

Mangrove Plantation at Abandoned Shrimp Ponds and New Mud-flat Areas for Restoration of Biodiversity and Climate Change Countermeasure

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Abstract

Mangrove forests are vital for both local communities and the global environment. Thriving in 128 tropical and sub-tropical countries, these ecosystems offer a wealth of benefits, supporting coastal livelihoods and well-being. Unfortunately, mangrove forests are facing significant decline worldwide. Recognized as crucial for sustainable development, mangroves play a pivotal role in achieving the 2030 Agenda goals.

Mangrove ecosystem studies have traditionally focused on the biodiversity of species such as mangrove trees, mudskippers, and commercially valuable organisms like mud crabs, mangrove crabs, cockles, sea catfish, and prawns. However, there has been a growing emphasis on understanding the benthic invertebrate community within these ecosystems.

Mangrove ecosystems serve as critical nurseries for juvenile fish and are essential for animal protein production. Many tropical fish species rely on these coastal forests during their life cycles. Pak Phanang Bay, renowned for its diverse marine life, supports a significant local economy. This study employed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope analysis of 47 fish species, 5 shell species, 5 crab species, 5 shrimp species, 2 squid species, an octopus, and 3 species of mangrove plant to investigate the bay's food web. *Rachycentron canadum* and *Acanthosquilla multifasciata* exhibited the highest trophic levels based on their $\delta^{13}\text{C}$ values. While *Rhizophora mucronata* and *R. canadum* showed a $\delta^{15}\text{N}$ difference of 4.6‰, traditional trophic level estimation based on $\delta^{15}\text{N}$ enrichment was inconclusive due to potential nitrogen loss in fish and shellfish tissues. Our findings indicate that mangroves form the foundation of the Pak Phanang Bay food web, supporting a complex trophic structure that progresses from herbivores to carnivores.

Keywords: Mangrove Forests, Food Chain, Stable Isotope

Introduction

Mangrove forests typically occur in intertidal regions of tropical and subtropical areas worldwide. Their northernmost distribution is Okinawa, Japan, while the southern limit encompasses Australia, New Zealand, and South Africa's east coast. Mangrove forests establish along estuaries, river deltas, continental

margins, and islands. They thrive within the tidal zone between mean sea level and mean high water spring tide level.

Mangrove ecosystems support a diverse array of both terrestrial (e.g., crabs, mollusks) and aquatic (e.g., root fouling organisms, fish) fauna. These ecosystems are characterized by a complex

Mangrove ecosystems offer a wealth of benefits to local communities, providing essential resources such as food and building materials, as well as crucial protection from coastal hazards [2]. To fully comprehend the value of these ecosystems, it is imperative to investigate entire food webs, considering the intricate relationships among species, their habitats, and the surrounding environment.

Natural food webs exhibit complex, interconnected relationships among diverse consumers and resources. Mangrove ecosystems are particularly important as nurseries for numerous fish species, many of which rely on these coastal forests for survival. Despite extensive research on estuarine organisms, the precise base of food webs supporting high productivity remains elusive. Traditional methods like gut content analysis and field observation have limitations. Stable isotope analysis offers a valuable alternative by tracing carbon and nitrogen flow through ecosystems. This technique provides insights into the dietary habits of organisms and the underlying food web structure over time.

comparing isotope ratios between producers and consumers. Effective application of this method requires distinct isotopic differences among primary producers.

Food chain length, a continuous variable, quantifies energy transfer and reflects the complexity of an ecosystem's structure. In mangrove ecosystems, it spans from primary producers, such as mangrove plants, to top predators. By measuring this length, we can assess the ecosystem's trophic organization.

Understanding the dynamics of fishery resources, such as population size, distribution, and migration, is crucial in fisheries management. The metabolic processes of digestion, absorption, and tissue growth, common to all organisms, involve isotopic fractionation. This phenomenon results in distinct stable isotope ratios between organisms and their food sources.

This study aims to determine carbon (^{13}C) and nitrogen (^{15}N) stable isotope ratios in key aquatic organisms, particularly fish, within the mangrove ecosystem of Pak Phanang Bay, Nakhon Si Thammarat, Southern Thailand. To elucidate food chain dynamics, we will analyze fish, shrimp, crabs, shells, and mangrove leaves from rehabilitated abandoned shrimp ponds and newly established mudflat areas.

Sampling Location and Stable Isotopes Analysis of Sample Preparation

The study was conducted in Pak Phanang Bay, Nakhon Si Thammarat, Thailand, focusing on areas within the "Green Carpet" mangrove restoration project. Initiated in 1997, this project has reclaimed approximately 1,500 hectares of degraded mangrove forest and former shrimp ponds (Figure 1). Pak Phanang Bay, renowned for its rich biodiversity and community-based conservation efforts, offers a unique environment shaped by ocean currents, sediment deposition, and a long history of sustainable resource management.



With the assistance of local fishermen, samples were collected from Pak Phanang Bay, encompassing 47 fish species, 5 shellfish species, 5 crab species, 5 shrimp species, 1 octopus, 2 squid, and 3 mangrove plant varieties. Collected samples were dried at 100°C for one week using an EO-700V drying oven (ASONE Co., Osaka, Japan), pulverized into a fine powder using a Wonder blender (Osaka Chemical Co., Japan), and stored in desiccators. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analyses were performed on these samples, including three mangrove species (*Rhizophora mucronata*, *R. apiculata*, and their hybrid) as primary producers.

Analysis of Stable Isotopes in Samples

Both stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in collected samples were analyzed by using Stable Isotope Mass Spectrometer (Flash EA1112-DELTA V ADVANTAGE ConFlo IV System, Thermo Fisher Scientific Japan).

The ratios of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ were expressed as the relative percentage difference between the samples and conventional standards, i.e. PDB (Pee Dee Belmnite) limestone carbonate for carbon and atmospheric N_2 for nitrogen as δ values, defined as [4, 5]:

$$\delta R = [(X_{\text{sample}} - X_{\text{standard}}) / X_{\text{standard}}] \times 10^3 [\text{‰}]$$

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

Results and Discussion

Table 1 presents stable isotope data ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and dietary classifications (carnivore, detritivore, omnivore, herbivore) for 47 fish, 5 shellfish, 5 crabs, 5 shrimp, 1 octopus, 2 squid, and 3 mangrove plants collected from the Green Carpet project and Pak Phanang Bay. A trophic structure became evident, with mangrove plants serving as the foundation, followed sequentially by herbivores, detritivores, omnivores, and carnivores. The prevalence of carnivorous species indicates a robust lower trophic level consumer base.

Table 1: Stable Isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Fishes, Crabs, Shellfishes and Mangrove Plants in Nakhon Si Thammarat

Sample	Scientific name	Feeding type	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Fish	<i>Alectis indica</i>	Carnivore	-16.0	10.3
Fish	<i>Ambassis nalus</i>	Carnivore	-23.2	11.4
Fish	<i>Brevitrygon imbricate</i>	Carnivore	-16.1	10.3
Fish	<i>Congresox talabon</i>	Carnivore	-17.6	12.1
Fish	<i>Coryphenoides sp.</i>	Carnivore	-16.6	11.2
Fish	<i>Eleutheronema tetradactylum</i>	Carnivore	-16.7	9.91
Fish	<i>Escualose choracata</i>	Carnivore	-23.2	11.4
Fish	<i>Gazza minuta</i>	Carnivore	-15.4	9.93
Fish	<i>Johnis belangerii</i>	Carnivore	-22.4	13.2
Fish	<i>Muraenesox cinereus</i>	Carnivore	-17.2	11.3
Fish	<i>Nemipterus peronei</i>	Carnivore	-15.3	10.8
Fish	<i>Opisthopterus tardoore</i>	Carnivore	-17.1	11.1
Fish	<i>Parastromateus niger</i>	Carnivore	-17.6	11.2
Fish	<i>Pennahia pawak</i>	Carnivore	-16.4	10.7
Fish	<i>Platycephalus indicus</i>	Carnivore	-16.2	11.2
Fish	<i>Portmus pelagicus</i>	Carnivore	-20.4	10.0
Fish	<i>Pseudorhombus malayanus</i>	Carnivore	-15.7	11.1
Fish	<i>Scomberoides lysan</i>	Carnivore	-17.8	10.9
Fish	<i>Scylla serrata</i>	Carnivore	-20.6	9.91
Fish	<i>Sphyaena putnamae</i>	Carnivore	-20.7	12.1
Fish	<i>Rachycentron canadum</i>	Carnivore	-14.2	10.6
Fish	<i>Taenioides cirratus</i>	Carnivore	-17.4	10.2
Fish	<i>Trichirus lepturus</i>	Carnivore	-17.1	11.8
Crab	<i>Leucosiidae sp.</i>	Carnivore	-16.1	7.48
Shrimp	<i>Acanthosquilla multifasciate</i>	Carnivore	-14.3	9.45
Shrimp	<i>Odontodactylus scyllarus</i>	Carnivore	-17.9	9.44
Squid	<i>Uroteuthis duvancelii</i>	Carnivore	-17.9	11.1
Fish	<i>Cynoglossus cynoglossus</i>	Detritivore	-18.9	11.5
Fish	<i>Gerres filamentosus</i>	Detritivore	-14.9	8.88
Fish	<i>Laeops guentheri</i>	Detritivore	-16.4	10.9
Fish	<i>Osteomugil cunnesius</i>	Detritivore	-17.7	10.8

Crab	<i>Uca arcuata</i>	Detritivore	-11.9	10.5
Shell	<i>Cerithidae cingulate</i>	Detritivore	-18.3	10.3
Shrimp	<i>Macrobrachium sp.</i>	Detritivore	-20.9	10.2
Shrimp	<i>Penaeus monodon</i>	Detritivore	-18.5	9.23
Fish	<i>Ambassis gymnocephalus</i>	Omnivore	-21.5	10.3
Fish	<i>A. nalua</i>	Omnivore	-16.7	10.4
Fish	<i>Anodontostoma chacunda</i>	Omnivore	-13.4	7.20
Fish	<i>Arius venosus</i>	Omnivore	-16.1	11.0
Fish	<i>Aurigeqnula fasciate</i>	Omnivore	-16.7	12.6
Fish	<i>Cynoglossus lingua</i>	Omnivore	-16.8	8.65
Fish	<i>Lutjamus russellii</i>	Omnivore	-25.0	11.5
Fish	<i>Netuma thalassina</i>	Omnivore	-20.1	10.2
Fish	<i>Pennahia anea</i>	Omnivore	-17.1	10.7
Fish	<i>Planiliza subviridis</i>	Omnivore	-18.1	6.33
Fish	<i>Plotosus lineatus</i>	Omnivore	-15.9	10.3
Fish	<i>Pomadasys argyreus</i>	Omnivore	-15.3	10.2
Fish	<i>Saurida micropectoralis</i>	Omnivore	-16.1	12.0
Fish	<i>Scatophagus argus</i>	Omnivore	-17.0	9.18
Fish	<i>Terapon jarbua</i>	Omnivore	-19.9	10.2
Octopus	<i>Amphioctopus aegina</i>	Omnivore	-15.4	10.1
Shrimp	<i>Metapenaeus stridulans</i>	Omnivore	-19.0	9.13
Shell	<i>Paguroidea sp.</i>	Omnivore	-21.4	6.92
Squid	<i>Sepia sp.</i>	Omnivore	-15.5	10.1
Fish	<i>Oreochromis niloticus</i>	Herbivore	-25.9	10.0
Fish	<i>Sacosteria edulis</i>	Herbivore	-25.5	6.72
Fish	<i>Sardinella albella</i>	Herbivore	-17.0	10.1
Fish	<i>Sardinella fimbriata</i>	Herbivore	-18.0	9.41
Fish	<i>Stolephorus indicus</i>	Herbivore	-18.9	10.2
Crab	<i>Aratus isonii</i>	Herbivore	-18.8	6.53
Crab	<i>Episesarma singararens</i>	Herbivore	-24.4	4.41
Crab	<i>Majidae sp.</i>	Herbivore	-17.0	8.13
Shell	<i>Cassidula nucleus</i>	Herbivore	-24.9	5.04
Shell	<i>Littoraria melanostoma</i>	Herbivore	-21.0	3.24
Shell	<i>Pythia plicata</i>	Herbivore	-24.4	2.52
Plant	<i>Rhizophora apiculate</i>	Mangrove	-29.4	7.73
Plant	<i>R. apiculate x R. mucronata Hybrid</i>	Mangrove	-29.9	5.39
Plant	<i>R. mucronata</i>	Mangrove	-31.4	6.05

The $\delta^{13}\text{C}$ Values of Mangrove Plants and Fishes

Mangrove plants are foundational to mangrove ecosystem food webs. Table 1 presents $\delta^{13}\text{C}$ values for three mangrove species: *Rhizophora apiculate* (-29.4‰), Hybrid of *R. apiculate* x *R. mucronata* (-29.9‰), and *R. mucronata* (-31.4‰). These $\delta^{13}\text{C}$ values classify all three as C3 plants [6]. Economically important for Thailand and Southeast Asia, *R. apiculate* and *R. mucronata* also provide crucial coastal protection. The hybrid species is a natural result of pollination between these two parent plants.

Previous studies have reported $\delta^{13}\text{C}$ values for mangrove plants ranging from -33‰ to -24‰ in southern Florida and Guade-

loupe [7, 8]. Hayase et al. found $\delta^{13}\text{C}$ values between -28.7‰ and -26.7‰ in the Matang mangrove forest, Malaysia [9]. These values align with the general $\delta^{13}\text{C}$ ranges of C3 plants (-32‰ to -27‰) and C4 plants (-11‰ to -17‰).

The $\delta^{13}\text{C}$ values of the collected samples ranged from -25.9‰ to -11.9‰. A clear trophic enrichment of $\delta^{13}\text{C}$ was observed from mangrove plants to higher trophic levels. This enrichment is likely due to the incorporation of heavy carbon from mangrove-derived organic matter into the food chain. Mangrove ecosystems, known for high sedimentation rates, contribute significantly to organic carbon accumulation. The soils of rehabilitated shrimp

ponds, derived from mangrove forests, likely contain organic-rich sediments. Consequently, organisms at higher trophic levels within these ponds may exhibit slightly elevated $\delta^{13}\text{C}$ values compared to those in the surrounding mangrove forest.

The herbivorous species *Pythia plicata* (shell) and *Episesarma singararensis* (crab) exhibited identical $\delta^{13}\text{C}$ values of -24.4‰. These organisms, directly consuming fresh *Rhizophora* spp. leaves and algal growth on mangrove trunks, show minimal isotopic enrichment. This suggests a direct dietary link to primary producers without significant trophic level elevation.

The omnivorous fish *Lutjanus russellii* (-25.0‰) and *Ambassis gymnocephalus* (-21.5‰) exhibited relatively low $\delta^{13}\text{C}$ values, likely due to their direct consumption of primary producers. However, the omnivorous *Arius venosus* (-16.1‰) and *Plotosus lineatus* (-15.9‰) showed significantly higher $\delta^{13}\text{C}$ values, despite similar dietary preferences. This suggests distinct metabolic processes or dietary preferences at the finer scale, influencing carbon isotope incorporation.

Stable isotope analysis indicates that *Cobia* (*R. canadum*) occupies a high trophic level, approximately 23-24 steps removed

from primary producers (mangroves). This apex predator highlights the complexity of the Pak Phanang Bay food web. The diverse array of fish, crabs, shells, and shrimp harvested from these waters supports local livelihoods. The high trophic level of *Cobia* underscores the importance of mangrove restoration efforts, as these ecosystems serve as critical nurseries and support rich biodiversity.

The $\delta^{15}\text{N}$ Values of Mangrove and Accumulation of Nitrogen into Fishes

The total nitrogen stable isotope ratio ($\delta^{15}\text{N}$) of living organisms reflects their trophic level and is a valuable tool for food web analysis. Amino acids, the building blocks of proteins, play a crucial role in this approach. Source amino acids (e.g., methionine, phenylalanine) exhibit minimal isotopic fractionation, while trophic amino acids (e.g., alanine, valine, isoleucine, proline, glutamic acid) undergo significant isotopic enrichment during metabolism. By comparing the $\delta^{15}\text{N}$ values of these amino acid groups, researchers can accurately estimate trophic position. Figure 2 illustrates the bioaccumulation of nitrogen isotopes in living organisms.

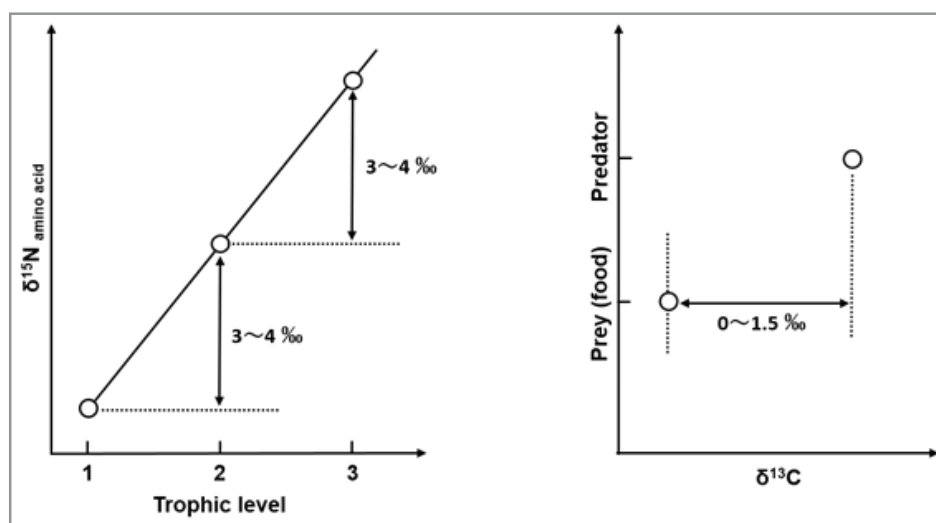


Figure 2: Relationship between the Trophic Level and $\delta^{15}\text{N}$ values of Amino Acids for Aquatic Ecosystems

The $\delta^{15}\text{N}$ value of *R. apiculata*, and *R. mucronata* leaves from Pak Poon mangrove forests were found to be 7.73‰, and 6.05‰, respectively. The $\delta^{15}\text{N}$ values of *R. apiculata* and *R. mucronata* of Ranong, Thailand was also reported to be 2.30‰ and 1.3‰. There's a significant difference in $\delta^{15}\text{N}$ values between the Pak Poon and Ranong mangrove forests for both *Rhizophora* species [10]. The difference in $\delta^{15}\text{N}$ values might indicate variations in environmental conditions, nitrogen sources, or ecological processes between the two locations. Higher $\delta^{15}\text{N}$ values in Pak Poon could suggest different nitrogen cycling patterns or potential anthropogenic influences in the area.

However, the Green Carpet Project site operates under non-tidal conditions within a closed water (sluice) gate system. Pond water is replaced every 3-4 weeks, potentially limiting the accumu-

lation of heavy nitrogen isotopes ($\delta^{15}\text{N}$) in growing mangrove plants, such as *R. apiculata*. Consequently, the $\delta^{15}\text{N}$ values of *R. apiculata* and *R. mucronata* leaves in these ponds (5.58‰ and 8.20‰, respectively) differ from those found in other abandoned shrimp ponds. These variations likely reflect differences in growth conditions, nutrient availability, and soil characteristics. Our findings suggest a simplified food chain structure in this system, with carnivorous fishes positioned only 4-5 trophic levels above primary producers. This contrasts with the more complex nitrogen cycling observed in natural mangrove and salt marsh ecosystems [11].

On the other hand, all samples were collected from the coastal waters of Pak Phanang Bay, Nakhon Si Thammarat, Thailand. Mangrove leaf samples, including *Rhizophora apiculata*, *R. mu-*

cronata, and Hybrid of *R. apiculata* x *R. mucronata*, exhibited $\delta^{15}\text{N}$ values of 7.73‰, 6.05‰, and 5.39‰, respectively. Notably, the $\delta^{15}\text{N}$ values of consumers, such as squids, octopuses, crabs, shells, shrimps, and fishes, were consistently higher than those of the primary producer mangrove leaves.

Trophic fractionation, the preferential excretion of lighter nitrogen isotopes (^{14}N) during metabolism, results in the enrichment of heavier nitrogen isotopes (^{15}N) in consumers at higher trophic levels. This isotopic enrichment is influenced by various factors, including starvation, age, and food quality [12-15].

Littoraria melanostoma, *Pythia plicata*, and *Episesarma singararens* exhibited relatively low $\delta^{15}\text{N}$ values of 3.24‰, 2.52‰, and 4.41‰, respectively. These low values suggest a high transaminase activity within these organisms, leading to the rapid deamination and subsequent loss of amino groups (primarily from alanine, leucine, valine, and glutamic acid) to the surrounding seawater. In contrast, amino acids like methionine and phenylalanine, with amino groups resistant to transaminase activity, are retained. This differential amino acid metabolism likely contributes to the observed low $\delta^{15}\text{N}$ signatures. Figure 3 shows supposed metabolisms of amino acids in living organisms.

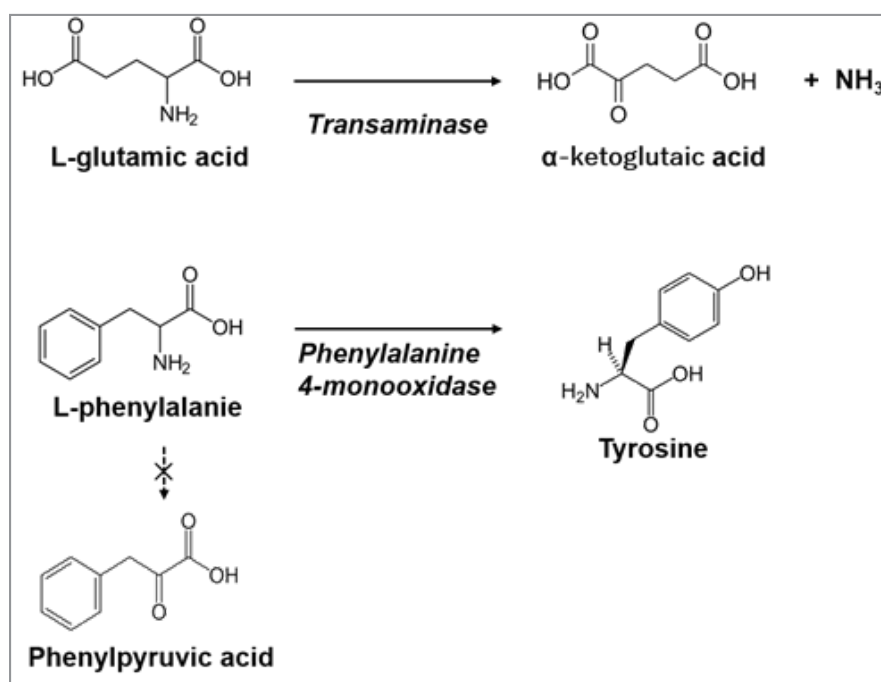


Figure 3: Nitrogen Stable Isotope Fractionation During Amino Acid Metabolism in Fishes

The $\delta^{15}\text{N}$ values of many fish, crabs, shells, and shrimp ranged from approximately 9 to 11‰, roughly double that of the mangrove plants. Notably, *Johnis belangerii*, a carnivorous fish, exhibited an exceptionally high $\delta^{15}\text{N}$ value of 13.2‰. This significant difference compared to herbivorous species like *Littoraria melanostoma*, *Pythia plicata*, and *Episesarma singararens* suggests potential variations in nitrogen metabolism among different organisms.

The present study demonstrates that this dataset accurately reflects the structure of a natural marine food chain. This analysis indicates that carnivorous fish occupy trophic levels at least four to five steps removed from primary mangrove producers. These findings align with previous research documenting the cycling of nitrogen (as ammonium, nitrate, and nitrite) within mangrove and salt marsh ecosystems [16].

Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of Food Chain in the Estuarine Ecosystems

The stable carbon isotope ratio ($\delta^{13}\text{C}$) in plants is influenced by geographic location and serves as a baseline for ecosystem-wide

isotopic patterns. Previous studies have reported consistent $\delta^{13}\text{C}$ enrichment of approximately 1.5‰ per trophic level in the Bering Sea and 0.7 to 1.4‰ per trophic level in the eastern Pacific [17]. Based on these findings, a general estimate of 0.8‰ enrichment per trophic level was adopted for this study [18].

Figure 4 illustrates the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the collected samples and mangrove leaves. *Rachycentron canadum* (Cobia), identified as a Carnivore (●), exhibited the most depleted $\delta^{13}\text{C}$ value (-14.2‰) among the fish samples, suggesting a trophic level of approximately 23-24 steps from primary producers. This apex predator status is evident in its position on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ plot. While $\delta^{15}\text{N}$ typically increases by 3-4‰ per trophic level, the observed $\delta^{15}\text{N}$ values in this study did not exhibit such a pronounced pattern. This discrepancy may be attributed to the predominance of straight-chain amino acids (e.g., glycine, alanine, aspartic acid, glutamic acid) in fish proteins, which are susceptible to deamination and thus less likely to accumulate heavy nitrogen isotopes. Conversely, the herbivorous *Pythia plicata* showed an unexpectedly low $\delta^{15}\text{N}$ value of 2.52‰, indicating efficient nitrogen excretion.

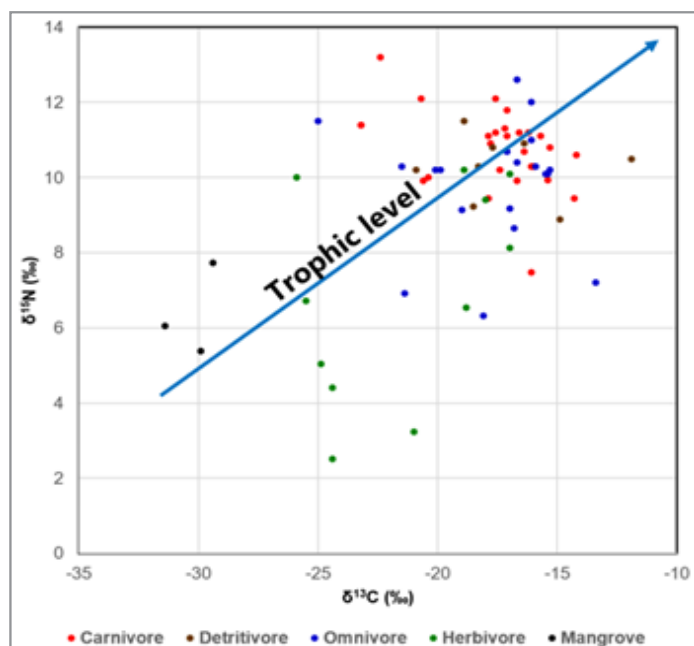


Figure 4: Relationship Between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Values in Fishes, Crabs, Shellfishes and Mangroves



Figure 5: Cobia, Trophic level 23 – 24 Steps of Top Rank in Pak Phanang Bay

Carnivores, primarily meat-eaters, such as the fish *Gazza minuta*, exhibited a $\delta^{13}\text{C}$ value of -15.2‰ . Omnivores (●), capable of consuming both plants and animals, including *Paguroidea sp.* (shell) and *Metapenaeus stridulans* (shrimp), displayed $\delta^{13}\text{C}$ values of -21.4‰ and -19.0‰ , respectively. Detritivores (●), organisms that feed on detritus, like *Gerres filamentosus* (fish) and *Penaeus monodon* (shrimp), had $\delta^{13}\text{C}$ values of -14.9‰ and -24.0‰ . Finally, Herbivores (●), primarily plant-eaters, such as *Episesarma singararens* (crab) and *Cassidula nucleus* (shell), showed $\delta^{13}\text{C}$ values of -24.4‰ and -24.9‰ .

Conclusion

Pak Phanang Bay supports a diverse fishery and is a vital economic resource for the local community. This study analyzed the stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios of 47

fish, 5 shellfish, 5 crabs, 5 shrimp, 1 octopus, 2 squid, and 3 mangrove plant species to investigate the bay's food web structure (Table 1).

Mangrove trees incorporate atmospheric carbon dioxide into their tissues through photosynthesis. However, the stable carbon isotope, ^{13}C , which constitutes 1.11% of atmospheric carbon, is fractionated during this process. Based on previous studies, C3 plants, including the loop-root mangroves examined here, typically exhibit $\delta^{13}\text{C}$ values ranging from -25 to -32‰ . In contrast, C4 plants have $\delta^{13}\text{C}$ values between -15 and -10‰ , while CAM plants fall between these two ranges.

Rachycentron canadum (fish) and *Acanthosquilla multifasciata* (shrimp) exhibited $\delta^{13}\text{C}$ values of -14.2‰ and -14.3‰ , respec-

tively. These exceptionally depleted values suggest that these species occupy the highest trophic levels within the investigated food chain.

In contrast, *Oreochromis niloticus* (fish) and *Pythia plicata* (shell) exhibited $\delta^{13}\text{C}$ values of -25.9‰ and -24.4‰, respectively. These relatively depleted $\delta^{13}\text{C}$ signatures suggest a predominantly herbivorous diet for both species.

The enrichment of the stable carbon isotope ($\delta^{13}\text{C}$) typically ranges from -0.80‰ to -1.00‰ per trophic level in a food chain. Based on a $\delta^{13}\text{C}$ enrichment rate of -0.80‰ per trophic level and comparing the $\delta^{13}\text{C}$ values of *R. canadum* (fish) and *R. mucronata*, *R. canadum* is estimated to occupy a trophic level of 23 to 24 steps within the Pak Phanang Bay ecosystem, indicating its apex predator status.

The $\delta^{15}\text{N}$ value of *Rhizophora mucronata* leaves was determined to be 6.05‰. In contrast, *Rachycentron canadum* (fish) exhibited a $\delta^{15}\text{N}$ value of 10.6‰, representing a significant enrichment compared to the primary producer. While typical trophic level studies suggest a $\delta^{15}\text{N}$ increase of 3-4‰ per trophic level, the observed enrichment in this study was less pronounced. This discrepancy may be attributed to the predominance of straight-chain amino acids (glycine, alanine, aspartic acid, and glutamic acid) in fish and shellfish proteins, which are susceptible to deamination and limit nitrogen accumulation.

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