Rehabilitation of Abandoned Shrimp Ponds through Mangrove Planting at Nakhon Si Thammarat, Southern Thailand: Investigation of a Food Chain System at a Newly Developed Mangrove Ecosystem

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ABSTRACT

The complete food chain system of mangrove plantations on shrimp ponds sites were studied using the stable nitrogen (¹⁵N) and carbon isotopes (¹³C) to complete food chain (web) system studies of mangrove plantations. The analyzed data clearly indicates that heavy nitrogen (¹⁵N) was gradually accumulated during each stage of the food chain system and finally in large fishes. On the other hand, heavy carbon (¹³C) increased only slightly during each stage of the food chain system. The δ¹⁵N values for carnivores were much higher than those of herbivores and omnivores. Carnivores consume nitrogen accumulated fishes and animals as their feed sources. Finally these carnivores gradually accumulate nitrogen in protein forms in their bodies from their metabolic activities. Herbivores eat only plants, whereas omnivores consume both plants and animals (including fishes). Usually, the δ¹⁵N values of herbivores and omnivores are low. This promising data truly represents the food chain system occurring in a natural marine ecosystem. The above analyzed data suggests that carnivorous fishes are at least 4 to 5 steps from the mangrove leaves. Mangrove forests can contribute to the reduction of greenhouse gases acting as a carbon sink and a rich biodiversity ecosystem.

Keywords: Mangrove planting, abandoned shrimp pond, food chain, carbon sink
INTRODUCTION

Forests have many and varied functions. Forests not only provide industrial timber and fuel, but are also increasingly critical in the preservation of national lands, the conservation of water resources, and human health, culture and education. Above all, mangrove forests are a unique ecosystem [1,2]. They are very important for subtropical and tropical people and the earth’s environment [3]. Mangrove forests cover approximately 18 million ha and are distributed at the river mouths and coastal areas of subtropical and tropical regions of the world [4]. The total area of abandoned shrimp ponds are between 24,000 and 32,000 ha in Thailand [5]. Abandoned shrimp ponds at Pak Phanang, Nakhon Si Thammarat occupy an area of approximately 4,000 ha. We have planted about 5 million mangrove plants (1,000 ha) at abandoned shrimp ponds since 1998 in Nakhon Si Thammarat, southern Thailand [6]. The planted mangrove plants are well grown. The amount of biomass of the planted mangrove trees has increased year by year. Many fishermen informed us that after the plantation of mangrove plants at the shrimp ponds sites there has been an increase in the population of species like crabs, shells, shrimps and fishes. Fishermen catching these fish from these mangrove planted ponds regularly send them to the city markets. Mangrove planted sites on shrimp ponds have already attained a significantly sustainable effect on the marine ecosystem.

Generally, food webs in nature have multiple, reticulate connections between a diversity of consumers and resources [7,8]. Despite years of research on the habitat use and feeding ecology of many abundant estuarine animals, we still do not know which autotrophs form the base of food webs that support high levels of secondary productivity in estuaries [9]. Traditional approaches to food web analysis include gut content analysis, direct observation in the field and the laboratory. Results of gut content analysis indicate feeding behavior and can indicate potential food sources, but they do not provide information about assimilation of food or necessarily identify the primary producers supporting consumers [10].

The stable isotope ratios of carbon ($^{13}$C/$^{12}$C) and nitrogen ($^{15}$N/$^{14}$N) have proven useful as tracers of organic matter sources and food web structure in a variety of aquatic systems [11-13], because unlike gut content analysis, they are directly related to assimilation [10]. This ratio, the stable isotope signature, is taken on by consumers and reflected in their tissues at whatever trophic level they occur [12]. 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 [9].

Environmental variation in the carbon isotope signatures of autotrophs is primarily affected by ratios of demand versus supply of carbon, differences in the photosynthetic modes of autotrophs and source of inorganic carbon. If supply of carbon in the form of CO$_2$ is high compared with demand, RUBISCO, the carbon fixing enzyme in photosynthesis will discriminate against $^{13}$C. Plants with C3 photosynthesis (including mangroves) discriminate strongly against $^{13}$C resulting in a relatively low
δ¹³C value of approximately −27% [14,15]. Plants with C4 pathways sequester carbon at high concentrations in the bundle sheath cells as little of this CO₂ leaks out. Thus RUBISCO, which processes CO₂ in the bundle sheath cells, has little opportunity to discriminate against ¹³C. As a result, C4 plants (e.g. salt marsh grass, corn, etc.) do not discriminate as strongly against ¹³C and have a relatively high δ¹³C value of approximately −13% [9,16].

The stable nitrogen isotope is also used to elucidate food webs, but their use is further complicated by trophic fractionation. Trophic fractionation of nitrogen occurs when physiological processes discriminate against an isotope. When a heterotroph consumes an autotroph or another heterotroph, ¹⁴N is preferentially excreted [17]. This results in consumers at higher trophic levels having enriched δ¹⁵N signatures. Peterson and Fry [17] reported that described fractionation of δ¹⁵N is 3 for each trophic level. This value has been used to assign trophic levels to consumers when the autotrophs supplying them were known. Fractionation of 3 is a mean about which there is considerable variation [18]. Fractionation of nitrogen, like that of carbon, is affected by supply. Consumers with limited nitrogen fractionate less. Levels of fractionation in consumers have been shown to be affected by starvation [19], age [20] and food quality [21].

In this study, we have surveyed and collected fishes, crabs, shrimps, molluses (shells) and mangrove leaves for the clarification of the food chain system (webs) at rehabilitating abandoned shrimp pond sites through a mangrove planting project named the “Green Carpet Project” [22]. Many scientists suggested that mangrove forests have a very good food chain system from mangrove plants to fishes [23-25]. The fallen mangrove leaves are firstly decomposed by microorganisms and these decomposed materials are mainly used by phytoplanktons and zooplanktons. These phytoplanktons serve as a food for crabs, shells, shrimps, finally to fishes and also to human beings. But, the previous studies on the detailed food chain system of mangrove plants are not detailed enough.

In order to get more detailed results, the complete food chain system of mangrove plantations on abandoned shrimp ponds sites were studied by nitrogen and carbon stable isotopes analysis (δ¹⁵N and δ¹³C) in fish samples and mangrove leaves [26].
MATERIALS AND METHODS

Sample Collection and Preparation

All samples used in this study were collected from Green Carpet Project sites at Nakhon Si Thammarat, southern Thailand. In total 6 species of fish, 2 species of crab, 1 species of shrimp, 2 species of shell and 2 species of mangrove plant were collected for $\delta^{15}$N and $\delta^{13}$C analysis at No. 34 shrimp pond planted in 1998. Twenty pieces of mature stage leaves for each species of mangrove were collected. In the field, measurements of $\delta^{15}$N and $\delta^{13}$C were taken from 10 matured green leaves of *Rhizophora apiculata* and *R. mucronata*. After collection these samples were dried under sunlight for a week at Nakhon Si Thammarat and kept in a drying oven at 90 °C for a week in the laboratory to achieve complete dryness (to constant weight) and finally powdered by a Wonder blender (Osaka Chemical Co.). These powdered samples were kept in desiccators in the presence of silica gel until further analysis.

Measurement of Stable Isotope

The ratios of $^{15}$N/$^{14}$N and $^{13}$C/$^{12}$C were expressed as the relative percentage difference between the samples and conventional standards, i.e. PDB (Pee Dee Belmnite) limestone carbonate for carbon [27] and atmospheric N$_2$ for nitrogen [28] as $\delta$ values, defined as:

$$\delta R = \left(\frac{X_{\text{sample}}-X_{\text{standard}}}{X_{\text{standard}}}\right) \times 10^3 \text{ [%]}$$

where $R = ^{13}$C or $^{15}$N, and $X = ^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N.

The levels of the nitrogen stable isotope of these powdered samples were analyzed using a Mass Spectrometer DELTAPLUSXL (Thermo Finnigan Inc.).

RESULTS AND DISCUSSION

The analysis results of the samples are shown in Table 1. We selected and planted 2 species of mangrove plant at the abandoned shrimp pond sites. One species is *R. apiculata* and other one is *R. mucronata*. We did not correct for natural mangrove leaves in the sample from the mangrove forest of Nakhon Si Thammarat in this study, because the mangrove forest of Nakhon Si Thammarat was planted around 20 years ago.
Table 1 δ^{15}N and δ^{15}C values of mangrove leaves and fishes at mangrove planting sites of Nakhon Si Thammarat, Thailand.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thai name</th>
<th>English name</th>
<th>Scientific name</th>
<th>Feeding type</th>
<th>δ^{15}N (%)</th>
<th>δ^{15}C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>Pla kluo (Big)</td>
<td>Four finger threadfin</td>
<td>Eleutheronema tetradactylum</td>
<td>Carnivore (Benthic feeder)</td>
<td>11.00</td>
<td>−16.0</td>
</tr>
<tr>
<td></td>
<td>Pla kluo (Small)</td>
<td>Four finger threadfin</td>
<td>Eleutheronema tetradactylum</td>
<td>Carnivore (Benthic feeder)</td>
<td>13.10</td>
<td>−21.6</td>
</tr>
<tr>
<td></td>
<td>Pla kluo</td>
<td>Four finger threadfin</td>
<td>Eleutheronema tetradactylum</td>
<td>Carnivore (Benthic feeder)</td>
<td>12.30</td>
<td>−20.7</td>
</tr>
<tr>
<td></td>
<td>Pla nil</td>
<td>Nile tilapia</td>
<td>Oreochromis niloticus</td>
<td>Herbivore</td>
<td>10.00</td>
<td>−25.9</td>
</tr>
<tr>
<td></td>
<td>Pla tagrub</td>
<td>Spotted scat</td>
<td>Scaphognathus argus</td>
<td>Omnivore</td>
<td>9.72</td>
<td>−23.1</td>
</tr>
<tr>
<td></td>
<td>Pla kluo koa</td>
<td>White sardine or Scalloped perchlet</td>
<td>Escualose choracata or Ambassis natalis</td>
<td>Carnivore (Zoo plankton feeder or Benthic feeder)</td>
<td>11.40</td>
<td>−23.3</td>
</tr>
<tr>
<td></td>
<td>Pla jaud</td>
<td>Belanger croaker</td>
<td>Johnius belangeri</td>
<td>Carnivore (Benthic feeder)</td>
<td>13.20</td>
<td>−22.4</td>
</tr>
<tr>
<td>Crab</td>
<td>Pu thale</td>
<td>Mud crab</td>
<td>Scylla serrata</td>
<td>Carnivore</td>
<td>9.91</td>
<td>−20.4</td>
</tr>
<tr>
<td></td>
<td>Pu ma</td>
<td>Swimming crab</td>
<td>Portunus pelagicus</td>
<td>Carnivore</td>
<td>10.00</td>
<td>−20.3</td>
</tr>
<tr>
<td>Shrimp</td>
<td>Kung ku la dam</td>
<td>Tiger prawn</td>
<td>Penaeus monodon</td>
<td>Detritivore</td>
<td>10.70</td>
<td>−24.0</td>
</tr>
<tr>
<td></td>
<td>Hoy nang rom</td>
<td>Oyster</td>
<td>Sacoostrea edulis</td>
<td>Herbivore (Filter feeder)</td>
<td>6.72</td>
<td>−25.5</td>
</tr>
<tr>
<td>Shrimp</td>
<td>Hoy song fa*</td>
<td>Oyster</td>
<td>Sacoostrea edulis</td>
<td>Herbivore (Filter feeder)</td>
<td>9.35</td>
<td>−19.9</td>
</tr>
<tr>
<td>Mangrove leaves</td>
<td>Hoy song fa*</td>
<td>Oyster</td>
<td>Rhizophora apiculata</td>
<td>Detritivore</td>
<td>3.00</td>
<td>−27.2</td>
</tr>
<tr>
<td></td>
<td>Rhizophora mucronata</td>
<td>Detritivore</td>
<td></td>
<td>5.12</td>
<td>−26.8</td>
<td></td>
</tr>
</tbody>
</table>

Hoy song fa*: no English name or Scientific name.

The δ^{15}N Values of Mangrove and Accumulation of Nitrogen into Fishes

The δ^{15}N value of Rhizophora apiculata and R. mucronata leaves were found to be 3.00 % and 5.12 %, respectively. The δ^{15}N value of R. mucronata was a little bit higher. If the plants grow under natural conditions, the δ^{15}N values of plants are usually a little smaller. The δ^{15}N values of R. apiculata and R. mucronata samples of Ranong were reported to be 2.3 % and 1.32 %, respectively [29]. This R. apiculata and R. mucronata of Ranong was collected from a tidal area (natural forest). However, the Green Carpet Project site is non-tidal. Ponds water is changed every 3 to 4 weeks by the owner of the ponds. As a result of the regular changes in the water of the ponds, heavy nitrogen ^15N may not be accumulated fast enough in the growing mangrove plants and thus the δ^{15}N value of R. apiculata will be small. As an example, the δ^{15}N value of R. apiculata and R. mucronata leaves of other abandoned shrimp ponds were found to be 5.58 % and 8.20 %, respectively. These δ^{15}N values were dependent on the growing place, water quality and soil conditions.
The δ¹⁵N values of Sacoostrea edulis (Oyster) and Pu ma (Thai name, crab) were 6.72 and 4.96 %, respectively. It is suggested that Sacoostrea edulis and Pu ma are the first stage (starting) of the food chain at this rehabilitating mangrove plantation. On the other hand, the δ¹⁵N values of Scylla serrata (Mud crab), Portunus pelagicus (Swimming crab) were 9.91 and 10.0 %, respectively. These 2 crabs are carnivores. This data suggests that these 2 crabs serve as a feed for small fishes and shells which accumulate heavy nitrogen in their bodies. We have to identify the scientific name and feeding characteristics of Pu ma. The δ¹⁵N value of Hoy song fa (Thai name, shell) of molluscs is 9.35 %. The corresponding value is higher than in Sacoostrea edulis. It indicates that Hoy song fa is a filter feeder herbivore and is not a herbivore directly involved in the higher stage of the food chain under natural conditions.

The δ¹⁵N value of Penaeus monodon (Tiger prawn) was 10.7 %. The feeding type of this Tiger prawn is classified as detritivore. This detritivore group feeds on organic sediment or accumulated materials, the dead bodies of planktons and others. These detritus usually contains high amount of nitrogen in the organic accumulated materials. The observed value of 10.7 % for Penaeus monodon indicates that the process may be least 3 or 4 steps from mangrove leaves. Mangrove leaves were decomposed by microorganisms and decomposed organic materials were fed on by phytoplanktons and zooplanktons. In their turn, the zooplanktons were food for the fishes (including shrimps).

The δ¹⁵N value of Eleutheronema tetradactylum (Four finger threadfin), Oreochromis niloticus (Nile tilapia), Scatophagus argus (Spotted scat), Escualose choracata or Ambassis nalua (White sardine or Scalloped perchlet) and Johnius belangerii (Belanger croaker) were 12.3, 10.0, 9.72, 11.4 and 13.2 %, respectively. Eleutheronema tetradactylum, Escualose choracata or Ambassis nalua and Johnius belangerii are classified as carnivores and the δ¹⁵N values of these 3 fishes are higher than herbivores and omnivores. The supposed food chain system at mangroves planted in abandoned shrimp pond is shown in Figure 1. As expected, Oreochromis niloticus which is a herbivore and Scatophagus argus which is an omnivore have lower δ¹⁵N values than carnivorous fishes. This data truly represents the food chain system in a natural marine ecosystem. The above analyzed data suggests that carnivorous fishes are at least 4 to 5 steps from mangrove leaves. Circulation of nitrogen (ammonium, nitrate and nitrite ions) of mangroves and salt marshes was reported [30]. Figure 1 shows clearly the accumulation stages of heavy nitrogen and heavy carbon from mangrove plants to large fishes through the food chain at the rehabilitated abandoned shrimp ponds.
The $\delta^{13}C$ Values of Mangrove and Fishes

The analytical results for $\delta^{13}C$ in the same samples are presented in Table 1. In this study, the $\delta^{13}C$ of Rhizophora apiculata and R. mucronata leaves were found to be $-27.2$ and $-26.8$ %, respectively. The $\delta^{13}C$ values of Rhizophora apiculata samples in Ranong were reported to be $-28.2$ and $-27.0$ %, respectively [29]. Previous studies have reported that $\delta^{13}C$ values of mangrove plants vary between $-33$ and $-24$ % in southern Florida and Guadeloupe, French West Indies [31,32]. Hayase and co-workers also reported stable isotope studies in the Matang mangrove forest in Malaysia with $\delta^{13}C$ values of mangrove leaves ranging from $-28.7$ to $-26.7$ % [33].

It was speculated that C4 plants are widely distributed in tropical areas of the world. We expected that C4 plants would contribute to organic matter more in this study. However, the average $\delta^{13}C$ values of the land plants were $-28.2$ %, suggesting only a little contribution of C4 plants at the rehabilitated abandoned shrimp ponds. Generally, the $\delta^{13}C$ values of C4 plants are around $-11$ %.

The $^{13}C$ values of shell fish (Hoy nang rom), shrimp (Kung ku la dam), crabs (Pu tha le, Pu ma) and fishes are gradually accumulated in their bodies within the food chain. The plot shows the distribution of $\delta^{15}N$ and $\delta^{13}C$ in the collected samples.
chain process. This may suggest that heavy carbon in organic matter increases in pond water and this organic matter is eaten by consumers and predators. Tropical mangrove swamps are known to cause high sedimentation rates in the surrounding areas, due to their high production rates and consequent accumulation of organic carbon. Rehabilitated abandoned shrimp ponds were constructed from natural mangrove forests. We can suppose that the original soil of the ponds is composed of naturally accumulated organic matter (including degraded organic matter of C4 plants or light carbon $^{12}$C of organic matters already degraded by microorganisms and released as carbon dioxide) in the soil. As a result, fishes higher in the food chain would be expected to have a little bit higher $^{13}$C values than mangrove plants.

**Food Chain System of Rehabilitated Mangrove Forests**

Figure 2 shows a proposed food chain system at the rehabilitated mangrove planting areas. Heavy nitrogen $^{15}$N concentration at each step gradually increases from the mangroves to the fishes. The $\delta^{15}$N values of mangrove plants are found to be only 3.00 and 5.12 %. The $\delta^{15}$N value for the final stage of the food chain in this rehabilitated mangrove forest are 13.1 % in Pla ka luo (Four finger threadfin, *Eleutheronema tetradaactylum*) and 13.2 % in Pla jaud (Belanger croaker, *Johnius belangerii*), respectively. We propose that the mangrove plants are the origin (start) of this food chain system. Almost all natural ecosystems are supported by the grazing food chain and microbial loop. The grazing food chain is connected from phytoplankton to zooplankton, consumers and predators. The microbial loop by bacteria is also very important for the utilization of dissolved organic matters and granular organic matters. These two are necessary systems for the mangrove ecosystem.

If a wide survey of fishes from Pak Phanang Bay in Nakhon Si Thammarat was undertaken, we would be able to make a more detailed and clear food chain process of the mangrove ecosystem in southern Thailand.
CONCLUSIONS

The $\delta^{13}C$ and $\delta^{15}N$ of biogeochemical samples from rehabilitated abandoned shrimp of Nakhon Si Thammarat, southern Thailand. From the above analysis we conclude that mangrove plants serve as very important food sources in the food chain system in the marine ecosystem. Figure 1 shows the trophic position model of food webs on our isotope-based estimates of trophic position. Figure 2 shows a proposed food chain system at the rehabilitated abandoned shrimp ponds. We observed the cycle takes place in at least 4 stages. At first, fallen mangrove leaves are decomposed by microorganisms, and decomposed materials are used by phytoplanktons and zooplanktons, and finally the fourth stage is that both these planktons are eaten by shells, crabs, and fishes. The obtained analysis data clearly indicates that heavy nitrogen ($^{15}N$) and carbon ($^{13}C$) are gradually accumulated at each stage of the food chain system and finally in large fishes (final stage of fish classification).
ACKNOWLEDGEMENTS

We especially thank KNCF (KEIDANREN Nature Conservation Fund, Tokyo, Japan) for 10 years of excellent support to the “Green Carpet Project at Nakhon Si Thammarat” in southern Thailand. We also thank the special coordination by Mr. Junichi Hagiya of REAGMAN (Research Association for Global Mangrove, Tokyo, Japan).

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MANGROVE PLANTING AT ABANDONED SHRIMP PONDS

บทคัดย่อ

ชิเกะ คาโตะ 1 สงบ พานิชชาติ 2 เศวตฉัตร บุญมิ่ง 2 วิโรจน์ธีรธนาธร 3 โนริโกะ ไซโตะ 1 โตชิโนริ โคจิมา 1 ทาชิมัตซู 3

การฟื้นฟูดาเนสชันลักษณะของการปลูกป่าชายเลนที่ถูกละทิ้งโดยการปลูกป่าชายเลนที่จังหวัดนครศรีธรรมราช ได้ศึกษาระบบห่วงโซ่อินทรีย์ของระบบนิเวศป่าชายเลน

ได้ศึกษาการทำการปลูกป่าชายเลนที่ถูกละทิ้งโดยการปลูกป่าชายเลนที่จังหวัดนครศรีธรรมราช โดยอาศัยการใช้ไอโซโทปของไนโตรเจน (15N) และคาร์บอน (13C) ข้อมูลที่วิเคราะห์ระบุว่าในไนโตรเจน (15N) สะสมมากขึ้นในแต่ละขั้นของระบบที่รับใช้การปลูกป่าชายเลนที่สุดในป่าชายเลนที่เกิดขึ้นใหม่ ส่วนคาร์บอน (13C) เพิ่มขึ้นเพียงเล็กน้อยในแต่ละขั้นตอนของระบบที่รับใช้การปลูกป่าชายเลนที่เกิดขึ้นใหม่ ของสัตว์กินเนื้อได้สูงกว่าในสัตว์กินพืชและสัตว์ที่กินทั้งสัตว์และพืช สัตว์กินเนื้อจะบริโภคโปรตีนและสัตว์ที่สะสมไนโตรเจนสัตว์กินเนื้อ อีกทั้งยังสะสมไนโตรเจนในร่างกายเพื่อให้ได้จากการปลูกป่าชายเลนที่เกิดขึ้นใหม่ สัตว์กินเนื้อสามารถสะสมไนโตรเจนในร่างกายมากกว่าสัตว์กินพืช ซึ่งมีผลต่อการสะสมไนโตรเจนในร่างกายของสัตว์กินเนื้อ ที่เกิดขึ้นใหม่ที่จะมีมูลค่าต่ำกว่าในสัตว์กินพืชและสัตว์ที่กินทั้งสัตว์และพืช 4 - 5 ขั้น ป่าชายเลนสามารถลดก๊าซเรือนกระจกได้มากกว่าสัตว์กินเนื้อ

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