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Seasonal Changes in Food Uptake by the Sea Cucumber *Apostichopus japonicus* in a Farm Pond: Evidence from C and N Stable Isotopes

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Abstract This study investigated the seasonal changes in carbon (C) and nitrogen (N) stable isotope values of several typical food sources of *Apostichopus japonicus* in a farm pond, including particulate organic matter (POM), macroalgae, benthic microalgae and animals such as nematode and copepod. The stable isotope technique was used to quantify relative contributions of various sources to the food uptake by *A. japonicus*. The results showed that significant changes occurred in the C and N stable isotope values of sea cucumber food sources due to the seasonality of micro- or macroalgae prosperity and the fluctuation of environmental conditions. The sea cucumber *A. japonicus* exhibited corresponding alterations in feeding strategy in response to the changes in food conditions. Calculation with a stable isotope mixing model showed that macroalgae was the principal food source for *A. japonicus* throughout the 1-yr investigation, with the relative contribution averaging 28.1%–63.2%. The relative contributions of other food sources such as copepod and nematode, POM, benthic microalgae to the total food uptake by sea cucumber averaged 22.6%–39.1%, 6.3%–22.2%, 2.8%–6.5%, and 2.8%–4.2%, respectively. Together these results indicated that the seasonal changes in food sources led to the obvious temporal differences in the relative contribution of various food sources utilized by *A. japonicus*. Such findings provide the basic scientific information for improving the aquaculture techniques of *A. japonicus*, particularly for optimizing the food environment of *A. japonicus* culture in farm ponds.

Key words sea cucumber; *Apostichopus japonicus*; stable isotope; feeding; macroalgae

1 Introduction

The sea cucumber *Apostichopus japonicus* (Skelenka) is a commercially important marine species for aquaculture in China. In the last two decades, the farming of *A. japonicus* has been rapidly developed (Chen, 2004). The total production of this species in China has reached 93000 tons in 2008 with a 19.4% annual increase compared with that in 2007 (Ministry of Agriculture of China, 2009). In order to meet the requirement for rapid extension of sea cucumber farming, extensive research has been conducted on *A. japonicus* regarding genetics (Li *et al*., 2009), energetics (Liu *et al*., 2009; Yuan *et al*., 2009), thermo-tolerance (Dong *et al*., 2010), immunology (Gu *et al*., 2011), and nutrology (Seo and Lee, 2011). However, few studies have investigated the feeding ecology and physiology of *A. japonicus* to date (Zhou *et al*., 2006; Ren *et al*., 2010). Previous research has shown that *A. japonicus* shows the state of dormancy, *e.g.*, aestivation and hibernation during winter due to the extreme temperature conditions, with the feeding and metabolic activities considerably depressed and even ceased. In contrast, *A. japonicus* exhibits active feeding and high growth rate in warm seasons such as spring and autumn (Liao, 1980; Yang *et al*., 2005; Yuan *et al*., 2007; Bao *et al*., 2010).

Understanding the feeding habit of sea cucumbers is of importance to the improvement of relevant aquaculture techniques. As an obligate deposit-feeding species, *A. japonicus* is fed by several food sources, mainly including benthic organisms such as microalgae, nematode, and copepod autochthonously produced in sediment, as well as the allochthonous items settled from water column such as planktonic microalgae and detritus of micro- and macroalgae (Hua, 1989; Zhang *et al*., 1995). The feeding behavior of sea cucumbers as well as their digestion and absorption of food nutrients are generally related to environmental factors of their habitat, particularly the quality

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and quantity of available food sources (Slater and Carton, 2010; Zamora and Jeffs, 2011). Paltzat *et al.* (2008) found that *Parastichopus californicus* Stimpson actively selects organic material from the sediment, thus significantly decreasing the content of sedimentary organic matter in a sea-cucumber-oyster polyculture system in British Columbia, Canada. Slater and Carton (2009) have reported similar results on the feeding activity of *Australostichopus mollis* in sea-cucumber-mussel poly-culture ecosystems in temperate regions. A recent field investigation by Slater and Carton (2010) have revealed the obvious differentiation in carbon (C) and nitrogen (N) stable isotope ratios of *A. mollis* in two habitats in Kenndey Bay, New Zealand. These authors indicated that different food sources were ingested and utilized by the sea cucumber in response to the changes in environmental conditions. More recently, a laboratory experiment by Gao *et al.* (2011) has demonstrated that the mixture of sedimentary substance containing microalgae and the macroalgae *Sargassum thunbergii* or *Gracilaria lemaneiformis* enhanced the growth of *A. japonicus* compared with the pure food ingredient of macroalgae.

Utilization of various food sources by aquatic animals has traditionally been evaluated by direct gut content analysis, *i.e.*, morphological identification of the food items taken up by the consumers (Lehane and Davenport, 2002; Akin and Winemiller, 2006). Ruiz *et al.* (2007), for example, investigated the feeding habit of the sea cucumber *Athyonidium chilensis* in a subtidal ecosystem and quantified various ingested food items *via* gut morphemetry *in situ*. These authors found that the macroalgae and micro-invertebrates were the main prey items of *A. chilensis*. Presently, quantitative evaluation on the uptake of potential food items by sea cucumbers are lacking, mainly because of the technical difficulties in taxonomical identification of the sea cucumber food items caused by the small-size and digestion damages to gut contents (Gao *et al.*, 2006; Wing *et al.*, 2008). Furthermore, application of direct gut content analysis only represents an instant snapshot of the food ingested by animals. This has led to an inevitable bias in quantifying the contribution of various potential items to the consumer's food assimilation due to different digestibility of the food items. As a result, the food items with low digestibility tend to be retained in the gut, thus can be identified as dominant food sources. The stable isotope approach offers distinct advantages over conventional dietary techniques because (1) evaluation of food sources by the stable isotope method is based on the assimilated instead of ingested constituents; and (2) assimilated matter represents the time-integrated utilization of food (Hobson and Welch, 1992). Accordingly, stable isotopes have increasingly been applied as tracers to follow the flux of organic matter or pollutants along food chains or food webs in terrestrial and aquatic environments (*e.g.*, Fry and Sherr, 1984; Riéra *et al.*, 1996; Riéra and Hubas, 2003; Shimoda *et al.*, 2007; Kharlamenko *et al.*, 2008).

The objectives of the present study were (1) to quantify the seasonal changes in the relative contribution of potential food sources to the feeding requirements of *A. japonicus* in a farm pond; and (2) to provide valuable data on the feeding habit of *A. japonicus* for improvement of sea cucumber farming techniques, particularly for optimization of the food environment in farm waters.

2 Materials and Methods

2.1 Study Area and Sample Treatment

This study was conducted in the Homey Group International, Rongcheng City, Shandong Province, eastern China (36˚54΄N, 122˚09΄E). The rectangular farm pond of *A. japonicus* was 1.3 km in length and 1.0 km in width, with water depth ranging from 2.0 to 3.0 m. There are no riverine inputs of freshwater to the pond and at least 1/3 water is exchanged weekly through tidal flushing. The farm pond is directly exposed to the sunlight without any shadow coverage. Juvenile sea cucumbers averaging 5 g in wet weight were released from the hatchery into the farm pond in spring every year.

The seabed of the pond was flattened artificially and covered with polypropylene membranes 2-yr prior to the experiment. The seabed sediment had spatially constant muddy characteristics and was composed of silt and clay mainly settled from the water column. Three sites were selected from the central part of the pond as the sampling stations and marked with floating buoys to ensure the exact positioning during each sampling cruise.

A preliminary investigation was conducted to confirm the potential food sources of *A. japonicus* at each sampling station. Sea cucumbers were collected by SCUBA diving and 10 middle-size individuals (~70 g in weight) were selected for further analysis. The sea cucumbers were transported to laboratory immediately after collection. To confirm the potential food items of *A. japonicus*, the sea cucumbers were dissected with the content collected from the entire digestive tract for microscopic examination. Direct microscopy demonstrated that the dominant food items included various microalgae species, microbenthic animals such as nematode and copepod and fragments of macroalgae.

Samples of sea cucumbers and potential food sources confirmed by microscopic observation were collected in four seasons, *i.e.*, September 2008 (summer), November 2008 (autumn), February 2009 (winter) and May 2009 (spring). During each sampling cruise, 36 adult sea cucumbers (71.85 g ± 17.44 g) were collected from the 3 sampling stations. In addition, approximately 10 kg of sediment was collected with a sediment sampler from the top 3 cm of the seabed for extraction of benthic animals and microalgae, as well as determination of typical pigment chlorophyll (Chl) *a* level.

The sea cucumbers were dissected in the laboratory, with the entire digestive tract rinsed with distilled double deionized water (DDDW). Then, the sea cucumbers were dried at 55℃ for > 72 h to constant weight and ground to fine, homogeneous powder using a sterile micro-grinder and a 0.1 mm sieve.

The potential food sources including benthic microalgae, nematodes and copepods were immediately separated from surface sediment upon collection. Benthic microalgae ($n=3$) were extracted following Couch (1989) and Riéra *et al.* (1996), Nematodes ($n=1-3$) extracted according to Couch (1988), and copepods ($n=1$) collected following Couch (1989). Thereafter, the nematodes and copepods were starved overnight in filtered seawater. Finally, all benthic microalgae, nematodes and copepods samples were collected on pre-combusted (450℃ for 6 h), ashless, glass fiber filters (Whatman GF/F) and weighed using a gentle vacuum suction (1/3–1/2 atmospheric pressure). The filters retaining microalgae, nematodes and copepods were dried at 55℃ for >72 h to constant weight. Two aliquots of the samples were prepared for C and N stable isotope analysis, respectively. For C stable isotope analysis, the samples were decarbonated using HCl (35%) fume for at least 24 h until no CO_2 bubbles were produced, as confirmed by a dissecting microscope. According to Lorrain *et al.* (2003), decarbonation via HCl-acidification removes inorganic carbonate without significantly affecting the stable isotope ratios of organic C. For N stable isotope analysis, the sample was directly measured without acid treatment. All samples were stored at −80℃ prior to stable isotope analyses.

Triplicate samples of suspended particulate organic matter (POM) were obtained from seawater by filtration and HCl treatment. Twenty-five liters of seawater was collected about 0.5 m above the seabed at each sediment sampling site with an acid-treated, pre-rinsed plexiglass water sampler (HL-CS1000 mL, Shanghai). Water samples were sieved through a 120 μm mesh to remove large-size zooplankton and fragments of macroalgal debris, and then filtered through glass fiber filters. Thereafter, the filter papers were quickly rinsed with DDDW water to remove salts adsorbed on the particle surface. Here, DDDW instead of isotonic salt solution was used to avoid potential salt contamination by seawater or the isotonic solution. No obvious cell rupture occurred under the short flushing time (<1 min) (Currin *et al.*, 1995; Gao *et al.*, 2006). Direct microscopy showed that the dominant constituents retained on the filters included various phytoplankton species and fine silty particles. Few pieces of debris retained on the filters were removed with forceps under microscope. For C stable isotope analysis, the filters containing suspended POM were decarbonated with HCl- treatment as that for benthic microalgae treatment.

To evaluate the relative contribution of macroalgae to food uptake by the sea cucumber, triplicate samples of dominant macroalgae species were collected by SCUBA diving during each sampling cruise, handpicked from the pond shore, or removed from farming facilities such as rafts and floating buoys connected with anchor. Preliminary analysis indicated that there were no obvious differences in the isotopic values among macroalgae species and their decayed detritus in the farm pond. Hence, fresh macroalgae instead of debris were sampled for stable isotope analysis. These macroalgae species commonly prosper in spring and depress in summer, with an obvious seasonal succession typical in temperate regions (Fong and Zedler, 1993; Shimoda *et al.*, 2007). The collected samples were examined under a dissecting microscope, and sediments and epibiota on the leaf surface were cleaned. The samples were rinsed with DDDW, dried at 55℃, and ground into powder (<0.1 mm) with a sterile mortar and pestle. The dried samples were stored at −80℃ prior to further analysis.

Our major objective was to quantify the relative contributions of potential food sources separated from surface sediment to the food taken up by *A. japonicus* using stable isotope techniques. Therefore, the isotopic ratios of potential food items were specifically measured in replacement of the bulk stable isotope analysis for the sediment.

2.2 Measurements of Environmental Conditions

During each sampling cruise, the temperature and salinity of seawater were measured *in situ* ~0.5 m above the seabed. One liter of sea water ($n=3$) was collected from the same water depth for typical pigment Chl *a* analysis. The Chl *a* was extracted from seawater and surface sediment with 90% acetone and measured with a spectrophotometer following Strickland and Parsons (1977).

2.3 Measurement of Stable Isotope and Elemental Concentrations

The C and M isotope ratios were determined using an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS, ThermoFinnigan MAT Deltaplus). Results of the isotope ratios were expressed in standard δ-unit notation as follows:

$$\delta X=[(R_{\text{sample}}/R_{\text{standard}})-1]\times 1\,000‰$$

where X is ^{13}C or ^{15}N, and R is $^{13}C:^{12}C$ ratio for C or $^{15}N:^{14}N$ ratio for N. The values were reported relative to the Vienna Pee Dee Belemnite standard (PDB) for C and to air N_2 for N. A laboratory working standard (glycine) was run for every 10 samples. Analytical precision was ±0.1‰ and ±0.2‰ for C and N, respectively. The C and N levels were determined using a CHNS/O Analyzer (PE2400 Series Ⅱ, PerkinElmer).

2.4 Statistical Analysis and Isotope Mixing Model

One-way analysis of variance (ANOVA) with Turkey's test for multiple comparisons was used to compare the differences of environmental parameters, *i.e.*, water temperature, salinity and Chl *a* levels of water and surface sediment among the four cruises and to examine the seasonal variability of isotopic values and potential food sources of *A. japonicus*. Prior to ANOVA analysis, raw data were tested for normality of distribution and homogeneity of variance with Kolmogorov-Smirnov test and Levene's test, respectively (Zar, 2009). All statistical analyses were performed with SPSS 16.0 (SPSS Inc., 2008).

The relative contribution of each potential food source to sea cucumber assimilation was evaluated with the software 'Isosource' (Phillips and Gregg, 2003). For C and N isotopes, average fractionation effects of 1‰ (Peterson and Fry, 1987; McClelland and Valiela, 1998; McCutchan *et al.*, 2003) and 1.6‰ (Vanderklift and Ponsard, 2003) were respectively used to correct stable isotope shifts for each trophic level. The N isotopic fractionation of 3‰–4‰ for each trophic level is widely used in the trophic relationship studies using stable isotope analysis. A recent review by Vanderklift and Ponsard (2003), however, concluded that the extent of isotopic discrimination of ammonotelic invertebrates, including *A. japonicus*, is lower than those of ureotelic or uricotelic organisms. On average, ammonotelic invertebrates show isotopic fractionation of 1.6‰ instead of 3‰–4‰ for each trophic level as used in this study.

3 Results

3.1 Environmental Conditions

The environmental conditions of the sea cucumber pond exhibited obvious seasonality (Table 1). The temperature significantly fluctuated in different seasons, with the highest value observed in summer and lowest value in winter ($F_{3,11}=33\,953.00$, $P<0.05$). The salinity was below 30 in summer and autumn, significantly lower than that in spring and winter ($F_{3,11}=261.33$, $P<0.05$). The seawater Chl *a* level was significantly higher in summer and autumn compared to those in spring and winter ($F_{3,11}=52.57$, $P<0.05$). However, the surface sediment showed no significant differences in Chl *a* level amongst four seasons, despite that the pigment level was higher in spring and autumn than in summer and winter ($F_{3,11}=1.09$, $P<0.05$).

3.2 Isotopic Composition and Food Contributions

The δ^{13}C and δ^{15}N values of *A. japonicus* ranged from −18.38‰ to −16.09‰ and 12.02‰ to 12.68‰, respectively (Table 2). Significant differences were observed in the δ^{13}C values of the sea cucumber among the four seasons ($F_{3,11}=61.53$, $P<0.01$), with ^{13}C enriched in summer and depleted in autumn. As for N stable isotope, the δ^{15}N values of *A. japonicus* showed no obvious seasonal fluctuations, except that the highest δ^{15}N value observed in autumn was significantly different from those in spring and summer ($F_{3,11}=12.21$, $P<0.05$).

Table 1 Environmental conditions in the sea cucumber farm pond of *Apostichopus japonicus* in four seasons during September 2008 to May 2009

Parameters	Sampling season			
	Summer (September 2008)	Autumn (November 2008)	Winter (February 2009)	Spring (May 2009)
Temperature (℃)	28.2 ± 0.1^a	15.0 ± 0.1^b	2.3 ± 0.1^c	17.5 ± 0.1^d
Salinity	28.9 ± 0.5^a	28.3 ± 0.2^a	34.9 ± 0.1^b	30.1 ± 0.1^c
Water Chl *a* (μg L^{-1})	8.58 ± 0.20^a	7.87 ± 0.29^a	3.68 ± 0.55^b	3.73 ± 0.32^b
Sediment Chl *a* (μg g^{-1})	2.10 ± 0.51^a	3.48 ± 2.55^a	1.64 ± 0.42^a	2.77 ± 0.35^a

Notes: data are presented as mean ± SD, $n=3$; different superscript letters within the same row mean significant difference ($P<0.05$).

Table 2 Seasonal changes in the isotopic composition (‰) of *Apostichopus japonicus* and its potential food sources in a farm pond during September 2008 to May 2009

Samples	Summer (September 2008)		Autumn (November 2008)		Winter (February 2009)		Spring (May 2009)	
	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
A. japonicus	−16.09 ± 0.35	12.68 ± 0.20	−18.38 ± 0.17	12.40 ± 0.12	−17.65 ± 0.23	12.08 ± 0.15	−17.79 ± 0.16	12.02 ± 0.13
POM	−22.18 ± 0.14	7.12 ± 0.15	−22.48 ± 0.13	7.29 ± 0.13	−25.63 ± 0.13	7.52 ± 0.33	−24.42 ± 0.04	5.52 ± 0.31
Benthic microalgae	−21.76 ± 0.76	4.10 ± 0.71	−24.26 ± 0.39	5.27 ± 0.40	−23.23 ± 0.09	5.82 ± 0.11	−23.15 ± 0.06	5.18 ± 0.26
Nematode	−22.28 ± 1.94	9.36 ± 0.24	−21.29 ± 1.05	10.75 ± 0.56	−24.43	8.73	−20.63	9.07
Copepod	–	–	−19.67	11.52	−20.66	11.43	−22.12	11.22
Chlorophyta								
Ulva prolifera	–	–	−16.43 ± 0.45	10.00 ± 0.04	–	–	−16.26 ± 0.37	10.93 ± 1.61
Ulva pertusa	–	–	−15.27 ± 0.02	13.05 ± 0.02	–	–	−11.54 ± 0.20	9.65 ± 0.48
Codium fragile	–	–	−15.75 ± 1.27	11.65 ± 1.46	–	–	−15.02 ± 0.01	12.49 ± 0.03
Rhodophyta								
Gracilaria bursapastori	–	–	–	–	–	–	−20.06 ± 0.23	7.82 ± 0.79
Gelidium amansii	–	–	−16.51 ± 0.18	11.47 ± 0.14	–	–	−16.51 ± 0.18	11.47 ± 0.14
Phaeophyta								
Laminaria sp.	–	–	–	–	–	–	−16.18 ± 0.02	10.69 ± 0.22
Ectocarpus sp.	–	–	–	–	−16.68 ± 0.23	10.52 ± 0.12	–	–
Sargassum thunbergii	–	–	−16.57 ± 0.62	9.39 ± 0.35	–	–	−16.24 ± 0.04	9.46 ± 0.33

Notes: POM: suspended particulate organic matter; for data presented as mean ± SD, n = 3 and for those presented as mean without SD, n = 1; '–' samples unavailable.

The stable isotopic ratios of POM showed significant seasonal fluctuations in both δ^{13}C and δ^{15}N values ($F_{3,11}=587.20$, $P<0.01$ for δ^{13}C; and $F_{3,11}=47.74$, $P<0.01$ for δ^{15}N). For C stable isotope of POM, the δ^{13}C values were higher in summer and autumn than those in winter and spring. As for N stable isotope of POM, ^{15}N was significantly depleted in summer as compared to the other three seasons. Similarly, the ^{13}C values of benthic microalgae extracted from surface sediment was most enriched in summer, with no significant differences from the other three seasons. The $\delta^{15}N$ values of benthic microalgae significantly varied in different seasons, with the lowest value observed in autumn ($F_{3,11} = 661.48$, $P < 0.01$ for $\delta^{13}C$; and $F_{3,11}=9.68$, $P<0.01$ for $\delta^{15}N$). For benthic animals extracted from surface sediment, harpacticoid copepod had higher C and N isotopic ratios than nematode.

As for the macroalgae, a total of 8 dominant species were collected during the four sampling cruises. Due to different growth seasons, the sampling of several species was not satisfactory in the depression seasons. Most macroalgae species were collected in autumn or spring only. Dominant macroalgae species was unavailable in autumn due to the degradation of most macroalgae species in summer. In winter, only the Phaeophyta *Ectocarpus* sp. was found in the pond. In general, the $\delta^{13}C$ values stabilized between −16‰ and −15‰, except the highest value (−11.54‰) in the Chlorophyta *Ulva pertusa* and lowest value (−20.06‰) in the Rhodophyta *Gracilaria bursa-pastori* in summer. The $\delta^{15}N$ values of most macroalgae species had no significant differences between autumn and spring, except that the ^{15}N value of *U. pertusa* was markedly depleted in summer compared to winter.

The dual C and N isotope plots for 3 sampling seasons illustrated the incorporation of food sources into the tissue of *A. japonicus* (Fig.1). Computation with "Isosource" (Phillips and Gregg, 2003) quantified the relative contributions of various food sources to the total food assimilation of *A. japonicus* in winter, spring and summer (Table 3). The calculation of food contributions in autumn was unavailable because the sea cucumbers stopped feeding in summer. In general, macroalgae was the dominant food source of sea cucumbers during the 1-yr investigation period, with the relative contribution averaging 54.4%−63.2% in winter and spring and 28.1% in autumn. Benthic microbenthos, *i.e.*, copepod and nematode, were also important food sources of sea cucumber, which respectively contributed 22.6%−39.1% and 6.3%−22.2% on average to the sea cucumber feeding. The relative contributions of other food sources, *i.e.*, POM and benthic microalgae were lower compared to those of macroalgae and benthic animals. The contributions of different food sources showed substantial seasonal changes, despite that macroalgae or copepod was the dominant food source of *A. japonicus*. Generally, the contributions of tested food sources (except for macroalgae) peaked in winter, while that of macroalgae increased in winter and spring.

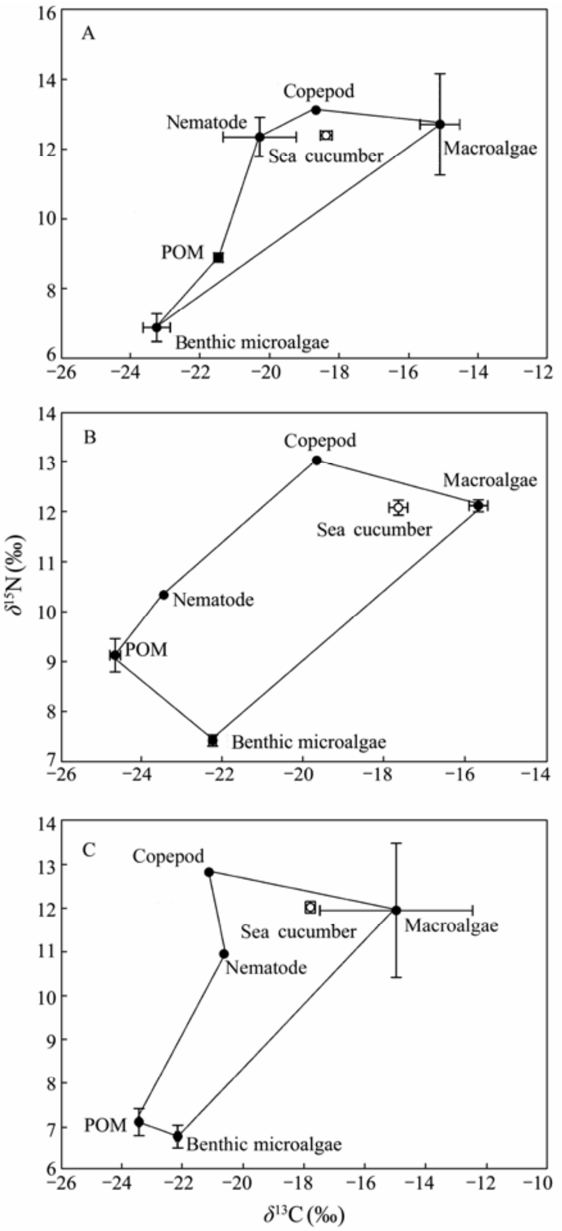

Fig.1 Dual stable isotope plots of $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) for the sea cucumber *Apostichopus japonicus* and its potential food sources in a farm pond in three sampling seasons during November 2008 to May 2009. POM, suspended particulate organic matter. The stable isotope ratios of food sources were corrected with the average fractionation effects of 1‰ and 1.6‰ for C and N, respectively. A, November 2008; B, February 2009; and C, May 2009.

Table 3 Contribution of potential food sources (%) to sea cucumber dietary consumption in a farm pond in three sampling seasons during November 2008 to May 2009

Food source	Autumn (November 2008)		Winter (February 2009)		Spring (May 2009)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Macroalgae	3.0−49.0	28.1 ± 8.1	43.0−80.0	63.2 ± 6.7	45.0−64.0	54.4 ± 4.0
Copepod	0−91.0	39.1 ± 19.6	0−54.0	22.6 ± 9.7	6−52.0	29.4 ± 7.2
Nematode	0−70.0	22.2 ± 15.4	0−27.0	6.3 ± 5.3	0−43.0	10.6 ± 8.6
POM	0−24.0	6.5 ± 5.0	0−19.0	4.4 ± 3.7	0−12.0	2.8 ± 2.2
Benthic microalgae	0−16.0	4.2 ± 3.4	0−15.0	3.5 ± 3.0	0−12.0	2.8 ± 2.5

Notes: POM, suspended particulate organic matter; average fractionation effects of 1‰ and 1.6‰ for C and N were respectively used to correct the stable isotope shifts between trophic levels.

4 Discussion

Results from the present study showed that substantial seasonal shifts occurred in the C and N stable isotope ratios of the sea cucumber *A. japonicus* and its potential food sources, *i.e.*, POM, macroalgae, benthic microalgae and benthic animals such as nematode and harpacticoid copepod. Such seasonal variations were mainly due to the changes in the abiotic environmental conditions and/or the biotic physiological processes (Canuel *et al*., 1995; Gannes *et al*., 1998; Schmidt *et al*., 1999; Gao *et al*., 2006; Kharlamenko *et al*., 2008).

The δ^{13}C values of *A. japonicus* showed significant differences among four seasons while the δ^{15}N values exhibited no obvious seasonal fluctuations. Both δ^{13}C and δ^{15}N values were highest in summer compared with those in other seasons. Such seasonal pattern of C and N stable isotopes could be attributed to the excessive expenditure of nutritional reserve in summer. Previous studies have shown that adult *A. japonicus* exhibits the state of dormant aestivation under extremely high water temperature in summer. During the dormancy, the alimentary tract of the sea cucumber degenerates, thus considerably depressing and even ceasing the feeding. As a result, the nutritional budget of *A. japonicus* shows a net consumption in the summer aestivation state, resulting in notable biomass losses (Liao, 1980; Yang *et al*., 2005; Yuan *et al*., 2007; Bao *et al*., 2010). During dormancy, the prior consumption of isotopically light ^{12}C or ^{14}N in the body wall or gut tissues leads to the enrichment of ^{13}C and ^{15}N in summer (Peterson and Fry, 1987; Peterson, 1999). On the other hand, biological components, *e.g.*, proteins, lipids and carbohydrates in various tissues potentially result in different isotopic fractionations. For instance, the tissues containing higher proportions of lipids usually exhibit higher negative C isotopic ratios than those with lower lipid contents, owing to the discrimination against ^{13}C during lipid synthesis (Tieszen *et al*. 1983; Sun *et al*. 2012). During the aestivation of sea cucumber, net consumption of energy substrates including lipids and proteins substantially reduces the contents of biological components in summer than in the other seasons, particularly spring and autumn when the sea cucumber grows rapidly with substantial accumulation of biological constituents (Bao *et al*., 2010). Hence, seasonal fluctuations in the biological composition of sea cucumber might be another factor contributing to the isotopic differences among the four seasons.

The temporal variations in δ^{13}C values of POM were consistent with those of POM derived from marine source reported by other studies (Peterson, 1999; Shimoda *et al*., 2007). In the present study, the δ^{13}C values of POM showed marked seasonal fluctuations, with the highest value observed in summer (September 2008) and the lowest in winter (February 2009). Generally, planktonic microalgae showed the highest primary productivity in summer and the discrimination of ^{13}C vs. ^{12}C during the processes of photosynthesis was reduced. As a result, δ^{13}C of POM showed the highest value in summer season. (Fry and Wainright, 1991; Canuel *et al*., 1995). Similarly, the δ^{13}C values of benthic algae extracted from surface sediment peaked in summer owing to the highest productivity of benthic microalgae in the warm season.

Assimilation of various food sources by marine consumers depends on both exogenic food availability and autogenic food preference of the animal (Wong and Cheung, 2001; Mangion *et al*., 2004; Gao *et al*., 2006). As a strategy to counteract the substantial fluctuations in the quantity and quality of organic matters potentially utilizable as food sources, marine animals show substantial feeding preference to various food components to optimize the energetic and nutritional requirements, such as the deposit-feeding holothurians species *Holothuria atra*, *Holothuria leucospilota* (Mangion *et al*., 2004), and *A. mollis* (Zamora and Jeffs, 2011).

Macroalgae are among the most productive plants in marine ecosystems. Up to 90% of the net production of macroalgae may end up as detritus ingestible by benthic deposit-feeders, including sea cucumber (Nadon and Himmelman, 2006). Hence, macroalgae and its decayed debris may be an important food source of sea cucumbers, which accounts for >50% of C utilized by benthic animals in coastal ecosystems (Nadon and Himmelman, 2006). Our results indicated that *A. japonicus* took up macroalgae as the principal food source throughout the investigation period. However, the relative contribution of macroalgae to the assimilation by *A. japonicus* was lower in spring and autumn than in winter, despite the prosperity of macroalgae in spring and autumn in the pond. This could be attributed to the co-existence of other food items in the pond, *e.g.*, benthic nematode and copepod, which commonly bloom in spring and autumn. The elevated production of such food sources potentially "diluted" the ingestion and uptake of macroalgae by sea cucumber (Franco *et al*., 2010; Debes and Eliasen, 2006; Yang *et al*., 2010).

Compared to macroalgae, benthic animals represented by nematode and copepod were of less importance to the food uptake by *A. japonicus.* However, the presence of animal-source protein including benthic animals in the pond food likely enhanced the metabolism and growth of the sea cucumber via secondary contribution. Recently, Wang *et al*. (2009) have found that the addition of an appropriate amount of fish meal to the food sources of *A. japonicus* was beneficial to increase its growth, indicating that the animal protein was an essential food component for sea cucumber.

In the present study, POM was the third important food source of *A. japonicus* compared to macroalgae and benthic animals. The relative contribution of POM to the food taken up by *A. japonicus* in autumn was higher than that in spring and winter. It was likely that the higher productivity of microalgae in autumn, as indicated by the higher Chl *a* level, elevated the assimilation of POM by sea cucumber compared to that in spring and winter. The decreased settlement of POM in spring and winter was likely caused by the enhanced wave activities associated with the strong southeast and northwest monsoon, which reduced the accumulation of POM for feeding the sea cucumber in spring and summer.

Of the tested food sources, benthic microalgae were of minimum importance to the food taken up by *A. japonicus*, possibly owing to its low digestibility. Previous studies indicated that benthic microalgae are mainly composed of diatom species in the coastal benthic environment in Shandong Peninsula (Jiang *et al*., 2007). Diatoms have been thought to be hardly digested by holothurians, because frustules of unicellular diatoms have evolved protection function and additional mechanical digestion is required to crush the diatom cells (Hamm *et al*., 2003). Despite of relatively low dietary contribution, benthic microalgae including various diatoms can be an essential food component for the sea cucumber. In one of our recent laboratory studies (Gao *et al*., 2011), we found that the addition of benthic microalgae to the macroalgae *Gracilaria lemaneiformis* and *Sargassum thunbergii* powder as the food sources improved the absorption of macroalgae by sea cucumber, although macroalgae was the preferential food source when the mixture of micro- and macroalgae was supplied.

In conclusion, this study suggested that despite the deposit-feeding habit, a substantial proportion of the food assimilated by *A. japonicus* was from macroalgae and POM settled from the water column. Hence, to increase the frequency of water exchange between the pond and open sea may enhance the quantity and/or quality of the food supply to sea cucumbers. As this study was conducted on the sea cucumber *A. japonicus* cultured in one farm pond, its representativeness needs to be tested and application of the results to other systems should be cautious.

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