Journal of the Marine Biological Association of the United Kingdom, 2016, 96(1), 197–204.
doi:10.1017/S002531541500168X

Fatty acid component in sea cucumber *Apostichopus japonicus* from different tissues and habitats

QINZENG XU[1,2], QIANG XU[1], XUELEI ZHANG[2], QUANCAI PENG[1] AND HONGSHENG YANG[1]

[1]Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China, [2]Key Laboratory of Marine Ecology and Environmental Science and Engineering, First Institute of Oceanography, State Oceanic Administration, Qingdao 266061, China

Fatty acids (FA) are a non-protein energy source and can act as trophic biomarkers in benthic food webs. We analysed the FA profiles of sea cucumber Apostichopus japonicus, *comparing tissues of body wall, gut sediment and ovaries in two habitats. Rongcheng Bay: kelp raft cultivation area with high organic matter in sediment; Laoshan Bay: strong current with low sediment organic matter. The results showed that body wall and ovary tissues were rich in long chain polyunsaturated FA (LC-PUFA), which contributed ~31% to the FA dissimilarity between the two tissues. SIMPER (similarity percentages routine) results showed that C20:5ω3 (EPA), C18:1ω7, C20:4ω6 (AA), C16:0, C14:1 and C20:1ω11 contributed to dissimilarity between the body wall and ovary tissues, while 16:1ω7, 20:5ω3, C16:0, C18:1ω7, C18:0 and C14:1 contributed more to the dissimilarity of body wall tissues between the two habitats. FA biomarkers showed that sea cucumbers from the two habitats had different food sources, with brown kelp and vascular plants being the main food for sea cucumbers in Rongcheng and diatoms for those in Laoshan. To better understand differences in FA composition in sea cucumbers, more research is needed examining a wider diversity of tissue types and habitats.*

Keywords: fatty acid, sea cucumber, *Apostichopus japonicus*, food resource

Submitted 12 December 2014; accepted 15 September 2015; first published online 23 October 2015

INTRODUCTION

Sea cucumbers *Apostichopus japonicus* (Selenka) are harvested as a dietary delicacy and medicinal cure with high nutritional value (Chen, 2004). Moreover, *A. japonicus* play an ecological role as suspension feeders, detritivores and prey for different marine species. Through their trophic activities, deposit feeding holothurians can change the size of benthic particles and turn over sediment (Uthicke, 1999; Anderson *et al.*, 2010). As an obligate deposit-feeding species, the sea cucumber *A. japonicus* plays an important trophic role in marine ecosystems (Slater & Carton, 2009; Ren *et al.*, 2010). The feeding activities of this species could effectively remove nutrient matter deposited in benthic habitats and consequently may reduce the nutrient loadings in coastal ecosystems (Zheng *et al.*, 2009; Sun *et al.*, 2012).

Lipids are a non-protein energy source, which provide energy and essential fatty acids for normal growth and survival of aquatic animals (Seo & Lee, 2011). Fatty acids (FA) are considered to serve a dietary function in benthic food webs (Kelly & Scheibling, 2012). FA and FA ratios are used as biomarkers to confirm and trace food resources and trophic relationships in different aquatic habitats (Penha-Lopes *et al.*, 2009; Coelho *et al.*, 2011).

Apostichopus japonicus is the main cultured species in northern China, and there are three main cultured ways: in ponds, at the coast and in cages. There has been much research on FA of *A. japonicus*, and many have shown that *A. japonicus* has different FA profiles in different sampling times and areas (Kasai, 2003; Dantong *et al.*, 2009; Gao *et al.*, 2011; Han, 2011; Lee *et al.*, 2012). The fatty acid values varied depending on the sampling time and collection region, however, an FA biomarker was not used as a biomarker for many studies.

In the present study we compare FA contents in the body wall and ovary of *A. japonicus* and also study FA biomarkers in sea cucumbers from two different habitats with organic matter in sediment (Rongcheng Bay: kelp raft cultivation area, high organic matter in sediment; Laoshan Bay: strong current area with low sediment organic matter) to determine habitat effects on FA content. Our hypotheses were that (1) fatty acid value especially polyunsaturated FA in ovary was higher than that in body wall and gut sediment; (2) FA composition were different and FA biomarkers may reveal main food resources for *A. japonicus* in the two areas.

MATERIALS AND METHODS

Sampling sites

Samples were taken at two different period in two different habitats. The first samples were taken in Laoshan Bay,

Corresponding author:
Q. Xu and H. Yang
Emails: xuqiangcas@126.com; hshyang@126.com

Qingdao in April 2011. Organic matter in sediment in this bay was about 1–2%, because of strong currents (0.6–0.8 m s^{-1}) and low organic matter input. The second samples were taken in Rongcheng Bay (37°15.29′N 122°35.25′E), Weihai in June 2012, and where the organic content in sediment was high and can reach 7% (Xu *et al.*, 2014) (Figure 1).

Sea cucumbers were taken in Laoshan Bay (36°15.07′N 122°40.80′E), while sea cucumber, sediment in the stomach as well as gonads from females were sampled in Rongcheng Bay. The sampled sea cucumber weight from both habitats was 200–300 g.

The sea cucumber samplings (12 individuals in each area) were dissected in the field after being washed with distilled water. The body wall, female gonad tissues and *in situ* sediment in the stomach were frozen and stored in the laboratory in a freezer at −20°C.

Fatty acid analysis

Tissue, sediment and gonad samples were ground after being lyophilized. The same amount of three samples was chosen randomly to create a mixture sample. Fatty acid analyses of the mixture sample were carried out as in Liu *et al.* (2011). Samples were placed in DCM: MeOH (dichloromethane: methanol) in a proportion of 2:1(v/v) with 0.01% BHT (butylhydroxytoluene) as an antioxidant. After sonication, the mixture was separated by centrifugation. The organic extracts were combined after three extractions and then evaporated under high purity nitrogen. Two millilitres of 6% KOH/MeOH was added to hydrolyse the samples at 80°C for 2 h under the protection of high purity nitrogen. After acidification to pH_2 with aqueous hydrochloric acid (1:1), 1 mL of 14% boron trifluoride-methanol was added and the samples were esterified at 80°C for an hour under the protection of high purity nitrogen. Fatty acid methyl esters (FAMEs) were recovered from the mixture by extraction with 3 × 2 ml of hexane.

Quantification of FA was carried out using an Agilent 7890A instrument with FID (Agilent Technologies, Wilmington, DE, USA) equipped with a DB-FFAP capillary column (30 m × 0.25 mm ID coated with 0.25 μm film thickness). The program of temperature was as follows: 70°C for 3 min, held at 220°C for 33 min after increasing at 3°C min^{-1} rate. Temperatures of the injector and detector were maintained at 220 and 280°C, respectively. FAME identification was performed by comparing relative retention times with those of known single (see Supplementary material Figure S1) and mixture standard (37-FAME Mix, 46-FAME Mix; Sigma, USA). Equivalent chain lengths were used as an aid in peak localization and identification. Each FAME area was corrected from the corresponding FID response factor and from the difference in weight between the FAME and its corresponding free fatty acid (Dubois *et al.*, 2014). We then used the quantitative method from Bai *et al.* (2010) to calculate the FA content.

FA biomarkers

Biomarkers (also expressed as ratios) indicate the relative importance of one food source over another. We used Alfaro *et al.* (2006) and other articles to calculate FA biomarkers. The odd and branch-chain FA (odd&br FA), the ratio of C18:1ω7/C18:1ω9 was used to show bacterial food sources (Budge *et al.*, 2001; Li *et al.*, 2007). The sum percentage content of C18:2ω6 and C18:3ω3 was used as a continental food source marker (above 2.5 implies a continental source) (Budge & Parrish, 1998; Cui *et al.*, 2012). $\sum$C16/$\sum$C18, C16:1/C16:0 and C20:5ω3/C22:6ω3 (EPA/DHA) ratios were used as markers for diatom food resources (Budge *et al.*, 2001). C18:1ω9 indicates brown kelp food resources (Alfaro *et al.*, 2006).

The statistics analysis

FA values were presented as means ± SD. FA and FA biomarkers mentioned above were subjected to a one-way ANOVA with *post hoc* Tukey test using SPSS 15.0 statistical software package. Significance was accepted at $P < 0.05$. One-way analysis of similarities (ANOSIM) were calculated on the Bray–Curtis similarity of body wall in the two habitats, sediment, body wall and gonad. Differences in FA (using percentage content) in the sediment, body wall and ovary in Rongcheng, body wall in Rongcheng and Laoshan were explored using the similarity percentages routine (SIMPER) based on the Bray–Curtis similarity (Hughes *et al.*, 2005; Budge *et al.*, 2007). All multivariate analysis was carried out using the PRIMER-E V6.0 (Clarke & Warwick, 2001; Clarke & Gorley, 2006).

RESULTS

Differences in FA among sediment, body wall and gonads of sea cucumbers in Rongcheng Bay

Total FA content was the lowest in gut sediment (700.96 ± 538.92 μg g^{-1}) and highest in the ovary (89164.13 ± 8363.35 μg g^{-1}) in Rongcheng Bay. FA in the body wall of sea cucumbers from Rongcheng Bay was 46746.99 ± 4196.47 μg g^{-1}. Absolute FA content is shown in supplementary material. FA profiles were dominated by polyunsaturated FA (PUFA) in body wall and ovary tissues, and by saturated FA (SFA) in the sediment (Figure 2).

Most FA, especially long chain PUFA (LC-PUFA, including 20 or more carbon atoms in polyunsaturated FA) were significantly different between body wall and gonad tissues, except for some monounsaturated FA (MUFA) and non PUFA (containing two or more double bonds, C14:1ω5, C16:2ω4, C16:4ω3, C20:1ω9, C22:6ω3). In gut sediment, C16:0 was the dominant FA, accounting for 20.46% of the total FA, followed by C18:0 (15.03%), C16:1ω7 (8.93%), C18:1ω7 (6.43%) and EPA (6.34%). EPA accounted for 15.24% of total FA in the ovaries, followed by C16:1ω7 (8.69%), C18:1ω7 (7.94%), C16:0 (7.92) and AA (7.35%).

The ANOSIM results show that percentage content of FA in the sediment, body wall and gonad in Rongcheng Bay were significantly different (Table 1). The dissimilarity between sediment and body wall was 33% (Table 2). The SIMPER results showed that C16:0, C18:0, EPA, C22:6ω3, C18:1ω7, AA (arachidonic acid, C20:4ω6), C16:0, C14:1 and C20:1ω11 contribute most to the dissimilarity between sediment and body tissue of *A. japonicus* in Rongcheng Bay (Table 2). However, EPA, C18:1ω7, AA, C16:0, C14:1 and C20:1ω11 contributed most to differences between the body

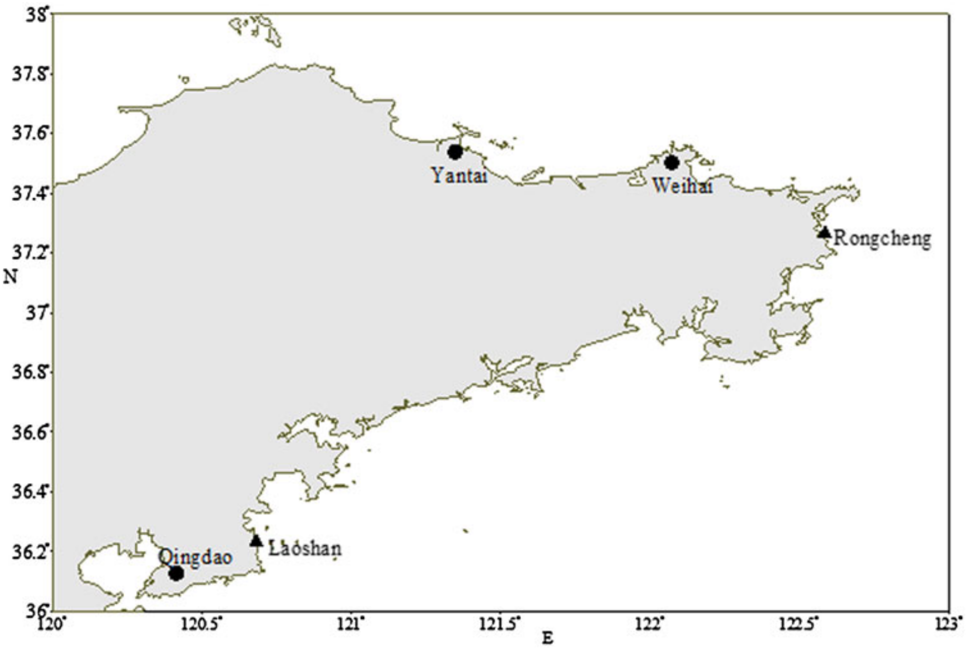

Fig. 1. The two sample areas for the sea cucumber *Apostichopus japonicus* in Shandong peninsula (▲indicates Rongcheng Bay and Laoshan Bay).

wall and ovary of *A. japonicus* in Rongcheng Bay (Table 3). LC-PUFAs contributed ~30.95% to the difference between the two tissues.

Differences in body wall FA

EPA was the most abundant FA in the body wall of sea cucumbers both in Rongcheng (11.51%, 5380.4 ± 607.11 μg g^{-1}) and Laoshan (15.29%, 2978.3 ± 1113.68 μg g^{-1}). C16:1ω7, C16:0, C18:0 and DHA were most abundant in Rongcheng sea cucumbers, accounting for 10.41, 8.92, 8.13 and 8.02% of total FA, respectively. However, C18:0, DHA, C16:0 and AA were most abundant in Laoshan sea cucumbers, accounting for 8.82, 8.61, 8.56 and 8.18% of total FA, respectively.

Results show that total FA, SFA, MUFA and PUFA in the body wall of sea cucumbers from Rongcheng were significantly higher than in Laoshan (see supplementary material). However, the percentage content of PUFA in Laoshan (44.67%) was higher than that in Rongcheng (35.83%, see Figure 2).

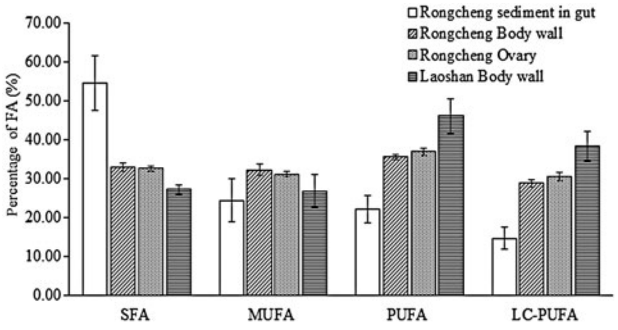

Fig. 2. Percentage of fatty acid (FA) in sediment, body wall and ovary of *Apostichopus japonicus* from Rongcheng Bay and in the body of *A. japonicus* from Laoshan Bay SFA: Saturated FA, MUFA, monounsaturated FA, PUFA, Polyunsaturated FA; LC-PUFA, long chain PUFA.

Absolute content of C14:1ω5, C16:1ω5, C16:2ω4, C16:3ω3, C16:4ω4, C18:2ω4, C18:4ω3, 20:1ω7, C20:3ω6, EPA and C22:0 were not significantly different in body wall tissues between Rongcheng and Laoshan while the other FAs were significantly different between the two habitats (Appendix 1). 16:1ω7, 20:5ω3, C16:0, C18:1ω7, C18:0 and C14:1 contributed most to the FA differences in the body wall from the two habitats (Table 4).

Food resources of sea cucumbers from different habitats

The odd&br FA content in Rongcheng was higher than that in Laoshan, while C18:1ω7/C18:1ω9 ratio (bacterial food sources) in the body wall of sea cucumbers from Laoshan (3.05 ± 0.54) was higher than that in Rongcheng (2.25 ± 0.13), but was not significantly different (Figure 3).

In Roncheng sediment, continental food source biomarker was 4.26, which implies FA was mainly from a continental source. But this biomarker in the body wall of sea cucumbers from the two habitats was below 2.5, which showed continental organic matter took little percentage.

The diatom food resource biomarkers had different trends in the two areas. The ratio of total C16 and C18 in Laoshan was slightly higher (1.18 ± 0.07), but not significantly different, than that in Rongcheng (1.07 ± 0.09). The ratio of C16:1/C16:0 was also higher in Laoshan (1.49 ± 0.39) than in Rongcheng (1.43 ± 0.13). The ratio of EPA/DHA

Table 1. Pairwise ANOSIM results of FA profiles in sediment in gut, body wall and gonad of *Apostichopus japonicus* from Rongcheng Bay.

Groups	Global R	Significance level %
Sediment, Body wall	0.875	2.9
Sediment, Gonad	0.927	2.9
Body wall, Gonad	1	2.9

Table 2. SIMPER results of sediment in gut (S) and the body wall (C) of *Apostichopus japonicus* from Rongcheng Bay (the dissimilarity of the two groups was 33.32%).

FA	Av. Abund of S	Av. Abund of C	Average dissimilarity	Dissimilarity/SD	Contribution %	Cumulative %
16:0	23.41	8.94	7.23	3.08	21.70	21.70
18:0	17.79	8.13	4.83	2.22	14.49	36.19
20:5ω3	5.62	11.50	2.94	4.09	8.82	45.01
22:6ω3	2.94	8.04	2.55	4.87	7.65	52.66
20:4ω6	3.23	6.75	1.76	3.26	5.29	57.94
16:1ω7	7.93	10.40	1.42	1.93	4.25	62.19
18:1ω7	5.39	7.28	1.15	2.45	3.47	65.66
18:2ω6	3.69	1.47	1.11	1.65	3.33	68.99
14:1	2.71	4.46	1.02	2.18	3.05	72.04
20:0	0.00	2.02	1.01	5.11	3.04	75.07
18:1ω9	4.92	3.24	0.84	1.72	2.53	77.60
20:1ω9	1.71	3.37	0.83	1.72	2.49	80.09
14:0	3.92	2.44	0.74	4.88	2.23	82.32
22:1ω9	0.00	1.06	0.53	46.26	1.59	83.91
14-anteiso	1.44	2.21	0.45	2.16	1.34	85.25
20:4ω3	1.29	0.51	0.39	1.66	1.18	86.43
20:1ω11	1.73	2.23	0.39	1.70	1.17	87.60
16:2ω4	0.00	0.76	0.38	5.00	1.14	88.74
16:1ω5	0.77	1.45	0.34	2.09	1.01	89.75
20:2ω6	1.65	1.17	0.32	2.65	0.95	90.70

had the same trend (Laoshan: 1.73 ± 0.30, Rongcheng: 1.43 ± 0.08). This series of ratios indicates higher variability in diatoms as a food resource of sea cucumbers from Laoshan than that of Rongcheng.

The percentage of brown kelp food resources biomarker (C18:1ω9) in body wall in Rongcheng (3.24 ± 0.17) was significantly higher than in Laoshan (2.12 ± 0.35, Figure 3).

DISCUSSION

FA differences between sediment, body wall, ovary and role of sea cucumbers

Sea cucumbers are suspension and deposit feeders (Massin, 1982). Deposit feeders have an important role in FA transformation from sediment and benthic creatures to the upper water ecosystem through eggs and larvae (Mileikovsky, 1974). Some deposit feeding sea cucumbers, such as *Holothuria* (*Halodeima*) *atra* and *Stichopus chloronotus* have bioturbate effects and can disturb the entire upper 5 mm of sediment once a year (4600 kg (dry wet) year^{-1} km^{-2}), significantly reducing the microalgal biomass in the sediment and playing a substantial role in the recycling of nutrients in oligotrophic environments where nutrients would otherwise remain trapped in the surface sediment (Uthicke, 1999). Uthicke (2001) also showed that a small sediment area may be fertilized by nutrients released via the body wall of sea cucumber, approximately 25% of which is in direct contact with the sediment.

Species of other feeding types also have the same ecological role. Sea urchin can deliver more LC-PUFA to the pelagic and benthic food webs through their gonads and subsequently in

Table 3. SIMPER results of the body wall (B) and the gonad (G) of *Apostichopus japonicus* from Rongcheng Bay (the dissimilarity of the two groups was 10.66%).

FA	Av. Abund of B	Av. Abund of G	Average dissimilarity	Dissimilarity/SD	Contribution %	Cumulative %
20:5ω3	11.50	15.22	1.86	5.26	17.43	17.43
22:6ω3	8.04	5.25	1.39	5.31	13.07	30.50
18:0	8.13	6.37	0.88	4.71	8.27	38.78
16:1ω7	10.40	8.69	0.85	1.46	8.01	46.79
20:1ω11	2.23	3.69	0.73	5.13	6.82	53.60
18:1ω9	3.24	2.16	0.54	3.98	5.04	58.65
16:0	8.94	7.94	0.50	2.23	4.70	63.35
22:0	0.94	1.85	0.46	4.97	4.30	67.64
20:4ω6	6.75	7.36	0.46	2.47	4.28	71.92
20:1ω9	3.37	2.68	0.44	1.44	4.15	76.07
20:0	2.02	2.86	0.44	1.49	4.15	80.22
18:1ω7	7.28	7.96	0.34	1.85	3.16	83.38
14:1	4.46	4.90	0.23	1.34	2.15	85.53
20:1ω7	1.28	1.65	0.19	1.19	1.82	87.35
16:2ω4	0.76	0.56	0.10	1.25	0.95	88.29
14:0	2.44	2.32	0.10	1.36	0.93	89.23
18:4ω3	0.80	0.70	0.08	1.39	0.78	90.01

Table 4. SIMPER results of the body wall of *Apostichopus japonicus* from Rongcheng Bay (RC) and Laoshan Bay (LC, the dissimilarity was 21.26%).

FA	Av. Abund of RC	Av. Abund of LC	Average dissimilarity	Dissimilarity/SD	Contribution %	Cumulative %
16:1ω7	10.40	7.02	2.79	1.55	13.12	13.12
16:1ω5	1.45	5.24	2.18	0.75	10.27	23.39
20:5ω3	11.50	15.00	1.75	2.03	8.23	31.62
22:3ω9	0.00	2.57	1.28	3.56	6.04	37.66
20:4ω6	6.75	8.80	1.19	0.94	5.61	43.27
20:1ω11	2.23	0.00	1.12	10.74	5.26	48.52
14:1	4.46	2.62	0.92	4.51	4.33	52.86
16:4ω3	0.17	1.67	0.75	0.87	3.54	56.40
20:1ω9	3.37	1.95	0.71	1.71	3.34	59.73
18:1ω9	3.24	2.12	0.56	3.33	2.62	62.35
22:4ω6	0.00	1.03	0.51	4.63	2.42	64.77
20:0	2.02	1.04	0.49	2.37	2.32	67.09
18:1ω7	7.28	6.34	0.47	3.61	2.20	69.30
14:0	2.44	1.63	0.42	1.43	1.98	71.27
22:6ω3	8.04	8.74	0.40	1.60	1.90	73.17
14-anteiso	2.21	1.48	0.36	3.74	1.70	74.87
20:2ω9	0.00	0.72	0.36	16.84	1.70	76.57
18:0	8.13	8.80	0.33	3.16	1.56	78.14
16:0	8.94	8.47	0.32	1.58	1.53	79.66
22:5ω3	0.61	0.00	0.31	25.13	1.44	81.11
20:1ω7	1.28	1.83	0.30	1.58	1.40	82.50
17:0	1.12	0.55	0.28	7.81	1.34	83.84
16:1ω9	0.97	0.44	0.27	1.79	1.25	85.09
20:3ω6	0.25	0.77	0.26	2.63	1.22	86.32
18:1ω11	0.00	0.49	0.25	7.09	1.16	87.48
22:1ω9	1.06	0.60	0.23	4.09	1.09	88.57
18:2ω6	1.47	1.01	0.23	3.64	1.08	89.65
20:4ω3	0.51	0.06	0.23	4.07	1.07	90.72

producing larvae, than they can through production of faecal pellets (Hughes *et al*., 2011).

Most sea cucumbers have the ability to select the organic food. Some study revealed that Mediterranean holothuroids show different selectivity for organic matter (Plotieau *et al*., 2014). In our study, the continental food source marker (sum of C18:2ω6 and C18:3ω3) in the body wall was lower than that in sediment, which indicates that *A. japonicus* have the ability to feed selectively.

In a benthic ecosystem, *A. japonicus* may select the much more organic sediment matter and then use some methods to transfer the nutrients. That is the same as sea urchins, related above. Sea cucumber may use their gonad to transfer nutrients. This study revealed that sea cucumbers can store more LC-PUFA in the ovary. The eggs released by sea cucumbers may be a suitable food source for other marine organisms especially carnivores living in upper water and can also provide abundant nutrients to the planktonic ecosystem.

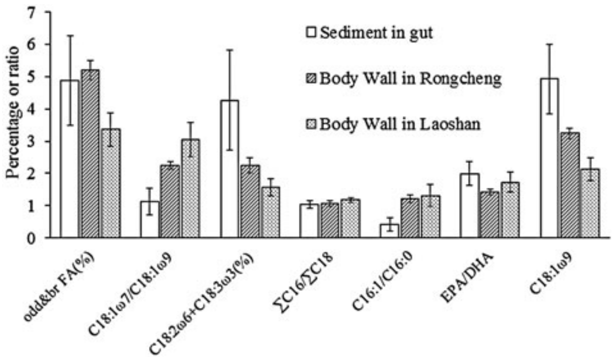

Fig. 3. Fatty acid biomarkers of the body wall of *Apostichopus. japonicus* from Rongcheng Bay and Laoshan Bay and sediment in gut in Laoshan Bay odd&br FA: odd and branch-chain FA, EAP/ DHA: C20:5ω3/C22:6ω3.

However, the ecological role of holothuroids is often neglected by researchers. Seagrass habitat research using carbon and nitrogen stable isotopes showed holothurians enhanced the sediment uptake of organic carbon and total nitrogen by up to 30× and 3×, respectively (Costa *et al*., 2014). Another study in a reef area revealed that sea cucumbers play an important ecological role in the coral reef $CaCO_3$ cycle (Schneider *et al*., 2013). So further laboratory and field simulation experiments are needed to fully understand the ecological effects of this species, which is the main cultured species in northern China coastal area.

FA in different habitats

FA composition, especially LC-MUFA, was different depending on sampling times, regions and age of species. Previous studies have shown differences in FA of *A. japonicus*. EPA, AA and DHA in the body wall of pond-cultured *A. japonicus* was between 7.24–14.45%, 4.54–8.16% and 2.41–4.45%, respectively over 1 year (Gao *et al*., 2011), while higher EPA levels (9.94–11.24%) and lower DHA levels (5.83–7.02%) have been shown for *A. japonicus* caught in Zhangzi Island coast (Dantong *et al*., 2009). The percentage of EPA and AA in the present study are in agreement with the ranges reported above, but the DHA was a little higher. DHA content had a larger difference.

The absolute FA contents in the body wall of sea cucumbers from the two studied habitats were both high, which could be partially due to the sampling time. *Apostichopus japonicus* will reduce feeding activities in cold water and enter aestivation in summer (Gao *et al.*, 2011). The sampling of the two areas was done in spring, which is the optimal season for feeding in *A. japonicus*. Another reason is the habitats. Sea cucumber living in open areas may feed on more detritus from kelp and other materials, so those individual may have higher MUFA from food than that inhabited in pond and cage.

FA is known to be a useful biomarker, with FA profiles indicating different food resources in different habitats (Braeckman *et al.*, 2012; Kelly & Scheibling, 2012). Some studies have used FA to indicate food supply in different habitats. Coelho *et al.* (2011) studied FA of mud snails *Hydrobia vulvae* from mudflats and seagrass meadows in the same estuary and found significant differences of food resources. SFA and MUFA were found in greater abundances in the eggs of crabs *Uca annulipes*' inhabiting a peri-urban mangrove subjected to domestic sewage discharges compared with those inhabiting pristine mangroves (Penha-Lopes *et al.*, 2009). Further, FA can be used to distinguish different geographic populations. Dong *et al.* (2013) showed that five kinds of FA, C18:1ω9, C20:1, C22:1ω9, C20:4ω6 and C20:5ω3, were effective in identifying six populations of crabs *Portunus trituberculatus* from the China Sea with a total discriminant accuracy of 88.46%.

Food supply has a significant effect on the FA profile of sea cucumbers (Alfaro *et al.*, 2006). Some studies have shown that FA composition may be influenced by the feeding and environmental conditions of the habitat (Iverson *et al.*, 1997). Neto *et al.* (2006) studied FA of three types of deep sea holothurians and suggested that the FA profile response to variations in food supply appears to depend on feeding mode. Lipid content of *Psychropotes longicauda* showed a strong positive correlation with the contents of lipids in the surficial sediments (Neto *et al.*, 2006). This may be because *P. longicauda* uses peltate tentacles to sweep sediments into the mouth to obtain food from the top ~5 mm of the sediment.

Similarly, the holothuria *A. japonicus* in the present study is also a surface sediment feeding species. Our results showed that some FA contents differed markedly between the body walls of *A. japonicus* from the two studied coastal areas. In this cultured environment, sea cucumber is often released and scatter-fed in the seabed. Rongcheng Bay is famous for raft cultivation of kelp *S. japonica* (previously known as *Laminaria japonica*), which can produce detritus on the seafloor (Zhang *et al.*, 2012). Eelgrass *Zostera marina* is very abundant in nearby coastal areas (Gao *et al.*, 2013; Zhou *et al.*, 2014). Another factor is the culture raft influence, which can slow down currents and retain much more organic matter. Detritus is a primary food source in subtidal soft-sediment habitats (Krumhansl & Scheibling, 2012). Sea cucumbers in this organic matter environment with a high content of sediment may eat detritus from brown kelp or eelgrass *Z. marina* that lives off the coastal areas of Rongcheng. Our results revealed that brown kelp food resource C18:2ω6 and C18:3ω3 in Rongcheng was significantly higher than in Laoshan. The diatom food resource was the main food resource in Laoshan Bay.

More research is needed to further examine the complicated relationship between the FA profile of *A. japonicus* and the environment that they inhabit.

CONCLUSION

This study reveals significant differences among FA from sediment, the body wall and the female ovary of sea cucumbers. LC-PUFA contributed the greatest differences in FA between the body wall and ovary. FA biomarkers show that sea cucumbers inhabiting Rongcheng and Laoshan have different food resources. Diatoms may contribute more to the diets of sea cucumbers in Laoshan, while brown kelp and vascular plants may play a more significant role in the diets of those in Rongcheng. It is necessary to do further research over longer time periods to better understand differences in FA composition in different tissues and habitats.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S002531541500168X

ACKNOWLEDGEMENTS

We are grateful to Dr Sigrun Jonasdottir for providing advice. We also thank D.S. Zhu and J.X. Wang for helping us to collect the sea cucumber samples.

FINANCIAL SUPPORT

This work was supported by the National Marine Public Welfare Research Project (No. 201305043), the Natural Science Foundation of China (No. 41406164), and the National Key Technology R&D Program (Nos 2011 BAD13B02, 2010BAC68B01).

REFERENCES

Alfaro A.C., Thomas F., Sergent L. and Duxbury M. (2006) Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine, Coastal and Shelf Science* 70, 271–286.

Anderson S.C., Flemming J.M., Watson R. and Lotze H.K. (2010) Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries* 12, 317–339.

Bai X.W., Song C.H., You J.M., Sun Z.W., Fu Y.Y. and Li G.L. (2010) Determination of fatty acids (C_1–C_{10}) from Bryophytes and Pteridophytes. *Chromatographia* 71, 1125–1129.

Braeckman U., Provoost P., Sabbe K., Soetaert K., Middelburg J., Vincx M. and Vanaverbeke J. (2012) Temporal dynamics in macrobenthic diet as inferred from fatty acid biomarkers. *Journal of Sea Research* 68, 6–19.

Budge S.M. and Parrish C.C. (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Organic Geochemistry* 29, 1547–1559.

Budge S., Parrish C. and McKenzie C. (2001) Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. *Marine Chemistry* 76, 285–303.

Budge S.M., Springer A.M., Iverson S.J. and Sheffield G. (2007) Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. *Marine Ecology Progress Series* 336, 305–309.

Chen J.X. (2004) Present status and prospects of sea cucumber industry in China. In Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J.-F. and Mercier, A. (Eds), *Advances in Sea Cucumber Aquaculture and Management*. Fisheries Technical Paper, vol. 463. Rome: FAO, pp. 359–371.

Clarke K.R. and Gorley R.N. (2006) *RIMER v6: user manual tutorial*. Plymouth: PRIMER-E.

Clarke K.R. and Warwick R.M. (2001) *Changes in marine communities: an approach to statistical analysis and interpretation*, 2nd edition. Plymouth: PRIMER-E.

Coelho H., Lopes da Silva T., Reis A., Queiroga H., Serôdio J. and Calado R. (2011) Fatty acid profiles indicate the habitat of mud snails *Hydrobia ulvae* within the same estuary: Mudflats vs. seagrass meadows. *Estuarine, Coastal and Shelf Science* 92, 181–187.

Costa V., Mazzola A. and Vizzini S. (2014) *Holothuria tubulosa* Gmelin 1791 (Holothuroidea, Echinodermata) enhances organic matter recycling in *Posidonia oceanica* meadows. *Journal of Experimental Marine Biology and Ecology* 461, 226–232.

Cui Y., Ying W., Jing Z. and Na W. (2012) Potential dietary influence on the stable isotopes and fatty acid compositions of jellyfishes in the Yellow Sea. *Journal of the Marine Biological Association of the United Kingdom* 92, 1325–1333.

Dantong L., Yaqing C., Zhenhai W., Wei C., JunYa W. and GuoDong C. (2009) Analysis of nutritive composition of body wall in wild sea cucumber *Apostichopus japonicus* Selenka at Zhangzi Island in spring and autumn. *Fisheries Science* (*Dalian*) 28, 365–369. [in Chinese with English abstract].

Dong Z., Shen S., Li X., Yan B. and Sun X. (2013) Fatty acid composition variation and fingerprint of the swimming crab *Portunus trituberculatus* from China Sea based on multivariate analysis method. *Journal of Fisheries of China* 37, 192–200. [In Chinese with English abstract]

Dubois S., Blanchet H., Garcia A., Massé M., Galois R., Grémare A., Charlier K., Guillou G., Richard P. and Savoye N. (2014) Trophic resource use by macrozoobenthic primary consumers within a semi-enclosed coastal ecosystem: stable isotope and fatty acid assessment. *Journal of Sea Research* 88, 87–99.

Gao F., Xu Q. and Yang H. (2011) Seasonal biochemical changes in composition of body wall tissues of sea cucumber *Apostichopus japonicus*. *Chinese Journal of Oceanology and Limnology* 29, 252–260.

Gao L., Wei Q., Fu F. and Shao H. (2013) Influence of outbreak of macroalgal blooms on phosphorus release from the sediments in Swan Lake Wetland, China. *Plant Biosystems* 147, 1175–1183.

Han H. (2011) Analysis and evaluation of nutritive composition in body walls for different age *Apostichopus japonicus* (Selenka). *Marine Environmental Science* 30, 404–409. [In Chinese with English abstract]

Hughes A.D., Catarino A.I., Kelly M.S., Barnes D.K.A. and Black K.D. (2005) Gonad fatty acids and trophic interactions of the echinoid *Psammechinus miliaris*. *Marine Ecology Progress Series* 305, 101–111.

Hughes A.D., Cook E.J., Orr H., Kelly M.S. and Black K.D. (2011) The transformation of long chain polyunsaturated fatty acids in benthic food webs: the role of sea urchins. *Journal of Experimental Marine Biology and Ecology* 409, 229–234.

Iverson S.J., Frost K.J. and Lowry L.F. (1997) Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. *Marine Ecology Progress Series* 151, 255–271.

Kasai T. (2003) Lipid contents and fatty acid composition of total lipid of sea cucumber *Stichopus japonicus* and *konowata* (salted sea cucumber entrails). *Food Science and Technology Research* 9, 45–48.

Kelly J.R. and Scheibling R.E. (2012) Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series* 446, 1–22.

Krumhansl K.A. and Scheibling R.E. (2012) Production and fate of kelp detritus. *Marine Ecology Progress Series* 467, 281–302.

Lee M.H., Kim Y.K., Moon H.S., Kim K.D., Kim G.G., Cho H.A., Yoon N.Y., Sim K.B., Park H.Y. and Lee D.S. (2012) Comparison on proximate composition and nutritional profile of red and black sea cucumbers (*Apostichopus japonicus*) from Ulleungdo (Island) and Dokdo (Island), Korea. *Food Science and Biotechnology* 21, 1285–1291.

Li Y.-L., Peacock A.D., White D.C., Geyer R. and Zhang C.L. (2007) Spatial patterns of bacterial signature biomarkers in marine sediments of the Gulf of Mexico. *Chemical Geology* 238, 168–179.

Liu M., Li C. and Sun S. (2011) Seasonal variation in fatty acid composition of seston and the copepod *Calanus sinicus* (Brodsky, 1962) in Jiaozhou Bay and its trophic implications. *Chinese Journal of Oceanology and Limnology* 29, 1164–1173.

Massin C. (1982) Food and feeding mechanisms: Holothuroidea. In Jangoux M. and Lawrence J.M. (eds) *Echinoderm nutrition*. Rotterdam: Balkema, pp. 43–55.

Mileikovsky S.A. (1974) On predation of pelagic larvae and early juveniles of marine bottom invertebrates by adult benthic invertebrates and their passing alive through their predators. *Marine Biology* 26, 303–311.

Neto R.R., Wolff G.A., Billett D.S.M., Mackenzie K.L. and Thompson A. (2006) The influence of changing food supply on the lipid biochemistry of deep-sea holothurians. *Deep-Sea Research Part I-Oceanographic Research Papers* 53, 516–527.

Penha-Lopes G., Torres P., Narciso L., Cannicci S. and Paula J. (2009) Comparison of fecundity, embryo loss and fatty acid composition of mangrove crab species in sewage contaminated and pristine mangrove habitats in Mozambique. *Journal of Experimental Marine Biology and Ecology* 381, 25–32.

Plotieau T., Lepoint G., Lavitra T. and Eeckhaut I. (2014) Isotopic tracing of sediment components assimilated by epibiontic juveniles of *Holothuria scabra* (Holothuroidea). *Journal of the Marine Biological Association of the United Kingdom* 94, 1485–1490.

Ren Y., Dong S., Wang F., Gao Q., Tian X. and Liu F. (2010) Sedimentation and sediment characteristics in sea cucumber *Apostichopus japonicus* (Selenka) culture ponds. *Aquaculture Research* 42, 14–21.

Schneider K., Silverman J., Kravitz B., Rivlin T., Schneider-Mor A., Barbosa S., Byrne M. and Caldeira K. (2013) Inorganic carbon turnover caused by digestion of carbonate sands and metabolic activity of holothurians. *Estuarine, Coastal and Shelf Science* 133, 217–223.

Seo J.Y. and Lee S.M. (2011) Optimum dietary protein and lipid levels for growth of juvenile sea cucumber *Apostichopus japonicus*. *Aquaculture Nutrition* 17, e56–e61.

Slater M.J. and Carton A.G. (2009) Effect of sea cucumber (*Australostichopus mollis*) grazing on coastal sediments impacted by mussel farm deposition. *Marine Pollution Bulletin* 58, 1123–1129.

Sun Z., Gao Q., Dong S., Shin P.K.S. and Wang F. (2012) Estimates of carbon turnover rates in the sea cucumber *Apostichopus japonicus* (Selenka) using stable isotope analysis: the role of metabolism and growth. *Marine Ecology Progress Series* 457, 101–112.

Uthicke S. (1999) Sediment bioturbation and impact of feeding activity of *Holothuria* (*Halodeima*) *atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. *Bulletin of Marine Science* 64, 129–141.

Uthicke S. (2001) Nutrient regeneration by abundant coral reef holothurians. *Journal of Experimental Marine Biology and Ecology* 265, 153–170.

Xu Q., Zhang L., Zhang T., Zhou Y., Xia S., Liu H. and Yang H. (2014) Effects of an artificial oyster shell reef on macrobenthic communities in Rongcheng Bay, East China. *Chinese Journal of Oceanology and Limnology* 32, 99–110.

Zhang J., Fang J., Wang W., Du M., Gao Y. and Zhang M. (2012) Growth and loss of mariculture kelp *Saccharina japonica* in Sungo Bay, China. *Journal of Applied Phycology* 24, 1–8.

Zheng Z., Dong S., Tian X., Wang F., Gao Q. and Bai P. (2009) Sediment-water fluxes of nutrients and dissolved organic carbon in extensive sea cucumber culture ponds. *CLEAN – Soil, Air, Water* 37, 218–224.

and

Zhou Y., Liu X., Liu B., Liu P., Wang F., Zhang X. and Yang H. (2014) Unusual pattern in characteristics of the eelgrass *Zostera marina* L. in a shallow lagoon (Swan Lake), north China: implications on the importance of seagrass conservation. *Aquatic Botany* 2014, 178–184.

Correspondence should be addressed to:
Q. Xu and H. Yang
Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences
email: xuqiangcas@126.com; hshyang@126.com