Determination of Trophic Transfer at a Created Intertidal Oyster (*Crassostrea ariakensis*) Reef in the Yangtze River Estuary Using Stable Isotope Analyses

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Abstract Oysters can create reefs that provide habitat for associated species resulting in elevated resident abundances, lower mortality rates, and increased growth and survivorship compared to other estuarine habitats. However, there is a need to quantify trophic relationships and transfer at created oyster reefs to provide a better understanding of their potential in creating suitable nekton habitat. Stable isotope analyses (δ^{13}C and δ^{15}N) were conducted to examine the organic matter sources and potential energy flow pathways at a created intertidal oyster (*Crassostrea ariakensis*; hereinafter, oyster) reef and adjacent salt marsh in the Yangtze River estuary, China. The δ^{13}C values of most reef-associated species (22 of 37) were intermediate between those of suspended particle organic matter (POM) and benthic microalgae (BMI), indicating that both POM and BMI are the major organic matter sources at the created oyster reef. The sessile and motile macrofauna colonizing the reef make up the main prey of transient nekton (e.g., spotted sea bass, Asian paddle crab, and green mud crab), thus suggesting that the associated community was most important in supporting higher trophic levels as opposed to the direct dietary subsidy of oysters. The created oyster reef consistently supported higher trophic levels than the adjacent salt marsh habitat due to the dominance of secondary consumers. These results indicate that through the provision of habitat for associated species, created oyster reefs provide suitable habitat and support a higher average trophic level than adjacent salt marsh in the Yangtze River estuary.

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Introduction

Oyster reefs introduce spatial heterogeneity to the environment and create complex biogenic structures (Coen et al. 1999), but have not been well-managed historically for ecological support (Beck et al. 2011). Healthy reefs not only have the ability to sequester carbon (Hargis and Haven 1999), protect fringing shorelines (Piazza et al. 2005), and provide important filtration services (Dame and Libes 1993), but they also create complex, biogenic structures that serve as essential fish habitat (Coen et al. 1999; Peterson et al. 2003). Because theory suggests that functional characteristics may be independent of the structure of the ecosystem (French McCay et al. 2003), it is appropriate to identify the role of created oyster reefs in mediating energy transfer. This is crucial to projects aimed at not only recovering oyster populations but those also hoping to enhance fisheries production.

Once ubiquitous features of the estuarine landscape (Kirby 2004), oyster reefs provide habitat for benthic

invertebrates, fishes, and mobile crustaceans (Dame and Patten 1981; Coen and Luckenbach 2000; Luckenbach et al. 2005). Oyster reefs support higher densities of fishes and crustaceans (resident species) than unstructured, mud bottom habitat (Stunz et al. 2010), and are utilized by mobile nekton in a variety of ways. For instance, some species are known to feed directly on the oysters that create reefs (e.g., mud crab *Panopeus herbstii*, oyster toadfish *Opsanus tau*, sheepshead *Archosargus probatecephalus*; Anderson and Connell 1999; Tolley et al. 2005; Tolley and Volety 2005), while others may feed primarily on the associated resident fauna within the reef complex (e.g., red drum *Sciaenops ocellatus*; Boothby and Avault 1971). Oyster reefs may also serve as spawning and nesting sites (Lehnert and Allen 2002; Tolley and Volety 2005) as well as provide spatial refuge for associated prey species (Grabowski 2004). Field investigations and experimental studies show that elevated nekton abundances at oyster reefs may be a result of lower mortality rates (Grabowski and Powers 2004), increased growth (Shervette and Gelwick 2008), and/or subsequent survivorship (Stunz et al. 2002; Minello et al. 2003) compared to other estuarine habitats.

Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) are commonly used to assess the relative contributions of primary producers assimilated by organisms and an organism's trophic position within a system. Stable carbon isotope ratios ($^{13}C/^{13}C$) vary substantially among primary producers with different photosynthetic pathways (e.g., C_3 vs. C_4 plants) and change little with trophic transfers (Peterson and Fry 1987). $\delta^{13}C$ can thus be used to determine ultimate sources of organic matter (Vander Zanden and Rasmussen 2001; Phillips and Gregg 2003). Stable nitrogen isotope ratios ($^{15}N/^{14}N$) show predictable stepwise enrichment with trophic transfers, and can thus be used to estimate an organism's trophic position within a food web (Peterson 1999). Therefore, plotting ratios of $\delta^{13}C$–$\delta^{15}N$ in space can reveal important aspects of trophic structure and allow a better understanding of species interactions. Compared to other estuarine biogenic habitats such as seagrass meadows (Vizzini et al. 2002; Lepoint et al. 2004), salt marshes (Kwak and Zedler 1997; Quan et al. 2007a), and non-vegetated bottom (Kang et al. 1999; Yokoyama and Ishihi 2003), relatively few studies have examined trophic transfer (using stable isotope analyses) at oyster reefs.

Creation of an intertidal oyster reef was initiated in April 2004 through transplanting hatchery seed oyster *Crassostrea ariakensis* to the intertidal artificial concrete modular (dikes and groins) as part of the Deepwater Navigation Channel Regulation Project (DNCRP) in the Yangtze River estuary (see Quan et al. 2006, 2007b, 2009b). The hatchery oyster was initially called *Crassostrea rivularis*, but the most recent classification (Wang et al. 2004) determined this to be the Asian oyster *C. ariakensis*.

Quan et al. (2009b) demonstrated that the created oyster reef supported sustainable oyster (*C. ariakensis*) populations that provide important ecosystem services and create complex habitat structure for motile epifaunal and nekton species. To better understand the effects of the created oyster reef on trophic transfer and local community structure, stable isotope analyses were used at the DNCRP site to: (1) determine the main organic matter sources assimilated by reef-associated organisms along a salinity gradient, (2) examine pathways of trophic transfer and resulting food web structure, and (3) compare this with nekton use of adjacent salt marsh habitat.

Materials and Methods

Study Site

The created intertidal oyster reef is located on the north passage of the south channel in the Yangtze River estuary, China (Fig. 1a). The estuary is well-mixed, ranges from oligo- to polyhaline, and has four major inlets that connect it to the East China Sea. Tides are semi-diurnal averaging 4.5 and 2.6 m at spring and neap tides, respectively. For a detailed description of the oyster reef, see Quan et al. (2009b). The cross-section of the artificial reef resembles an isosceles trapezoid, with a height of 2.5 m above mean low water level (MLW), a width of 4 m for the short parallel side and 18.4 m for the long parallel side (Fig. 1b). Dense oysters and typical 3D reef structure (dead and live oyster matrix) only appeared in the lower (0.1 m MLW) and middle (1.2 m MLW) intertidal zone, while sporadic oysters distributed in the high (2.5 m MLW) intertidal zone of the created reef (Quan et al. 2009b).

We sampled the oyster reef 5 years after creation in June 2009. To account for possible spatial variations, three sampling sites were determined along the reef in an increasing salinity gradient. Depending on the tide, runoff flow and climate conditions, salinity typically ranges from 0.6‰ to 7.3‰ at the sampling site "S2", from 2.5‰ to 15.7‰ at the sampling site "S5", from 8.9‰ to 23.4‰ at the sampling site "S8" (Quan et al. 2007b, 2009b).

Sample Collection and Processing

In order to determine the potential food source contribution of marsh plants to secondary production at the created oyster reef, three marsh vascular plants (common reed *Phragmites australis*, bulrush *Scirpus mariqueter*, and Atlantic cordgrass *Spartina alterniflora*) were sampled from Jiuduansha Wetland Nature Reserve (JWNR) that is located approximately 0.1 km to the south (Fig. 1a). In the salt marsh zone within JWNR, each of two native C_3 plants (*S. mariqueter* and *P. australis*) and an exotic C_4 plant

Fig. 1 Locations of the created intertidal oyster reef in the Yangtze River estuary (**a**) and schematic drawing of cross section of the artificial reef (**b**). *MLW* mean low water
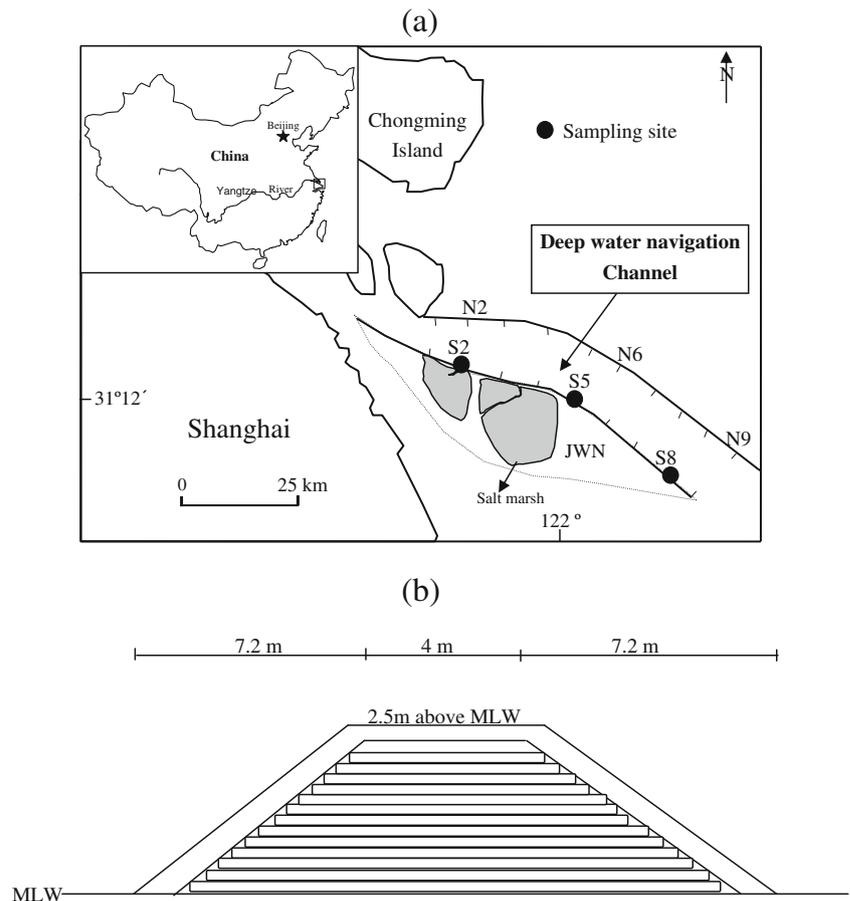
(*S. alterniflora*) forms a dense monoculture (Quan et al. 2007a, 2011). Based on the results from remote sensing analysis in 2004 (Huang and Zhang 2007), *S. alterniflora*, *S. mariqueter*, and *P. australis* occupied 30.1%, 53.2%, and 16.7% of the total salt marsh area, respectively. For each species, leaves of 10 randomly selected plants were sampled by hand, cleaned with distilled water to remove attached matter, and then pooled as a composite sample.

Samples of suspended particulate organic matter (POM), benthic microalgae (BMI), macroalgae, macroinvertebrates, and fishes were collected from each of the three sampling sites (S2, S5, S8) at the oyster reef. POM samples were collected during high tide at each sampling site. Water samples (2L) were filtered onto pre-combusted Whatman glass fiber filter papers (GF/F; 47 mm Ø) under low vacuum (Riera et al. 2002; Quan et al. 2007a). The visible zooplanktons on the filter were removed using tweezers. BMI samples were collected in the high intertidal zone of the oyster reef using procedures similar to Riera et al. (1999) as modified by Quan et al. (2007a, 2009a). The reef surface (with dense microalgal mats) was covered with a 5-mm thick layer of precombusted sand (soaked in 10% hydrochloric acid for 24 h, combusted at 450°C for 4 h), a piece of 63-μm nylon mesh, and another 5-mm thick

layer of precombusted sand. After 3 h in situ incubation, the top 2 mm of sand was gently scraped and mixed with prefiltered seawater. The water–sand mixtures were shaken and the supernatants were filtered through pre-combusted Whatman GF/F filters. Macroalgae (*Enteromorpha* sp.) only appeared at one of the three sampling sites (S2) and samples were taken by hand, cleaned of epibionts and attached matter, and rinsed with distilled water.

Sessile and motile epibenthic macrofauna (defined here as those organisms remaining within the reef matrix at low tide) at the oyster reef were sampled using a quadrat method (Quan et al. 2009b). All the excavated material in each quadrat (0.2×0.2 m) was sieved using 1.0-mm mesh and the retained material was examined for macrofauna (i.e., oyster, barnacles, decapods, mollusks, annelids, and fishes). Reef-associated nekton species were collected using a gillnet (mesh, 30 mm; height, 1.5 m; length, 100 m) and fish traps that was composed of 30 rectangular cages (0.5×0.3×0.3 m) with steel bars and plastic-coated wire at each of the three sampling sites (see Quan et al. 2009b). At slack low tide at each sampling site, six fish traps (4-mm nylon mesh) were deployed at 50-m intervals along a straight line to the bottom of the oyster reef. At high tide, a gill net was deployed at each sampling site for 3 h to collect transient nekton species.

After shells of approximately 20 individuals of each shellfish species (*C. ariakensis*, *Vignadula atrata*, *Rapana bezoar*, *Cancellaria mangeloides*, and *Barbatia bistrigata*) were removed, soft muscle tissues were pooled and homogenized as one sample, respectively. For crabs, muscle and gill fractions of two individuals of each species were pooled as one sample. For prawns, both the entire cuticle and the head were removed and the remaining tissue of approximately 10 individuals was pooled as one sample. Fish dorsal muscle tissue was dissected from two to five individuals of each species and pooled as one sample.

Fish and macroinvertebrate tissue, benthic microalgae, macroalgae, and marsh vegetation samples were dried in an oven at 60°C to constant mass, and ground to a fine powder with a mortar and pestle. Dried samples were treated with 1 N hydrochloric acid to eliminate carbonates (Jacob et al. 2005). The treated samples were again oven dried at 60°C and ground to a fine powder.

Additionally, we analyzed organic matter sources and trophic positions of 31 consumers (20 macroinvertebrate and 11 fish) in the Jiuduansha marsh adjacent to the created intertidal oyster reef using stable isotope analysis in 2007 (Quan et al. 2009a). There were nine co-occurring organisms between these two habitats.

Organic samples were analyzed for ^{13}C and ^{15}N stable isotope ratios and C/N values at Stable Isotope Ratio Mass Spectrometry Lab (Delta V IRMS with Flash EA 1112 Series, Thermo Finnigan), Research Institute of Forestry, Chinese Academy of Forestry. Urea and glycine were analyzed as accuracy and precision standards for isotopic ratios. Stable isotope abundance is expressed in δ notation as per mille difference from an international standard (Vienna Pee Dee Belemnite for carbon, air for nitrogen), which indicates depletion or enrichment of the heavy isotope relative to the light isotope in the following relationship:

$$\delta X = \left(\left[R_{sample} / R_{standard} \right] - 1 \right) \times 10^3$$

where, X is ^{13}C or ^{15}N, and R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The analytical precision of these measurements was 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

Data Analysis

The trophic position (TP) of an organism was calculated based on the $\delta^{15}N$ difference between the species and baseline biota. The formula used for calculating TP of a species was:

$$TP_{organisms} = \left(\left(\delta^{15}N_{organisms} - \delta^{15}N_{baseline} \right) / TEF \right) + 2$$

where, $TP_{organisms}$ and $\delta^{15}N_{organisms}$ are the trophic position and the $\delta^{15}N$ value of the tested organism, respectively.

$\delta^{15}N_{baseline}$ is the $\delta^{15}N$ value of the baseline biota (primary consumer). In this study, bivalve *V. atrata* was recognized as the baseline biota because it had the most depleted $\delta^{15}N$ among all animal organisms. TEF is the trophic enrichment factor and is generally considered to be 3.4‰ (e.g., Peterson and Fry 1987; Post 2002).

Differences in $\delta^{13}C$, $\delta^{15}N$ and C/N among six organic matter sources (*P. australis*, *S. mariqueter*, *S. alterniflora*, POM, BMI, and macroalgae) and spatial variations in the average $\delta^{13}C$ of POM, BMI, and dominant consumers were tested using one-way analysis of variance (ANOVA; followed by Turkey's honestly significant difference post hoc test). The average $\delta^{13}C$, $\delta^{15}N$, and trophic position between species were determined to calculate the difference in organic matter sources and trophic structure between the created oyster reef and adjacent salt marsh. To meet assumption of ANOVA, the stable isotope data were log transformed prior to analysis. Non-multidimensional scaling (NMDS), based on Bray–Curtis dissimilarity indices (PRIMER software package, version 6.0), calculated from $\delta^{13}C$ and C/N was used to determine the main organic matter sources of macroinvertebrates and fishes at the oyster reef.

Results

Organic Matter Sources

The C$_4$ marsh plant *S. alterniflora* had a significantly more enriched $\delta^{13}C$ value (-12.6 ± 0.1‰) than the other organic matter sources (Table 1). Two C$_3$ plants *P. australis* and *S. mariqueter* were the most depleted in $\delta^{13}C$, and were not significantly different from one another (Table 1). The $\delta^{13}C$ values of BMI and macroalgae (*Enteromorpha* sp.) were statistically similar and were significantly more enriched than that of POM (Table 1). The average $\delta^{15}N$ values of the potential organic matter sources ranged from 5.0‰ to 8.7‰ (Table 1). Macroalgae (*Enteromorpha* sp.) was significantly more enriched in $\delta^{15}N$ than the other primary producers, while *S. mariqueter* had the most depleted $\delta^{15}N$ values. Three marsh vascular plants had significantly greater C/N than the other organic matter sources (Table 1). The C/N of POM and BMI were statistically similar and were significantly lower than that of macroalgae (Table 1).

The average $\delta^{13}C$ values of 37 animal species (22 macroinvertebrates, 15 fishes; Table 2) ranged from -23.1‰ for the Asian oyster (*C. ariakensis*) to -12.3‰ for parasites (*Gnorimosphaeroma rapi*; Table 2, Fig. 2). Three bivalves (*C. ariakensis*, *B. bistrigata*, and *V. atrata*) had the most depleted $\delta^{13}C$ values and were most similar to $\delta^{13}C$ values for POM (Fig. 2). Stable carbon isotope values of most consumers (22 of 37) were intermediate between those of

Table 1 Average $\delta^{13}C$, $\delta^{15}N$, and C/N values (± 1 SE) of potential organic matter sources of the oyster *C. ariakensis* and reef-associated organisms at the created intertidal oyster reef in the Yangtze River estuary

Significant differences ($p<0.05$) in stable isotope ratios and C/N among organic matter sources are indicated by *different letters*; $n=$sample size

Food sources	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N	n
Marsh macrophytes				
P. australis (PA)	−27.3±0.4 a	6.9±0.5 b	24.0±0.1 a	3
S. mariqueter (SM)	−27.7±0.5 a	5.0±0.1 d	25.0±0.7 a	3
S. alterniflora (SA)	−12.6±0.1 d	5.8±0.2 c	23.0±1.3 a	3
POM	−21.9±0.5 b	5.7±0.1 c	5.2±1.0 c	10
Benthic microalgae (BMI)	−18.3±0.1 c	6.5±0.1 b	5.1±0.2 c	9
Macroalgae				
Enteromorpha sp. (EN)	−18.0±0.3 c	8.7±0.4 a	7.2±0.3 b	4

POM and BMI (Fig. 2). Eleven species had relatively more enriched $\delta^{13}C$ values than BMI, but the average $\delta^{13}C$ differences between BMI and most (nine species) of these species were within 1.8‰. NMDS ordination of $\delta^{13}C$ and C/N values of consumers shows that all the plots for consumers are clustered with those for BMI, POM and macroalgae, but are completely separated from those for three marsh plants (Fig. 3).

BMI $\delta^{13}C$ did not differ among the 3 sampling sites (Fig. 4, one-way ANOVA, $p>0.05$). Although POM $\delta^{13}C$ exhibited a gradual ^{13}C-depletion from the upstream (S2) to the downstream (S8) along an increasing salinity gradient (Fig. 4), no significant difference was found among three sampling sites. A gradual enrichment trend was observed for the average $\delta^{13}C$ of the oyster *C. ariakensis*, and two dominant nekton species (oriental shrimp *Palaemon macrodactylus* and Shokihaze goby *Tridentiger barbatus*) along the increasing salinity gradient. The oyster had relatively greater $\delta^{13}C$ values at sampling sites S5 and S8 than at S2 (Fig. 4, $p<0.05$, one-way ANOVA).

Trophic Structure and Energy Flow Pathway

The average $\delta^{15}N$ values of 37 consumer species associated with the created intertidal oyster reef varied between 5.7‰ and 13.3‰ (Table 2), which suggested an average of three trophic levels (Fig. 5). Four fishes and nine macroinvertebrates represented primary consumers, with a mean trophic position of 2.6. Most organisms (13 macroinvertebrates and 9 fishes) were recognized as secondary consumers with a mean trophic position of 3.3 (Fig. 5). Two fishes, red eel goby (*Odontamblyopus lacepedii*) and spotted sea bass (*Lateolabrax maculatus*), were the top predators at the created oyster reefs (Fig. 5). Generally, fishes were more enriched in $\delta^{15}N$ and occupied higher trophic positions ($\delta^{15}N$ 10.0‰, TP 3.3) than macroinvertebrates ($\delta^{15}N$ 9.1‰, TP 3.0; Fig. 5).

Combining the abundance data and stable isotope analyses, a simplified food web diagram was constructed for the created intertidal oyster reef (Fig. 6). POM and BMI constituted the nutritional base of the oyster reef food

web. Three main energy flow pathways for the created intertidal oyster reef can be described as: (1) POM→ bivalves (mainly oyster)→amphipod→carnivores. (2) BMI→reef resident crab→mud crab (Asian paddle crab *Charybdis japonica*, green mud crab *Scylla paramamosain*). (3) Complex organic matter sources from POM and BMI→barnacles, anchovy→omnivores (e.g., shrimps, snails, polychaetes)→carnivores (e.g., spotted sea bass, red eel goby).

Discussion

Organic Matter Sources

Our results indicate that POM and BMI constitute the main organic matter source fueling the food web at the created intertidal oyster reef in the Yangtze River estuary, China. Although macroalgae ^{13}C values were close to that of most species, strong dependence and direct assimilation by organisms is not thought to occur due to macroalgae's relatively enriched ^{15}N values (8.7‰) as well as its limited areal cover and low biomass. Our results are corroborated by a similar consumer reliance on POM and BMI at a natural oyster reef in Texas, USA (Wrast 2008), as well as an artificial rocky shore in the Westerschelde estuary, Netherlands (Riera et al. 2004). This indicates that the presence of a biogenic structure and its proximity to sources (e.g., microalgae colonization of oyster shells), regardless of it being natural or created/restored, affects basal trophic relationships. Although the study concludes that POM and BMI constituted the main organic matter sources of the oyster reef food web, we cannot totally eliminate the food source contribution of marsh plants and macroalgae to the food web of the created oyster reef.

The main organic matter source fueling aquatic food webs may vary significantly among habitat types at seemingly small spatial scales (Deegan and Garritt 1997; Paterson and Whitfield 1997). Within our study system, Quan et al. (2009a) found that the C_4 marsh plant and BMI fueled the aquatic food webs in the salt marshes (JWNR)

Table 2 Average $\delta^{13}C$, $\delta^{15}N$, and C/N values (±1 SE) of macroinvertebrates and fishes collected from the created intertidal oyster reef in the Yangtze River estuary

Family	Species	Code	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N	n
Invertebrate						
Alpheidae	*Alpheus japonicus* (shrimp)	ALJ	−21.0±0.8	9.5±2.2	4.66±0.76	2
Arcidae	*B. bistrigata* (bivalve)	BAB	−21.1±0.5	5.8±0.0	5.81±1.71	2
Balanidae	*Balanus albicostatus* (barnacle)	BAA	−19.8±1.7	8.2±1.0	3.66±0.64	2
Cancellariidae	*C. mangeloides* (snail)	CAM	−18.9±0.2	9.4±0.2	5.30±1.85	2
Gammaridae	*Gammarus* sp. (amphipoda)	GAS	−23.0	10.2	4.66	1
Grapsidae	*H. sinensis* (crab)	HES	−16.6	7.8	4.47	1
	Eriochier leptognathus (crab)	ERL	−20.9±0.5	8.2±0.6	5.30±0.62	3
	Metopograpsus latifrons (crab)	MEL	−17.2±1.2	8.7±0.5	4.31±0.71	4
	Hemigrapsus penicillatus (crab)	HEP	−17.4±1.7	10.2±1.0	4.15±0.69	3
Leucosiidae	*P. pisum* (crab)	PHP	−17.9	8.3	6.92	1
Microcotylidae	*G. rapi* (parasite)	GNR	−12.3	10.1	6.49	1
Muricidae	*R. bezoar* (snail)	RAB	−19.5±0.2	9.3±0.3	3.76±0.08	4
Mytilidae	*V. atrata* (bivalve)	VIA	−21.9	5.7	7.45	1
Nereididae	*Perinereis aibuhitensis* (polychaetes)	PEA	−19.6±0.6	10.6±1.0	5.619±0.691	9
Ostreidae	*C. ariakensis* (bivalve)	CRA	−23.1±0.2	7.5±0.4	5.79±0.48	24
Palaemonidae	*Macrobrachium nipponense* (shrimp)	MAN	−20.6±0.8	11.7±0.6	3.64±0.14	3
	P. macrodactylus (shrimp)	PAM	−18.8±0.2	10.5±0.3	3.52±0.04	9
	E. carinicauda (shrimp)	EXC	−19.0±0.4	9.7±0.3	3.35±0.05	6
	Exopalaemon annandalei (shrimp)	EXA	−20.7±0.7	8.7±0.3	3.49±0.29	5
Portunidae	*S. paramamosain* (crab)	SCP	−17.3±1.3	10.1±0.2	3.54±0.03	4
	C. japonica (crab)	CHJ	−17.9±0.1	10.1±0.6	3.49±0.04	3
Xanthidae	*Pilumnus scabrisculus* (crab)	PIS	−17.5±0.8	9.6±0.9	5.42±0.72	5
Fish						
Ariidae	*Arius sinensis*	ARS	−20.0±0.6	10.7±0.7	3.65±0.07	6
Clupeidae	*Coilia mystus*	COM	−19.5±0.3	8.5±0.3	3.43±0.17	8
Cynoglossidae	*Cynoglossus gracilis*	CYG	−19.9	9.4	3.97	1
Gobiidae	*Liciogobius guttatus*	LIG	−19.1±0.6	10.1±0.7	3.65±0.09	3
	O. lacepedii	ODL	−21.2	13.3	3.63	1
	Trypauchen vagina	TRV	−18.3	10.1	4.35	1
	T. barbatus	TRB	−19.1±0.6	11.4±0.4	3.41±0.07	6
Mugilidae	*C. haematocheilus*	CHH	−14.6±0.5	9.5±0.3	3.41±0.09	5
Polynemidae	*Eleutheronema rhamdinum*	ELR	−18.9±1.3	9.0±0.2	3.53±0.11	2
Sciaenidae	*Johnius distinctus*	JOD	−19.2±0.2	9.3±0.2	3.35±0.05	3
Serranidae	*L. maculatus*	LAM	−20.2±1.4	12.5±0.2	3.33±0.05	2
Synodidae	*Harpodon nehereus*	HAN	−18.1	7.6	3.40	1
	C. lucidus	COL	−19.1±0.4	8.3±0.7	3.77±0.12	5
	Argyrosomus argentatus	ARA	−20.0	9.9	3.21	1
Tetraodontidae	*Takifugu bimaculatus*	TAB	−16.7±2.0	10.7±0.6	3.63±0.18	2

surrounding the oyster reef. Results from the present study indicate that fish and macroinvertebrates at the oyster reef had more depleted ^{13}C values than those collected in the salt marsh habitat (Fig. 7); organisms at the oyster reef depend more on POM and BMI compared with those in the surrounding salt marsh habitat. This is important because it shows the main organic matter source fueling the created

oyster reef food web is different than that at the adjacent salt marsh habitat.

The oyster *C. ariakensis* in the created reef had more depleted ^{13}C values (1.3‰) than POM. This may be explained by two mechanisms. Firstly, the existence of lipid in the oyster tissue may have resulted in approximately 1‰ depletion in stable carbon isotope ratio (Lorrain et al.

Fig. 2 $\delta^{13}C$ values of consumers overlaid on those of primary producer values. Values are mean±1 SE for consumers. The breadth of the rectangular boxes represents±1 SE of $\delta^{13}C$ values of primary producers. The abbreviations are shown in Tables 1 and 2
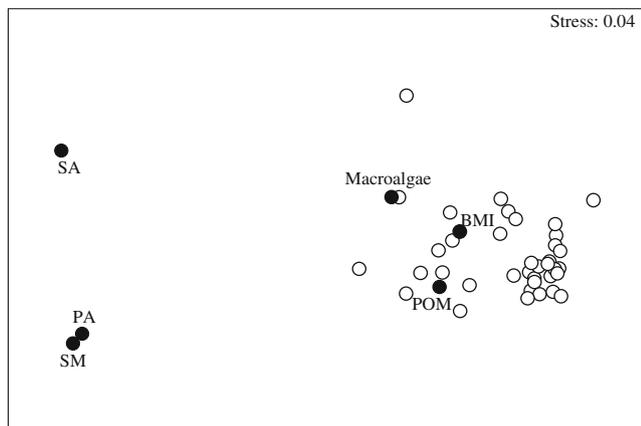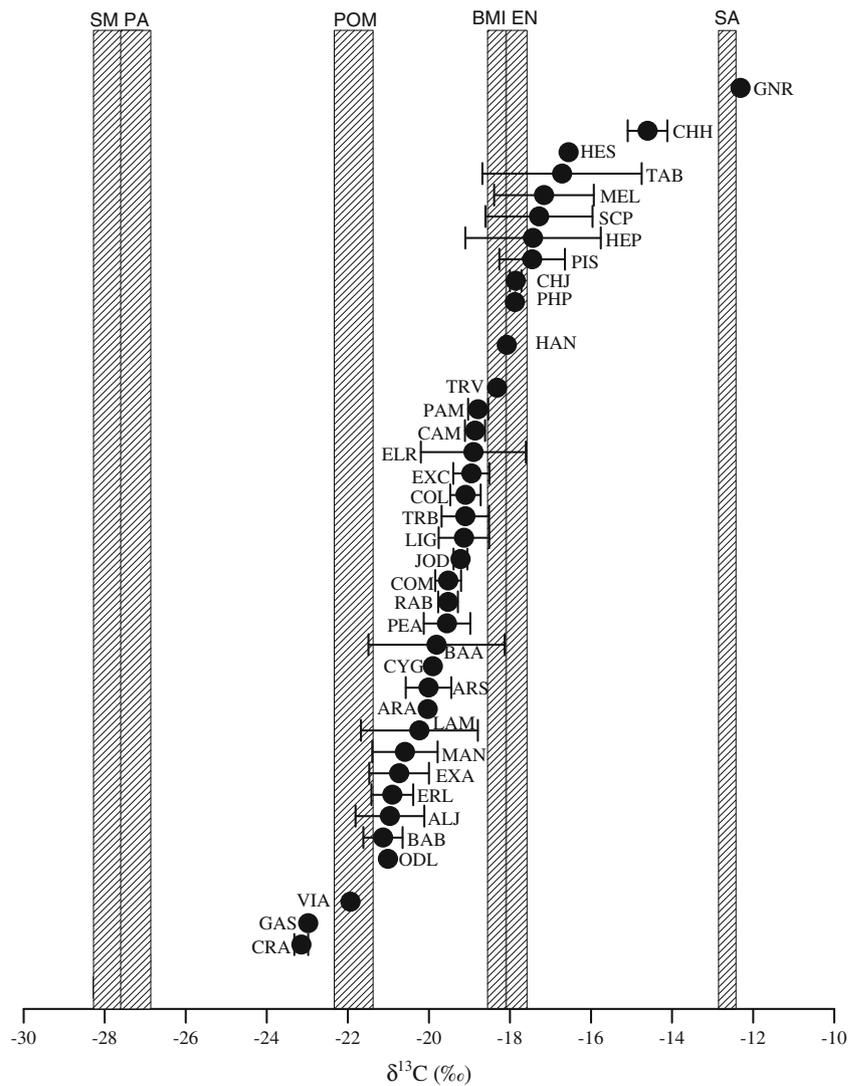
Fig. 3 Non-multidimensional scaling (NMDS) ordination of organic matter sources (*full circle*) and consumers (*empty circle*) at the created intertidal oyster reef based on their $\delta^{13}C$ and C/N values

2002). Secondly, POM is a complex mixture of phytoplankton, detritus and metazoans, and oyster selective feeding could easily account for the difference from the POM isotopic signature (Cognie et al. 2001; Riera et al. 2002). Therefore, we suggest that the oyster primarily assimilated the organic carbon from estuarine phytoplankton, while marsh plants (C₃ plants *P. australis* and *S. mariqueter*, C₄ plant *S. alterniflora*), BMI, and macroalgae *Enteromorpha* sp. played minor roles in providing nutrition for the oyster. Average $\delta^{13}C$ values for *C. ariakensis* in this study followed the range of the $\delta^{13}C$ values reported for other oyster species (Table 3). In most cases, phytoplankton is considered to be the major organic matter source assimilated by oysters due to its high food availability and quality (Haines and Montague 1979; Wrast 2008). However, carbon from benthic microalgae (Riera and Richard 1996; Riera 1998; Cognie et al. 2001), attached microalgae (Fukumori et al. 2008), and

Fig. 4 Spatial variations of $\delta^{13}C$ values of BMI, POM, oyster (*C. ariakensis*), oriental shrimp (*P. macrodactylus*) and Shokihaze goby (*T. barbatus*) at the created intertidal oyster reef. Bars represent±1 SE. Significant differences (*p*<0.05) in stable isotope ratios among sampling sites are indicated by *different letters*

enriched ^{13}C values than bivalve mollusks (*B. bistrigata*, *V. atrata*, and *C. ariakensis*). The resident crabs directly grazed the benthic microalgae on the created oyster reef, and thus integrated the relatively heavy ^{13}C values from autotrophs. Resident bivalves likely assimilated the organic matter from POM with relatively depleted ^{13}C values. This result is consistent with a report from Estero Bay, Florida, USA, that found resident crab and shrimp species depend heavily on benthic microalgae (Abeels et al. 2010). In general, benthic feeders had slightly more enriched $\delta^{13}C$ values than their pelagic counterparts.

There are significant effects of temporal and spatial variation in food availability and quality reflected by variations in stable carbon isotope signatures (Riera and Richard 1997; Chanton and Lewis 2002; Piola et al. 2006). Several studies have observed a gradual enrichment trend in organisms' $\delta^{13}C$ values along a salinity gradient within an estuary (e.g., Riera and Richard 1997; Riera 1998; Piola et al. 2006). These studies indicate a change in organic matter sources from terrestrial input in upper reaches of the estuary, to benthic diatoms, phytoplankton, eelgrass, and macroalgae (^{13}C enriched) in the estuarine mouth and marine littoral (Riera and Richard 1997; Riera 1998; Piola et al. 2006). The average $\delta^{13}C$ values of the oyster species (*C. ariakensis*) and two dominant nekton species (*P. macrodactylus* and *T. barbatus*) at our created reef generally showed a similar estuary-to-ocean gradient. The gradual enrichment may be explained by a change in relative contribution of different food sources (Riera and Richard 1997; Piola et al. 2006) or consumption of phytoplankton with different ^{13}C isotope values (Deegan and Garritt 1997; Piola et al. 2006). DIC $\delta^{13}C$ values typically change from a depleted signature (approx. −12‰) in the upper freshwater portions of an estuary, to an enriched signature (~0‰) at the marine end (Peterson 1999). Therefore, corresponding phytoplankton and bivalves are also likely to integrate this varied estuarine ^{13}C signature.

Trophic Structure and Ecological Implications

Most species (22 of 37) associated with the created oyster reef represent secondary consumers, while primary consumers dominated the aquatic food web in the adjacent salt marsh (Quan et al. 2009a). Species assemblages at our created intertidal oyster reef had higher trophic positions than individuals of the same species at the adjacent salt marsh (Fig. 7). For instance, spotted sea bass *L. maculatus* (4.0 vs. 3.5), ridgetail prawn *Exopalaemon carinicauda* (3.2 vs. 3.0), so-iny mullet *Chelon haematocheilus* (3.1 vs. 2.6), and big head croaker *Collichthys lucidus* (2.8 vs. 2.4) all occupied higher trophic positions at the created oyster reef. Consistent results have also been reported by previous studies in other areas (Simonsen 2008; Wrast 2008). For

terrestrial/marsh detritus (Hackney and Haines 1980; Incze et al. 1982; Riera and Richard 1996) was found to contribute significantly to filter-feeding oysters in many estuarine ecosystems. Although oysters are able to ingest preferentially nutritious food particles (algae cell) and reject non-nutritious particles (detritus) as pseudofaeces (Newell and Jordan 1983; Piola et al. 2006), the relative importance of different organic matter sources in the nutrition of oysters may depend heavily on study location, freshwater inflow and food availability (Simenstad and Wissmar 1985; Langdon and Newell 1990; Riera et al. 2002).

The present study found that reef-resident crabs (Chinese shore crab *Hemigrapsus sinensis*, *Philyra pisum*) had more

Fig. 5 Average $\delta^{15}N$ values and trophic position of macro-invertebrates (*black circle*) and fishes (*empty square*) at the created intertidal oyster reef. Trophic position of a consumer was calculated by its mean $\delta^{15}N$ value
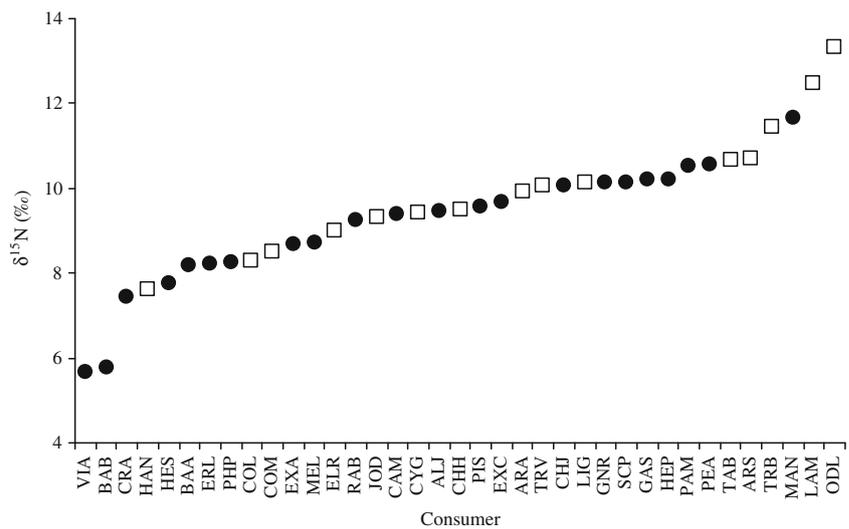
example, Wrast (2008) indicated that a subtidal oyster reef supported a more robust food web with greater number of links and higher level predators as compared to salt marsh and non-vegetated bottom. Simonsen (2008) found that Atlantic croaker had higher $\delta^{15}N$ values at an artificial oyster reef than mud bottom.

There is some uncertainty in the exact mechanisms behind why more transient nekton species (secondary consumers) may select oyster reefs over other potential habitat types. It may be a result of the increased foraging opportunities a reef
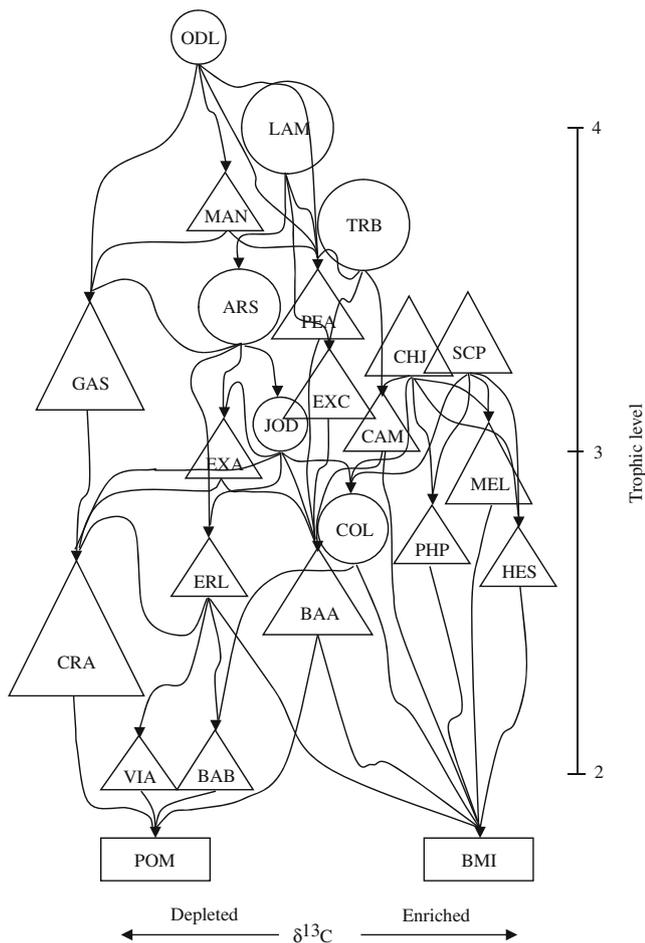
Fig. 6 Simplified food web diagram for the created intertidal oyster reef. The size of symbols (*circle*, fish; *triangle*, macroinvertebrates; and *square*, basal food source) represents the relative abundance
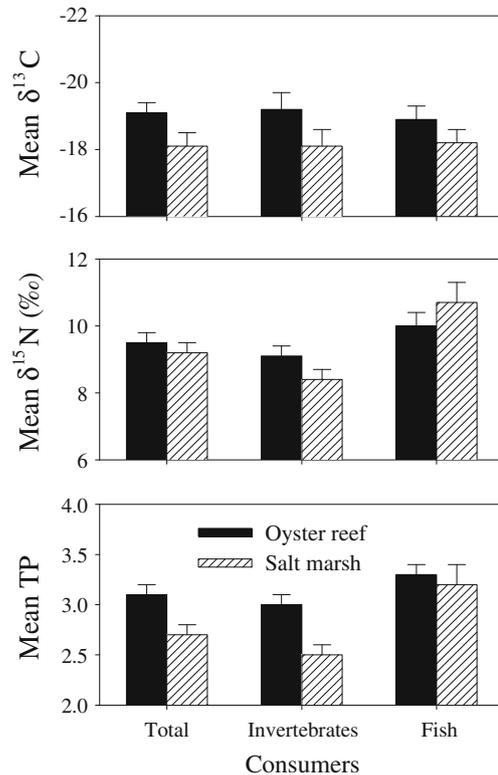
Fig. 7 Comparison of mean $\delta^{13}C$, $\delta^{15}N$ and trophic position (TP) of consumers between the created intertidal oyster reef and adjacent salt marshes in the Yangtze River estuary. Mean values between species were calculated for comparison

Table 3 Comparisons of average $\delta^{13}C$ values (±1 SE) of oysters from various study locations worldwide

Site	Species	$\delta^{13}C$ (‰) of oysters	$\delta^{13}C$ (‰) of food sources	Source
Estero Bay Aquatic Preserve, Florida	*Crassostrea virginica* (Eastern oyster)	−22.45±0.12		Abeels et al. 2010
Uwa Sea, Japan	*Pinctada fucata martensii* (Pearl oyster)	−17.5 to −16.8	POM −20.2 to −19.1 Attached algae −13.0	Fukumori et al. 2008
Lavaca Bay, Texas	*Crassostrea virginica* (Eastern oyster)	−22.79±0.39	SOM −17.61±0.60 POM −20.34±0.99	Wrast 2008
Manning River, Australia	*Saccostrea glomerata* (Sydney rock oyster)	−24.6 to −25.9	POM −26.2±0.5 SPOM −26.4±0.4 Seagrass −11.4±0.2 Mangrove −27.3±0.5 Epiphytes −24.3±0.4	Piola et al. 2006
Westerschelde estuary, Netherlands	*Crassostrea gigas* (Pacific oyster)	−22.0 to −20.9	SOM −24.7 to −24.0 POM −24.7 to −23.8 BMI −18.0 to −15.6	Riera et al. 2004
Southern coast of Korea peninsula	*Crassostrea gigas* (Pacific oyster)	−17.4	POM −19.8 BMI −13.7	Kang et al. 2003
Oosterschelde estuary, Netherland	*Crassostrea gigas* (Pacific oyster)	−17.3±0.3	POM −23.2±0.6 BMI −11.3±0.1	Riera et al. 2002
Apalachicola Bay, Florida	*Crassostrea virginica* (Eastern oyster)	−24.5		Chanton and Lewis 2002
Marennes-Oléron, France	*Crassostrea gigas* (Pacific oyster)	−24.7 to −19.7 (mid-estuary) −20.3 to −15.9 (estuarine mouth) −21.5 to −17.0 (marine littoral)	River POM −36.7±2.3 Estuarine POM −23.5±1.5 BMI −16.1±0.7	Riera and Richard 1997
Marennes-Oléron, France	*Crassostrea gigas* (Pacific oyster)	−22.2 to −21.4(mid-estuary) −19.7 to −16.1 (estuarine mouth) −20.2 to −19.2 (marine littoral)	River POM −29.2 to −27.4 Marine POM −21.8 to −19.5 BMI −16.0	Riera and Richard 1996
Hood Canal, Washington	*Crassostrea virginica* (Eastern oyster)	−19.7±1.8	POM −23.4±2.6 SOM −23.5±1.3	Simenstad and Wissmar 1985
Yangtze River estuary	*C. ariakensis* (Asian oyster)	−23.1±0.2	POM −21.9±0.5 BMI −18.3±0.1	The present study

may provide because of its refuge or nursery function for resident species (Coen and Luckenbach 2000; Harding and Mann 2001; Tolley and Volety 2005; Shervette and Gelwick 2008). Enhanced prey abundance at oyster reefs may also increase predator foraging resources by reducing interference interactions, thus mitigating trophic transfer and resulting community structure (Harding and Mann 2001, 2003; Grabowski and Powers 2004). For instance, bluefish (*Pomatomus saltatrix*) and striped bass (*Morone saxatilis*) fed on a higher percentage of teleost prey at oyster reefs than mud bottom habitat (Harding and Mann 2001, 2003). However, because we did not manipulate species assemblages or observe behavior, we can only speculate on mechanisms causing higher average trophic levels at our created oyster reef versus the adjacent salt marsh.

Oysters are dominant components of many shallow marine systems (Beck et al. 2011) and they form an important trophic linkage between the benthos and surrounding environment (Langdon and Newell 1990; Riera and Richard 1997; Mann 2000). Successful management and restoration/

creation of oyster reefs is likely to improve with a better understanding of how physical structure might influence trophic structure, and it is therefore important to understand how energy is transferred through the system. The present study found that POM and BMI provide the main basal resources for the local food web and that the created oyster reef supports a higher trophic level than adjacent salt marsh habitat. The oyster *C. ariakensis* had the most depleted ^{13}C values among the 37 animal species collected and indicates that few organisms directly consume the oysters. Instead, many reef-resident fauna (e.g., crab, snail, barnacles, and polychaetes) constitute the main prey item of commercially important nekton species. Similar results have been reported on the dietary importance created by oyster reefs and the resulting resident species assemblages rather than the direct dietary benefit of oysters themselves (e.g., Grabowski et al. 2005; Tolley and Volety 2005; Shervette and Gelwick 2008); however, this is the first study to our knowledge that quantifies this metric. This suggests that the structure created by oyster reefs and its resulting associated species assem-

blage is an important factor-mediating trophic transfer in the Yangtze River estuary, China. This direct use indicates that it is possible to create oyster reefs that may serve as suitable nekton habitat and support higher trophic level assemblages than adjacent salt marsh with time.

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