3 ^a
3 ⁺	108-125	24	-18.3 ± 0.4 ^a	8.1 ± 0.2 ^b
4 ⁺	116-127	22	-18.2 ± 0.4 ^a	8.3 ± 0.1 ^b

Column means followed by a different letter superscript are significantly different at $P < 0.05$

Table 3

Mean trophic position of scallops grouped by age

Age class (in Years)	<i>N</i>	Mean $\pm$ SD	Range
1 ⁺	13	2.5 $\pm$ 0.3	2.1–3.2
2 ⁺	24	2.3 $\pm$ 0.1	2.1–2.4
3 ⁺	24	2.5 $\pm$ 0.2	2.2–2.9
4 ⁺	22	2.7 $\pm$ 0.2	2.4–3.0

Table 4

Percentage $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ contributions of micro- (100–330 μm), meso-zooplankton (>330 μm) and suspended particulate organic matter (SPOM) to scallops' diet in different age classes. Values represent mean (range).

Age class (in Years)	Food sources		
	SPOM	Zooplankton	
		100–330 μm	>330 μm
1 ⁺	68 (65-70)	18 (0-35)	15 (0-30)
2 ⁺	46 (41-50)	30 (0-59)	25 (0-50)
3 ⁺	19 (12-25)	44 (0-88)	38 (0-75)
4 ⁺	8 (0-15)	50 (0-100)	43 (0-85)

Table S1

Summary table showing the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) values of suspended particulate organic matter (SPOM) sampled from 2007 to 2009 at Tokoro seabed, Okhotsk Sea

Date	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
2007 ($n = 18$)	-24.7 ± 0.8	5.4 ± 1.7
2008 ($n = 24$)	-24.0 ± 0.9	4.4 ± 1.3
2009 ($n = 12$)	-22.7 ± 0.5	6.0 ± 1.0
Mean	-23.8	5.3
SD	1.0	0.8

Table S2

Summary table showing the tissue-diet isotopic fractionation values ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) among ages of *Mizuhopecten yessoensis* grown at Tokoro seabed, Okhotsk Sea. The difference between isotopic ratios of gut content and that of muscle tissue was used as representation of tissue-diet isotopic fractionation.

Age (in Years)	$\Delta\delta^{13}\text{C}$	$\Delta\delta^{15}\text{N}$
1 ⁺ (<i>n</i> = 12)	3.3 ± 0.7	1.6 ± 1.0
2 ⁺ (<i>n</i> = 12)	3.3 ± 0.9	1.3 ± 0.8
3 ⁺ (<i>n</i> = 12)	3.4 ± 0.7	1.6 ± 1.3
4 ⁺ (<i>n</i> = 12)	3.7 ± 0.6	2.3 ± 1.2
Mean	3.4	1.7
SD	0.2	0.4

Table S3

Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) values of muscle tissues from different cohorts of *Mizuhopecten yessoensis* grown at Tokoro seabed, Okhotsk Sea

Cohorts	Area	Date (mo/yr)	Recalculated Age (in years)	Shell Height (mm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
2007	B	10/2008	1.42	74.94	-18.3 ± 0.0	7.2 ± 0.1
		11/2008	1.47	78.56	-18.2 ± 0.0	7.2 ± 0.3
		12/2008	1.53	76.20	-18.3 ± 0.1	7.5 ± 0.2
		5/2009	1.95	89.20	-18.8 ± 0.1	7.3 ± 0.1
2006	C	10/2007	1.40	80.30	-18.2 ± 0.4	7.0 ± 0.3
		11/2007	1.49	82.60	-18.0 ± 0.1	6.9 ± 0.2
		4/2008	1.88	94.66	-18.1 ± 0.2	7.4 ± 0.2
		5/2008	1.95	93.81	-17.7 ± 0.0	7.4 ± 0.3
		6/2008	2.06	98.10	-17.8 ± 0.1	7.7 ± 0.2
		7/2008	2.15	103.54	-18.1 ± 0.1	7.5 ± 0.1
		8/2008	2.21	101.39	-17.9 ± 0.3	7.7 ± 0.2
		9/2008	2.28	106.38	-18.0 ± 0.3	7.6 ± 0.1
		10/2008	2.42	108.12	-17.9 ± 0.3	7.2 ± 0.3
		11/2008	2.47	112.02	-17.6 ± 0.1	7.2 ± 0.0
		12/2008	2.53	108.86	-17.6 ± 0.1	7.4 ± 0.2
		5/2009	2.95	115.60	-18.5 ± 0.2	7.1 ± 0.1
2005	A	10/2006	1.38	77.20	-18.2 ± 0.4	6.9 ± 0.3
		11/2006	1.50	86.60	-18.0 ± 0.1	7.2 ± 0.1
		12/2006	1.55	83.60	-18.1 ± 0.2	7.0 ± 0.1
		4/2007	1.88	87.25	-17.7 ± 0.0	7.6 ± 0.3
		5/2007	1.95	90.75	-17.8 ± 0.1	7.5 ± 0.2
		6/2007	2.03	101.25	-18.1 ± 0.1	7.5 ± 0.1
		7/2007	2.13	102.00	-17.9 ± 0.3	7.5 ± 0.1
		8/2007	2.19	100.75	-18.0 ± 0.3	7.4 ± 0.1
		9/2007	2.27	107.50	-17.9 ± 0.3	7.5 ± 0.2
		10/2007	2.40	109.50	-17.6 ± 0.1	7.6 ± 0.1
		11/2007	2.49	108.38	-17.6 ± 0.1	7.4 ± 0.3
		12/2008	2.88	114.46	-18.5 ± 0.2	7.7 ± 0.2
		5/2008	2.95	112.21	-18.2 ± 0.4	7.5 ± 0.2
		6/2008	3.06	118.77	-18.0 ± 0.1	8.0 ± 0.2
		7/2008	3.15	121.94	-18.1 ± 0.2	8.0 ± 0.1
		8/2008	3.21	117.89	-17.7 ± 0.0	8.0 ± 0.0
		9/2008	3.28	118.87	-17.8 ± 0.1	7.9 ± 0.2
		10/2008	3.42	123.39	-18.1 ± 0.1	7.7 ± 0.4
		11/2008	3.47	121.34	-17.9 ± 0.3	7.6 ± 0.1
		12/2008	3.53	121.55	-18.0 ± 0.3	8.3 ± 0.1
		5/2009	3.95	124.58	-17.9 ± 0.3	7.5 ± 0.1
2004	D	7/2006	2.11	95.20	-19.3 ± 0.0	7.7 ± 0.3
		8/2006	2.19	96.60	-18.9 ± 0.0	7.9 ± 0.2
		9/2006	2.27	99.20	-18.8 ± 0.1	7.8 ± 0.4
		10/2006	2.38	96.90	-18.6 ± 0.3	7.9 ± 0.1
		11/2006	2.50	103.80	-18.2 ± 0.4	8.0 ± 0.2

Cohorts	Area	Sampling Month	Recalculated Age (in years)	Shell Height (mm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
2004	D	12/2006	2.55	105.10	-18.3 ± 0.1	7.9 ± 0.0
		4/2007	2.88	111.00	-18.1 ± 0.2	7.8 ± 0.2
		5/2007	2.95	110.00	-18.8 ± 0.2	7.9 ± 0.2
		6/2007	3.03	112.00	-18.9 ± 0.2	7.4 ± 0.3
		7/2007	3.13	115.75	-18.7 ± 0.3	8.0 ± 0.2
		8/2007	3.19	117.75	-18.9 ± 0.1	7.9 ± 0.2
		9/2007	3.27	122.25	-18.6 ± 0.1	8.2 ± 0.1
		10/2007	3.40	119.50	-18.7 ± 0.2	7.9 ± 0.1
		11/2007	3.49	118.13	-18.4 ± 0.2	8.1 ± 0.1
		4/2008	3.88	123.74	-18.1 ± 0.0	8.1 ± 0.1
		5/2008	3.95	123.16	-17.8 ± 0.1	8.5 ± 0.1
		6/2008	4.06	121.92	-17.7 ± 0.1	8.4 ± 0.2
		7/2008	4.15	124.32	-18.1 ± 0.1	8.4 ± 0.4
		8/2008	4.21	122.80	-18.0 ± 0.3	8.3 ± 0.2
		9/2008	4.28	125.18	-17.9 ± 0.0	8.3 ± 0.1
		10/2008	4.42	125.07	-17.7 ± 0.1	8.1 ± 0.3
		11/2008	4.47	125.85	-17.8 ± 0.1	7.9 ± 0.4
		12/2008	4.53	127.36	-17.6 ± 0.4	8.0 ± 0.4
2003	B	7/2006	3.11	108.40	-18.5 ± 0.4	8.2 ± 0.2
		8/2006	3.19	108.80	-19.0 ± 0.1	8.2 ± 0.2
		9/2006	3.27	106.80	-18.7 ± 0.0	8.4 ± 0.1
		10/2006	3.38	110.60	-18.7 ± 0.1	8.3 ± 0.3
		11/2006	3.50	109.60	-18.6 ± 0.3	8.1 ± 0.2
		12/2006	3.55	112.90	-18.3 ± 0.1	8.2 ± 0.1
		4/2007	3.88	115.50	-18.0 ± 0.1	8.2 ± 0.3
		5/2007	3.95	107.00	-18.4 ± 0.0	8.0 ± 0.3
		6/2007	4.03	116.25	-18.9 ± 0.5	8.2 ± 0.3
		7/2007	4.13	124.50	-18.5 ± 0.2	8.3 ± 0.1
		8/2007	4.19	119.25	-19.1 ± 0.2	8.3 ± 0.1
		9/2007	4.27	118.75	-18.2 ± 0.2	8.3 ± 0.0
		10/2007	4.40	116.75	-18.5 ± 0.1	8.2 ± 0.1
		11/2007	4.49	115.00	-18.3 ± 0.1	8.4 ± 0.1
		4/2008	4.88	124.44	-18.0 ± 0.1	8.7 ± 0.2
		5/2008	4.95	121.75	-17.6 ± 0.1	8.5 ± 0.2
2002	C	7/2006	4.11	119.40	-18.6 ± 0.0	8.1 ± 0.1
		8/2006	4.19	125.40	-18.3 ± 0.5	8.3 ± 0.5
		9/2006	4.27	120.40	-18.3 ± 0.0	8.4 ± 0.2
		10/2006	4.38	119.20	-18.0 ± 0.2	8.6 ± 0.5
		11/2006	4.50	113.20	-18.3 ± 0.3	8.4 ± 0.1
		12/2006	4.55	118.40	-18.6 ± 0.1	8.3 ± 0.3
		5/2007	4.95	124.00	-18.5 ± 0.2	8.3 ± 0.2