Determination of Food Web in Intertidal Mudflat of Tropical Mangrove Ecosystem Using Stable Isotope Markers: A Preliminary Study

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Abstract: Present study utilized stable isotope markers of carbon-13 (^{13}C) and nitrogen-15 (^{15}N) to indicate existing food web in an intertidal mudflat of Sungai Janggut, Selangor, Malaysia and also the relative contribution of primary producers to the diets of consumers. The $\delta^{13}C$ values of algae was -18.69 ± 0.7‰, detritus -24.38 ± 0.9‰, invertebrates -15.25 ± 0.1 to -21.39 ± 0.1‰ and fishes -16.17 to -21.45 ± 0.2‰. The $\delta^{15}N$ values of algae was 2.52 ± 0.1‰, detritus 1.53 ± 0.1‰, invertebrates 4.33 ± 0.4 to 8.97 ± 0.5‰ and fishes 9.54 ± 0.3 to 12.81 ± 0.4‰. This showed the assimilation of carbon and nitrogen from variety of sources in mangrove ecosystem. In general, organisms had more positive value of carbon than algae and detritus, indicating a metabolic shift in isotope ratios. This was particular; the average carbon in animal isotope ratio was 0.4‰ and 5.9‰ more positive than mean ratio of algae and detritus. Although there have no obvious systematic trophic enrichment in $\delta^{13}C$, the value of $\delta^{15}N$ is good enough to demonstrate the existence of a food web in mangrove ecosystem of Sungai Janggut. Further investigations are needed to gather enough information in order to design an accurate and comprehensive model of the food web in a mangrove ecosystem.
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1. Introduction

Mangrove forests have unique ecosystem, are very important coastal resources to the environment food web cycle. They provide primary source of food for aquatic animal when the leaves and branches of a mangrove fall to the ground. This productivity has direct impact on the health and function of the marine food chain (Saenger et al., 1983). One of the significant characteristics of mangrove ecosystem is the intertidal mudflat, which are characterized by high biological productivity and abundance of organisms, but low diversity with few rare species and support large numbers of predatory birds and fish. This area provides important permanent and temporary habitats for a large number and range of marine and terrestrial fauna. The researches has shown that the area support wide range of benthic marine fauna (Ismail et al. 1991. 1995; Ismail and Ramli 1997; Riak et al. 2003; Zulkifli et al. 2010a,b; Mohamat-Yusuff et al. 2010, 2011, 2012; Khodadoust et al. 2013).

Stable isotope analysis is an important technique for characterizing food web structure and following the pathways of energy flow through food webs (Yoshioka et al., 1994; Grey et al., 2001). The ^{13}C and ^{15}N isotope composition of consumer tissues are thus function of each species, the relative proportions of each prey species assimilated, isotope fractionation associated with converting prey tissue into consumer and in certain cases, scavenging location. Moreover, the stable isotope signatures of tissues generally reflect the diet over the period during which the tissues was synthesized (Hobson and Clark, 1992; Bearhop et al., 2002; Zulkifli et al., 2012), such that tissues with different turnover rates will integrate dietary information over different temporal periods.

The intertidal mudflat of Sungai Janggut consists of various coastal organisms which have interrelated between them in the formation of food web. In present study, $\delta^{13}C$ and $\delta^{15}N$ values were used to investigate the extent to which carbon and nitrogen derived from producer is used by animal in intertidal by using the stable isotope marker in order to determine the food webs in mangrove area. This statement were supported by Minagawa and Wada (1984) and Michener and Schell (1994), who mentioned that the stable isotope studies offer an alternative technique for trophic studies.

It allows better understanding about the

nutrition contribution sources that contribute to the food web by knowing the food web track among intertidal organisms using the isotope marker. The data obtained can be used in indicate consumption of the producer carbon. The ^{13}C and ^{15}N stable isotope ratio at the base of food webs may also vary spatially, and this is reflected in spatial variability in isotopic composition among food web (Hobson, 1999). Hence, this study aimed to provide a reference record for conducting further stable isotope analysis studies.

2. Material and Methods

Samples for isotopic analysis were collected between July to August 2012 at from Sungai Janggut (3°10′N, 101°18′E), a coastal area of Selangor, Malaysia (Figure 1). This area is located in vicinity with a coal generated power plant. Riak et al. (2003) reported that the intertidal areas between Kapar to Pantai Remis (including Sungai Janggut) are rich with macrobenthic fauna.

Figure 1. Sampling location for sediments and biological samples from Sungai Janggut, Malaysia

Collections of samples were made randomly during low tide at the selected intertidal area and inlets, respectively. Surface sediments (about 2 cm depth) were collected by using a clean plastic scoop from the intertidal area. Algae was sampled by vigorously shaking a sample of the submerged parts of the plant found in the water in algal free water, sufficient to remove most attached algae (Jones et al., 1999). Other available fauna were also collected. All samples were stored in clean zip lock plastic bags and transported back to laboratory. In the laboratory, samples were stored in a freezer. Prior to chemical treatments, biological samples were sorted according to species. All samples were cleaned with Mili-Q water. The stable isotope analysis was based on a method reported by Nakamura et al. (2008) and Zulkifli et al. (2012). All samples were dried by using an air-circulating oven at 60°C for 24 h or until constant weight obtained. Each of the samples was pulverized to form a fine powder by using a mortar and pestle. To eliminate lipid component in the tissue, the modified Bligh and Dyer (1959) method was conducted by homogenizing 1g of biological tissue with 3ml chloroform : methanol (2:1 ratio) mixture for the 3 h. The mixture was then centrifuged at 760x g (4°C) for 10 minutes using a high-speed refrigerated centrifuge. Once completed, the supernatant was discarded and the remaining pellet was dried in a vacuum desiccator for 1 hour. All samples were then fumed with 12M HCl for 10 hours to remove inorganic carbonates. The excess acid was subsequently removed in a vacuum desiccator with some pellets of NaOH for 3 hours. The samples were dried at 60°C before analysis.

Carbon (C) and nitrogen (N) stable isotope compositions were measured with an elemental analyser (EA) connected on-line to an isotope-ratio mass spectrometer (IR-MS). Isotopic compositions of C and N were expressed in δ notation ($\delta^{13}C$, $\delta^{15}N$) as part per thousand (‰) differences from an international standard (Vienna- PeeDee Belemnite (V-PDB) for carbon; atmospheric N_2 for nitrogen). The analytical precision for the isotopic analyses was better than ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope data were reported as the relative difference between ratios of a sample and standards in standard notation as:

$$\delta X\,(‰) = \left[\left(\frac{R_{sample}}{R_{std}}\right) - 1\right] \times 1000$$

where, R is ^{13}C / ^{12}C or ^{15}N / ^{14}N of sample or standard, X is $\delta^{13}C$ or $\delta^{15}N$ in per-mil (‰) deviation of that sample from the recognized isotope standards. In order to give better valuation of the variation in stable isotope values, the values were determined for separable organisms for animal samples, than combining organisms for analysis within the area (Thimdee et al., 2004).

3. Results

A total of ten different biological samples were able to be collected in this study. Results on $\delta^{13}C$ and $\delta^{15}N$

of present study showed various degree of carbon and nitrogen isotopes in intertidal habitat. Table 1 summarizes $\delta^{13}C$ and $\delta^{15}N$ vales in samples collected from intertidal area of Sungai Janggut, Selangor, Malaysia.

Table 1. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in collected samples from Sungai Janggut

Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Surface sediment	-24.38 ± 0.9	1.53 ± 0.1
Algae	-18.69 ± 0.7	2.52 ± 0.1
Cerithidea cingulata	-19.50 ± 0.9	7.26 ±1.0
Chicoreous capucinus	-19.68 ± 0.2	6.64 ± 0.6
Macrobrachium equidens	-18.92 ± 0.1	6.62 ± 0.5
Marcia marmorata	-15.56 ± 0.2	6.95 ± 1.1
Nerita lineata	-16.69 ± 1.4	4.43 ± 0.8
Thais gradata	-15.25 ± 0.1	8.97 ± 0.5
Uca vocans	-21.39 ± 0.1	4.33 ± 0.4
Boleophthalmus boddarti	-16.17 ± 0.1	12.81 ± 0.4
Periophthalmus novemradiatus	-21.45 ± 0.2	9.54 ± 0.3

4. Discussions

Algae as primary producer are abundant in mangrove area. The mean $\delta^{13}C$ and $\delta^{15}N$ value for the algae species was -18.69 ± 0.7‰ and 2.52 ± 0.1‰, respectively (Figure 2). The $\delta^{13}C$ value was within the range of -8 to -27‰ reported for epiphytic algae by Haines and Montague (1979).

Mangrove or other vascular plant carbon was apparent in the sediments and larger suspended particulate matter of the area. One sediment sample (top 2 cm from hand collected cores) from mangrove had value -24.38 ± 0.9‰ and 1.53 ± 0.1‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The isotopic composition of detritus in mangroves was closed to the algae. This result showed that sedimentary organic matter at mangroves area originated from photosynthesis resident, dominating each area.

In Malaysia, one of the most common crab species in and near the mangrove forest is the fiddler crabs (*Uca* spp). The $\delta^{13}C$ value of -21.39 ± 0.1‰ for Uca vocans was within the range of -15.4 to -23.6‰ reported for fiddler crab (*Uca*) by Rodelli et al. (1984) and suggested that they feed on microscopic algae. In contrast, Jenekitkarn (1995) found through gut content analysis that *Uca* spp. feed on detritus. In this study, we found that the $\delta^{15}N$ value of *U. vocans* (4.33 ± 0.4‰) was ~1.81‰ and ~2.8‰ higher than algae and detritus. This result suggests that both algae and detritus could be a major carbon source of *U. vocans*. The difference in $\delta^{13}C$ composition between algae and *U. vocans* would not be consistent with the assumption that they feed on algae. Hence, it is likely that *U. vocans* could potentially feed on other types of algae which are not collected in this study.

The five species of molluscs *Nerita lineata*, *Chicoreous capucinus*, *Marcia marmorata*, *Cerithidea cingulata* and *Thais gradata*, showed little difference in $\delta^{13}C$ composition. *N. lineata* grazes on algae (Ng and Sivasothi, 1999) that thrive on the rocks, scraping this off with their radula. In the case of *Mar. marmorata*, like many other bivalves, this clams are filter feeders which filter tiny bits of plant and animal materials out of the water they live in. *N. lineata* had mean $\delta^{13}C$ value of -16.69 ± 1.4‰ close to the value of algae (-18.69 ± 0.7‰) and its $\delta^{15}N$ value also showed ~1.91‰ higher than algae (Figure 2). This result indicates that algae matter is an important part of its diets. The pond clams *Mar. marmorata* showed $\delta^{15}N$ enrichment pattern compared to algae and detritus, while the $\delta^{13}C$ value of *Mar. marmorata* showed ~3.1‰ and ~8.8‰ higher than algae and detritus, respectively. These findings could potentially suggest that detritus is not a major food source of *Mar. marmorata*, on the other hand, the clams could heavily feed on algae.

Together with *Ch. capucinus*, *T. gradata* prey on a wide variety of food sources from barnacles, nest-building mussels to snails and clams hide in the mud and worms in rotten wood (Ng and Sivasothi, 1999; Mohamat-Yusuff et al. 2010, 2011, 2014). The mean $\delta^{13}C$ and $\delta^{15}N$ values of *Ch. capucinus* is -19.68 ± 0.2‰ and 6.64 ± 0.6‰, respectively. As for *T. gradata*, it has $\delta^{13}C$ value of -15.25 ± 0.1‰ which was ~4.4‰ more positive than *Ce. cingulata*. Both snails species have higher $\delta^{15}N$ values compare to the class species of detrivores and herbivores. It is likely that *Ch. capucinus* and *T. gradata* fed heavily on the detrivores and herbivores feeder (*N. lineata* ~7.8‰). According to Tan and Woo (2010), *Ce. cingulata* grazes on tiny things growing or settling on the muddy bottom, such as diatoms, bacteria and detritus. The small different in $\delta^{13}C$ value for *Ce. cingulata* living in mangrove area (-19.50 ± 0.9‰), which feed on various tiny things suggests that algae was a major food sources of *Ce. cingulata*. Whereas, *Ce. cingulata* has $\delta^{15}N$ value of 7.26 ±1.0‰ which was ~5.7‰ higher than detritus, suggesting that detritus was not major food source in mangrove area.

The prawns, *Mac. equidens*, are omnivorous and are known to feed on a variety of foods including cyanobacteria, microheterotrophic protozoans, meiofauna, benthic diatoms, filamentous alga and algae (Chong and Sasekumar, 1981). The $\delta^{15}N$ value of shrimp is 6.62 ± 0.5‰. The $\delta^{15}N$ value was 4.1 and 5.09‰ higher than algae and detritus, respectively. This result indicates that algae were

important food sources for shrimps living in the mangrove are rather than detritus, and suggest that they have possibly feed on other sources like plankton.

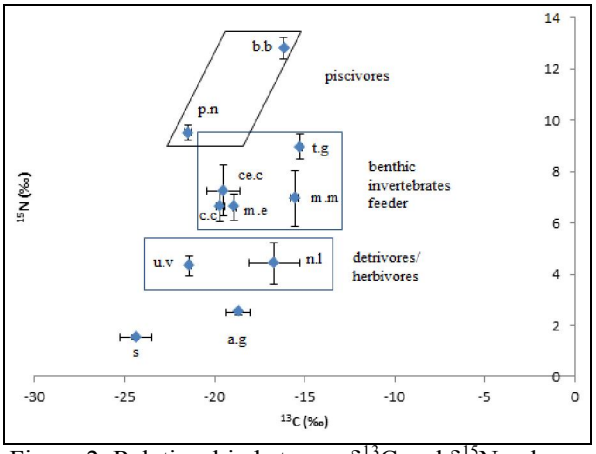

Figure 2. Relationship between $\delta^{13}C$ and $\delta^{15}N$ values of primary producer and aquatic animals collected from mangrove area. (Primary producer algae = a.g. Surface sediment = s. Molluscs: *Marcia marmorata* = m.m, *Cerithidea cingulata* = ce.c, *Thais gradata* = t.g, *Chicoreous capucinus* = c.c, *Uca vocans* = u.v, *Nerita lineata* = n.l, *Macrobrachium equidens* = m.e. Fishes: *Periophthalmus novemradiatus* = p.n, *Boleophthalmus boddarti* = b.b.)

The range of $\delta^{13}C$ and $\delta^{15}N$ in amphibious fish species collected in Sungai Janggut intertidal area were -21.45 ± 0.2 to -16.17‰ and 9.54 ± 0.3 to 12.81 ± 0.4‰, respectively. The small range in isotopic composition indicates that fishes fed on similar foods and occupied same trophic levels. *P. novemradiatus* is carnivores, feeding on small crab, worms and insects (Ng and Sivasothi, 1999; Clayton, 1993). Assuming dietary inputs from invertebrates and detritus found in mangroves, a combination of in vertebrates and detritus can yield a ^{13}C similar to this fish. However, the $\delta^{15}N$ value of the *P. novemradiatus* was ~8.0‰ higher than the detritus, suggesting that detritus was not a major food source of the *P. novemradiatus*. On the other hand, its $\delta^{15}N$ value was 0.6-2.9‰ higher than invertebrates, suggesting that *P. novemradiatus* heavily fed on various type of invertebrates (examples, *Mar. marmorata*, *Ce. cingulata*, *Ch. capucinus*, *T. gradata*, *Mac. equidens*) (Figure 2). Therefore, these species can be classified in molluscivore feeders. On the other hand, *B. boddarti* emerges when the tide recedes to graze on algae and detritus (Ng and Sivasothi, 1999). The muscle tissues of these fishes showed relatively positive in $\delta^{13}C$ values compared to *P. novemradiatus*. Coinciding with the large differences in $\delta^{15}N$ value detritus compare to algae (11.28‰ and 10.29, respectively), the $\delta^{13}C$ data indicate that algae were major food sources for this fish compare to detritus (Figure 2).

Present study found the $\delta^{13}C$ values of about 1‰ to show prey-predator relationship in intertidal area is doubtful. Similar result has been reported by Marguilier et al. (1997) from Gazi Bay, Kenya. This suggests that mangrove carbon can make a significant contribution to most aquatic animals inhabiting mangroves. In present study, we found no systematic trophic enrichment in $\delta^{13}C$ and some showed depletion although most of the predators showed enrichment in $\delta^{13}C$ relative to their prey. Due to the variability in $\delta^{13}C$ within potential food types and migration of the organisms in the areas, the interpretation of $\delta^{13}C$ measurements may not accurately resolve the trophic structure in this ecosystem. Compare to $\delta^{15}N$ which the values were excellent in become trophic indicator, its values were enriched by 3-4‰ relative to their diets. Different species but sames genus may have been feeding on different diets (e.g. *T. gradata* and *Ch. capucinus*).

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