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The Contribution of Mangrove Detritus to the Production of Commercially Important Shrimp Species.

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Progress Report on Research Undertaken

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INTRODUCTION

This report is divided into three sections. The first deals with growing and preparing the radiolabelled mangrove detritus at the Horn Point Environmental Laboratory. The second and third details the feeding studies and collection of material for stable isotope analysis from our first research trip to Malaysia between January 15 and February 18, 1989.

1) Production of ^{14}C Mangrove detritus

Malaysian seeds of the mangrove, Rhizophora apiculata, were sent in July 1988 to Horn Point Environmental Laboratory. A fast draining soil was prepared by mixing 9 parts of a coarse grained sandy garden soil with 1 part rotted farmyard manure. Plastic garden pots (24 cm diameter and 30 cm deep) were filled with this soil and placed in shallow plastic pans. The soil was soaked in 12 ppt salinity water and the height of the water in the drain pan was adjusted to a depth of approximately 4 cm. Henceforth only evaporative losses were made up by watering the soil surface with distilled water to maintain the level in the drain pan. The seeds, approximately 20 cm long, were planted vertically in pots with 5 cm buried in the soil. The seeds were grown in a controlled environment chamber (25 °C: 95% Relative Humidity: Photoperiod 14H light:10 h dark) until each plant was growing vigorously with approximately 20 large leaves (after about 6 months). The pots were then transferred to an air-tight plexiglas growth chamber designed by Ferguson and Williams (1974) to uniformly label plant material with gaseous $^{14}CO_2$. A $^{14}CO_2$ atmosphere was produced by adding sufficient 1 M citric acid solution to liberate 0.5 mCi $^{14}CO_2$ per day from 8 mCi of $NaH^{14}CO_3$, buffered in 100 ml 30 ppt seawater, contained in a stirred flask in the chamber. The atmospheric ^{14}C specific activity was monitored by withdrawing 10 ml gaseous samples via a septum in the chamber wall using a gas tight syringe. The gaseous $^{14}CO_2$ was absorbed for 2 h in 10 ml of scintillation cocktail (Oxifluor-CO_2: New England Nuclear) before counting on a LKB Beta Rack scintillation counter (LSC). When no further $^{14}CO_2$ was liberated by the addition of citric acid, 20 ml of concentrated HCL was added to ensure that all ^{14}C had been driven off. The plants were grown for a further 5 days in a $^{14}CO_2$ free atmosphere, although CO_2 levels

were maintained by adding $NaHCO_3$ to the flask in the chamber.

The leaves were harvested and divided into 3 groups; a) the youngest leaves that had grown substantially in the chamber, b) those that had made a small amount of growth and, 3) mature leaves that exhibited no growth. For the purposes of these experiments only the youngest leaves was used as this material had the highest specific activity. The leaves were homogenized to a particle size of between 100 to 200 um in a polytron tissue homogenizer (Brinkman Instruments)

Crude fibre was prepared from the Rhizophora apiculata leaves using a method similar to that employed by Newell and Langdon (1986) to prepare Spartina alterniflora detritus. This involved a combination of organic solvent extraction and the acid/alkali extraction of Strickland and Parsons (1972).

2) Sample collection for stable isotope analysis

Samples for analysis of the stable isotopes of carbon, sulfur, and nitrogen were collected from the Selat Kelang Utara estuary on the east coast of Malaysia, approximately 30 miles west of Kuala Lumpur. Two locations were within the tidal creeks draining the mangrove swamps (3° 3'N : 101° 18'E and 3° 4'N : 101° 22'E) which is the nursery area for the banana prawn, Penaeus merguiensis. Two other sampling areas were offshore [6 km offshore (3° 11'N 101° 14'E) and 12 km offshore (3° 12'N 101° 19'E)] where adult shrimp of many species are commercially harvested. In the shallows the young Penaeus merguiensis shrimp were collected using a fine mesh cast net. Penaeus merguiensis adults, and both juvenile and adult rainbow prawns, Parapeneopsis sculptilis, were collected from the offshore sampling sites by trawling from a boat. At the same time other animal species (listed in table 2) were also collected. All animals were transported on ice to the laboratory where they were rinsed in distilled water. Only muscle tissue was dissected out, so as to avoid the stable isotope results possibly being contaminated by material remaining in the gut. The muscle tissue from 10 individuals was

pooled to make a single sample for that locality. Concurrently with the animal collections, mixed phytoplankton/seston samples were obtained by prefiltering seawater through a 53 um screen to remove the zooplankton and then filtering it through Whatman GF/C filters. Large pieces of mangrove leaf detritus were hand sorted from material dredged at the two offshore stations. This detritus was washed gently in distilled water. A bottom grab was used to collect sediment samples from the offshore site; in the estuary sediment samples were collected by hand from mud banks exposed at low water. Mixed filamentous algae was collected by hand from the estuary. Senescent mangrove leaves were taken from ten separate trees on the creek banks. All material was oven dried at 60 °C and ground in a mortar before being washed in 10% V:V HCL to remove salts and carbonates, rinsed twice in distilled water and dried at 60C for 7 days. These samples have been sent to the Ecosystem Center, Marine Biological Laboratory for stable isotope analysis.

3) In vivo ^{14}C feeding studies

Two species of shrimp were selected for these studies based on the different distributions of their juvenile and adult stages. One, the banana prawn, Penaeus merguiensis (de Man), has its juvenile life stages within the mangrove dominated estuaries. The other, the rainbow prawn, Parapeneopsis sculptilis (Heller), has its juvenile nursery grounds off the mouth of the estuary. Adult shrimp of both species co-exist in offshore feeding grounds. Juvenile and adult shrimp were freshly collected as described above. The shrimp were rapidly transported to the laboratory in aerated insulated containers. They were then held at 28 °C and 32 ppt in 200 L aquaria. Water quality was maintained with a biological gravel filter supplemented by a aquarium filter pump system. All experimental shrimp were held unfed for 48 h to ensure that they would feed during the experiments.

Preliminary experiments showed that both species of shrimp would actively feed on pellets made from 75% W:V gelatin containing 1% V:V finely homogenized bivalve tissue (the cockle). The ^{14}C mangrove leaf material was directly incorporated into the gelatin as it was being

prepared. The warm gelatin was extruded from a plastic syringe just before it solidified and the 2 mm wide ribbon was then cut into 15 mm long pieces.

The minimum and maximum gut passage time of adult and juvenile shrimp was determined in preliminary experiments by feeding the shrimp gelatin pellets containing 3um fluorescent microspheres (Polyscience Inc.). The time between the shrimp being first fed the beads and the beads first appearing in the fecal pellets was variable and ranged from 3 to 8 h. All beads were voided from the animal within 24 h.

Individual shrimp were placed in 10 aquaria containing 4 L of aerated sea water and allowed to acclimatize under conditions of low light intensity for 4 h. Weighed ^{14}C pellets were then added to the 10 experimental aquaria and 2 control aquaria maintained without shrimp. The feeding activity of individual shrimp was carefully observed for 5 h. Shrimp that did not feed on the ^{14}C pellets within this time were discarded from the experiment. Once a shrimp had completely, or substantially, ingested a pellet it was immediately removed, rinsed in seawater, and transferred to another aquaria to depurate. These shrimp were provided with non-^{14}C labelled pellets to act as a "chase". All fecal material was collected with a large bore pipette and preserved with formaldehyde (2% V:V). Shrimp were depurated for 26 h before being removed and frozen at 25 °C.

The amount of ^{14}C respired in both the feeding and depuration aquaria was measured using the procedure described by Crosby et al (1989). This involved raising the pH of a 20 ml sample of seawater to 10 with NaOH to prevent the loss of any respired $^{14}CO_2$. This water was filtered through 0.22 um nuclepore filters. The ^{14}C concentration in 4 ml samples of this water was measured using LSC, with Packard Insta-Gel as the scintillation fluid. A second 4 ml sample of this filtrate was acidified (pH <1) with HCL to drive off any CO_2. The ^{14}C remaining in this sample was counted and the difference in activity between the basic and acidified samples was the amount of respired ^{14}C. Samples from the two control aquaria were

processed in the same way to assess the degree of ^{14}C leaching from the pellet and bacterial respiration of the ^{14}C mangrove leaf material.

Fecal samples were made up to 4 ml with distilled water and sonicated to disrupt the fecal ribbons. In order to ensure that each shrimp had been completely depurated the intestines were dissected from each shrimp and then homogenized in distilled water. Packard Insta-gel was added to both the fecal and intestine samples and vigorously shaken to ensure that all particles were uniformly suspended in the resulting gel and the ^{14}C activity determined using LSC. The shrimp were cut in half to facilitate freeze drying. The freeze dried shrimp were transported to my laboratory in Maryland where they will be ground to a fine powder. Weighed samples will be taken for LSC using Protosol to dissolve the tissue and reduce quenching. All ^{14}C counts were corrected for quenching using a quench curve constructed using internal standards.

The amount of mangrove leaf carbon ingested by each shrimp will be calculated by summing the amounts present as body burden, feces, and respired. The amount of mangrove carbon assimilated will then be calculated by expressing the body burden and respired $^{14}CO_2$ as a percentage of that ingested. The data will then be analyzed using SAS ANOVA programs to determine if there are any significant differences between either shrimp species or the two life stage's ability to utilize carbon from non-refractory or refractory mangrove leaf material.

References

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Table 1. Details of samples collected for stable isotope analysis. At least 10 individual specimens of animals or leaves were pooled to form a composite sample.

Sample Description	Sites
Penaeus merguiensis juveniles	estuary
Parapeneopsis sculptilis juveniles	6 km offshore
Penaeus merguiensis adults	12 km offshore
Parapeneopsis sculptilis adults	12 km offshore
Phytoplankton/seston	12 km offshore
Sea Cucumber	6 and 12 km offshore
Filamentous algae	estuary
Dredged mangrove leaves	6 and 12 km offshore
Sediment samples	estuary and 12 km offshore
Mangrove leaves (*Rhizophora apiculta*)	land
Herbivorous gastropod	land