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Food sources and trophic structure of fishes and benthic macroinvertebrates in a tropical seagrass meadow revealed by stable isotope analysis

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ABSTRACT

Stable carbon and nitrogen isotope analysis was used to examine the food sources and trophic structure of 17 fish species and six groups of benthic macroinvertebrates in a seagrass meadow in North Sulawesi, Indonesia. The seagrass, their associated epiphytes, sediment organic matter (SOM) and particulate organic matter (POM) were identified to be the food sources, with $\delta^{13}C$ values ranging from −19.49 (POM) to −9.66‰ (seagrass). The $\delta^{13}C$ of the 23 fauna taxa were between −18.57 (*Arothron manilensis*) and −11.62‰ (*Protoreaster* sp.). For five of the six groups of benthic macroinvertebrates, seagrass and their epiphytes contributed more than 69.4%. For 14 of the 17 fish species, seagrass and their epiphytes are the main contributors. For 15 of the 17 fishes, the trophic levels inferred from SIA are lower than those from the previously reported diet composition analysis. These findings show that seagrass and their epiphytes are consumed by most of the fish and benthic macroinvertebrates, and are important for a large portion of the food web in seagrass meadows in the Coral Triangle area.

Introduction

Seagrass meadows are one of the typical marine ecosystems. They form important and readily accessible fishing grounds and provide trophic subsidy to adjacent fisheries and a critical nursery habitat for commercial species (Nagelkerken et al. 2012). In parts of the Coral Triangle area, the fauna associated with seagrass provide a local food supply, contributing at least 50% of fish-based food (Unsworth et al. 2014). Seagrass material is consumed by a large number of species and is important for a large portion of the food web in tropical seagrass meadows (Vonk et al. 2008). Moreover, resource utilization inside and adjacent to seagrass meadows do not differ significantly, with seagrass-associated inputs extending beyond the borders of vegetation patches (Vafeiadou et al. 2013). Although most food web analyses in seagrass meadows have shown that assimilation of seagrass material is minimal (Smit et al. 2005, 2006), observations and grazing experiments indicate that a large number of species consume large amounts of seagrass biomass (McRoy & Helfferich 1980; Heck & Valentine 2006). This may be because seagrass material has a low assimilation and a high excretion in fauna (Mateo et al. 2006).

Stable isotope analysis (SIA) is a popular method to estimate the trophic interactions in natural systems (Peterson & Fry 1987; Post 2002; Lassalle et al. 2014). Carbon and nitrogen stable isotope ratios in particular have been effectively proven to be a valuable source of dietary information when feeding is too difficult to observe. Examples of stable isotope analysis performed on most representative species of a given ecosystem are becoming more and more prevalent in the scientific literature (Papiol et al. 2013). The $\delta^{13}C$ values are broadly used to analyse the food sources of animals, while $\delta^{15}N$ values are used to calculate the trophic levels (TLs) (Fry 2007).

In seagrass meadows, potential food sources for primary consumers are very diverse, including seagrass and their epiphytes, detritus, macroalgae and phytoplankton (Smit et al. 2005, 2006). Different types of producers have distinct $\delta^{13}C$ ratios (Smit et al. 2005; Benstead et al. 2006). The consumers in seagrass meadows utilize different food sources depending on feeding habits and the composition of food sources in the habitats. Several studies have examined food utilization by fish and benthic macroinvertebrates in seagrass meadows (Lebreton et al. 2011; Ouisse et al. 2012), but

information about the relative importance of resources is still inconclusive (Vafeiadou et al. 2013), and the findings are diverse. In temperate meadows, seagrass detritus, epiphytes and benthic algae are all main carbon sources in Mediterranean seagrass meadows (Vizzini et al. 2002), while seagrass-associated carbon sources like epiphytic microalgae and seagrass detritus are equally as important as microphytobenthos (MPB) and particulate organic matter (POM) for the diet of estuarine benthic macroinvertebrates in southwest Portugal (Vafeiadou et al. 2013). In contrast, the organic carbon of consumers in seagrass systems are epiphytic algae, and the contribution of seagrass is very low in the northern Gulf of Mexico (Moncreiff & Sullivan 2001). In tropical meadows, half of the 55 fauna species off the west coast of South Sulawesi fed on seagrass material directly or indirectly (Vonk et al. 2008).

Southeast Asia is one of the most biodiverse hotspots due to its enormous species richness (Sodhi et al. 2004), and the Indonesian coasts located at the heart of the Coral Triangle area harbour an exceptionally high seagrass and fish diversity (Vonk et al. 2008, 2010; Pogoreutz et al. 2012). However, studies on seagrass and related fish have focused mostly on the Caribbean region (Unsworth et al. 2007), and only a few works have examined Indo-Pacific seagrass beds (Hutomo & Martosewojo 1977; Erftemeijer & Allen 1993; Nakamura & Sano 2004; Unsworth et al. 2007, 2014; Vonk et al. 2010; Pogoreutz et al. 2012), especially the food sources of fauna in seagrass beds (Vonk et al. 2008; Pogoreutz et al. 2012). Therefore, considering the economic importance of seagrass beds and associated fisheries, it is necessary to understand the nutritional food web structure for the management of seagrass meadows in the Indo-Pacific region.

This study investigates the food web structure and expounds the contribution of potential carbon sources to fish and benthic macrofauna in North Sulawesi seagrass meadows using stable isotope analysis (SIA). The study also addresses the main carbon source contributors to fish and benthic macrofauna in this area and whether there is significant variation in resource utilization by different fauna species.

Materials and methods

Study area

The present study was carried out in Kema (1°20′44.42″N, 125°4′ 34.52″E), North Sulawesi. North Sulawesi has a typical equatorial climate, and the mean temperatures at sea level are uniform, varying by only a few degrees throughout the region and the year between 20 and 28℃. Tides in this area are mixed and mainly semi-diurnal and fluctuated slightly with an annual tidal range of 2.4 m. In the seagrass meadows, *Enhalus acoroides* (Linnaeus f.) Royle and *Thalassia hemprichii* (Ehrenberg) Ascherson are the most dominant species, with a coverage of about 65.12%, and the sediment characteristics of the area are mixed mud and sand. This area is subjected to strong influences from two monsoons: the wet northwest monsoon from December to February and the dry southeast monsoon from May to August. This study was carried out at the end of May during the dry season, and there is no land run-off here during this season.

Field collection

All the samples were collected during low tide in May 2014. Fish were caught with seine nets at two stations (Figure 1), and three seine nets were assigned at each site. All specimens retained by the seine net were immediately preserved in an ice box. After identification, fish were counted and weighed, and the surface area of the seine was used to express the biomass and abundance. For invertebrates, samples were collected at random from within an area of about 10 m² at each site using a box corer with dimensions of 25 cm × 25 cm × 30 cm.

Cylindrical PVC pipes with a diameter of 10 cm were used to collect sediment organic matter (SOM), and the top 1–2 cm of the sediments were collected and passed through a 63 µm mesh, then used for isotope analysis. There are in total three samples, two samples from station 1 and one sample from station 2, with a distance of 50 m between the two stations. About 2 l of surface seawater was collected and passed through a 77-µm mesh. The filtrate was further filtered using Whatman GF/F filters that were pre-combusted for 4 hours at 450℃. The epiphytes were scraped carefully from the seagrass surface using a scalpel blade and collected onto the filters. Four samples of *Enhalus acoroides* and *Thalassia hemprichii* were collected and analysed together, and the leaves cleaned with distilled water several times to remove the attachments such as SOM and epiphytes from the surface of the seagrass.

Stable isotope analysis

The SIA was performed on the five types of organic sources (Table I), as well as the 23 consumer species (Table II). In total 101 samples covering a wide range of representative taxa and organic sources of the Kema seagrass food webs were analysed, including seagrass, epiphytes, SOM, POM, fish and invertebrates (total
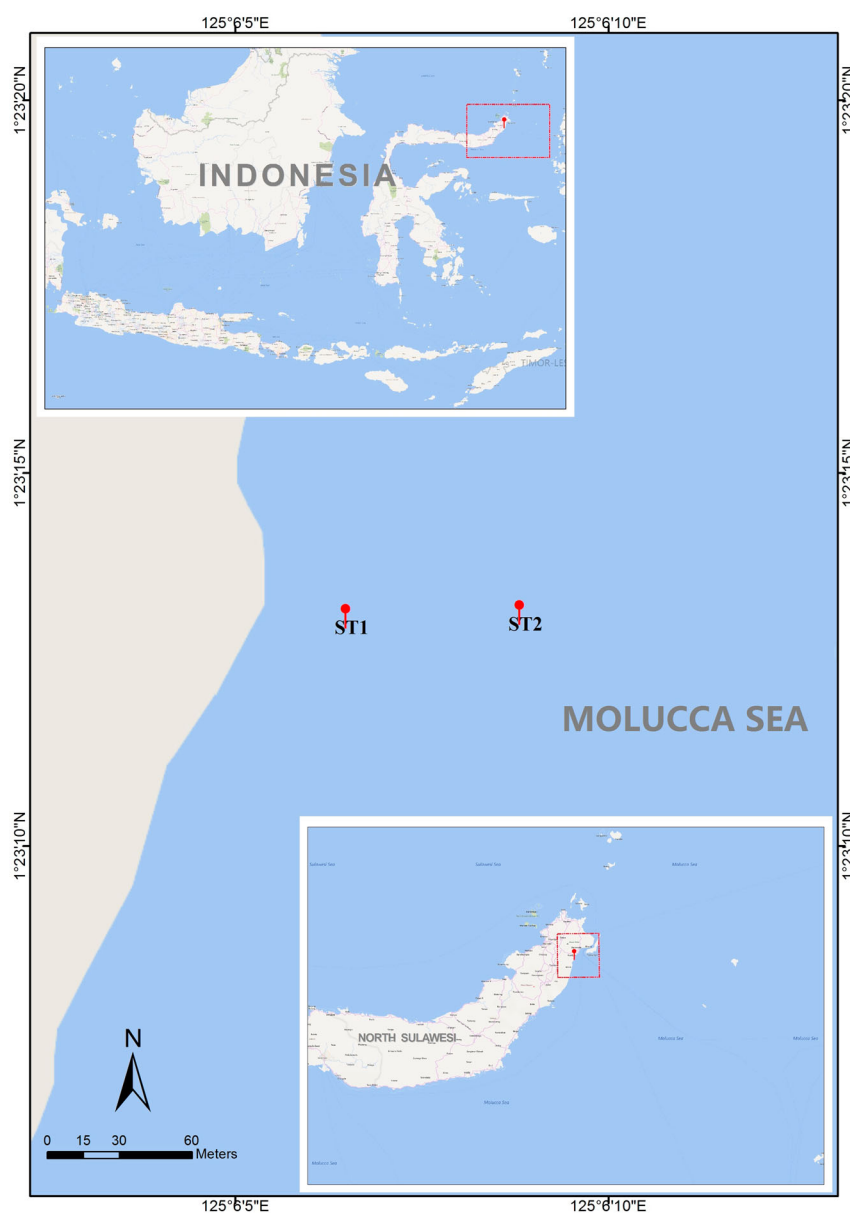
Figure 1. Study area, showing the location and the survey stations along the west coast of North Sulawesi.

mentioned in Tables I and II). Given that numerous types of organic materials contain inorganic carbonates that are known to be more enriched in δ^{13}C than other components, the POM, epiphytes, seaweed and SOM were acidified before analysis. Filters were exposed to HCl vapour for 12 hours to remove carbonates. Sediments were also acidified by applying 1-mol l^{-1} HCl solution for 2 hours to SOM. Muscle samples were taken from the dorsal musculature of fish. Many invertebrates such as polychaetes are very small and sampling the musculature of invertebrates with high carbonate content like echinoderms and sponges is difficult, so either a part of the body or its entirety were used for SIA instead of only the muscles. All of the samples were washed very carefully with distilled water to prevent any

contamination by sediment carbonates. The echinoderms and sponges were soaked in 1-mol l^{-1} HCl solution for 2 hours to remove the carbonates.

The δ^{13}C and δ^{15}N signals of samples were measured using an isotope ratio mass spectrometer (IRMS) attached to a Flash EA1112 HT Elemental analyser. The sample was burned into CO_2 and N_2 under high temperature. The ratios of ^{13}C/^{12}C and ^{15}N/^{14}N were detected by IRMS and then compared with international standards (Pee Dee Belnite and atmospheric N_2). δ^{13}C and δ^{15}N were then calculated using the following equation:

$$\delta^{13}C/\delta^{15}N = \frac{(R_{sample} - R_{standard})}{R_{standard}} \times 1000 \qquad (1)$$

Table I. $\delta^{13}C$ and $\delta^{15}N$ values (mean value ± SE) of organic carbon sources in Kema seagrass meadow.

Organic matter sources	$\delta^{13}C$/‰	$\delta^{15}N$/‰	n
Seagrass	−9.66 ± 0.93 a	5.08 ± 0.77 a	4
Epiphytes	−14.50 ± 1.75 b	0.27 ± 0.85 b	4
Sediment organic matter (SOM)	−18.33 ± 0.14 c	3.10 ± 0.10 c	3
Particulate organic matter (POM)	−19.49 ± 0.80 c	5.57 ± 0.52 a	4
Seaweed	−19.76 ± 0.93 c	3.08 ± 0.60 c	3

Note: The differences of $\delta^{13}C$ or $\delta^{15}N$ values among organic matter sources were analysed by ANOVA with a post hoc Tukey's test. Different letters in the columns (a, b, and c) represent significant differences among organic carbon sources ($P < 0.05$). 'n' represents the replicates for stable isotope analysis.

The detection limits were 0.1‰ and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. R represents either $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

IsoSource analysis

All the data were analysed using SPSS 16.0. Differences in $\delta^{13}C$ or $\delta^{15}N$ among food sources were analysed by ANOVA with a post hoc Tukey's test. The contributions of the four food sources to the 23 consumers were calculated using IsoSource 1.3 software (Phillips & Gregg 2003, 2014). The trophic enriched factor (TEF) is assumed to be 0.47‰ for $\delta^{13}C$ between two neighbour TLs and 2.52‰ for $\delta^{15}N$ (Zanden & Rasmussen 1999). A principal component analysis (PCA, SPSS 16.0) was conducted on current data for 17 fish species containing four food sources to examine the associations

between the food sources and to categorize the species.

Trophic level from SIA

Trophic levels of each organism were estimated as follows (Post 2002):

$$TL_{consumer} = TL_{basis} + \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{basis})}{TEF} \quad (2)$$

where TL_{basis} is the trophic position of a primary consumer used to estimate the TLs of other consumers in the food web (Zanden & Rasmussen 1999; Post 2002) and is assumed to equal 2. $\delta^{15}N_{consumer}$ is the value measured for the consumer, and $\delta^{15}N_{basis}$ is $\delta^{15}N$ of an organism that is as close as possible to truly herbivorous and is either sessile or has very limited mobility. In the present study, *Ophiarachna* sp. was identified as the most relevant for directly reflecting all organic matter at the base of food webs.

Results

$\delta^{13}C$ and $\delta^{15}N$ of organic carbon sources

The five main organic carbon sources for the animals in the Kema seagrass meadow are seagrass, their associated epiphytes, seaweed, SOM and POM. Significant differences were found in $\delta^{13}C$ and $\delta^{15}N$ among the types of organic matter ($P < 0.01$), with $\delta^{13}C$ values

Table II. $\delta^{13}C$ and $\delta^{15}N$ values (mean value ± SE) of consumers in Kema seagrass meadow.

Consumers	B/g m^{-2}	$\delta^{13}C$/‰	$\delta^{15}N$/‰	TL_{SIA}	$TL_{Fishbase}$	ML1/mm	ML2/mm	n
Fish								
Sillago sihama (Forsskål, 1775)	0.0819	−13.61 ± 0.10	9.70 ± 0.22	3.18 ± 0.065	3.30 ± 0.10	115.1	225	4
Plotosus lineatus (Thunberg, 1787)	0.1249	−12.49 ± 0.04	8.46 ± 0.11	2.81 ± 0.033	3.50 ± 0.60	115.1	140	4
Syngnathoides biaculeatus (Bloch, 1785)	0.05755	−12.36 ± 0.15	8.14 ± 0.31	2.72 ± 0.092	3.60 ± 0.60	20.1	19	4
Siganus sp.	0.02965	−14.88 ± 1.02	7.24 ± 1.11	2.46 ± 0.33	2.30 ± 0.13	10.0	18	4
Ostorhinchus margaritophorus (Bleeker, 1855)	0.0094	−13.29 ± 0.69	7.85 ± 0.12	2.63 ± 0.035	3.40 ± 0.50	35.0	48	4
Atherinomorus sp.	0.0142	−15.17 ± 0.73	8.61 ± 0.25	2.86 ± 0.075	3.30 ± 0.50	56.6	110	4
Terapon jarbua (Forsskål, 1775)	0.01415	−18.28 ± 0.08	8.37 ± 0.19	2.79 ± 0.055	3.50 ± 0.47	66.9	130	4
Aeoliscus strigatus (Günther, 1861)	0.03955	−15.58 ± 0.04	6.38 ± 0.20	2.2 ± 0.059	3.50 ± 0.48	119.0	150	4
Pardachirus pavoninus (Lacepède, 1802)	0.05385	−13.57 ± 0.68	8.52 ± 0.11	2.83 ± 0.031	3.10 ± 0.26	165.6	250	4
Parajulis poecilepterus (Temmink & Sclegel, 1845)	0.012	−12.32 ± 0.89	7.03 ± 0.17	2.39 ± 0.049	3.40 ± 0.50	120.0	154	4
Acreichthys tomentosus (Linnaeus, 1758)	0.00797	−13.96 ± 0.69	6.93 ± 0.41	2.36 ± 0.12	3.00 ± 0.39	70.0	77	4
Arothron manilensis (Marion de Procé, 1822)	0.05785	−18.57 ± 0.03	7.29 ± 0.13	2.47 ± 0.039	3.50 ± 0.50	99.8	310	4
Diodon holocanthus Linnaeus, 1758	0.91355	−13.72 ± 0.02	11.03 ± 0.06	3.57 ± 0.018	3.30 ± 0.50	120.3	150	4
Monacanthus chinensis (Osbeck, 1765)	0.0701	−12.58 ± 0.08	6.37 ± 0.17	2.20 ± 0.050	2.60 ± 0.30	149.4	380	4
Gerres oyena (Forsskål, 1775)	0.08385	−11.87 ± 0.29	6.93 ± 0.17	2.36 ± 0.051	3.10 ± 0.20	62.4	200	4
Gerres erythrourus (Bloch, 1791)	0.0287	−13.95 ± 0.86	7.48 ± 0.08	2.53 ± 0.025	3.30 ± 0.43	56.7	250	4
Sphyraena barracuda (Edwards, 1771)	0.0177	−16.62 ± 0.10	10.50 ± 0.23	3.41 ± 0.069	3.60 ± 0.53	95.6	660	4
Invertebrates								
Ophiarachna sp.		−11.75 ± 2.32	5.70 ± 0.65	2.00 ± 0.19				3
Oligochaeta		−13.33 ± 0.10	3.77 ± 0.10	1.43 ± 0.029				2
Polychaeta		−13.44 ± 0.14	6.67 ± 1.41	2.29 ± 0.42				2
Protoreaster sp.		−11.62 ± 3.07	7.97 ± 0.79	2.67 ± 0.23				3
Alpheus sp.		−12.61 ± 1.45	8.80 ± 0.28	2.91 ± 0.083				2
Porifera		−17.38 ± 0.45	7.51 ± 1.44	2.53 ± 0.42				3

B: biomass investigated in this study using the seine net; ML1: max length in this study; ML2: maturity length in FishBase. TL_{SIA} and $TL_{Fishbase}$ represent the trophic levels estimated by SIA and from FishBase (Froese & Pauly 2015).

ranging from −9.66 ± 0.93‰ for seagrass to −19.49 ± 0.80‰ for POM and δ¹⁵N values ranging from 0.27 ± 0.85‰ for epiphytes to 5.57 ± 0.52‰ for POM (Table I).

δ¹³C and δ¹⁵N of consumers

A total of 23 species of animals were collected at two sampling sites, including six invertebrate species and 17 fish species. The majority of invertebrate species (except Porifera) were rich in δ¹³C, with δ¹³C values ranging from −17.38 ± 0.45‰ for Porifera to −11.62 ± 3.07‰ for *Protoreaster* sp. The invertebrates show high variation in δ¹⁵N enrichment (Table II, Figure 2) from 3.77 ± 0.10‰ for Oligochaeta to 7.97 ± 0.79% for *Protoreaster* sp. Fish are widely varied in δ¹⁵N from 6.37 ± 0.17‰ for *Monacanthus chinensis* (Osbeck, 1765) to 11.03 ± 0.06‰ for *Diodon holocanthus* Linnaeus, 1758. Similarly, the δ¹³C values varied widely from −18.57 ± 0.03‰ for *Arothron manilensis* (Marion de Procé, 1822) to −11.87 ± 0.29‰ for *Gerres oyena* (Forsskål, 1775).

Possible contribution of four primary food sources to fish and invertebrates

The possible contributions of the four primary food sources to fish and invertebrates are shown in Table III. For six benthic invertebrate taxa collected in the Kema seagrass meadow, seagrass contributed 63.7% (47–74%), 65.5% (49–75%) and 51.6% (29–65%) to *Ophiarachna* sp., *Protoreaster* sp. and *Alpheus* sp., respectively, while POM and SOM, largely composed of the microalgae, jointly contribute more than 90% to Porifera by posterior aggregation. For the Oligochaeta and Polychaeta samples, the IsoSouce model with four end-members cannot obtain definite results, since the δ¹³C values are in the middle of the range of the four food sources.

For most of the fish species, seagrass and their epiphytes are the main contributors. For example, seagrass contributes 47.9% (23–63%), 50.3% (27–64%), 53.1% (31–66%), 50.5% (27–65%) and 59.6% (41–71%) to *Plotosus lineatus* (Thunberg, 1787), *Syngnathoides biaculeatus* (Bloch, 1785), *Parajulis poecilepterus* (Temminck & Schlegel, 1845), *Monacanthus chinensis* and *Gerres oyena*, respectively. For *Sillago sihama* (Forsskål, 1775), *Pardachirus pavoninus* (Lacepède, 1802), *Acreichthys tomentosus* (Linnaeus, 1758), *Diodon holocanthus* and *Gerres erythrourus*, epiphytes contribute more than 33%. For *Siganus* sp., *Atherinomorus* sp. and *Aeoliscus strigatus* (Günther, 1861), the ranges of feasible contribution of the four food sources were very broad and overlapped each other (Table II).

Therefore, it was not possible to determine with certainty the major carbon sources assimilated by these fish species using only the IsoSource model. However, their δ¹³C values were closest to epiphytes, indicating a possible high contribution of epiphytes to their diet (Figure 2).

The median diet contributions of POM to *Terapon jarbua* (Forsskål, 1775) and *A. manilensis* were quite high, more than 84% of the total, with feasible ranges of 64–96% for *T. jarbua* and 76–98% for *A. manilensis*. The contribution was also high for *Sphyraena barracuda* (Edwards, 1771) at more than 40% of the total, but relatively low for other species.

The trophic levels of fish and invertebrates

Of all the analysed consumers, *Diodon holocanthus* is the top predator and was richer in δ¹⁵N than other species (Table II). However, with the exception of Oligochaeta, only 1.5 TLs separated *D. holocanthus* from the baseline organism *Ophiarachna* sp.

Discussion

Stable isotope ratios of organic sources

The δ¹³C values of POM are close to typical values for marine phytoplankton, which range from −22 to −18‰ (Goering et al. 1990; Middelburg & Nieuwenhuize 1998; Kang et al. 2003; Gaston & Suthers 2004; Shang et al. 2008). This shows the dominance of phytoplankton in the POM in the Kema seagrass meadow. The closeness of the δ¹³C values between SOM and POM suggested that the SOM may have mostly originated from POM. However, the contribution of MPB is also highly possible because of the high transparency and shallow waters in Kema seagrass beds. Previous studies showed that the δ¹³C values of MPB are only slightly higher than those of phytoplankton (Moncreiff & Sullivan 2001). Unfortunately, this study did not collect MPB samples, so these possibilities cannot be excluded.

Stable carbon isotope values for *Enhalus acoroides* and *Thalassia hemprichii* seagrass in the Kema seagrass beds fell within the ranges of previously reported values (Kitting et al. 1984; Vonk et al. 2008; Miyajima et al. 2015). The epiphytes attached to *T. hemprichii* were mainly composed of MPB with δ¹³C values similar to those for MPB in many previous studies (Moncreiff & Sullivan 2001; Kang et al. 2003; Choy et al. 2008). The δ¹³C values of epiphytes were comparable to those of the epiphytes on the thalli of *Spartina alterniflora* (Loisel) in North American salt marshes and of *Halodule*
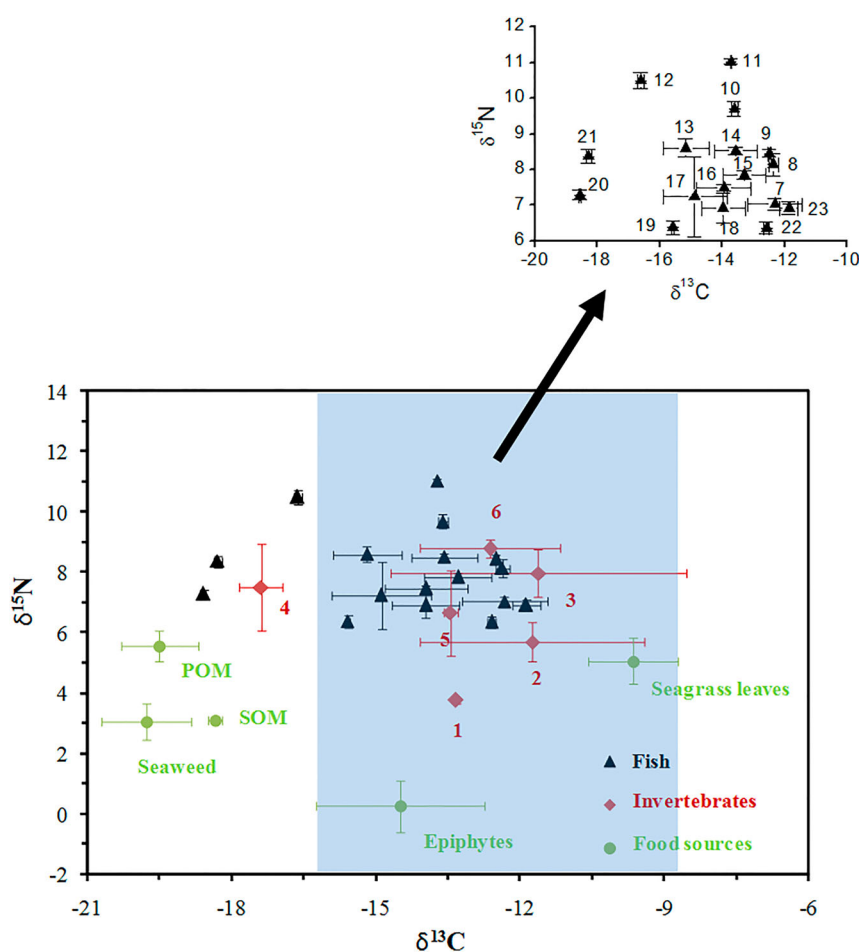
Figure 2. Dual-isotope plots of $\delta^{13}C$ and $\delta^{15}N$ for consumers and their potential food sources; Invertebrates: (1) Oligochaeta; (2) *Ophiarachna* sp.; (3) *Protoreaster* sp.; (4) Porifera; (5) Polychaeta; (6) *Alpheus* sp.; Fishes: (7) *Parajulis poecilepterus*; (8) *Syngnathoides biaculeatus*; (9) *Plotosus lineatus*; (10) *Sillago sihama*; (11) *Diodon holocanthus*; (12) *Sphyraena barracuda*; (13) *Atherinomorus* sp.; (14) *Pardachirus pavoninus*; (15) *Ostorhinchus margaritophorus*; (16) *Gerres erythrourus*; (17) *Siganus* sp.; (18) *Acreichthys tomentosus*; (19) *Aeoliscus strigatus*; (20) *Arothron manilensis*; (21) *Terapon jarbua*; (22) *Monacanthus chinensis*; (23) *Gerres oyena*.

wrightii Ascherson near Horn Island in the Gulf of Mexico (Currin et al. 1995; Moncreiff & Sullivan 2001).

The food source of invertebrates

The $\delta^{13}C$ values of Porifera are close to those of SOM and POM, which contribute 38.6% and 43.3% respectively to the diet of Porifera; this agrees with the *in situ* result that POM was the main source for some sponge species (Ribes et al. 1999). The $\delta^{13}C$ values for Oligochaeta and Polychaeta were closest to those of seagrass and epiphytes (Figure 2), which shows the important contribution of seagrass and epiphytes to these fauna (Table III). This agrees with the findings in the southeast of Hainan Island that two species of Polychaeta, *Dasybranchus caducus* (Grube, 1846) and *Marphysa sanguinea* (Montagu, 1813) (Fan et al. 2011), as well as findings in the southwest of Portugal that *Terebellidae* sp. and

Nephtyidae sp. (Vafeiadou et al. 2013), mainly feed on seagrass and epiphytes. The $\delta^{13}C$ values for shrimp in Kema are close to the results from Florida (Vaslet et al. 2012), but much lower than those from South Sulawesi and Hainan Island (Vonk et al. 2008; Fan et al. 2011). However, the carbon sources of *Alpheus* sp. in the Kema, South Sulawesi and Hainan Island seagrass beds were all mainly from seagrass. The higher $\delta^{15}N$ values showed that *Alpheus* sp. is not a primary consumer.

For *Ophiarachna* sp. and *Protoreaster* sp., two species of echinoderms, the $\delta^{13}C$ values were between those of seagrass and their associated epiphytes, indicating that they assimilated a proportion of carbon from both sources. The different $\delta^{13}C$ values showed the different contribution of seagrass and their associated epiphytes to both species. However, seagrass is obviously the most important carbon source to both species, although the value is

Table III. The ranges of possible contribution of four primary food sources to the carbon assimilated by fish and invertebrates in the Kema seagrass meadow.

Species	Seagrass	Epiphytes	SOM	POM
Fish				
Sillago sihama	29.8 (0–29)	34.3 (0–98)	19.1 (0–5)	16.8 (0–51)
Plotosus lineatus	47.9 (23–63)	25.7 (0–77)	14.1 (0–43)	12.4 (0–37)
Syngnathoides biaculeatus	50.3 (27–64)	24.4 (0–73)	13.5 (0–41)	11.8 (0–36)
Siganus sp.	20.6 (0–40)	32.3 (0–79)	24.9 (0–68)	22.1 (0–60)
Ostorhinchus margaritophorus	38.0 (9–55)	30.5 (0–91)	16.8 (0–51)	14.7 (0–45)
Atherinomorus sp.	17.0 (0–35)	29.0 (0–70)	28.4 (0–74)	25.6 (0–65)
Terapon jarbua	1.1 (0–4)	2.5 (0–8)	12.4 (0–36)	84.0 (64–96)
Aeoliscus strigatus	16.5 (0–34)	28.4 (0–68)	29.0 (0–75)	26.2 (0–66)
Pardachirus pavoninus	32.5 (1–51)	33.2 (0–99)	18.3 (0–55)	16.1 (0–49)
Parajulis poecilepterus	53.1 (31–66)	23.1 (0–69)	12.7 (0–38)	11.1 (0–34)
Acreichthys tomentosus	30.2 (0–50)	34.1 (0–98)	19.0 (0–57)	16.7 (0–50)
Arothron manilensis	0.6 (0–2)	1.6 (0–5)	8.4 (0–24)	89.4 (76–98)
Diodon holocanthus	26.4 (0–46)	34.6 (0–92)	20.7 (0–61)	18.3 (0–54)
Monacanthus chinensis	50.5 (27–65)	24.4 (0–73)	13.4 (0–40)	11.7 (0–36)
Gerres oyena	59.6 (41–71)	20.0 (0–59)	10.9 (0–33)	9.6 (0–29)
Gerres erythrourus	29.3 (0–49)	34.4 (0–97)	19.3 (0–58)	17.0 (0–51)
Sphyraena barracuda	6.5 (0–18)	13.1 (0–35)	38.6 (0–93)	41.8 (0–82)
Invertebrates				
Ophiarachna sp.	63.7 (47–74)	17.9 (0–53)	9.8 (0–30)	8.6 (0–26)
Oligochaeta	41.4 (14–58)	28.8 (0–86)	15.9 (0–48)	13.9 (0–42)
Polychaeta	39.8 (12–57)	29.6 (0–88)	16.3 (0–49)	14.3 (0–43)
Protoreaster sp.	65.5 (49–75)	17.1 (0–51)	9.3 (0–28)	8.1 (0–28)
Alpheus sp.	51.6 (29–65)	23.8 (0–71)	13.1 (0–40)	11.5 (0–35)
Porifera	6.0 (0–17)	12.1 (0–33)	38.6 (0–95)	43.3 (0–83)

Note: The values are median and ranges in parentheses are 1st and 99th percentile values.

not as high as for *Hemicentrotus pulcherrimus* (A. Agassiz, 1863) in Xincun Bay (Fan et al. 2011).

The food source of fish

The $\delta^{13}C$ ranges for fish were also quite wide, from -18.57 ± 0.029‰ (*Arothron manilensis*) to -11.87 ± 0.29‰ (*Gerres oyena*), illustrating that the food sources of the fish in Kema seagrass beds are very diverse. The 17 species are divided into four groups based on the result of principal component analysis in this study.

Group 1 includes *Monacanthus chinensis*, *Plotosus lineatus*, *Sygnathoides biaculeatus*, *Parajulis poecilepterus* and *G. oyena*. The contribution of seagrass to these fish is more than 50%, which indicates that the seagrass is their main food source in the Kema seagrass meadow. Some of the feeding habits of the fish are different. For example, *M. chinensis* feeds on seagrass, whereas *G. oyena* feeds on zoobenthos (Froese & Pauly 2015). It is worth noting that *M. chinensis* feeds on seagrass directly, while *G. oyena* and other species feed on small fish and invertebrates that assimilate the seagrass. This is the same as the findings that *G. oyena* indirectly depends on seagrass carbon in South Sulawesi (Vonk et al. 2008).

Group 2 includes *Terapon jarbua*, *A. manilensis* and *Sphyraena barracuda*, where POM is the main food source for these fishes, especially for *T. jarbua* and *A. manilensis*. While the piscivorous predator

S. barracuda is partly dependent on carbon originating from seagrass in South Sulawesi (Vonk et al. 2008), which is quite different from the species in North Sulawesi, that may be because the individuals in this study (95.6 mm) were much smaller than in South Sulawesi (217 mm), and they feed on small fishes and shrimps (Froese & Pauly 2015) that assimilate different carbon sources, or the species may move from reefs and associated areas.

Group 3 includes *Sillago sihama*, *Ostorhinchus margaritophorus* (Bleeker, 1855), *Pardachirus pavoninus*, *Acreichthys tomentosus*, *Diodon holocanthus* and *Gerres erythrourus*, these mainly feeding on seagrass and epiphytes. The importance of seagrass and epiphytes is similar to the findings for South Sulawesi. For example, *A. tomentosus* may depend on epiphytes either directly as a part of the diet or indirectly through benthic invertebrates that feed on epiphytes in the region. However, *P. pavoninus* indirectly depends on seagrass carbon (Vonk et al. 2008).

Group 4 included *Aeoliscus strigatus*, *Atherinomorus* sp. and *Siganus* sp. There is no significant difference in the contributions of seagrass, epiphytes, SOM and POM to these fish. However, it is worth noting that the food source of *Siganus* spp. is dependent on the food availabilities in different areas. For example, the main food source of *Siganus canaliculatus* (Park, 1797) is seagrass in Xincun Bay (Fan et al. 2011), while it is macroalgae in Qinglan Bay, possibly because the
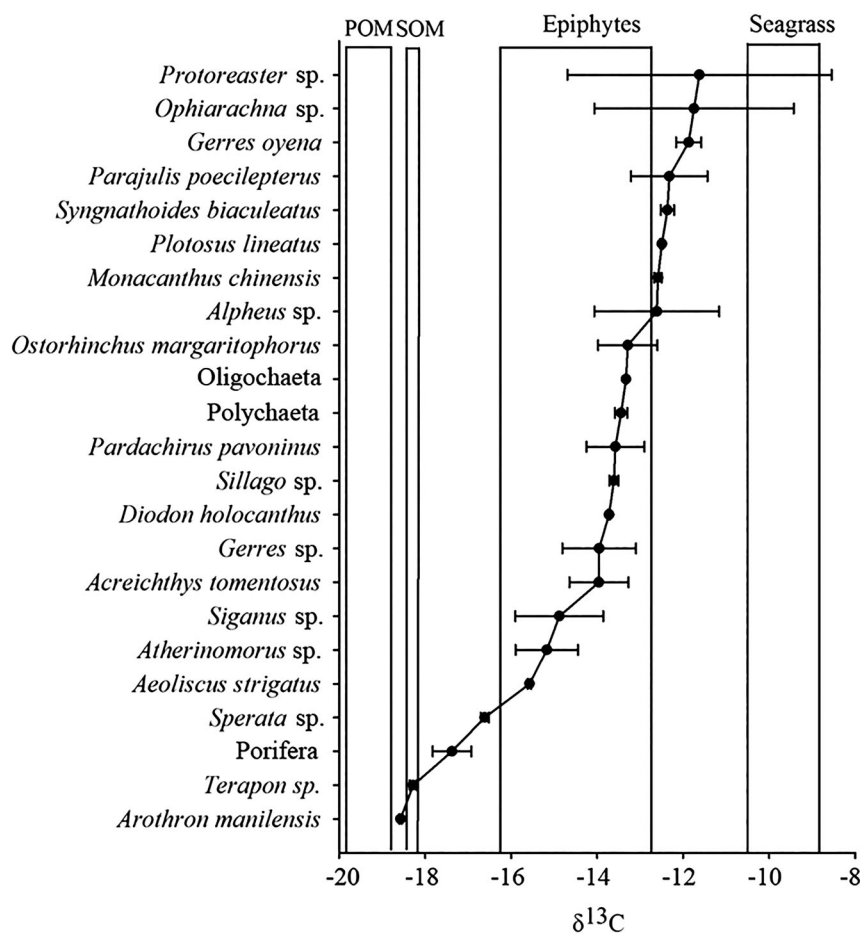
Figure 3. $\delta^{13}C$ values of consumers overlaid on food resource values (mean ± SE).

biomass of macroalgae here is more than just seagrass (Cui et al. 2013). Moreover, *Siganus doliatus* Guérin-Méneville, 1829–38 mainly feeds on epiphytes, while *S. canaliculatus* in South Sulawesi partly feeds on epiphytes (Vonk et al. 2008).

With the exception of *Siganus* sp. and *D. holocanthus*, the TLs of all other fish species inferred from SIA are smaller than those from the diet composition analysis (Froese & Pauly 2015). This may be due to the samples collected in the Kema seagrass meadow being juvenile and sub-adult fish (Table II); especially for *A. strigatus*, the TL should be 3.50 for adult fish, but was only found to be 2.20 in this study. The TLs of fish will change with ontogenetic and diet changes and the juvenile TLs are smaller than those of adults (Pauly et al. 2001; Zhang & Tang 2004), as in, for instance, *Tridentiger barbatus* (Günther, 1861), *Chelidonichthys kumu* (Cuvier, 1829), *Muraenesox cinereus* (Forsskål, 1775) (Liao et al. 2015), *Sardinella zunasi* (Bleeker, 1854) and *Setipinna taty* (Valenciennes, 1848) (Wang et al. 2013). In South Sulawesi, juvenile fish comprised 26% of the fish catch, but gear type significantly influenced this proportion (Unsworth et al. 2014). In Kema, with the exception of

S. biaculeatus, all 16 fish species collected consisted of juveniles. This demonstrates that the seagrass meadow is an important nursery for the organisms that live there.

In summary, this study analysed the food sources and trophic structure of 17 species of fish and six groups of benthic macroinvertebrates in a tropical seagrass meadow. The results show that seagrass and their epiphytes are the main contributors to five of the six groups of benthic macroinvertebrates and 14 of the 17 fishes and that seagrass and its epiphytes are important for a large portion of the food web in seagrass meadows in the Coral Triangle area. Protection of the seagrass meadows is essential for fisheries and sustainable resource utilization.

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