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# Trophic connectivity between intertidal and offshore food webs in Mirs Bay, China

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**Summary**  Trophic interactions are common worldwide, both within and between ecosystems. This study elucidated the trophic connectivity between intertidal and offshore zone, in Mirs Bay, China. The contributions of offshore suspended particulate organic matter (SPOM), intertidal macroalgae and epiphytes to consumer biomass were assessed, and the trophic pathways were identified through the use of stable isotope ratios of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) of basal sources and consumers. Mean $\delta^{13}$C values of basal sources had a wide range ($-19.6$‰ to $-11.8$‰) and were generally well separated in Mirs Bay. The average $\delta^{13}$C of consumers in Mirs Bay ranged from $-19.2$‰ to $-11.8$‰, reflecting a carbon source integrated from different primary producer signals. IsoSource model solutions indicated consumers assimilated organic carbon from a mixture of basal sources. Offshore SPOM carbon was the primary carbon source supporting most consumers in both intertidal and offshore zones. Intertidal macroalgae and epiphytes also accounted for a large fraction for some consumers. $\delta^{15}$N data indicated 5 trophic levels in Mirs Bay. Intertidal consumers, except for *Capitulum mitella*, had a TP (trophic position) between 2 and 3, and mainly included filter-feeders and grazers. In contrast, almost all offshore consumers had a TP of between 3 and 4 except for filter-feeders (zooplankton), planktivores (*Clupanodon punctatus* and *Sardinella aurita*) and piscivores (*Gymnura japonica*). The basal sources fueled consumers

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through two trophic pathways, each of which involved organisms of both intertidal and offshore zones, implying trophic connectivity between them in the Mirs Bay ecosystem.

## 1. Introduction

Food webs among different habitats vary greatly in the abundances of their basal sources, community composition and productivity, leading to food webs being spatially heterogeneous. Trophic interactions are common among habitats because the nutrients, detritus, prey and consumers that comprise food webs often cross-habitat boundaries (Polis and Strong, 1996). Cross-habitat exchanges of materials can be of great importance to effective natural source dynamics both within and between ecosystems (Polis et al., 1997; Savage et al., 2012). For instance, in a deciduous forest and stream ecotone the populations of birds and fish, subsidized by aquatic insect and terrestrial invertebrate input, respectively, accounted for 25.6% and 44.0% of the annual total energy budget of the bird and fish assemblages (Nakano and Murakami, 2001). Abundant mangrove leaf litter from the intertidal zone promoted populations of the commercially important fisheries in adjacent coastal waters (Day et al., 2012).

Bays are complex ecosystems consisting of different habitats such as intertidal and offshore zones, which can be considered as interacting habitats (Jansson, 1988). The rhythmic movement of the tide is responsible for the materials exchange between intertidal and offshore zones and carries a diverse and abundant potential food source for consumers (Polis et al., 1997). In addition, consumers can move to forage across habitats according to prey availability (Mittelbach and Osenberg, 1993; Randall, 1965). Elucidating the trophic connectivity between intertidal and offshore zones is urgent because bay ecosystems are impacted by human activities in many regions of the world. For example, a large amount of intertidal areas has been destroyed by reclamation, which often changes the living environment and community structure of intertidal organisms (Lu et al., 2002). The result may be damage to the trophic interactions between intertidal and offshore zones. Thus, a current goal in ecological research is to quantitatively assess trophic relationships among spatially heterogeneous and contiguous, connected habitats (Claudino et al., 2015; Conway-Cranos et al., 2015). Knowledge of trophic relationships is crucial for researchers' understanding of bio-population ecology and natural resource management.

It is, however, difficult to elucidate trophic relationships using traditional methods. Stable isotopes have recently been used successfully to study trophic connectivity (Claudino et al., 2015; Selleslagh et al., 2015). Carbon stable isotope ratios ($\delta^{13}$C) change predictably between diet and consumer, and have been used in ecological studies to trace the flow of sources of organic matter in marine and freshwater ecosystems (Fry and Sherr, 1984; Peterson and Fry, 1987). In ecosystems, $\delta^{13}$C values differ among phytoplankton (offshore zone) and macroalgae and epiphytes (intertidal zone) (Kang et al., 2008; Ouisse et al., 2012). Thus, $\delta^{13}$C values can distinguish the basal sources of consumers, and researches can estimate the relative contribution of these primary producers to consumer biomass through IsoSource model of Phillips and Gregg (2003). In contrast, consumers are typically enriched in $^{15}$N by 3—4‰ relative to their diet (Deniro and Epstein, 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987). Therefore, stable nitrogen isotope ratios ($\delta^{15}$N) can be used to define their trophic position (TP), based on the pathways of energy flow (Post, 2002; Vander Zanden and Rasmussen, 1999).

In this context, the aims of this study were to (1) quantitatively assess the basal sources that support consumer biomass of intertidal and offshore zones to determine whether there is trophic interaction between these two zones in Mirs Bay; and (2) identify the main trophic pathways between the intertidal and offshore zones to highlight the importance of trophic connectivity.

## 2. Material and methods

### 2.1. Site description

Mirs Bay is northeast of Hong Kong and south of Shenzhen, south China. It is close to the Pearl River Estuary to the west and Daya Bay to the east. The Bay, part of the South China Sea, is semi-enclosed and has an area of 320 km$^2$ and a mean depth of 18 m. The coastline extends about 100 km, and is mostly rocky (Zhang et al., 2013). The sediment is sandy (Xia et al., 2005). The water movement in Mirs Bay is influenced primarily by the tide because no large river flows into it.

### 2.2. Sample collection and preparation

This research was undertaken at three intertidal zones and four offshore stations of Mirs Bay (Fig. 1). Samples were collected from intertidal zones and offshore stations representing different bio-community. The basal sources and consumers sampled at intertidal zones mainly included macroalgae, epiphyte and bivalves, barnacles, chitons, limpets, crabs as well as whelks. In contrast, the samples collected at offshore stations consisted of phytoplankton, zooplankton, fishes, shrimps, mantis shrimps, squids and crabs. Because the intertidal zones can submerge for several hours during high tides, so basal sources can exchange and consumers can forage between intertidal zones and offshore stations. Sampling was conducted in September 2013.

The most abundant basal sources (macroalgae and epiphytes) and the most common consumers (macroinvertebrates) were sampled during low tide at intertidal zones. All macroalgae and macroinvertebrates samples were collected by hand. Epiphyte samples were gently scraped from
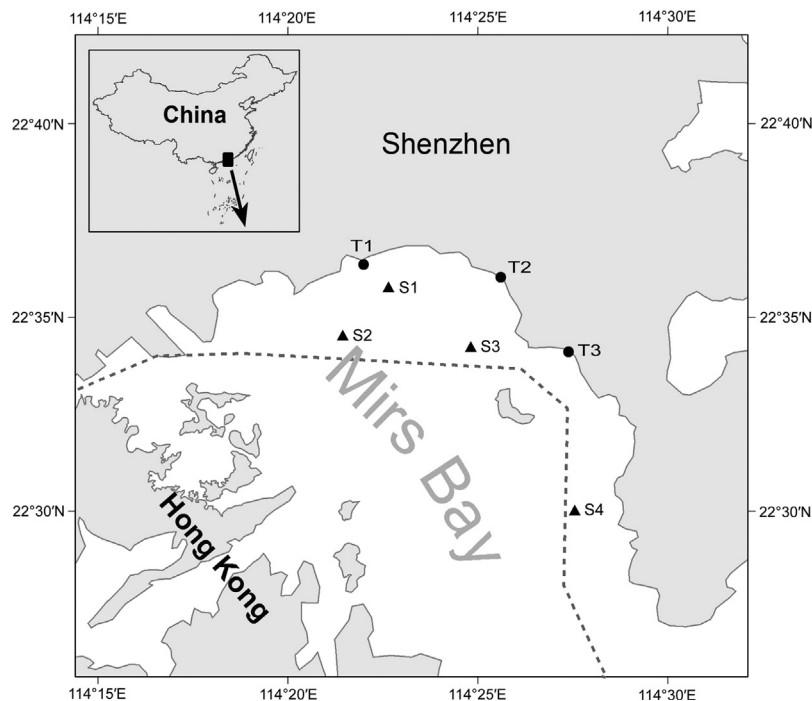
**Figure 1**   Map of Mirs Bay, China, showing the location of sampling stations and zones. Black triangles indicate location of offshore sampling stations; black circles indicate intertidal sampling zones.

rocks using a wire or nylon brush and carefully re-suspended in filtered seawater. They were then filtered onto precombusted (500°C, 4 h) Whatman GF/F filters. Experimental trawling was undertaken in four off-shore stations (ranging in depth from 12 to 19 m) from a commercial trawler. The catches (fish and macroinvertebrates) from each haul were stored in the field. Suspended particulate organic matter (SPOM) samples were obtained by filtering 5—7 L water at two depths (0.5 m and 10 m) using 4 L Niskin bottles from offshore stations through pre-combusted (500°C, 4 h) Whatman GF/F filters, followed by manual removal of all visible zooplankton and other contaminants under a dissecting microscope (40× magnification). The SPOM samples were used to represent phytoplankton (Hill et al., 2006; Kaehler et al., 2000; Markel and Shurin, 2015). Mesozooplankton and macrozooplankton were sampled from vertical tows at off-shore stations using a zooplankton net with 505 μm mesh size. Zooplankton samples were maintained alive in filtered seawater for 2 h to allow gut evacuation. All samples were stored on ice and transported to the laboratory and frozen at −20°C.

In the laboratory, as copepods accounted for the majority of zooplankton abundance in Mirs Bay (unpublished data), they were handpicked under a dissecting microscope and transferred to pre-combusted tin cups immediately. Fish and macroinvertebrate samples were defrosted and then sorted, with specimens being identified to the lowest possible taxonomic level. A small piece of white muscle tissue was dissected from the dorsum of large fish. For small fish, the head and viscera were removed, and the remainder of the body (consisting primarily of white muscle) was retained for isotope analysis. Only muscle tissue was processed for macroinvertebrate samples. Because of low individual weight, a composite tissue sample from more than one individual of the same taxon was used for some macroinvertebrates, including

small mollusks and crabs. All samples except zooplankton were washed with distilled water, dried (60°C, 48 h), ground to a fine powder and stored in glass vials for later isotope analysis.

### 2.3. Stable isotope analysis

Stable isotope analysis was conducted at the Chinese Academy of Agricultural Sciences Environmental Stable Isotope Laboratory, Beijing, China. To determine the $\delta^{13}C$ and $\delta^{15}N$ values for each sample, approximately 1—2 mg ground tissue was combusted using an NCE elemental analyzer (Vario PYRO Cube, Elementar) interfaced via continuous flow to an Isoprime 100 isotope-ratio mass spectrometer. Stable isotope ratios were expressed in the delta ($\delta$) notation, defined as parts per thousand (per mil, ‰) deviation from a certified standard:

$$\delta^{13}C \text{ or } \delta^{15}N = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000,$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. For carbon isotopes, the standard was Vienna Pee Dee Belemnite limestone, and atmospheric nitrogen was the nitrogen standard. Analytical errors were 0.08‰ for both $\delta^{13}C$ and $\delta^{15}N$.

### 2.4. Data analysis

Many species were collected from only one or two intertidal zones and offshore stations, preventing analysis of spatial variability within them. Therefore, data from different intertidal zones or offshore stations were pooled.

IsoSource model of Phillips and Gregg (2003) was used to estimate the percent contribution of basal sources to con-

sumer biomass. It allows multiple sources to be evaluated for each consumer using $\delta^{13}$C measurements, and to apply this approach the $\delta^{13}$C measurements of consumers were corrected for trophic fractionation (Post, 2002) using the TP assignments discussed below. Trophic fractionation of $\delta^{13}$C for consumers was set at 0.4‰, representing the average value from reported fractionations (Post, 2002). Source increment was set at 1% and tolerance at 0.1%. In Mirs Bay the macroalgae *Amphiroa zonata* and *Enteromorpha* spp. had similar $\delta^{13}$C values and were combined into the variable macroalgae.

The TP of each consumer was calculated as:

$$TP = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.4,$$

where $\delta^{15}N_{consumer}$ was the $\delta^{15}$N value of the consumer being evaluated, $\delta^{15}N_{baseline}$ was the average $\delta^{15}$N value of the consumers used to estimate the base of the food web (*i.e.* mollusk species in this study) and 3.4‰ was the per trophic level fractionation of $\delta^{15}$N according to Post (2002).

## 3. Results

### 3.1. Isotope signatures of basal sources

Three major potential basal sources for consumers were identified in Mirs Bay: the most abundant macroalgae species (*Enteromorpha* spp. and *Amphiroa zonata*) and epiphytes (consisting mainly of epilithic diatoms) collected from intertidal zones, and SPOM collected from offshore zones. The mean $\delta^{13}$C values of basal sources had a wide range (−19.6‰ to −11.8‰): SPOM had the most depleted $\delta^{13}$C value, and epiphytes had the most enriched values (Fig. 2). Macroalgae values were intermediate and had similar mean values between species (*Enteromorpha* spp. = −16.1‰ and *A. zonata* = −14.9‰).

SPOM samples had similar $\delta^{15}$N values to epiphyte samples, and both of them were lower than those of the two macroalgae species (*Enteromorpha* spp. = 9.6‰ and *A. zonata* = 9.1‰).

### 3.2. Isotope signatures of consumers

A total of 32 species of consumers was collected and subjected to stable isotope analysis, comprising 9 intertidal macroinvertebrate taxa and 23 offshore species of animals.

The $\delta^{13}$C signatures of consumers sampled at intertidal zones were relatively more variable than those collected from adjacent offshore zones (Fig. 2). The intertidal consumers had a range of $\delta^{13}$C signatures from −17.9 (*Perna viridis*) to −11.8‰ (*Liolophura japonica*), whereas the offshore consumers varied between −19.2 (zooplankton) and −14.4‰ (*Gymnura japonica*). In intertidal zones, the bivalves (*P. viridis*, *Crassostrea rivularis* and *Arcopsis sculp-*
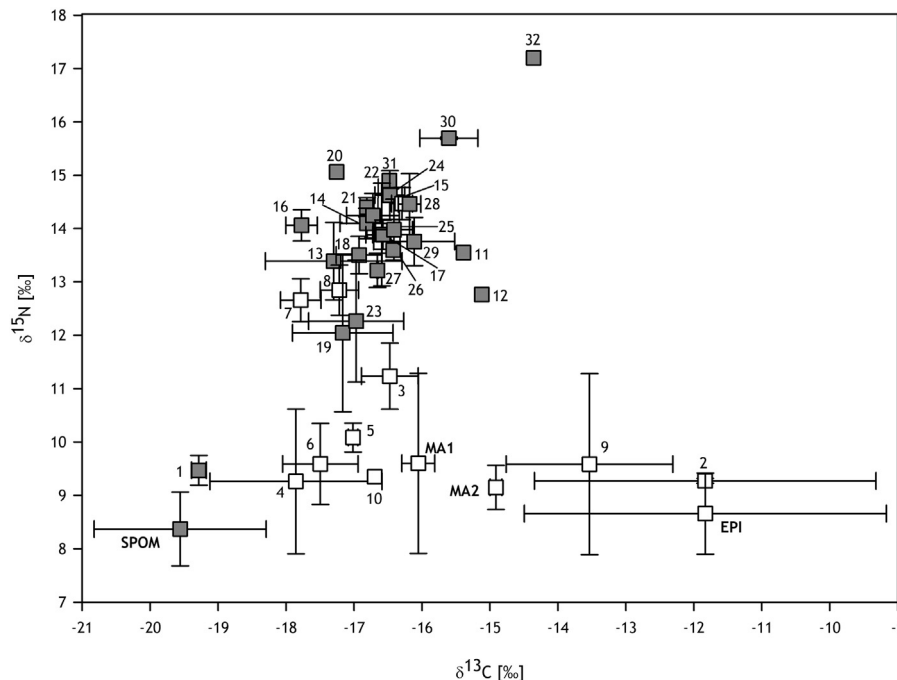


**Figure 2** Mean $\delta^{13}$C and $\delta^{15}$N values (±SD) in basal sources and consumers from Mirs Bay, China. White squares and gray squares represent samples from intertidal zones and offshore stations, respectively. Abbreviations are as follows, SPOM = suspended particulate organic matter; MA1 = macroalgae *Enteromorpha* spp.; MA2 = macroalgae *Amphiroa zonata*; EPI = epiphytes. Numbers correspond to consumers discussed within the text. 1 = Zooplankton; 2 = *Liolophura japonica*; 3 = *Thais clavigera*; 4 = *Perna viridis*; 5 = *Crassostrea rivularis*; 6 = *Arcopsis sculptilis*; 7 = *Balanus* sp.; 8 = *Capitulum mitella*; 9 = *Cellana grata*; 10 = *Gaetice depressus*; 11 = *Charybdis feriatus*; 12 = *Portunus trituberculatus*; 13 = *Metapenaeus affinis*; 14 = *Oratosquilla oratoria*; 15 = *Loligo duvaucelii*; 16 = *Collichthys lucidus*; 17 = *Decapterus maruadsi*; 18 = *Caranx kalla*; 19 = *Clupanodon punctatus*; 20 = *Thrissa dussumieri*; 21 = *Nemipterus japonicus*; 22 = *Leiognathus brevirostris*; 23 = *Sardinella aurita*; 24 = *Polynemus sextarius*; 25 = *Trichiurus haumela*; 26 = *Gastrophysus spadiceus*; 27 = *Apogon striatus*; 28 = *Pagrosomus major*; 29 = *Rhabdosargus sarba*; 30 = *Gerres japonicus*; 31 = *Muraenesox cinereus*; 32 = *Gymnura japonica*.

**Table 1**   Means and ranges of basal source contributions (%) to consumer biomass in Mirs Bay, China, from IsoSource models. Bold values indicate high contributions from a single source.

| Location | Species | SPOM | | Macroalgae | | Epiphytes | |
|---|---|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range | Mean | Range |
| Intertidal zone | *Liolophura japonica* | 2.5 | 0—6 | 5.8 | 0—13 | **91.8** | 87—96 |
| | *Thais clavigera* | **53.3** | 37—69 | 30.7 | 0—63 | 15.9 | 0—33 |
| | *Perna viridis* | **73.8** | 64—83 | 17.3 | 0—36 | 8.9 | 0—19 |
| | *Crassostrea rivularis* | **60.8** | 47—74 | 25.7 | 0—53 | 13.4 | 0—28 |
| | *Arcopsis sculptilis* | **68.3** | 54—79 | 20.9 | 0—43 | 10.8 | 0—23 |
| | *Balanus* sp. | **81.3** | 74—88 | 12.3 | 0—26 | 6.4 | 0—14 |
| | *Capitulum mitella* | **70.1** | 59—80 | 19.8 | 0—41 | 10.1 | 0—21 |
| | *Cellana grata* | 14.0 | 0—29 | 29.9 | 0—62 | **56.0** | 38—73 |
| | *Gaetice depressus* | **53.3** | 37—69 | 30.7 | 0—63 | 15.9 | 0—33 |
| Offshore zone | Zooplankton | **99.0** | 98—100 | 1.0 | 0—2 | 0.0 | 0—1 |
| | *Charybdis feriatus* | 38.5 | 18—58 | **40.4** | 0—82 | 21.1 | 0—43 |
| | *Portunus trituberculatus* | 31.0 | 8—53 | **45.4** | 0—92 | 23.6 | 0—48 |
| | *Metapenaeus affinis* | **73.8** | 64—83 | 17.3 | 0—36 | 8.9 | 0—19 |
| | *Oratosquilla oratoria* | **66.3** | 54—78 | 22.2 | 0—46 | 11.4 | 0—24 |
| | *Loligo duvaucelii* | **57.1** | 42—71 | 28.2 | 0—58 | 14.7 | 0—30 |
| | *Collichthys lucidus* | **83.1** | 76—89 | 11.3 | 0—24 | 5.6 | 0—12 |
| | *Decapterus maruadsi* | **60.8** | 47—74 | 25.7 | 0—53 | 13.4 | 0—28 |
| | *Caranx kalla* | **66.3** | 54—78 | 22.2 | 0—46 | 11.4 | 0—22 |
| | *Clupanodon punctatus* | **68.3** | 57—79 | 20.9 | 0—43 | 10.8 | 0—23 |
| | *Thrissa dussumieri* | **75.6** | 66—84 | 16.3 | 0—34 | 8.1 | 0—17 |
| | *Nemipterus japonicus* | **66.3** | 54—78 | 22.2 | 0—46 | 11.4 | 0—22 |
| | *Leiognathus brevirostris* | **64.5** | 52—76 | 23.5 | 0—48 | 12.0 | 0—25 |
| | *Sardinella aurita* | **64.5** | 52—76 | 23.5 | 0—48 | 12.0 | 0—25 |
| | *Polynemus sextarius* | **60.8** | 47—74 | 25.7 | 0—53 | 13.4 | 0—28 |
| | *Trichiurus haumela* | **60.8** | 47—74 | 25.7 | 0—53 | 13.4 | 0—28 |
| | *Gastrophysus spadiceus* | **57.1** | 42—71 | 28.2 | 0—58 | 14.7 | 0—30 |
| | *Apogon striatus* | **57.1** | 42—71 | 28.2 | 0—58 | 14.7 | 0—30 |
| | *Pagrosomus major* | **55.2** | 39—70 | 29.4 | 0—61 | 15.3 | 0—32 |
| | *Rhabdosargus sarba* | **51.6** | 35—67 | 31.7 | 0—65 | 16.7 | 0—34 |
| | *Gerres japonicus* | **47.9** | 30—65 | 34.2 | 0—70 | 17.9 | 0—37 |
| | *Muraenesox cinereus* | **60.8** | 47—74 | 25.7 | 0—53 | 13.4 | 0—28 |
| | *Gymnura japonica* | 27.4 | 3—51 | **47.7** | 0—97 | 25.0 | 0—51 |

*tilis*) and barnacles (*Balanus* sp. and *Capitulum mitella*) had lower $\delta^{13}C$ signatures while the chiton *L. japonica* and limpet *Cellana grata* had higher values. The $\delta^{13}C$ values of whelk *Thais clavigera* and crab *Gaetice depressus* were intermediate. By contrast, offshore consumer $\delta^{13}C$ values were mainly distributed between −18.0 and −15.0‰ except for zooplankton (−19.2‰) and *G. japonica* (−14.4‰).

The $\delta^{15}N$ ranges of intertidal consumers were more depleted and less variable than those of offshore consumers, with values ranging from 9.3 (*P. viridis*) to 12.8‰ (*C. mitella*) and 9.6 (zooplankton) to 17.2‰ (*G. japonica*).

### 3.3. Contributions of basal sources supporting consumers

IsoSource solutions showed that consumers assimilated organic carbon from a mixture of basal sources. In offshore zones, SPOM was the most important production source supporting the biomass of most consumers (Table 1). Macroalgae also accounted for a large fraction, especially for the crabs *Portunus trituberculatus* and *Charybdis feriatus* and

the fish *G. japonica*. In contrast, model results suggested that epiphytes likely made minor contributions.

In intertidal habitats, epiphytes accounted for significant fractions of the *L. japonica* and *C. grata* biomass and contributed smaller fractions to other consumers. SPOM was the main basal source of organic carbon for most consumers except *L. japonica* and *C. grata* similar to patterns in offshore zones. Consumers also appeared to rely on macroalgae as part of their carbon source.

### 3.4. TP of consumers

$\delta^{15}N$ data indicated ∼5 trophic levels in Mirs Bay (Fig. 3). Apart from *C. mitella*, intertidal consumers had a TP of between 2 and 3. The consumers with the lowest trophic levels were mainly bivalves (*P. viridis*, *A. sculptilis* and *C. rivularis*), chiton (*L. japonica*) and limpet (*C. grata*) as well as crab (*G. depressus*). In contrast, almost all offshore consumers had a TP of between 3 and 4 except for the zooplankton and fish *Clupanodon punctatus*, *Sardinella aurita* and *G. japonica*. Trophic-level 2 included the zooplankton, fish *C.*
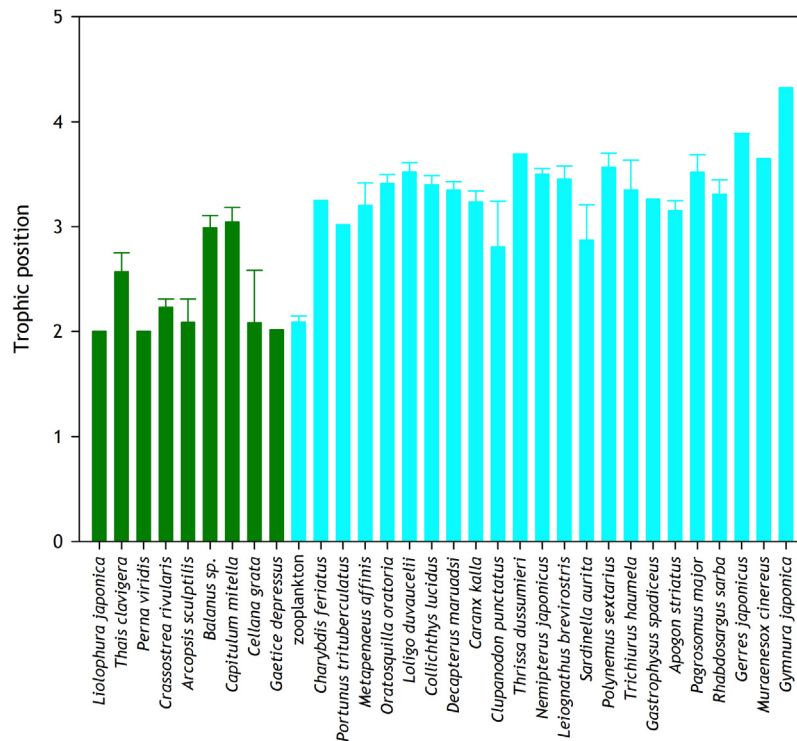
**Figure 3**   Mean trophic positions ($\pm$ SD) of consumers in Mirs Bay, China, based on $\delta^{15}$N values. Green bars and blue bars represent samples from intertidal zones and offshore stations, respectively.

*punctatus* and *S. aurita*, and the highest TP was occupied by *G. japonica* (TP = 4.32).

## 4.  Discussion

### 4.1.  Carbon isotopic composition of basal sources

Along the rocky shores of Mirs Bay, the most abundant macroalgae in the low- and mid-intertidal zones were *Enteromorpha* spp. and *Amphiroa zonata*. Their $\delta^{13}$C signatures were in the range of values documented in intertidal beds in a mega-tidal system near Roscoff, France (Ouisse et al., 2012) and coastal areas on the Mexican Eastern Pacific coast (Nava et al., 2014). SPOM displayed similar $\delta^{13}$C signatures to those demonstrated by Deegan and Garritt (1997), Douglass et al. (2011) and Kang et al. (2008). Epiphytes of the intertidal zones also had $\delta^{13}$C values close to those reported in Uchiumi and Fukuura Bays, Japan (Doi et al., 2008) and Shark Bay, Australia (Belicka et al., 2012). These $\delta^{13}$C values of basal sources had a wide range (−19.6‰ to −11.8‰) and were generally well separated, which created favorable conditions for assessing the relative contributions of carbon sources to consumer biomass in Mirs Bay.

### 4.2.  Potential use of basal sources by consumers

In intertidal zones, the filter-feeders were mostly represented by the bivalves *Perna viridis*, *Arcopsis sculptilis* and *Crassostrea rivularis*, and by the barnacles *Balanus* sp. and *Capitulum mitella*, and showed high dependence on offshore SPOM,

while intertidal macroalgae and epiphytes also contributed to them. Such species live on rocks, Kang et al. (2008) and Little and Kitching (1996) reported that, on rocky shores, suspension-feeding invertebrates depended on composite sources of macroalgae and phytoplankton. Filter-feeders may feed on macroalgal detritus or exudates in the interface of the water-rock boundary (Kang et al., 2008; Schaal et al., 2010). In addition, epiphytes (as detritus in SPOM) might be part of the diet of filter-feeders (Doi et al., 2008). These findings also indicated the presence of suspended macroalgal and epiphyte detritus in the water column in intertidal zones. For the grazer chiton *Liolophura japonica*, the similarity in $\delta^{13}$C signatures and trophic enrichment of $\delta^{15}$N with respect to epiphytes clearly revealed that it depended on materials from epiphytes as its food source. The IsoSource solutions confirmed that epiphytes showed the highest contribution to *L. japonica* nutrition. The chiton scraped off epiphytic organic matter from hard surfaces with radulae (Takai et al., 2004). Another grazer in the current study was the limpet *Cellana grata*, which did not only rely on epiphytes (like *L. japonica*) but also on some macroalgae, according to the IsoSource solutions. This species is generally referred to as a generalist grazer on many rocky shores, grazing on epilithic biofilm and macroalgae (Burnett et al., 2014). The intertidal varunid crab *Gaetice depressus* is considered to be an omnivore, relying on mixed SPOM, macroalgae and epiphytes as well as on heterotrophic detritus as its food source, as already observed in a boulder shore ecosystem (Wahyudi et al., 2013). However, Wahyudi et al. (2013) found that *G. depressus* used macroalgae as its main diet, which differed from the finding in the present study. This disagreement may be explained by local availability of food sources. Wahyudi et al. (2013) stated that

*G. depressus* preferred to feed upon SPOM and epiphytes when macroalgae were insufficiently abundant. Carbon in the whelk *Thais clavigera* also originated from a mixture of sources, as it did in the crab *G. depressus*; however, *T. clavigera* did not use these sources directly. Although the TP of *T. clavigera* was 2.57 in this study, it is generally referred to as a carnivore, preying primarily on bivalves, limpets and barnacles (Blackmore, 2000; Wada et al., 2013; Wai et al., 2008). Its prey fed on SPOM, macroalgae and epiphytes, as discussed above, and in turn contributed to the diet of *T. clavigera*. A similar result for this species was also found in another ecosystem (Wai et al., 2008). This result also suggested that a lower $\delta^{15}N$ trophic fractionation of 3.4‰ might be present for *T. clavigera*. McCutchan et al. (2003), compiling published studies, concluded that consumers feeding on invertebrate diets had the $\delta^{15}N$ trophic fractionation of 1.4 $\pm$ 0.20‰, which was significantly lower than that for those consumers feeding on other high-protein diets (vertebrates; microbes; and animal-based prepared diets). If we had used the $\delta^{15}N$ trophic fractionation of 1.4‰ to calculate the TP of *T. clavigera* in the present study, its mean TP would have been 3.36, which conformed to its feeding habit.

In offshore zones, the $\delta^{13}C$ signatures of zooplankton closely matched that of SPOM, with TP (2.09) clearly suggesting that they filtered SPOM. IsoSource solutions showed that SPOM accounted for almost all zooplankton biomass, implying that zooplankton depended on organic matter from SPOM almost entirely as its sole food source. The dominant zooplankton species during the investigation were *Subeucalanus subcrassus* and *Temora turbinata* (unpublished data), both known to be small-particle filter-feeders (Li et al., 2012). SPOM mainly comprised of phytoplankton in the offshore zone (Ouisse et al., 2012; Schaal et al., 2010) compared with the SPOM of intertidal zone, which was composed of a greater amount of macroalgal debris (Duggins and Eckman, 1997; Golléty et al., 2010) and epiphytic algae detritus (Doi et al., 2008), as well as phytoplankton. According to Bouillon et al. (2000), quantities of debris and detritus in the intertidal water column were generally greater than those of locally produced phytoplankton. This may explain why filter-feeders in offshore zones relied less on macroalgae and epiphytes than those in intertidal zones. The planktivorous fishes *Clupanodon punctatus* and *Sardinella aurita* displayed lower $\delta^{15}N$ values than other consumers, except for zooplankton in offshore zones. Their TP (2.81 and 2.87, respectively) indicated that these organisms mostly fed on primary consumers and basal sources, and confirmed that they mainly ate diatoms, copepods, Macrura larvae and fish larvae (Huang et al., 2008; Wang and Qiu, 1986). IsoSource solutions showed that SPOM-derived carbon was the major contributor for these two planktivorous fishes. Macroalgae made an important contribution to the carbon source of the crabs *Portunus trituberculatus* (TP = 3.02) and *Charybdis feriatus* (TP = 3.25) and of butterfly ray *Gymnura japonica* (TP = 4.32), followed by SPOM and epiphytes. The $\delta^{13}C$ values of these two crabs were similar to those of the macroalgae, suggesting that they mainly relied on energy derived from macroalgae. However, their TP indicated an indirect use of macroalgae. Their $\delta^{13}C$ values were similar to those of the grazer (*C. grata*), omnivore (*G. depressus*) and carnivore (*T. clavigera*) as well as to piscivore fishes (*Gerres japonicus* and *G. japonica*), which also consumed macroalgae as their

important carbon source. This suggests that there is probably a trophic relationship between these two crabs and those organisms. The remaining consumers (17 of 23 species including shrimps, mantis shrimps, squids and fishes) sampled in offshore zones depended more heavily on SPOM, and to a lesser extent on intertidal macroalgae and epiphytes. Such species were mainly carnivorous and had a TP above 3, indicating that they can incorporate integrated basal sources indirectly through trophic interaction.

### 4.3. Major trophic pathways in Mirs Bay

Trophic connectivity between intertidal and offshore zones mainly followed either of two carbon pathways in Mirs Bay (Fig. 4).

The first and most enriched carbon pathway involved a combination of intertidal macroalgae and epiphytes, grazers, omnivores and their predators, and this provided the first clue about the degree of trophic linkage. Grazers included the intertidal limpet *C. grata* and chiton *L. japonica*, which mainly relied on epiphytes and macroalgae (see above 4.2). The intertidal varunid crab *G. depressus* and whelk *T. clavigera* were classified as an omnivore and a carnivore, respectively, and *G. depressus* fed on macroalgae and epiphytes while *T. clavigera* ate limpets as part of their diet. The offshore crabs *P. trituberculatus* (TP = 3.02) and *C. feriatus* (TP = 3.25) were predators in the present study and their major prey items included mollusks, crustaceans and fishes (Huang, 2004; Jiang et al., 1998). According to the $\delta^{13}C$ values and TP, the intertidal limpet *C. grata*, varunid crab *G. depressus* and whelk *T. clavigera* were assumed to provide the diet for these two crabs, and they might be further preyed on by silver biddy (*G. japonicus*) and butterfly ray (*G. japonica*). The silver biddy was already observed to feed on benthic invertebrates almost exclusively (Cyrus and Blaber, 1982), and the butterfly ray mainly ate teleosts, crustaceans and cephalopods (Capapé, 1986; Jacobsen et al., 2009; Raje, 2003).

Another carbon pathway mainly comprising offshore SPOM, which was incorporated by filter-feeders, omnivores and their predators. The filter-feeders consisted of planktonic zooplankton and intertidal bivalves, both of them consuming SPOM as their main biomass. These filter-feeders acted as trophic intermediates between primary producers and consumers. Zooplankton were preyed on not only by planktivorous fishes such as *C. punctatus* (Huang et al., 2008) and *S. aurita* (Wang and Qiu, 1986) which also fed on SPOM, but also by intertidal barnacles (Lu et al., 1996). The intertidal omnivore *G. depressus* and carnivore *T. clavigera* were also involved in the second carbon pathway besides the first, since *G. depressus* mainly fed on SPOM while *T. clavigera* preyed on bivalves and barnacles. The predators (including shrimps, mantis shrimps, crabs, squids and some fishes at trophic-level 3) conformed well to their known feeding habits. For example, shrimp *Metapenaeus affinis* mainly preys on polychaetes, crustaceans, fish larvae and bivalves (Yu et al., 2016); and mantis shrimp *Oratosquilla oratoria* mainly consumes bivalves, crabs, shrimps and fishes (Ning et al., 2016). Squid are considered to be carnivores as they not only eat fishes, crustaceans and Mollusca, but also show cannibalistic behavior each other (Castro and Huber, 2007). For the fishes, most teleosts are carnivores (Castro and
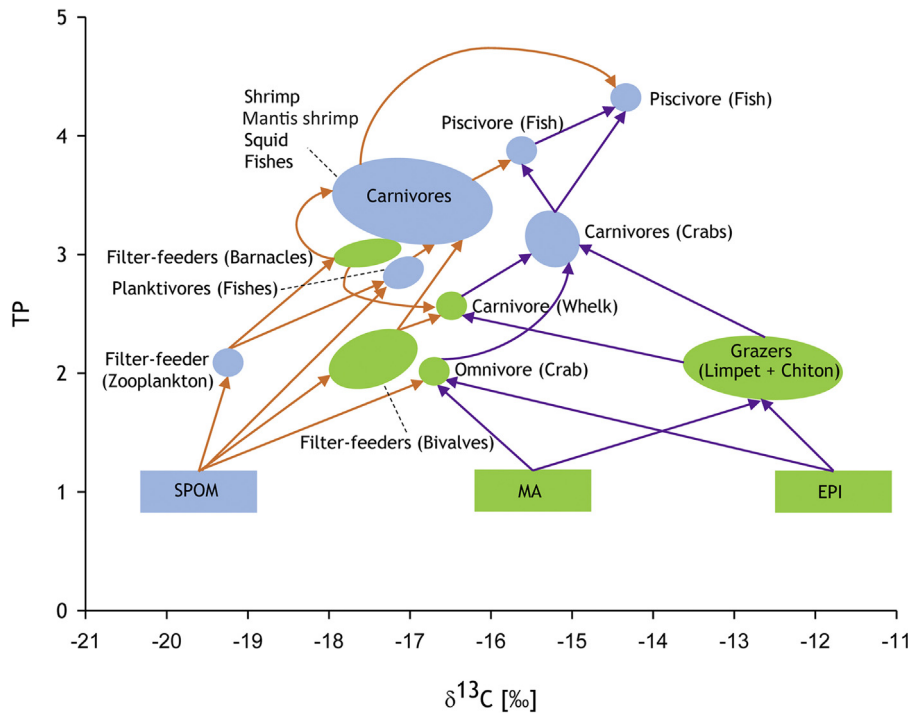
**Figure 4** Trophic model of the food web in Mirs Bay, China, delineating the two major carbon pathways. SPOM = suspended particulate organic matter epiphytes; MA = macroalgae; EPI = epiphytes. Green and blue areas represent samples from intertidal zones and offshore stations, respectively. Purple arrows represent the first carbon pathway; orange arrows represent the second carbon pathway.

Huber, 2007), including all the teleosts here except for the planktivorous fishes *C. punctatus* and *S. aurita*. These upper trophic level predators were fueled by mixed basal sources through integrated multiple trophic pathways thought to be common in food webs (Belicka et al., 2012; Rooney et al., 2006). They were further eaten by the piscivore fishes *G. japonicus* and the top predator *G. japonica*.

## 5. Conclusions

Offshore SPOM carbon was the primary carbon source supporting most consumers not only in the offshore zone, but also in intertidal zones, emphasizing the importance of offshore primary production to intertidal consumers. Intertidal filter-feeders play an important role in retaining offshore primary production. Similarly, intertidal macroalgae and epiphytes also make some contributions to offshore consumers through transfer from prey. The basal sources fueled consumers through two trophic pathways, each of which involved organisms of both intertidal and offshore zones. The coupling of basal sources from different habitats to consumers suggested trophic interaction between intertidal and offshore zones in Mirs Bay. Future protection and preservation of bay ecosystems should consider the whole food web, not just offshore or intertidal food webs, to maintain ecological structure and function.

Further studies should carry out fatty acid biomarkers analysis because this will provide detailed tracking of carbon substrates in food webs not available to stable isotopes. Seasonal analysis should also be included, to enhance understanding of the temporal interactions of trophic dynamics.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.oceano.2018.10.001.

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