δ^{13}C and δ^{15}N Stable Isotope Ratio Analysis of Fish Species and Mangrove Leaf on Mangrove Ecosystem of Muara Badak Coastal Area-East Kalimantan, Indonesia

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Abstract:** The ecological structure link between mangrove ecosystem and aquatic food production is of great importantly for fisheries management. The objectives of the research to know well about are to :(1) determine the stable C and N isotopic compositions of fish species and mangrove leaf (2) use bulk and compound-specific isotopic ratios to determine relative tropic positions for fish species in mangrove ecosystem of MuaraBadak coastal area – East Kalimantan, Indonesia. A few of fish species found in mangrove ecosystem was examined on the basis of carbon and nitrogen stable isotope distributions. Result of the study revealed that significant differences in stable isotope signatures (C and N) exist in some species from different habitats in that coastal area, which could be used to delineate feeding habitats of fishes. The space mangrove area and dense mangrove area do not appear to be direct sources of carbon in the diets of studied fish species; rather, they probably serve as refuge as well as a substratum for a variety of primary producers and consumers that are important in the food webs of these habitats. All fishes showed higher δ^{15}N values suggesting with a higher trophic level. Some fish species of similar feeding guilds showed some degree of segregation by feeding on different food resources. The food web in the mangrove ecosystem of this coastal area showed various trophic levels. The fishes were the most enriched in δ^{15}N. Also, clear gradients in both δ^{13}C and δ^{15}N could be observed for different feeding guilds of fishes and for different habitats such as mangrove area and rivermouth of this coastal area. Based on analysis of stable isotope ratio (both δ^{13}C and δ^{15}N) of fish species showed that the mangrove plant have basically food chain on mangrove ecosystem. Nevertheless, the biodiversity and community structure of mangrove had been decreasing presently in MuaraBadak coastal area, it that reason to keep and to save mangroves ecosystem and also promote the sustainable use management of mangrove ecosystem.

***Keywords:** δ^{13}C and δ^{15}N ratios; carbon sources; community structure; fish and mangrove communities; stable isotope signature.*

1. INTRODUCTION

The vegetation in MuaraBadak coastal area is predominantly mangrove forests. Prior to 1980, mangrove vegetation in that coastal area were pristine and about 60% of the area was covered by Nypa. Considerable changes in the extent of mangrove ecosystem occurred in 1990-2002, with peak degradation on of 1996 to 2000. Until 2001, about 63% mangrove areas were deforested mainly due to conversion for shrimp ponds. Loss of more than half the mangrove forests caused environmental impacts and affected aquatic productivity, social and economic condition and the livelihoods of communities living in this area. Conflicts among various resource users were a frequent occurrence in the local communities, particularly related to land ownership and water pollution issues.

The importance of mangrove ecosystems as nursery ground for juvenile fish. Many tropical fish species spend part of their life cycles in mangrove swamps or are dependent on food chains which can be traced back to these coastal forests. Numerous studies have documented the fish assemblages of such environments; however, the exact role that mangroves, as an ecosystem, play in sustaining the juvenile fish remains unknown. Initial findings from Mahakam Delta indicate a wide diversity and an abundance of fish in the mangrove habitats. Furthermore, most small juveniles (< 5 cm) were found in mangrove habitats, whereas larger specimens of the same species were found in deeper waters.

The power of stable isotope analysis as a tool in the investigation of aquatic food web structure and dietary patterns is based on the significant and consistent differences in isotopic composition of different types of primary producers due to different photosynthetic pathways or different inorganic carbon sources. This method present time-integrated information on organic carbon sources for heterotrophs and feeding relationships, mainly using carbon and nitrogen stable isotope ratios in organism [1] as like fish and plankton communities. So that, recent studies have used natural abundance by stable isotopes for food web analyses. Analysis of the stable isotopes of carbon and nitrogen can provide a clearer understanding of diets because they reflect the actual assimilation of organic matter into consumer tissue rather than merely its consumption and provide an average of the diet over periods.

Stable isotope analyses are powerful tools for tracing energy flow in food webs and for the study of trophic positions of higher consumers. Using this method, it is possible to assess the importance of different primary producers, such as phytoplankton, micro- and macro-algae and mangrove leaves, as the baseline source of energy for higher trophic levels on Mahakam Delta [2]. This is possible because different primary producers have different stable isotope carbon and nitrogen values. The ecological structure link between mangrove ecosystem and aquatic food production is of great importantly for fisheries management. However, the complexity and diversity of natural food webs make it difficult to demonstrate this ecological link. It is possible that not only phytoplankton community but also other organic sources may contribute carbon to the fish and mangrove community. The important to clarify trophic dynamics in marine ecosystem for fishery activities management mainly for fishing ground. Organic carbon sources and trophic position of fish species in mangrove ecosystem of MuaraBadak coastal area, were examined on the basis of carbon and nitrogen stable isotope distributions.

The objectives of the research to know well about carbon-nitrogen stable isotope ratio for major aquatic biota such as fish community on mangrove ecosystem in MuaraBadak coastal area - East Kalimantan, Indonesia. Major objectives of this research are to: (1) determine the stable C and N isotopic compositions of fish species and mangrove leaves (2) use bulk and compound-specific isotopic ratios to determine relative tropic positions for fish species in mangrove ecosystem.

2. MATERIALS AND METHODS

2.1. Sampling and Location

The research had been done on MuaraBadak coastal area, the Northern-side of Mahakam Delta, KutaiKertanegara Regency-East Kalimantan, Indonesia (Figure 1). Sampling was carried out during January 2018 to June 2018. Fish samples had been done on fishing ground area that consists of area such as mouth of river have high salinity, mid area have mid salinity and inside of river have low salinity level condition.

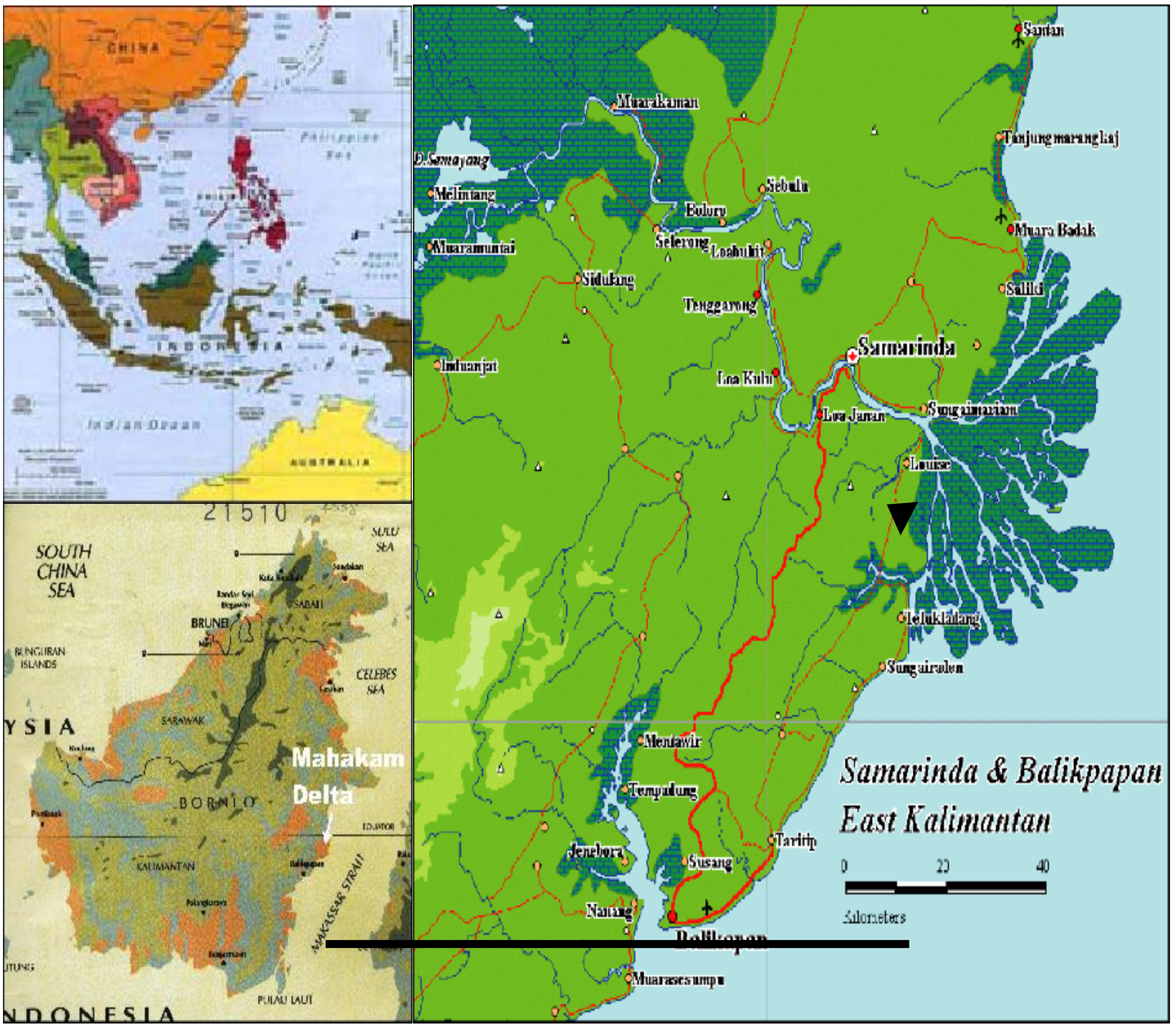

Figure 1. Study Area of MuaraBadak Coastal Area – East Kalimantan, Indonesia

2.2. Sample Extraction

Mangrove leaf samples have collected on mangrove plant area dried by vacuum-dry oven (in 10 minutes) and re-dried by auto dry oven (1 day). The sample were later ground to a fine powder. The mangrove samples have consist of mangrove leaf and branch with 7 mangrove species dominancy.Total of Fish sample was carried out of 47 fishes of14 fish species onboth sampling time. The sampling collected fish species sample in mangrove area, in order to know contrast the isotopic distribution of the fish species. The fishes were collected using passive gear such as trap net (*belat*) and gill net (*rengge*) on fishing ground area and fish samples were kept in a cool box on board. The muscle of fish samples were excised from the trunk behind a pectoral fin of adult fishes after storage at -20^{o}C. The tissues (meat fish) of fish samples were washed and dried at 60^{o}C for at least 24 h. The sample were later ground to a fine powder. All meat samples were subsequently defatted with a chloroform-methanol solution (2:1), in order to eliminate the influence of variation in content of lipids that are more depleted in C13 than amino acids and carbohydrates.

2.3. Stable Isotope Analysis

Stable isotope ratios of carbon and nitrogen were measured with an ANCA-GSL mass spectrometer Version 4.0 (PDZ Europa Ltd/Integrated Analytical Solutions, Cheshire, UK) and a Delta Plus Advantage Mas Spectrometer (ThermoFinnigan, Bremen-Germany) coupled with an elemental analyzer (Flash EA 1112, ThermoFinnigan). Analysis of stable isotope ratio will be done in the Center of Natural Environmental Sciences, Nihon University-Fujisawa (Japan). Then, for stable isotope analysis, 0.75-0.76 mg of standard (alanine) and homogenous material sample were weighted into 4x6 tin capsules. Isotope ratios, the δ^{13}C and δ^{15}N, are expressed as per mil deviations from the standards as defined by equation:

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

Where, R = 13C/12C or 15N/14N, Belemnite (PDB) and atmospheric nitrogen were used as the isotope standards for carbon and nitrogen, respectively. Analytical precision for isotope analyses was + 0.32 ‰ for δ^{13}C and + 0.29 ‰ for δ^{15}N.

3. RESULTS

The Carbon and Nitrogen Stable isotope ratio analysis to the mangrove leaves in mangrove ecosystem of MuaraBadak coastal area were presented on Table 1.

Table 1. The δ^{13}C and δ^{15}N stable isotope signature of mangrove leaves in MuaraBadak Coastal

No	**Name of Mangrove Plants**	**δ^{13} C**	**δ^{15} N**
1	Avicennia alba (White mangrove) 1	-31.7	3.8
2	Avicennia alba (White mangrove) 2	-29.4	2.0
3	Avicennia alba (White mangrove) 3	-29.4	2.0
4	Avicennialanata (Ridlev mangrove)	-29.4	3.7
5	Avicennia marina (Forsk mangrove)	-32.4	3.3
6	Bruguieraparviflora (Small Flower mangrove)	-33.7	3.6
7	Rhizophoraapiculata (Tall-still mangrove) 1	-32.1	0.9

8	Rhizophoraapiculata (Tall-still mangrove) 2	-29.9	2.0
9	Rhizophoramucronata (Asiatic mangrove)	-29.3	2.2
10	Sonneratiacaesiolaris (Mangrove apple)	-29.7	0.9

The Carbon and Nitrogen Stable isotope ratio analysis to the fish species in mangrove ecosystem of MuaraBadak coastal area were presented on Table 2.

Table 2. The δ^{13} C and δ^{15} N stable isotope signature of fish species on variance of salinity level

Salinity Level	**Name of Fishes**	**δ^{13}C**	**δ^{15}N**
High salinity area	Caranxignobilis (Giant Trevally)	-23.0	8.3
	Chandasp (Glass fish)	-16.9	8.3
	Chanoschanos (Milk Fish)	-22.7	4.8
	Clupeasp (Herrings)	-17.2	10.4
	Caranxignobilis (Giant Trevally)	-18.9	9
	Gerresfilamentosus (Mojarra)	-23.8	7.3
	Johniushypostoma (Small Croakers)	-19.0	10.0
	Johniushypostoma (Small Croakers)	-22.6	7.6
	Leiognathuselongatus (Slender Pony)	-17.5	7.8
	Lutjanussp (Red Snappers)	-20.7	8.3
	Lutjanussp (Red Snappers)	-16.2	9.5
	Leiognathuselongatus (Slender Pony)	-17.5	7.8
	Mugilsp (Mullets)	-20.8	5.7
	Mugilsp (Mullets)	-17.9	7.5
	Otolithessp (Croakers)	-21.9	8.6
	Sillagoanalis (Rough Sillago)	-17.4	7.6
	Scatophagussp (Spotted Scat)	-26.1	7.1
	Trichiuruslepturus (Cutlass fish)	-16.7	10.4
Mid salinity area	Clupeasp (Herrings)	-20.2	8.4
	Caranxignobilis (Giant Trevally)	-17.9	9
	Chandasp (Glass fish)	-13.8	6.8
	Johniushypostoma (Small Croakers)	-19.3	9.7
	Leiognathuselongatus (Slender Pony)	-16.7	8.5
	Lutjanussp (Red Snappers)	-18.2	9.1
	Mugilsp (Mullets)	-18.2	6.8
	Sillagoanalis (Rough Sillago)	-17.4	9.4
Low salinity area	Chandasp (Glass fish)	-17.9	4.2
	Chanoschanos (Milk Fish)	-20.2	9.4
	Caranxignobilis (Giant Trevally)	-24.5	8.5
	Caranxignobilis (Giant Trevally)	-17.6	9.5
	Clupeasp (Herrings)	-19.0	9
	Gerresfilamentosus (Mojarra)	-18.4	8.0
	Johniushypostoma (Small Croakers)	-26.1	5.7

	Johniushypostoma (Small Croakers)	-19.1	10.4
	Lutjanussp (Red Snappers)	-21.4	9.3
	Lutjanussp (Red Snappers)	-18.3	9.2
	Leiognathuselongatus (Slender Pony)	-17.6	7.8
	Mugilsp (Mullets)	-29.0	6.9
	Mugilsp (Mullets)	-17.1	5.7
	Scatophagussp (Spotted Scat)	-18.2	10.3
	Sillagoanalis (Rough Sillago)	-17.6	9.6
	Trichiuruslepturus (Cutlass fish)	-16.7	10.1
	Upeneusrittatus (Goat Fish)	-21.6	7.8

4. DISCUSSION

4.1. Carbon and Nitrogen Stable Isotope Ratio of Mangrove Leaf

Mangrove forests are productive ecosystems and support a high abundance and diverse variety of wildlife that as a result of high leaf production, leaf fall and rapid breakdown of the detritus. Litter fall has been estimated to account for 30–60% of total primary production [3]. The importance of mangrove leaf litter in the maintenance of detrital-based food webs in the coastal environment and their significance for coastal fisheries has been indicated for some time. However, mangrove forests are disappearing at alarming rates due to various human impacts like reclamation for aquaculture, farming and industrial development, the greatest losses occurring in MuaraBadak Coastal Area - Mahakam Delta, East Kalimantan. Since period of 1980 and 2000 had lost 70 % of its mangrove forests.

Mangrove leaves had been collected on MuaraBadak Coastal Area-East Kalimantan for Stable Isotope Analysis consist of 7 mangrove species such as Avicennia alba or white mangrove, Avicennialanata or ridlev mangrove, Avicennia marina or forks mangrove, Bruguieraparviflora or small flower mangrove, Rhizophora apiculate or tall-still mangrove, Rhizophoramucronata or Asiatic mangrove and Sonneratiacaesiolaris or mangrove apple. A part of mangrove had been carrying out for isotope analyzed that were leaves of mangrove plant, including leaf and dead leaf of mangroves presently.

Mangrove leaves of different species on MuaraBadak coastal area showed little variation in $\delta^{13}C$, with values ranged -29.9 ‰ to -33.7 ‰. The $\delta^{13}C$ of drifting mangrove leaves agreed well the value measured for a mixture of mangrove leaves from different species, weighted by species biomass in the study area. Mangrove leaves were the main carbon source for estuarine POM and estuarine sediments.

Stable C isotope ratios for mangrove leaves of -33.7 %o to -28.4 ‰a classify them with the dominant land plants that use the C3 photosynthetic pathway [4]. Compared to mangrove leaves, microphytes from the riverine mangrove were relatively enriched in $\delta^{13}C$ (-23.8‰ for the phytoplankton or <35 fJ.m fraction and -24.2‰ for epiphytic algae) and are within the range of - 19 to -24‰ for marine algae [4].

The $\delta^{13}C$ of drifting mangrove leaves agreed with the value measured for a mixture of mangrove leaves from different species, weighted by species biomass in the study area. Mangrove leaves were the main carbon source for estuarine POM and estuarine sediments. The $\delta^{13}C$ data support the concept ofmangrove forests as important sources of suspended particulate organic matter, coarse detritus and sediment (SOM) on Mahakam Delta. The last twenty years show that stable isotope analyses have been used at different locations in the world to determine the contribution of mangroves as a source of organic carbon to adjacent estuarine environments. The general conclusion is that mangrove detritus utilization is often quite restricted in spatial scale, and carbon derived from mangroves that it has most nutritional importance in MuaraBadak coastal area.

4.2. Carbon and Nitrogen Stable Isotope Ratio of Fish

Fish species had been collected on MuaraBadak of Mahakam Delta for Stable Isotope Analysis consist of 14 fish species such as Caranxignobilis (Giant Trevally), Chandasp (Glass fish), Chanoschanos (Milk Fish), Clupeasp (Herrings), Gerresfilamentosus (Mojarra), Johniushypostoma (Small Croakers), Leiognathus elongates (Slender Pony), Lutjanussp (Red Snappers), Mugilsp (Mullets), Otolithessp (Croakers), Sillagoanalis (Rough Sillago), Scatophagussp (Spotted Scat), Trichiuruslepturus (Cutlass fish) and Upeneusrittatus (Goat Fish).

The overall of fish species had $\delta^{13}C$ values ranges -26.05 ‰ till -16.65 ‰ on mangrove area. The distribution of $\delta^{15}N$ values for fish species collected in this survey show that considering a trophic enrichment of 5.47 ‰ till 10.27 ‰ for fish species. The Overall of fish species had $\delta^{13}C$ values between -19,0 ‰till - 16.9 ‰ on fishing ground of river mouth area (high salinity level), ranged between - 20.2 ‰ till -13.8 ‰ on fishing ground of middle river area (mid salinity level), and ranged between -19.1 ‰ till -17.1‰ on fishing ground of inside river area (low salinity level). The overall distribution of $\delta^{15}N$ values for fish species collected in this survey show that considering a trophic enrichment of 7.5 ‰ till 10.4 ‰ on fishing ground of river mouth area (high salinity level), ranged between 6.8‰ till 9.4 ‰ on fishing ground of middle river area (mid salinity level), and ranged between 4.2 ‰ till 10.4 ‰ on fishing ground of inside river area (low salinity level). The position of the marine living including fishes based on the relationship between the carbon stable isotope ratio presented a clear gradient in $\delta^{13}C$ could discerned for fishes as well as food items from the mangrove habitats to the river mouth.

Fish and food items on the mangrove habitats were significant depleted to those from the river mouth area by on average from 5.47 ‰ to 10.44 ‰ for fishes and food items, respectively. There were no significant differences in $\delta^{13}C$ between the two habitats of mangrove area and river mouth. Individual fish species on the river mouth had more depleted in $\delta^{13}C$ in comparison to those of the same species from mangrove habitats of MuaraBadak area. The $\delta^{13}C$ depletion of individual species was generally in the order: river mouth area < mangrove area.

4.3. Trophic Relationship on Mangrove Ecosystem

The food web in the mangrove ecosystem of MuaraBadak coastal area has various trophic levels. Based on the stable isotope ratio (both $\delta^{13}C$ and $\delta^{15}N$) related to the food web on the fish species and mangrove leaves show that mangrove plant as a base of the food chain on Mahakam Delta.

Trophic levels ranged from detritus feeder and herbivores to carnivores in MuaraBadak coastal area based on trophic position by the literature (http/www.fishbase.org) ranged from 2 to 2.5 as like detritus feeder and herbivores, more than 2.5 to 3.0 as like the omnivore, more than 3.0 to 3.5 as like low carnivore, more than 3.5 to 4.0 as like middle carnivore, and more than 4.0 as like highly carnivores. Overall fish species showed that the trophic level and habitat show that 62.78 % were carnivores, 16.11 % omnivores, 21.22 % herbivores and detritivores.

Isotopic composition of fish revealed significant differences among trophic classes on each. Herbivores on the sparse mangrove had significantly lower $\delta^{13}C$ values than the dense mangrove area. Generally, herbivore has lower $\delta^{13}C$ and $\delta^{15}N$ values than omnivore and carnivore also. However, herbivores such as Milkfish (C. chanos) had significantly higher $\delta^{13}C$ and $\delta^{15}N$ values than omnivores and carnivores. These conditions indicated that had been the changes of food sources on the sparse mangrove area caused by mangrove converted and environmental changes. These changes of the habitat conditions caused some of euryphagic fish species could be consumption some kind of food availability.

Herbivores had $\delta^{13}C$ values similar to detritus feeder. Based on $\delta^{13}C$ values, trophic classes did not differ much within area. The $\delta^{15}N$ composition of fish species on the mangrove area ranged from 5.5 ‰ to 10.4 ‰. Low $\delta^{15}N$ values were normally associated with detritivores and herbivores such as Mullet (Mugilsp) and Milkfish (C. chanos), while higher $\delta^{15}N$ ratios were found in carnivore species such as Red Snappers (Lutjanussp), Giant Trevally (C. ignobilis) and Cutlassfish (T. lepturus). Nitrogen isotopic composition had the highest $\delta^{15}N$ values until 10.4 ‰ among the four trophic levels.

The $\delta^{15}N$ values and number of trophic levels for fish species measured in this study are similar to those reported in other food web studies[4]. However, trophic levels calculated using detritus feeder. The $\delta^{15}N$ mean values as a reference where one or two levels lower than those estimated using $\delta^{15}N$ mean values between sediment and mangrove litter. This suggests that the mean value between sediments and mangrove litter is likely a better $\delta^{15}N$ reference. Previous food web studies carried out in tropical rivers have successfully used the mean of sediment as a $\delta^{15}N$ reference [5].

The majority of fishes were secondary heterotrophy (trophic level from 2.0 to 3.0, with $\delta^{15}N$ values ranging from 4.8 ‰ to 5.7 ‰). The $\delta^{15}N$ values of tertiary consumers (trophic level 3.0 to 4.0) ranged from 5.5 ‰ to 8.6 ‰, and this group was comprised primarily of adult fishes. Estimates of trophic positions of 4.0 were only observed for three species (Lutjanussp, C. ignobilis and T. lepturus), with $\delta^{15}N$ values ranged from 8.30 ‰ to 10.5 ‰, respectively.

Isotopic composition of fish revealed significant differences among trophic classes. On average, herbivores had significantly lower $\delta^{13}C$ values than omnivores, but herbivores had $\delta^{13}C$ values similar to detritus feeder. Based on $\delta^{13}C$ ratios, trophic classes did not differ much within the area.

Generally, trophic levels calculated using $\delta^{15}N$ as an indicator was two levels lower than the calculated in the literature using gut content analyses. It had been reported that the $\delta^{15}N$ metric may underestimate the importance of linkages in the microbial loop, reducing the apparent food web length by one or two trophic levels [4]. Regardless of the metric used to estimate trophic levels in the CGSM complex, the lengths of food webs are small. It showed that an adaptive response of fish assemblages to strong seasonal environmental fluctuations that

affected the availability of food resources,as typically occurs in tropical estuaries and rivers [5]–[7].
Nevertheless, other mangrove faunas most notably crab had observed to consume significant amounts of mangrove leaves [3], [8], [9]. For the sake of accuracy, conservationists may therefore describe the crabs and others fauna, but not for penaeid-shrimps, as mangrove detritus feeders in the reason to save mangroves.

5. CONCLUSIONS

Mangrove leaves of different species on MuaraBadak coastal area showed little variation in δ^{13}C, with values ranged -29.9 ‰ to -33.7 ‰. The δ^{13}C of drifting mangrove leaves agreed well the value measured for a mixture of mangrove leaves. The distribution of δ^{15}N values for mangrove leaves collected in this survey show that considering a trophic enrichment of 0.9 ‰ till 3.8 ‰.
The overall of fish species had δ^{13}C values ranges -26.05 ‰ till -16.65 ‰ on mangrove area. The distribution of δ^{15} N values for fish species collected in this survey show that considering a trophic enrichment of 5.47 ‰ till 10.27 ‰ for fish species. The Overall of fish species had δ^{13}Cvalues between -19,0 ‰till - 16.9 ‰ on fishing ground of river mouth area (high salinity level), ranged between - 20.2 ‰ till -13.8 ‰ on fishing ground of middle river area (mid salinity level), and ranged between -19.1 ‰ till -17.1 ‰ on fishing ground of inside river area (low salinity level). The overall distribution of δ^{15}N values for fish species collected in this survey show that considering a trophic enrichment of 7.5 ‰ till 10.4 ‰ on fishing ground of river mouth area (high salinity level), ranged between 6.8 ‰ till 9.4 ‰ on fishing ground of middle river area (mid salinity level), and ranged between 4.2 ‰ till 10.4 ‰ on fishing ground of inside river area (low salinity level).
The food web in the mangrove ecosystem of MuaraBadak coastal area has various trophic levels. Based on the stable isotope ratio (both δ^{13}C and δ^{15}N) related to the food web on the fish species and mangrove leaves show that mangrove plant as a base of the food chain on MuaraBadak coastal area. Nevertheless, the biodiversity and community structure of mangrove had been decreasing presently. To repairing, the decreasing of mangrove and to increase of fishery productivity in MuaraBadak coastal area – East Kalimantan, Indonesia must be going the optimum and sustainability of mangrove management.

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