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Functional groupings and food web of an artificial reef used for sea cucumber aquaculture in northern China

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ABSTRACT

Artificial reef is considered as a useful tool to remodel habitats in coastal and estuarine area. Some artificial reefs (ARs) were conducted in Shandong Peninsula for sea cucumber *Apostichopus japonicus* integrated multi-trophic aquaculture (IMTA). Little is known about the main feeding type and food resources of living organisms in this IMTA ecosystem. Neither is the information about other animals competing food with *A. japonicus*. Functional group (FG) and their food resources of mobile organisms and epifauna in ARs area were investigated. There were three types of food resources and five FGs within two trophic levels in studied area. Particle organic matter (POM), seaweed detritus and sediment were considered to be the main food resources. The first three FGs were primary consumers and were mainly epifauna, while the other two FGs were secondary consumers. FG 1 species were filter feeders, and group 2 was all deposit feeders and *A. japonicus* was in this group. FG 2 contained few species and this indicated that *A. japonicus* had few food competitors. FG 3 contained most epifauna species which were detritus feeders and this result implied that the artificial oyster shell reed can retain detritus effectively. The food sources of group 4 were complex. Species of group 5, mostly fish, occupied the top trophic level and fed primarily on species of FG 1 and FG 2. This kind of ARs can retain detritus effectively and provide suitable habitat to epifauna and surrounding natural fauna community.

1. Introduction

Artificial reefs (ARs) are constructed to model natural reef ecosystems with the purpose of providing habitat (Lira et al., 2010; Seaman, 2000). A wide range of materials including rock, concrete, wood, steel, and old ships have been used as ARs (Baine, 2001). Oceanic platforms, piers, breakwaters, groins, low crested structures, and shipwrecks can also be considered ARs (Martins et al., 2009).

Once a new reef structure is placed underwater, it is rapidly colonized by many types of organisms. Organisms living on the surfaces of these underwater structures are called epifauna. Mobile fauna commonly inhabiting AR structures include burrowing species (infauna) and free-living species that shelter in crevices (sedentary species) (Lira et al., 2010). Associated epifauna and mobile fauna may increase the food chain length of the AR in part by promoting the development of higher trophic levels (Danovaro et al., 2002; Krohling et al., 2006).

The sea cucumber *Apostichopus japonicus* Selenka, whose natural range in Asia covers the coasts of Russia, Japan, China, and Korea from 35°N to 44°N (Yuan et al., 2009), has become an important component of the mariculture sector in northern China (Zhang et al., 2011). Soaring prices were responsible for stimulating the development of *A. japonicus* aquaculture in China.

ARs are considered best for sea cucumber aquaculture in coastal and pond ecosystems (Chen, 2003), as they provide protection from predators (Ambrose and Anderson, 1990; Zhang et al., 2011), food resources, and habitat for aestivation and hibernation (Chen, 2005). Among other materials, stone and concrete structures have been widely used as artificial substrates or reefs for sea cucumber aquaculture in China (Zhang et al., 2015). Oyster shells, common waste of seafood, are abundantly available in coastal area. ARs made of bags of oyster shells have been introduced as a potentially appropriate habitat for sea cucumber cultivation (Zhang et al., 2015), and have been used in integrated multi-trophic aquaculture (IMTA) systems, because Holothurians are deposit feeders and have been commonly used in IMTA co-culture systems to ingest detritus (MacDonald et al., 2013; Nelson et al., 2012; Slater and Carton, 2007, 2009; Yu et al., 2014b; Zamora and Jeffs, 2012; Zhou et al., 2006).

Stable isotope analysis is a useful tool for following energy flow and organic matter cycling within food chains and food webs (Carlier et al., 2007; Sun et al., 2012). Compared with conventional gut content analysis, stable isotopes reflect integrated food assimilation over a long time

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period corresponding to the turnover time of the tissues analyzed (Post, 2002).

In this study, stable isotope analysis was used to investigate the food web structure of the faunal community of an IMTA system in Shandong peninsula, which used the artificial oyster-shell reef as the major habitat, and cultured *A. japonicus* with seaweed *Saccharina japonica*. Two questions were raised: 1) which feeding type was dominant in this IMTA ecosystem; 2) does *A. japonicus* have food competitors?

2. Material and methods

2.1. Study area

Artificial oyster-shell reefs were constructed in Rongcheng Bay to create an IMTA system for the aquaculture of *A. japonicus* (Xu et al., 2014; Zhang et al., 2015). This bay is widely known for raft and rope cultivation of the seaweed *S. japonica*. This bay was specifically chosen for the IMTA system because of the large quantity of algal detritus, which may increase food resources available to other organisms in the AR (Zhang et al., 2012).

2.2. Sampling

We studied the faunal community on the oyster shell reef every month from March to June 2011. The epifauna (including sea cucumber) from different artificial reef surface area were scraped off carefully and collected in a bag by a scuba diver.

An underlying fishing net was used to collect fish inhabiting the reef. Sea cucumbers, fish, and large mollusks were dissected in the field after rinsing with distilled water. All biological samples were frozen in clean test tubes.

Seaweed and seagrass debris that settled on the reef surface (classified as moderately fresh or rotten) was also collected by net, rinsed in fresh water, and stored. Particulate organic matter (POM) was measured using pre-combusted (450 °C, 2 h) glass-fiber filter (Whatman GF/F type) after being sieved through a 125 μm mesh sieve. The sediment on the reef was scrapped from the surface of the reef. The sediment was grounded with a mortar and pestle, passed through a 200-μm screen sieve. Total organic carbon (TOC) and total nitrogen (TN) content were measured using stable isotope analysis as described below.

2.3. Isotopic analysis

Samples of fauna and sediment were freeze-dried and acidified with 1 mol l^{-1} HCl to remove carbonates (GF/F filter was acidified by acid steaming with 37% HCl), after which they were dried again and homogenized without rinsing (Jacob et al., 2005).

Lipids were not extracted to avoid potential negative impacts on nitrogen isotopes of the samples. Analyses of stable isotopes (δ^{13}C and δ^{15}N), TOC, and TN were performed using a Flash EA 1112 elemental analyzer and a Delta V Advantage isotope ratio mass spectrometer (Thermo Fischer, Waltham, MA, USA).

Stable isotope ratios were expressed in δ-unit notation, which is defined as follows:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000$$

where X = ^{13}C or ^{15}N, and R is either $^{13}C/^{12}C$ ratio for carbon or $^{15}N/^{14}N$ ratio for nitrogen. $R_{standard}$ for the ^{13}C and ^{15}N tests are the Pee Dee Belemnite standard (PDB) and Atm-N_2, respectively. To correct any instrument drift (rarely necessary), at least 2 laboratory working standards (protein, glycine or urea) were run regularly during the tests. Analytic precision was ±0.1‰ for δ^{13}C and 0.2‰ for δ^{15}N, respectively.δ^{13}C and δ^{15}N content were expressed by mean ± SD.

2.4. Data analysis

The trophic level (TL) of each species was evaluated on the basis of its δ^{15}N content, which is generally acknowledged as a reliable indicator of trophic position (Post et al., 2000). TL was calculated using the following formula:

$$TL_i = \left[\left(\delta^{15}N_i - \delta^{15}N_{pc}\right)/3.4\right] + 2$$

where TL_i is the average trophic level of species *i*; $\delta^{15}N_i$ is the average δ^{15}N of species *i*; $\delta^{15}N_{pc}$ is the average δ^{15}N of primary consumers, commonly filter feeders; and 3.4 is the mean δ^{15}N trophic enrichment occurring per trophic level(Post et al., 2000; Ying et al., 2015).

Euclidean distances between each paired sample combination were computed for TOC, TN, δ^{13}C, and δ^{15}N content using normalized data. Cluster analysis was then performed. Functional groups were determined from the results of cluster analysis.

IsoSource was used in this study to calculate the proportional contribution of POM, seaweed detritus, and sediment to the food sources of each functional group based on δ^{15}N content (Phillips and Gregg, 2003) using 1% increments and 0.05 tolerance. In addition, IsoSource was used to determine the food resources of the sea cucumber *A. japonicus* from δ^{13}C and δ^{15}N content.

A number of isotope mixing models have been proposed to identify the relative contributions of food resources, including geometric approaches, linear and Bayesian mixing models, and spatially based approaches (Layman et al., 2012). IsoSource, which was used in this study, was developed by Phillips and Gregg (2003) and has become one of the most common analytical tools in the field (Layman et al., 2012).

In typical study, δ^{13}C represents food source, while δ^{15}N represents trophic level. In this study, the Group 4 and 5 were secondary consumer. Food source of those two groups were the primary consumer. The value of δ^{15}N can represent the feature of each primary group L1–L3, as said above δ^{15}N represent trophic level and can represent. The value used for calculating was the average value of δ^{15}N of each group.

Sea cucumber was the main cultured species in this IMTA system, so both δ^{13}C and δ^{15}N were used for precise food resource result.

3. Results

3.1. Food resources

The δ^{13}C and δ^{15}N contents of potential food sources in artificial oyster shell reef area were shown in Table 1. The δ^{13}C and δ^{15}N contents of POM were low (Table 1). The δ^{13}C and δ^{15}N contents of moderately fresh seaweed detritus (MKELP) was −18.60 ± 0.37‰ and 8.26 ± 0.96‰ respectively. While the value of decomposed detritus (OKELP), contained a variety of macroalgae and eelgrass, was −17.82 ± 0.45‰ and 8.41 ± 1.37‰ respectively. The sediment on the reef that settled from the upper water column had an average of −19.28 ± 0.23‰ δ^{13}C and 5.60 ± 1.23‰ δ^{15}N.

Table 1
Food resources of an artificial oyster reef in Rongcheng Bay, China.

Food resources	δ^{13}C (‰)	δ^{15}N (‰)
POM	−23.28 ± 0.23	2.60 ± 1.50
MKELP	−18.60 ± 0.37	8.26 ± 0.96
OKELP	−17.82 ± 0.45	8.41 ± 1.37
Sediment	−19.28 ± 0.23	5.60 ± 1.23

POM: particulate organic matter; MKELP: moderately fresh seaweed detritus; OKELP: degraded seaweed detritus.