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Shigeru Kato⁴, Savettachat Boonming⁵, Kan Chantrapromma⁶, Suthira Thongkao⁷, Sangob Panichart⁸, Sanit Aksornkoae⁹, Prasert Tongnunui⁷, Woraporn Tarangkoon⁸ and Toshinori Kojima⁹

¹,²Department of Materials and Life Science, Seikei University, Japan
³Nakhon Si Thammarat Regional Forest Division, Thailand
⁴Hat Yai University, 125/502 Polpichai Rd., Hatyai, Songkhla, Thailand 90110
⁵Walailak University, 222 Thaiburi, Thasala, Nakhon Si Thammarat, Thailand 80161
⁶Thailand Union for Mangrove Rehabilitation and Conservation, Thailand
⁷,⁸Rajamangala University of Technology Srivijaya, Thailand
¹kato00001@cc.seikei.ac.jp, ⁹kojimaseikei@gmail.com

ABSTRACT

Mangrove forests provide numerous ecosystem services, including nutrient cycling, sediment trapping, protection from cyclones and tsunami, habitat for numerous organisms and wood for fuel and lumber. It is being a unique habitat for several fresh and saline tolerant species. The present research aims to study the carbon accumulation and food cycle system in the rehabilitated mangrove site of Nakhon Si Thammarat, southern part of Thailand. The mangrove rehabilitation sites, at the abandoned shrimp ponds and new mud flat areas, have been taking place since 1998 by participatory planting activity. Almost seven million mangrove trees were planted in 1,200 ha. It is observed that the rehabilitated mangrove site can be a habitat for increasing the population of the species like crab, shell, shrimp and fish. It is also found that the δ¹⁵N content in the living organism is gradually increased from small phytoplankton to large fishes in the food chain system. Conversely, it is observed that there is significant change in the δ¹³C value during the food chain system. The data analysis reveals that the carnivorous fishes involve in 12th to 13th step of the food chain system which starts from the falls of mangrove leaves of the rehabilitated mangrove forest. Carbon content of soil in the rehabilitated mangrove forest was gradually increased with planting age from the state of the abandoned shrimp ponds and new mud flat areas. The rehabilitated mangrove forest would be as a sink source for atmospheric carbon and rich biodiversity of the estuaries ecosystem.

Keywords: mangrove rehabilitation; mangrove forest; stable isotopes; food cycle; carbon sink.

1. INTRODUCTION

Tropical deforestation represents approximately 25 percent of total anthropogenic GHG emissions worldwide. Deforestation, forest degradation or changes in land management practices can cause of releasing the carbon dioxide from soil to the atmosphere.
Mangrove forests cover large areas of tropical and subtropical along the coastline in the world. There are many and multiple functions as ecosystem services including fisheries and fiber production, sediment regulation, and storm/tsunami protection in mangrove forest (Alongi, 2002; FAO 2007). Furthermore, mangrove forest can be the conservation of water resources, human health, culture and education for students and people. Above all, it is a unique ecosystem (Luiz et al. 2001). It is also very important for subtropical and tropical people and earth environment (FAO Forestry report 147, 2006). Mangrove forest distributes around 15 million ha at estuary and coastal of subtropical and tropical regions in the world (Mark, Kainuma & Collins, 2010). Total area of abandoned shrimp ponds are between 24,000 and 32,000 ha in Thailand (Sanit, Ruangrai, Wattana & Suthawan, 2004). Mangrove trees were planted about 7 million mangrove plants in abandoned shrimp ponds around the 1,200 ha since 1998 at Nakhon Si Thammarat province, southern Thailand (Shigeru et al., 2006). All of planted mangrove plants are well grown. Biomass amount of the planted mangrove trees were concretely increasing year by year. This increasing biomass can decrease CO₂, greenhouse gas from atmosphere.

Food webs in natural ecosystem have multiples, reticulated connections between a diversity of consumers and resources (Boon, Bird & Bunn, 1997). Traditional approaches to food web analysis include gut content analysis which is a direct observation in the consumer animals. Results of gut content analysis indicate feeding behavior and potential food sources, but they do not provide information about assimilation of food or necessarily identify the primary production for support consumers (Thomas et al., 1993).

The stable isotope ratios of Carbon (13C/12C) and Nitrogen (15N/14N) have proven useful as tracers of organic matter sources and food web structure in a variety of aquatic systems (Bouillon et al., 2002). This is because unlike gut content analysis, they are directly related to assimilation (Thomas & Cahoon, 1993). The stable isotope ratios in consumers is indicated on by consumers and reflected in their tissues at whatever trophic level they occur. The use of stable isotopes to determine food web structure involves the comparison of stable isotope ratios between autotrophs and consumers and requires distinct differences in the isotopic signatures among autotrophs (Bouillon, Koedam, Raman & Dehairs, 2002; Vander & Rasmussen, 2001; Overman & Parrish, 2001).

Soil carbon is the last major pool of the carbon cycle. Carbon is fixed by photosynthesis process of plants. It is transferred to the soil via dead plant matter including dead roots and leaves. This dead organic matter is a substrate which is transformed to the atmosphere as carbon dioxide or methane by the respiration of decomposer depending on the availability of oxygen in the soil. Soil carbon can also be oxidized by combustion and returned to the atmosphere as carbon dioxide.

Large carbon stores in mangrove soils occur because carbon deposition in mangrove soils is high. Due to high primary production rates of some mangrove forests and the conspicuous root systems and highly organic soils, it has been proposed that mangrove allocate a large portion of the their fixed carbon to roots (Twilly, Chen & Hargis, 1992; Komiyama, Ogino, Sanit & Sabhasri, 1987).

Soil carbon is primarily composed of biomass and non-biomass carbon sources. Biomass carbon primarily includes various bacteria and fungi. Some of the carbon compounds are easily digested and respired by the microbes resulting in a relatively short residence time in
the soil. Others, like lignin, humic acid or substrate encapsulated in soil aggregates, are very difficult for the biomass to digest and have very long residence times in the soil.

In this study, fishes, crabs, shrimps, molluscs (shells) and mangrove leaves have been surveyed and collected for the clarification of food chain system (webs) at rehabilitating abandoned shrimp pond sites through mangrove planting project. Carbon storage is also quantified at rehabilitating abandoned shrimp pond sites and new mudflat areas through mangrove planting.

2. MATERIALS AND METHOD

2.1 Preparation of food chain study at rehabilitated mangrove forests

All fish samples along canal of planted mangrove, abandoned shrimp ponds and Pak Poon estuary in Nakhon Si Thammarat were caught. 0.8 mm mesh seine net was used for fish sampling. All collected fish samples were dried at 80°C in electric drying oven (Model 2-2050, ISUZU, Tokyo, Japan) for 1 week. After drying, each fish sample was crushed to fine powder by using a pestle and motor. Each fish powder sample was degreased and de-oil by chloroform extraction for stable isotopes analysis.

Stable isotopes $^{13}$C and $^{15}$N in collected fish samples were analyzed by Flash EA1112-DELTA V ADVANTAGE Con Flo IV System of Thermo Fisher Scientific Company Japan as Stable isotope Analysis Mass-spectrometer.

2.2 Soil sampling from mangrove forests

Soil samples were collected from planted mangrove area at abandoned shrimp ponds and new mudflats in Nakhon Si Thammarat province, respectively. Soil samples were collected from surface soil and each 10cm depth from surface to until 1m depth by soil sample stick. All collected soil samples were dried at 105°C in electric drying oven (Model 2-2050, ISUZU, Tokyo, Japan) for 1 week and each soil sample was crushed to powder by using a pestle and motor. Soil samples analyzed the amount of Carbon and Nitrogen in soil by NC analyzer (Automatic High Sensitive NC Analyzer SUMIGRAPH NC-22A, Sumitomo Chemical Co., Osaka, Japan).

3. RESULTS AND DISCUSSION

3.1 Food web at planted mangrove forests

$\delta^{13}$C and $\delta^{15}$N values in sample of fishes, shrimp, crab and shell from canal along planted mangrove area at abandoned shrimp ponds and new mudflat areas in Nakhon Si Thammarat province, Thailand is shown in Figure 1. $\delta^{13}$C and $\delta^{15}$N values in two mangrove species (Rhizophora apiculata and Rhizophora mucronata) are also shown in Figure 1. Table 1 shows information of fish samples. Two species of mangrove plants were used as starting material of key food web in rehabilitated mangrove ecosystem. First of all, carbon is fixed from carbon dioxide in the atmosphere through photosynthesis of mangrove plants. Nitrogen (ammonium ion and nitrate ion as example) is absorbed from soil as nutrients for plant grow. $^{13}$C and $^{15}$N are gradually accumulated in to mangrove tree (trunk, branch, leaves, and roots) as chemical components.
Figure 1: Distribution of $\delta^{13}$C and $\delta^{15}$N values of fish samples and mangrove plants.

Table 1: Information of fish samples.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>English common name</th>
<th>Feeding habits</th>
<th>Forage guilds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambassidae</td>
<td>Ambassas dussumieri</td>
<td>Malaysian Glassy Perchlet</td>
<td>Carnivore</td>
<td>Crustaceans / fish larvae / insects</td>
</tr>
<tr>
<td>Ariidae</td>
<td>Arius maculatus</td>
<td>Spotted catfish</td>
<td>Carnivore</td>
<td>Invertebrates / small fishes</td>
</tr>
<tr>
<td>Atherinidae</td>
<td>Atherinomorus lacunosus</td>
<td>Tropical silverside</td>
<td>Carnivore</td>
<td>Zooplankton</td>
</tr>
<tr>
<td>Eleotridae</td>
<td>Butis holomatoxodon</td>
<td>Mud sleeper</td>
<td>Carnivore</td>
<td>Crustaceans / small fishes</td>
</tr>
<tr>
<td>Mugilidae</td>
<td>Clupea lusitana</td>
<td>Greenback mullet</td>
<td>Carnivore</td>
<td>Zooplankton / zoobenthos / detritus</td>
</tr>
<tr>
<td>Cynoglossidae</td>
<td>Cynoglossus puncticeps</td>
<td>Spotted tongue sole</td>
<td>Carnivore</td>
<td>Benthic invertebrates</td>
</tr>
<tr>
<td>Gerreidae</td>
<td>Gerres filamentosus</td>
<td>Whiptail silverbiddy</td>
<td>Carnivore</td>
<td>Small crustaceans / polychaetes / forams</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>Gobiosoma fasciatum</td>
<td>Tank goby</td>
<td>Carnivore</td>
<td>Small insects / crustaceans / small fish</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>Gobiosoma stellatum</td>
<td>Color croaker</td>
<td>Carnivore</td>
<td>Invertebrates / small fishes</td>
</tr>
<tr>
<td>Lagocephalidae</td>
<td>Lagocephalus deactis</td>
<td>Decon red ponyfish</td>
<td>Carnivore</td>
<td>Small crab / polychaetes / detritus</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>Odonus marmoratus rubrinus</td>
<td>Red eelgoby</td>
<td>Carnivore</td>
<td>Brachi-peduncle / cephalopods / small fishes</td>
</tr>
<tr>
<td>Opilchidae</td>
<td>Pseudopus canrigens</td>
<td>Burrowing snail eel</td>
<td>Carnivore</td>
<td>Prawns / small fish</td>
</tr>
<tr>
<td>Periidae</td>
<td>Pristurus monodon</td>
<td>Time savior</td>
<td>Carnivore</td>
<td>Small crabs / shrimp bivalves and squids</td>
</tr>
<tr>
<td>Scombridae</td>
<td>Rastrelliger kanagura</td>
<td>Indian mackerel</td>
<td>Omnivore</td>
<td>Phytoplankton / zooplankton</td>
</tr>
<tr>
<td>Osteidae</td>
<td>Scomborus laticauda</td>
<td>Oyster</td>
<td>Herbivore</td>
<td>Mainly diatoms / detritus</td>
</tr>
<tr>
<td>Serranidae</td>
<td>Scatophagus argus</td>
<td>Spotted scar</td>
<td>Omnivore</td>
<td>Worms / crustaceans / forams / plant matter</td>
</tr>
<tr>
<td>Percidae</td>
<td>Siluella serrata</td>
<td>Mud cab</td>
<td>Carnivore</td>
<td>Crustaceans / detritus / molluscs / small crabs / fish</td>
</tr>
<tr>
<td>Engraulidae</td>
<td>Septinoga melaenochir</td>
<td>Dusky-hawk anchovy</td>
<td>Carnivore</td>
<td>Insect larvae / small fishes</td>
</tr>
<tr>
<td>Sillaginidae</td>
<td>Sillago sihama</td>
<td>Silver sillago</td>
<td>Carnivore</td>
<td>Polychaetes / small prawns / shrimps / amphipods</td>
</tr>
<tr>
<td>Engraulidae</td>
<td>Stenotomus chacoensis</td>
<td>Anchoa anchovy</td>
<td>Carnivore</td>
<td>Zooplankton</td>
</tr>
<tr>
<td>Tetraodontidae</td>
<td>Tetraodon nigrovittatus</td>
<td>Spotted green pufferfish</td>
<td>Carnivore</td>
<td>Molluscs / crustaceans</td>
</tr>
<tr>
<td>Engraulidae</td>
<td>Thunnus thynnus</td>
<td>Bonito</td>
<td>Carnivore</td>
<td>Prawns / cephalopods / polychaetes / amphipods</td>
</tr>
<tr>
<td>Trichiuridae</td>
<td>Trichiurus lepturus</td>
<td>Largetooth tuna</td>
<td>Carnivore</td>
<td>Euphausiids / zooplankton / crustaceans / fishes / squids</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>Trygonoptera vagina</td>
<td>Burrowing eel</td>
<td>Carnivore</td>
<td>Zoobenthos</td>
</tr>
</tbody>
</table>
δ¹³C values in mangrove plant of *R. apiculata* and *R. mucronata* were -27.200‰ and -26.800‰, respectively. Generally, δ¹³C value of C3 plants indicates from -30 to -25‰. These two mangrove species evidently indicated C3 plant. δ¹³C values of C4 plants indicates from -15 to -10‰. δ¹³C value of CAM plants indicates between C3 plant and C4 plants.

δ¹³C values of *Leiognathus decorus* and *Odontamblyopus rubicundus* were -15.549‰ and -15.704‰, respectively. These values are indicated final consumer in this mangrove planted study site. Secondly, δ¹³C values of *Stolephorus ardhraensis* and *Atherinomorus duodecimalis* were -17.710‰ and -17.855‰, respectively. On the other hand, δ¹³C values of Oystrea (Sacoostrea) *edulis*, *Penaeus monodon* and *Chelon subviridis* were -25.500‰, 24.000‰ and -23.135‰, respectively. δ¹³C of these species was not pretty much concentrated in the fish body.

Generally, an increase 1‰ of δ¹³C in sample indicates one step of food web (chain) in natural ecosystem (Kato et al. 2008). Fallen mangrove leaves and branches are degraded to small molecule organic materials by microbes and these degraded organic materials are consumed by phytoplankton and zooplankton. Furthermore, these phytoplankton and zooplankton are consumed by fishes, crabs, shells, shrimps, sea animals. And ¹³C is gradually transported and accumulated into each step of food linkage. For instance, δ¹³C value of Oystrea (S.) *edulis* was 25.500‰. This is supposed that Oystrea (S.) *edulis* consumes phytoplankton and mangrove leaves as food resources. The result is that Oystrea (S.) *edulis* was an indicator in very low accumulation coefficient of ¹³C in the body. Oystrea (S.) *edulis* is supposed to be a part of the bottom of food chain in this planted mangrove forest area from ¹³C accumulation. Its trophic level is supposed to 1 or 2 steps only of food linkage from mangrove plants.

On the other hand, *L. decorus* of δ¹³C value -15.549‰ and *O. rubicundus* of δ¹³C value -15.704‰ are supposed 12 to 13 steps of food linkage from mangrove plants in this mangrove planted sites. But, *C. subviridis* of δ¹³C value -23.135‰ and *Thryssa hamiltonii* of δ¹³C value -23.437‰ are supposed only 3 to 4 steps of food linkage from mangrove plants. These results mean that mangrove ecosystem at after mangrove planting areas are gradually recovering to natural mangrove ecosystem condition.

δ¹⁵N values indicate the trophic level position of various marine organisms. Generically, higher organisms in the trophic pyramid have accumulated higher levels of δ¹⁵N (means higher δ¹⁵N values in sample) relative to their prey and others before them in the food web. δ¹⁵N values of *R. apiculata* and *R. mucronata* in planted mangrove tree at abandoned shrimp ponds were 3.00‰ and 5.12‰, respectively. Lowest δ¹⁵N value of *O. rubicundus* was 3.416‰. After that, δ¹⁵N values of in *Scatophagus argus*, *C. subviridis* and Restrelliger kanagurta were 5.89‰, 7.82‰ and 7.845‰, respectively. On the other hand, the highest δ¹⁵N value indicated that *L. decorus* and *Sillago sihama* were 12.898‰ and 12.187‰, respectively.

Numerous studies on marine ecosystems have shown that on average there is a 3.2‰ enrichment of δ¹⁵N vs. diet between different trophic level species in ecosystems. But, these δ¹⁵N data from this study did not relate to 3.2‰ enrichment of δ¹⁵N vs. diet between different trophic level species in ecosystems. We could not reach food web (chain) linkage from relationship between accumulation of δ¹⁵N and δ¹³C into fishes.

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δ¹³C and δ¹⁵N values of *L. decorus* were -15.549‰ and 12.898‰. δ¹³C and δ¹⁵N values of *O. rubicundus* were -15.704‰ and 3.416‰. δ¹³C value of *L. decorus* and *O. rubicundus* was similar value. It is supposed that these both fishes have strong decarboxylation enzyme in the fish body. On the other hand, δ¹⁵N value of *L. decorus* and *O. rubicundus* was much different value by around 4 times higher. Maybe, physiological (metabolism in fish) process of *O. rubicundus* and *L. decorus* are much different. Deamination enzyme of *O. rubicundus* abounds in fish. This resulted in Nitrogen including organic compounds degraded to ammonium, nitrate and nitrite ions, nitrogen gas through very strong physiological processes and all these produced decomposed compounds (metabolites) are excreted (discharge) fluid (waste) to outside of fish body. On the other hand, physiological (metabolism in fish) process of *L. decorus* is not strong and nitrogen including organic compounds is gradually accumulated in fish body with ¹³C (Kuramoto & Minagawa, 2001; Chikaraishi, Kashiyama, Ogawa & Ohkouchi, 2007; Sugisaki et al., 2013).

### 3.2 Carbon accumulation in the mangrove planted soil

Forest soils constitute a large pool of carbon and releases of carbon from pedosphere, caused by anthropogenic activities such as deforestation, may significantly increase the concentration of carbon dioxide (GHG) into the atmosphere. The request of UNFCCC is that countries must estimate and report GHG emissions and removals, including changes in carbon stocks in all five pools (above- and belowground biomass, dead wood, litter and soil carbon) and associated emissions and removals from land use, land-use change and forestry activities according to the Intergovernmental Panel on Climate Change’s good practice guidance (Nellemann et al., 2009).

Soil carbon is the terminative major pool of the carbon cycle. The carbon that is fixed as biomass by photosynthesis of plants is transferred to the soil via dead plant matter including dead roots, leaves as and fruiting bodies as litters. This dead organic matter creates a substrate which decomposers (consumers) respire back to the atmosphere as carbon dioxide or methane depending on the availability of oxygen in the soil and reduction condition of soil. Soil carbon can also be oxidized by combustion and returned to the atmosphere as carbon dioxide.

Some of the carbon compounds are easily digested and respired by the microbes resulting in a relatively short residence time in the pedosphere. Others, like lignin, humic acid or substrate encapsulated in soil aggregates, are very difficult for the biomass to digest and have very long residence times as carbon in the pedosphere.
Carbon concentration certainly increased at abandoned shrimp pond mangrove planted sites. Carbon concentration of abandoned shrimp pond soil (not planted) indicated 0.77% at 0 to 10 cm depth, 0.56% at 41 to 50 cm and 0.63% at 91 to 100 cm, respectively. Carbon concentration of mangrove planted site soil showed higher than unplanted mangrove in abandoned shrimp pond. Especially, carbon amount of soil depth 0 to 10 cm was higher than other soil depth at abandoned shrimp pond mangrove planted in 1998. Figure 2 shows very high carbon concentration in soil depth of 0 – 10 cm and 11 -20 cm. And carbon is slowly moving (transferring) to deep soil layer.

![Figure 2: Carbon concentration of 1998 mangrove planted abandoned shrimp pond soil](image)

Carbon certainly increased mangrove planted along canal side in 2000 and new mudflat mangrove planted sites of Pak Poon. This data indicates that especially, carbon is certainly accumulating into pedosphere. Carbon amount at new mudflat mangrove planted sites of Pak Poon area in 2008 and 2009 did not have so much change from soil surface to 1 m soil depth.
This is because of this mangrove planted sites are receiving regular ebb and flow of sea water in a day. At diverse time, these new mudflats are hit by abnormal high sea water flood tide (abnormal sea water level and retaining period for two weeks more in April, 2012) and very large quantity soil (including debris) is transferred to this new mudflat mangrove planted sites from other areas. Carbon is gradually accumulating into mangrove planted soil by mangrove litters. This result indicates that mangrove planting is very important for sequestration of carbon dioxide as “blue carbon” at salt marsh areas from atmosphere tree can store carbon as biomass (tree trunk, branch, roots and leaves) for several decades (Nellemann et al., 2009).

4. CONCLUSIONS

Stable isotope study of $^{13}$C and $^{15}$N in fish samples is very helpful skill for food web (chain) research of mangrove ecosystem. Especially, $\delta^{13}$C value is very important for comprehension (understanding) of food web (chain) at mangrove ecosystems. Of course, $\delta^{15}$N value is very important for comprehension (understanding) of food web (chain) at mangrove ecosystems. We need more circumstantial discussion of $^{15}$N behavior in fish sample at mangrove ecosystems. Mangrove planting is very important for sequestration of carbon dioxide from atmosphere and mangrove tree can store carbon as biomass (tree trunk, branch, roots and leaves).

5. ACKNOWLEDGEMENTS

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