POY/CDIE

Final Report

Covering Period: 1988 - 1993

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

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

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Project Number: 8.054

A.I.D. Grant Project Officer: Dr. John Wilson

Project Duration: 1988 - 1991

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1. Executive Summary:

Yields of commercially valuable prawn species are appreciably higher along tropical coastlines where mangrove forests are most extensive. This is thought to be due to mangrove forests serving as essential nursery and feeding areas for juveniles of many species of prawns. Thus, the ongoing conversion of mangrove forests fringing many tropical shorelines to other uses has possible severe adverse consequences on adjacent aquatic systems. Only by fully understanding such complex ecological relationships can informed management decisions be made concerning the development and use of these coastal wetlands.

We used stable isotope tracer techniques to evaluate the relative importance of algal and mangrove carbon in the nutrition of commercially valuable rainbow and banana prawns on the western coast of peninsular Malaysia. Prior gut content analyses indicated that mangrove detritus is ingested in large amounts by all life stages living both inshore and in coastal waters. Our analyses indicated that this mangrove detritus did indeed contribute to the nutrition of the juvenile banana prawns that are restricted to the tidal creeks draining the mangrove forest but not to prawns captured offshore. Laboratory radiotracer experiments, however, demonstrated that prawns can only digest mangrove detritus with a low efficiency (ca. 10%). This indicates that juvenile banana prawns are only utilizing some of the ingested mangrove detritus directly. Mangrove detritus that is small enough to be ingested by prawns is likely to be highly refractory because it has been through the "shredder" organisms, such as the grapsid crab that feed directly on fallen mangrove leaves. Instead, we suggest that prawns within the creeks are primarily ingesting and digesting microbial intermediaries that are either utilizing particulate or dissolved organic carbon that is originating from the mangroves.

The ability of juvenile P. merguiensis living inshore to directly assimilate refractory cellulosic material was no higher than for adult P. sculptilis living offshore. Thus, because the amount of mangrove detritus found in the guts of prawns in these two habitats does not differ substantially (Leh and Sasekumar 1984) it suggests that juvenile P. merguiensis are not utilizing the mangrove material directly. are perhaps digesting microorganisms that are either utilizing particulate or dissolved organic carbon that is originating from the mangroves. Such microbial intermediaries are likely to be most abundant in the tidal creeks where the mangrove detritus is most concentrated and hence are responsible for the strong mangrove stable isotope signal in the juvenile P. merguiensis.

Mangrove forests serve to trap large amounts of sediment and hence contribute to the stabilization and development of the intertidal mudflats necessary for benthic algal production. In turn, benthic microalgae contribute a major proportion of the diet of prawns living both inshore and offshore of the mangrove forests of Malaysia. This relationship between degree of benthic algal production and shrimp nutrition is an aspect of the ecology of the region that was previously little understood yet is important to the commercial prawn fishery. This knowledge is being integrated by the two Malaysian PI's into their ongoing fisheries programs at the University of Malaya. The information will be dissen ..iated by

them to help national and regional fisheries management programs understand the ecology of commercially valuable species.

2: Research Objectives:

The world area of mangroves is approximately 48 million hectares, with almost half located in Asia and Oceania, the remainder distributed between Africa and the western hemisphere tropics (Rodin et al. 1975). These forest ecosystems are inexorably being converted to other uses, including aquaculture ponds, agriculture, industrial development, urbanization, etc. For example, mangrove forests have been reduced by 20% in Peninsular Malaysia (Ong, 1982) and in many other regions of the tropics mangrove forests are in precipitous decline (reviewed by Hatcher et al. 1989)

The potential adverse consequences of mangrove forest destruction have been investigated in numerous studies of possible interrelationships between forest and coastal ecosystems. Such studies have clearly established that fishery yields, including commercially valuable prawn species, are often appreciably higher along coastlines where mangrove forests are most extensive (see review by Marshall 1994). Recent research suggests a complex ecological relationship between mangrove and coastal ecosystems in which shallow-water mangrove forests and creeks serve as essential nursery and feeding areas for juveniles of many species of prawns (Chong et al. 1990, Vance et al. 1990, for review see Hatcher et al. 1989).

The exact role of detritus derived from mangrove trees in providing nutriments to organisms within the mangrove systems or offshore is uncertain. Originally, it was believed that high offshore fish production largely resulted from the "outwelling" of mangrove detritus transported offshore (for review see Hatcher et al. 1989). However, there is perhaps little quantitative nutritional importance to this material once it becomes dispersed in adjacent coastal waters (Nixon et al. 1984). For example, Roberston et al. (1991) report that outwelled mangrove carbon provides only about 16% of the carbon required for sediment bacterial production in the Great Barrier Reef lagoon.

Several studies have examined the potential significance of mangrove detritus in the nutrition of animals from tidal creeks and coastal waters of Peninsular Malaysia. For example, microscopic examination has demonstrated that identifiable mangrove detritus in addition to remains of animal and benthic algae are present in the guts of many animal species, including prawns, collected from both inshore and offshore (Chong and Sasekumar 1981, Leh and Sasekumar 1984, Sasekumar and Chong 1987). The penaeid banana prawn Penaeus merguiensis (de Man), has been classified as an omnivore with a preference for animal food (Chong and Sasekumar 1981; Leh and Sasekumar 1984; Sasekumar and Chong 1987), in which detritus forms a food supplement for all life stages, within the creeks draining mangrove forests as well as offshore (Chong and Sasekumar, 1981). Although there is less published data on the diet of the rainbow prawn, Parapeneopsis sculptilis (Heller), it also seems to an opportunistic omnivore (Leh and Sasekumar 1984)

Results obtained from such gut content analysis must be interpreted with extreme caution, however, because even though material may be ingested it is not necessarily digested efficiently. Indeed, a study comparing the stable carbon isotope ratios of mangrove leaves with the tissue of various consumers did not indicate a significant role of mangrove detritus in the carbon budget of any consumer species collected offshore of western Malaysia (Rodelli et al. 1984). In the same study, several species of juvenile prawns collected from within the creeks draining the mangrove forest did have carbon isotope values consistent with about 65% of their tissue carbon being derived from mangrove leaves. In addition, eleven species of fish collected by Rodelli et al (1984) in the mangrove creeks were also found to derive up to 60% of their carbon from mangroves. Because of this discrepancy between the results of gut content analyses and stable isotope analyses for prawns collected offshore, Rodelli et al. (1984) postulated that the mangrove detritus offshore was highly refractory and not available to these higher trophic levels.

In a detailed analysis of the distribution of the juveniles and adults of nine species of prawn on the central east coast of Peninsular Malaysia, Sasekumar et al. (1992) suggested that some species may be more dependent than others on mangrove estuaries as habitat. Our objective was to determine the possible importance of a mangrove detritus in the nutrition of prawns living within the creeks draining mangrove forests and in coastal waters offshore from such forests. Two species of prawn were selected for these studies based on the different distributions of their juvenile and adult stages. One, P. merguiensis, develops as a juvenile within the mangrove dominated estuaries (Roberston 1988, Vance et al. 1990, Sasekumar et al. 1992). The other, P. sculptilis has its juvenile nursery grounds off the mouth of the estuary. Adult prawns of both species co-exist in offshore feeding grounds.

The overall hypothesis we tested was that juveniles prawns living within mangrove creeks derive significantly greater nutritional benefits from mangrove detritus than do juveniles and adults living offshore. We used a multiple isotope approach (carbon, nitrogen, and sulfur) to elucidate the degree to which juvenile and adult prawn collected from the various habitats were utilizing mangrove detritus. The advantage of this triple isotope technique compared with the single carbon isotope analysis of Rodelli et al. (1984) is that it potentially allows more positive discrimination between different food sources in the diet of test organisms (Peterson et al. 1985, 1986, Peterson and Fry 1987, Peterson and Howarth 1987).

In order to determine the extent to which mangrove lignocellulose can be degraded to nutritionally valuable molecules and assimilated we also performed feeding studies. Chemically defined lignocellulosic crude fibre prepared from radiolabeled (^{14}C) mangrove leaves was fed to the different life stages of the prawn. Digestion of such cellulose requires a suite of three enzymes. Consequently, studies that attempt to estimate the ability of animals to digest cellulose but that rely on chemical assays of the activity of a single one of these cellulase enzyme frequently overestimate cellulose digestion efficiency (see Newell and Langdon 1986).

3: Methods

Sample collection for stable isotope analysis

Samples for analysis of the stable isotopes of carbon, sulfur, and nitrogen were collected mainly during January 1989 and December 1990 at a number of locations on the east coast of Peninsular Malaysia (see Fig 1). Two locations were within the tidal creeks draining the mangrove swamps which is the nursery area for the banana prawn, P. merguiensis. Three other sampling areas were 3, 6 and 12 km offshore where adult prawn of many species are commercially harvested.

At inshore sampling sites, juvenile P. merguiensis prawns were collected using a fine mesh cast net. Penaeus merguiensis adults, and both juvenile and adult P. sculptilis, were collected by trawl from the offshore sampling sites. Prawns were characterized as being either juvenile or adult, based on degree of development of external genitalia. The other animals listed in Table 1 were also collected along the creeks or in the trawls offshore. All specimens were transported to the laboratory on ice. To avoid tissue samples being contaminated by material remaining in the gut care was taken to ensure that only muscle was taken for stable isotope analysis and this was rinsed copiously with distilled water.

Inshore, freshly dropped senescent leaves were collected from under ten separate mangrove trees adjacent to the creek banks. Mixed filamentous algae was collected by hand from the banks of the tidal creeks. Offshore, large distinct pieces of mangrove leaf detritus were hand-sorted from material dredged at the trawl stations. All material was washed 4 times vigorously in distilled water to remove extraneous sediment and debris. Both the leaf detritus and macroalgae were then microscopically cleaned of any epibionts using a fine scraper and forceps.

Mixed phytoplankton/seston samples were obtained from a tidal creek and offshore by prefiltering seawater through a 53 μm screen to remove any zooplankton and then filtering it through Whatman GF/C filters and rinsing the filtrate three times with distilled water. A bottom grab was used to collect sediment samples from the offshore site; in the estuary surficial sediment samples were collected by hand from mud banks exposed at low water. This material was rinsed 5 times with distilled water (4:1 ratio, water to sample) and then it was allowed to settle and the supernatant decanted to waste.

All samples were oven dried at 60 °C and ground in a mortar before being washed for 1.5 h in 10% V:V HCl to remove salts and carbonates, rinsed twice in distilled water and dried at 60 C for 7 days. Samples for stable isotope analysis consisted of aggregates of a number of individuals (see Table 1) of the same species that had been collected concurrently at the same site. Such pooling of samples was needed in order to obtain the large amount (at least 1 g) of material required for sulfur analyses. For food web studies such pooling of samples has the advantage that potential intraspecific variability in the isotopic signal is reduced (Montague et al. 1981, Stephenson et al. 1984, Schleser 1992).

Samples were analyzed by the Ecosystem Center, Marine Biological Laboratory, Woods Hole for carbon, nitrogen and sulfur stable isotope ratios using a high precision mass spectrometer. The ratios of heavy to light stable isotopic abundance are expressed using the standard δ notation (Craig 1957, Fritz and Fontes 1980, 1986) where positive values indicate enrichment and negative values indicate depletion of the heavy isotope relative to a standard reference sample. Values were calculated according to the formula:

$$\delta X(‰) = \ldots\ldots\ldots\ldots ([R_{sample} / R_{standard}] - 1) \times 1000$$

Where X is either ^{13}C, ^{34}S, or ^{15}N and R is either $^{13}C:^{12}C$, $^{34}S:^{32}S$, or $^{15}N:^{14}N$ compared, respectively to a carbon standard of Peedee Belemnite, sulfur standard of Canyon Diablo troilites, and nitrogen from the atmosphere.

In vivo ^{14}C feeding studies

Propagules of the Malaysian mangrove, Rhizophora apiculata, were planted vertically to a depth of 5 cm in plastic pots filled with 25 cm of soil (9 parts sand:1 part composted cow manure). These pots were placed to a depth of 6 cm in 12 ppt salinity water. Subsequently, only evaporative losses were made up by watering the soil surface with distilled water to maintain the level in the drain pan. The propagules were grown in a controlled environment chamber (25 °C: 95% relative humidity: photoperiod 14 h light:10 h dark) until, after about 6 months, each plant was growing vigorously with approximately 20 large leaves.

The plants were then transferred to an air-tight plexiglas plant growth chamber designed by Ferguson and Williams (1974) in which 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, and 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 an LKB 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 d in a $^{14}CO_2$ free atmosphere, although CO_2 levels were maintained by adding $NaHCO_3$ and HCl to the flask in the chamber.

Only the older leaves lower on the plant that had grown a little were used for this study because these had a higher proportion of refractory cellulose than the youngest leaves. The selected leaves were homogenized to a particle size of between 100 to 200 μm in a polytron tissue homogenizer (Brinkman Instruments). Crude fibre was prepared from the homogenized R. apiculata leaves using a combination of organic solvent extraction and acid/alkali extraction (Strickland and Parsons 1972, Newell and Langdon 1986). The resulting highly refractory lignocellulosic crude fibre was designed to mimic mangrove leaf

material that had been subject to leaching and breakdown during the period of offshore transport. During this offshore transport the more labile compounds will have been leached or utilized by microorganisms.
The powdered mangrove detritus was incorporated in pellets made of gelatin following the technique of Moriarty (1976). We used a 75% W:V gelatin to DI water to which finely homogenized meat from the cockle, Anadora granosa, was mixed at 1% V:V to serve as a phagostimulant. The powdered ^{14}C mangrove leaf material was directly incorporated into the gelatin as it was being prepared. The warm gelatin was poured on aluminum foil and refrigerated until it solidified. This sheet of gelatin was cut into pieces approximately 2 x 15 mm long. Non-radiolabeled mangrove crude fibre was also incorporated into gelatin pellets that were fed to prawns prior to experiments and to serve as food during purging of the radiolabeled food.

In preliminary experiments using the same gelatin pellets into which 3 μm fluorescent microspheres (Polyscience Inc.) were incorporated, we ascertained the minimum and maximum gut passage time of adult and juvenile prawns. The time between the prawn being fed the pellet and the beads first appearing in the fecal pellets was variable and ranged from 3 to 8 h. By collecting all fecal pellets sequentially, we determined that beads were entirely voided within 24 h from prawns of all sizes.

In preparation for feeding with labelled pellets, Juvenile and adult P. merguiensis and P. sculptilis were freshly collected in January and February 1991 either by trawl or cast net, as described above. These prawns were rapidly transported to the laboratory in well-aerated insulated containers. They were then held at 28 °C and 32 ppt in 200 L tanks and fed to satiation with pellets containing non-radiolabeled mangrove detritus and were allowed to graze on epibionts growing in the tank. Water quality was maintained with a biological gravel filter supplemented by an aquarium filter pump system. Prior to use in the feeding studies all prawns were held for 24 h in glass aquaria where they were fed control gelatin pellets ad libitum

Seawater (32 ppt) was sterilized by adding 3 ml of commercial 5% hypochlorite bleach l^{-1} and holding for 12 h. The bleach was neutralized by adding 150 mg l^{-1} sodium thiosulfate; aerated for 2 h; and then adjusted to pH to 8.3 using $NaH^{14}CO_3$. Four liters of sterile seawater was added to individual aquaria. A prawn was placed in each aquaria and allowed to acclimate for 1 h under conditions of low light intensity. A weighed ^{14}C pellet was then added to each experimental aquaria with prawns and to control aquaria maintained without prawns. The feeding activity of individual prawns was carefully observed for 5 h; prawns that did not feed on the ^{14}C pellets within this time were removed from the experiment. After a prawn had either substantially or completely ingested a pellet the prawn was immediately removed, rinsed in seawater, and transferred to another aquaria to depurate with non-^{14}C labelled pellets as food. Voided fecal material from each aquarium was collected

individually with a large bore pipette and preserved with formaldehyde (2% V:V). After a prawn had been depurated for 26 h it was frozen at -25 °C.

Fecal samples were made up to 4 ml with distilled water and sonicated to disrupt the fecal ribbons. Packard Insta-gel was added to each vial and vigorously shaken to ensure that all particles were uniformly suspended in the resulting gel and the ^{14}C activity determined using LSC. In order to ensure that any unassimilated material remaining in the intestines did not contaminate the body-burden analyses, guts from all prawns were dissected and homogenized in distilled water. Vials were treated as described above for fecal samples.

Prawns were cut in half to facilitate freeze drying and were then ground individually to a fine powder. Weighed triplicate sub-samples were taken for LSC using Packard 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 ^{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 seawater sample from each aquarium to 10 with NaOH to prevent the loss of any respired $^{14}CO_2$. This water was filtered through 0.22 μm 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 determined and the difference in activity between the basic and acidified samples was defined as 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. These control rates were then used to adjust the results from the experimental aquaria.

The amount of mangrove leaf carbon ingested by each prawn was calculated by summing the amounts present as body burden, feces, and respired. The amount of mangrove carbon assimilated was calculated by expressing the body burden and respired $^{14}CO_2$ as a percentage of the amount of isotope ingested. Assimilation efficiencies were arcsine transformed to ensure normality. Differences in assimilation between species of prawn and the two life stages were tested by ANOVA followed by SNK multiple range test.

4: Results and Discussion

Stable Isotope Analysis of Primary Producers

The $\delta^{13}C$, ^{34}S, and ^{15}N values for the various primary producers and consumers found within the mangrove forests, and at various distances offshore, on the east coast of Peninsular Malaysia are listed in Table 1. These values are also presented as dual isotope plots in Figs 2 - 4. Because such plots separate the stable isotope ratios of organisms along two axes, it

enables visual discrimination between the elemental composition of consumers and potential food sources.

The carbon, sulfur, and nitrogen ratios of seston samples collected both in the tidal creeks and offshore had ratios similar to those in the literature (summarized by Peterson and Howarth 1987) for marine phytoplankton (Figs 2 - 4). This indicates that phytoplankton, and not detritus from mangroves or other upland plants, was the major source of suspended organic material in the < 53 μm size fraction.

Generally, the four species of macroalgae (numbers 24 - 27) collected from the sediment surface on the creek banks had stable isotope values closer to phytoplankton than mangrove leaves. The only inconsistency was the very negative del carbon values for the two Graçilaria sp (samples 25 and 26). This suggests that these macrolagae have a strong discrimination against $\delta^{13}C$ and maybe taking up atmospheric carbon dioxide which is more negative than inorganic carbon available from seawater. The $\delta^{34}S$ and $\delta^{15}N$ values of the macroalgae are consistent with these nutrients being obtained from seawater. Seawater sulfate $\delta^{34}S$ value is about +20‰ which is considerably more positive than the sulfate available to upland plants from precipitation ($\delta^{34}S$ +2 to approximately +14 ‰; Fry et al. 1982, Peterson et al. 1986). In contrast, if estuarine plants take up reduced sulfur directly or indirectly their $\delta^{34}S$ values will be negative because sediment sulfide has values of approximately - 24‰ (Fry et al. 1982). Porewater sulfate values are variable depending on bacterial processes but were found by Fry et al. (1982) to be only slightly less positive (mean +16.7‰) than seawater. Plants absorbing dissolved inorganic nitrogen directly from seawater have $\delta^{15}N$ values of + 6 to + 10‰ (Peterson and Howarth 1987). This is considerably more positive than plants that obtain the majority of their nitrogen from the atmosphere, which, by definition, is assigned a standard value of zero.

There was strong similarity between mangrove leaf ratios for all three stable isotopes irrespective of the species of tree or if the material consisted of highly degraded leaves dredged from offshore. This is unlike the situation in Florida reported by Zieman et al. (1984) for mangrove leaves in litter bag decomposition experiments where nitrogen ratios changed from about δ +6‰ to -5‰ as decomposition occurred over a 6 week period, although their carbon ratios did not alter from a value of about δ -26‰. Our lowest mangrove nitrogen values was +2.24 (sample 13) which was for freshly dropped leaves that had not even been subject to decay in the water.

Our mean $\delta^{13}C$ value for mangroves of -28.34‰ was very close to the mean value of -27.1‰ reported by Rodelli et al. (1984) for a number of species of mangroves collected from the same mangrove forests that we studied. These values are close to the $\delta^{13}C$ -29.3‰ reported by Peterson and Howarth (1987) for other C-3 upland plants obtaining inorganic carbon from the atmosphere.

Interestingly, in our study the mean mangrove values for $\delta^{34}S$ of +4.57‰ and for $\delta^{15}N$ of +5.4‰ were more positive than the values of +1.8‰ for $\delta^{34}S$ and 0.4‰ for $\delta^{15}N$ reported

by Peterson and Howarth (1987) for other rooted upland plants. Our sulfur value for mangrove leaves was also more positive than the single value of -0.2 obtained by Fry et al. (1982) for Avicennia nitidia collected from Texas. They report that samples from the interior of these mangrove roots were even more negative (-3.2 ‰) than the leaves due to the active incorporation of reduced sulfides from the sediments. These comparisons suggest that the mangroves in our study area were obtaining sulfur and nitrogen from seawater or interstitial porewater to a greater extent than is typical for other species of rooted plants in such habitats.

The fine detritus and living organisms (e.g., bacteria, diatoms, meiofauna, etc.) in the surficial layers of the sediment is an important food source for prawns (Leh and Sasekumar 1984) that is technically difficult to sample. Our stable isotope values for sediments (19 and 20) had extremely negative $\delta^{34}S$ values of -11.6‰ and -8.85‰. The $\delta^{15}N$ values of +4.11‰ and +2.12‰ were among the most negative of all our samples. These extremely negative values suggest a strong contribution by inorganic minerals to the sediment isotope signal. The sediment carbon values were in the range suggesting they may have originated from a mixture of phytoplankton and mangrove detrital carbon. Such sampling of bulk sediments, however, ignores the possible contribution of many types of living organisms including benthic diatoms, cyanobacteria, microheterotrophic protozoans, and meiofauna. We attempted to circumvent this problem by collecting the stomach contents from the mud skipper Boleopthalmus boddaerti, (sample 23) that selectively feeds on small organic particles in the surface layer of water on the creek banks. It is apparent from the $\delta^{34}S$ and $\delta^{13}C$ (Fig. 2) and $\delta^{15}N$ and $\delta^{13}C$ (Fig. 3) double plots that its stomach contents were more negative than our mangrove material and seston values, as well as literature phytoplankton values. These stomach content values suggest that there is a third primary producer within the coastal waters of Malaysia that is a source of food.

The waters in our study region were extremely turbid with secchi depths of generally less that 0.5 m. Under such conditions phytoplankton production is likely to be light limited. The study area was characterized by extensive poorly drained intertidal mud flats, however, which provide conditions highly conducive to benthic diatom production. Preliminary studies have shown that dense populations of benthic microflora, identified chiefly as diatoms, occur on the surfaces of intertidal sediments within, and adjacent to, the mangrove forests of our sampling area (Thong et al. 1993). The sulfur utilized by such microalgae would most likely be derived from within the sediments. The material taken from the mud skipper stomachs had sulfur values similar to mangroves and were considerably more negative than phytoplankton that utilize sulphate from seawater. The nitrogen values of the stomach contents were similar to mangroves suggesting that the microalgae, like the mangroves, derive their nitrogen almost equally from both sediments and seawater. The carbon values were considerably more positive that the mangrove signal suggesting that atmospheric CO_2 was not an important carbon source. These benthic microalgae must derive inorganic carbon from the surface seawater even though the carbon values were slightly more positive than the literature values for phytoplankton (Fig. 2). Pelagic diatoms are known to fractionate carbon differently from nanoplankton. For example, Gearing et al. (1984) report that in a temperate

estuary diatoms from the spring bloom had a mean (± S.E.) $\delta^{13}C$ value of -20.3 ± 0.6‰ (n = 27), which was almost 2‰ more positive than the value when nanoplankton species were blooming (-22.2 ± 0.6‰; n = 29).
Because of the technical difficulties of separating sufficient biomass of benthic microalgae from sediment samples there is little literature data available for stable isotope ratios for field-collected benthic microalgae. Haines (1976) reported $\delta^{13}C$ values of -16.2 to -17.9‰ for a sample of benthic algae, described as "mostly diatoms", collected from a Georgia saltmarsh. Peterson and Howarth (1987) collected unidentified "creekbank algae" from the same saltmarsh system and found almost identical $\delta^{13}C$ value of -16.7‰ and +3.5‰ for ^{15}N. Peterson et al. (1986) reported that saltmarsh benthic algae exhibited variable $\delta^{34}S$ values that were dependent on the proximity of the specimens to sulfide within the sediment. Thus, purple sulfur bacteria collected directly from the surface of waterlogged sediments exhibited $\delta^{34}S$ of between -10 to -13‰. In a mat of filamentous cyanobacteria, those living furthest away from the sediment had $\delta^{34}S$ of between +13‰ and values for those close to the sediment were more negative. Conversely, a filamentous epibenthic alga had a $\delta^{34}S$ of +18.2‰, suggesting that the majority of its sulphate was being obtained from seawater.

In an attempt to circumvent the difficulty of collecting benthic microalgae from the creeks of peninsular Malaysia, Rodelli et al. (1984) analyzed the carbon stable isotope ratio of benthic diatoms cultured in the laboratory. The mean (± S.E.) $\delta^{13}C$ values for 5 strains of benthic diatoms was -17.8 ± 0.85‰ (plotted as a histogram in Fig. 2) which is in close agreement with the values obtained by Haines (1976) for diatoms collected from a saltmarsh..

Stoner and Zimmerman (1988) obtained a $\delta^{13}C$ value of -14.2‰ for a mat forming blue-green alga Spirulina sp. collected from a lagoon adjacent to mangrove forests of Puerto Rico. Stribling (1994) collected a mat of an unidentified pennate diatoms from a saltmarsh creek in the mesohaline region of Chesapeake Bay field that provided sufficient biomass for both carbon and sulfur analysis. Her values of -14.85‰ for $\delta^{13}C$ and of +5.4‰ for $\delta^{34}S$ are extremely distinct from both phytoplankton and mangrove values (Fig. 2). The blue-green algae carbon values from Stoner and Zimmerman (1988) and Stribling (1994) are similar but both are slightly more negative than those obtained by Haines (1976) and Rodelli et al. (1984) for unicellular microalgae.

Sullivan and Moncreiff (1990) collected small motile pennate diatoms (Sullivan and Moncreiff (1988) living on or within sediments beneath the canopy of vascular plants in a Mississippi saltmarsh. They attempted to separate from non-algal contaminants sufficient algal biomass for a single analysis of three stable isotope ratios. They report $\delta^{13}C$ values of -20.6, $\delta^{34}S$ values of +14.3‰ and $\delta^{15}N$ values of +6.1 ‰. Their carbon value is considerably more negative than any other values for benthic algae quoted above. This is most likely due to a terrigenous organic carbon (-27‰) from riverine inputs contaminating their samples. Such a pattern of more negative carbon isotope ratios in sediments and consumers from this part of the north-central Gulf coast has been observed frequently (for review see Fry 1983). Thus, because their stable isotope values are so different from our

own values for mud skipper gut contents and literature values for benthic algae, we will not consider them further in our discussion.

Gut Content Analysis

Based on extensive gut content analysis it has been shown that like most penaeid shrimps, both juvenile and adult Penaeus merguiensis and Parapeneopsis sculptilis, are opportunistic omnivores (Chong and Sasekumar 1981, Leh and Sasekumar 1984, Sasekumar and Chong 1987, Robertson 1988). Penaeus merguiensis collected from inshore and offshore of Peninsular Malaysia have gut contents that comprise about 70% by volume animal matter. For Parapeneopsis sculptilis, which only occurs offshore, this increases to about 80% (Leh and Sasekumar 1984). In both species the balance of the diet is plant material with over 95% being recognizable mangrove detritus, and the remainder comprising benthic diatoms and algae (Leh and Sasekumar 1984, Robertson 1988).

Prey items of juvenile Penaeus merguiensis living inshore are diverse but include many benthic species such as foraminiferans, polychaetes and small crustaceans that are active consumers of benthic microalgae (Dall 1968, Chong and Sasekumar 1981, Leh and Sasekumar 1984). Adults of both prawn species living offshore actively prey upon small and large crustaceans, molluscs and fishes (Chong and Sasekumar 1981, Leh and Sasekumar 1984, Robertson 1988).

Robertson (1988) reported that juvenile Penaeus merguiensis living within the creeks draining mangrove forests from NE Australia have a much lower percentage of animal prey within their guts than those studied by Chong and Sasekumar (1981) and Leh and Sasekumar (1984) in Peninsular Malaysia. Robertson (1988) found the majority of food in the gut to be detrital flocs (identified as organic aggregates and small particles from fecal pellets bound in a matrix with diatoms and dead algal filaments), These flocs comprised 74% of gut volume for the smallest prawns (7 - 10 mm carapace length but this volume decreased to 54% for 18 - 20 mm individuals. Recognizable mangrove detritus formed less than 8.5% of gut volume for all size prawns within the creeks.

Stable Isotope Analysis of Consumers

One impediment to using stable isotope ratios for identifying food resources of consumers is isotopic fractionation that occurs as carbon, sulfur and nitrogen are metabolized and passed up through the food web. Peterson and Fry (1987) summarized from numerous literature reports the magnitude of possible fractionation and reported that only small isotope shifts of about +0.2‰ were likely between successive trophic levels for carbon and sulfur. For nitrogen, the shift was larger and quite variable but averaged 3.2‰.

Fortunately, however, in our study there were large isotopic differences between the major primary producers that enable discrimination between potential food sources even if some

isotopic fractionation does occur. The carbon, sulfur and nitrogen ratios of mangrove material were always more negative than seston samples collected either in tidal creeks or offshore and generally more negative than the macroalgae growing on the creek banks. Literature data available for benthic diatoms, and our values for benthic material obtained from mud skipper stomach contents, have carbon values more positive than phytoplankton and considerably more positive than mangroves (Fig. 2). Sulfur values for mud skipper stomach contents and benthic microalgae were similar to mangrove but more negative than phytoplankton. Because of these differences in isotopic ratios between the various primary producers it is possible to critically evaluate the ratios of the various consumers we sampled and discern their main food sources.

When interpreting stable isotope ratios it is important to obtain "end member" isotopic values for consumers known to feed predominately on a certain food item. In this way it is possible to compare these known values to those of species for which the diet is unknown. In our study the organism most likely to have stable isotope ratios close to those of mangrove leaves is the grapsid mangrove crab, Sesarma versicolor. This species is an almost obligate consumer of fallen mangrove leaves which it retrieves and consumes within its burrows on the forest floor. Such "shredder" organisms are very important in reducing the leaves to small particles that are then available to other consumers. This crab (sample 28) had tissue carbon, sulfur and nitrogen isotopic composition essentially identical to that of mangrove material (Figs 2, 3, and 4). Conversely the offshore sardine, Sardinella fimbriata (sample 33), feeds on pelagic prey and has isotopic ratios entirely consistent with phytoplankton being the initial source of organic matter, with some degree of isotopic fractionation.

Stable isotope values for prawns we collected fall into two relatively distinct domains, neither of which coincide with values exhibited by the "end-members" feeding exclusively on either mangrove or phytoplankton. The juvenile and adult P. sculptilis and adult P. merguiensis collected from offshore had more positive carbon, sulfur, and nitrogen values than juvenile P. merguiensis collected from within the tidal creeks. There was an indication that the isotopic ratios of both prawn species collected farthest offshore (Fig 3; samples 5, 8, and 10) clustered together, with slightly more positive $\delta^{13}C$ and slightly less positive $\delta^{15}N$ values than prawns collected 3 and 6.5 km offshore. Such a pattern would be consistent with isotopic fractionation of carbon in offshore prawns due to them feeding on prey items at higher trophic levels. If that was the correct explanation then the nitrogen values would also be expected to exhibit a positive shift and not the observed slight negative shift. The reasons for these isotopic shifts can only be elucidated by further detailed sampling of prawns at varying distances offshore.

Based on the sulfur and carbon data (Fig. 2) it is apparent that mangrove detritus does not contribute either directly or via microheterotrophic intermediaries to the nutrition of prawns collected offshore. Instead, because the prawn's stable isotope signal is intermediate between phytoplankton (= seston) and benthic microalgae it indicates that both species of prawns living offshore appear to rely equally on benthic and pelagic microalgae. As discussed above, stomach content analysis indicates that less than 5% of the plant material ingested by

both species of prawn collected offshore is algal cells. Thus, in order for prawns to have isotopic values similar to these algal groups it is likely that they are actively preying upon secondary consumers of phytoplankton and benthic microalgae.

Sasekumar et al. (1992) report that adult Penaeus merguiensis, but not Parapeneopsis sculptilis, migrate inshore at high tide to prey on organisms on the intertidal mudflats. Thus in order for Parapeneopsis sculptilis to exhibit stable isotope values so divergent from our seston values and average phytoplankton values it must be preying upon other species that migrate inshore to feed on benthic microalgae.

Considering the sulfur and carbon double plot (Fig. 2) it is apparent that juvenile Penaeus merguiensis collected inshore obtain some of their nutriments either indirectly via microbial intermediaries or directly from mangrove detritus. Benthic microalgae also must contribute to these prawns nutrition to a greater extent than phytoplankton. The carbon and sulfur stable isotope ratios of these juvenile prawns are very similar to the stomach contents obtained from the mud skipper suggesting that the prawns and fish are feeding on a very similar mix of organic material (Fig. 2).

Our findings provide direct experimental evidence of Dall's (1968) hypothesis, based on his analysis of the gut content of penaeid prawns from eastern Australia, that microorganisms are the prawns major food items. Moriarty (1976), however, collected penaeid prawns Metapenaeus bennettae over seagrass beds in the same estuary as Dall (1968) and also reported a high abundance of bacteria in the gut, but very low abundance of microalgae. Using radiotracer techniques Moriarty (1976) demonstrated that these prawns could assimilate a range of bacterial species with > 80% efficiency. The same prawns also assimilated a blue-green alga with high efficiency (63% n = 6).

Our triple stable isotope results also support some of the conclusions of Rodelli et al. (1984) based on carbon stable isotope analysis of material collected from the same region. They estimated that P. merguiensis collected inshore obtained on average 65% of their carbon from mangrove detritus with the remainder coming from a combination of phytoplankton and benthic microalgae. They could find no isotopic evidence that mangrove detritus was used as a carbon source by prawns offshore but they suggested that phytoplankton carbon was the major source. However, our sulfur isotope data (Fig. 2) indicates a much greater importance of benthic microalgae in the nutrition of prawns both inshore and offshore than postulated by Rodelli et al. (1984).

Our conclusions are supported by previous work on the nutrition of penaeid prawns living in close proximity to mangrove forests in other regions. For example, our $\delta^{13}C$ mean values for prawns collected offshore of -15.51 was almost identical to those recorded in the Caribbean for two penaeid species of -15 to -18.1 by Stoner and Zimmerman (1988). Based on gut content analysis and carbon stable isotopes they concluded that the prawns were mainly predators feeding on organisms that were utilizing the filamentous blue-green alga Spirulina sp. as a primary carbon source and not mangroves or phytoplankton.

Conversely, Gleason and Wellington (1988) suggest that small penaeid prawns Penaeus aztecus in a Texas saltmarsh are omnivores, feeding primarily on demersal fauna and phytoplankton. They suggest that the prawns stable carbon isotope values of -18.8 $\pm$ 0.13 (S.E. n = 4) reflects a diet that is primarily derived either directly from plankton ($\delta^{13}C$ value of -22.0 $\pm$ 2.15 (S.D. n = 30) or via the consumption of demersal prey. However, because they did not perform multiple isotope analyses, and they did not sample benthic microalgae, their results can not exclude the possibility that benthic microalgae were also contributing to the prawns nutrition.

Rodelli et al. (1984) determined ^{13}C values for a variety of species collected from the same geographic location (Table 1). There was generally close agreement for the $\delta^{13}C$ values from both studies with the exception of two species of molluscs. In our study, the bivalve Polymesoda erosa (sample 29) was 3‰ more negative than reported by Rodelli et al. 1984. This indicates that the specimens we collected had a stronger mangrove carbon signal than those sampled by Rodelli et al. (1984). The sulfur signal for this bivalve (Fig. 2) was over 5‰ more negative that mangrove leaves and was the most negative of all consumers we analyzed. This suggests that benthic microalgae and microorganisms that obtain their sulfur from interstitial waters were important in the clam's diet. Conversely, in our study the herbivorous gastropod Littorina melanostoma (sample 31) had $\delta^{13}C$ values less negative than reported by Rodelli et al. (1984) suggesting a greater reliance on benthic microalgae than mangrove detritus. The reasons for these differences are not apparent, but may relate to site-specific variations in food availability to these organisms.

Radiotracer Feeding Studies

The efficiency with which prawns assimilated chemically defined lignocellulosic crude fibre is presented in Table 2. Adult P. merguiensis and juvenile and adult P .sculptilis collected from offshore, and juvenile P. merguiensis collected from within the creeks, assimilated mangrove carbon with the same efficiency (mean 11.68 $\pm$ 1.13 (S.E. n = 47). The assimilation efficiency of adult P. merguiensis was significantly lower (SNK $P < 0.01$) . We have no explanation for the significantly lower utilization of the mangrove material by adult Penaeus merguiensis. Experimental conditions were not altered and so the difference may reflect either an ontogenic loss of cellulase enzymes in this species or possibly a seasonal reduction in utilization of mangrove detritus and hence a reduction in the production of cellulase. Feeding studies for all species and life stages were, however, performed concurrently during January and February.

These results indicate that prawns both inshore and offshore can digest mangrove detritus albeit at a relatively low assimilation efficiency, compared with their ability to digest microorganisms and animal prey reported by Moriarty (1976) and Dall and Moriarty (1983). Previous studies by Chong and Sasekumar (1981) and Leh and Sasekumar (1984) have shown that over 95% of the plant material within the guts of both juveniles and adults of both species of prawn is recognizable mangrove detritus. Thus, it would seem likely that even

though mangrove detritus is ingested it can only make a limited direct contribution to the carbon requirements of prawns both inshore and offshore.

The potential role of cellulosic compounds in the nutrition of prawn has not received a great deal of attention. Early nutritional studies (reviewed by Dall and Moriarty 1983) suggest that some species of crustaceans, including some isopods and mysids, have the capacity to digest cellulose. A recent radioisotope study by Crosby (1985) demonstrated that the grass shrimp Palaemonetes pugio could assimilate carbon from marsh cordgrass Spartina alterniflora detritus with approximately 40% efficiency. Our results indicate that both P. merguiensis and P. sculptilis have the capacity to utilize refractory lignocellulosic albeit with a lower efficiency.

5: Impact, Relevance and Technology Transfer

The Malaysian participants in this project have developed an international reputation based on their last 15 years research into the interrelationship between mangrove forests and commercial fisheries. Currently there is an ongoing debate concerning the consequences of reclaiming tropical mangrove forests on coastal fisheries. Thus, the results from our study of the nutrition of commercial prawn species will be invaluable in advising coastal zone managers in Malaysia. Also, because both Dr. Sasekumar and Dr. Chong participate in many regional programs they will be able to transfer the knowledge gained from our study to help guide management decisions and provide a basis for future research in S.E. Asia.

Dr. Sasekumar and Dr. Chong came to the University of Maryland as part of this project for 4 weeks in June 1990. Dr. Marshall accompanied them on a research trip to Florida with a visit to mangrove forests bordering the Everglades National Park. During this trip they also had discussions with leading scientists working on the management of these extensive mangrove environments. This enabled them to develop contacts and discuss their research with American scientists. While at the University of Maryland they also had opportunities to discuss issues relating to the conservation and development of tidal wetlands with a large group of scientists. These contacts have helped build their expertise and enabled them to continue to put their research into a broader context.

Another benefit of this project on the Department of Zoology is that about $3,000 of the funds were used to renovate an aquarium facility at the University and purchase new collecting gear.

6: Project Activities/Outputs

Meetings: Prof. Marshall and Dr Newell have both made two extended research trips to work with Dr. Sasekumar and Dr. Chong at the University of Malaya. The first trip was undertaken jointly between January 15 and February 18 1989. On the second trip Dr.

Marshall went for 4 weeks November 1990 in order to collect specimens. Dr. Newell went for 5 weeks in January 1991 to continue seasonal experiments initiated in 1989.

Dr. Sasekumar and Dr. Chong came to the University of Maryland as part of this project for 4 weeks in June 1990. During this period we worked on some aspects of this project and Dr. Marshall accompanied them on a tour of the extensive mangrove tracts in the Everglades National Park. This provided an opportunity to discuss management issues with scientists working in these ecosystems.

Publications: A version of this report has been submitted for publication to the international journal "Marine Biology".

7: Project Productivity

The overall aims and specific objectives that we listed in our proposal were:

1: Use a combination of defined feeding studies with ^{14}C labelled mangrove material and stable isotope tracer techniques to further ascertain the extent and rate at which commercially important prawns utilize mangroves as a source of nutrition.

2: To use these data to elucidate the reasons for a possible discrepancy between the large quantities of mangrove detritus ingested by offshore prawns and their apparent low levels of assimilation of mangrove carbon.

3: To interpret from such information the relative significance of the mangrove detritus pathway for the major prawn fisheries that are economically important in S.E. Asian countries.

As described in detail in section 3 above, and briefly summarized below, we achieved our three objectives. We also went beyond what we proposed by elucidating the key role that benthic microalgae play in the nutrition of prawns both inshore and offshore.

Using multiple stable isotope analysis, we substantiated our hypothesis that mangrove detritus contributed strongly to the nutrition of juvenile P. merguiensis living within the tidal creeks draining the mangrove forests, but not to juveniles and adults living offshore. However, the ability of juvenile P. merguiensis living inshore to directly assimilate refractory cellulosic material was only 13.5%. This was not significantly greater than the average value of 10.5% for juvenile and adult P. sculptilis living offshore. Thus, because the amount of mangrove detritus found in the guts of prawns in these two habitats does not differ substantially (Leh and Sasekumar 1984) it suggests that juvenile P. merguiensis living inshore are not utilizing the mangrove material directly. Mangrove detritus that is small enough to be ingested by prawns is likely to be highly refractory because it has been through the "shredder" organisms such as the grapsid crab that feed directly on fallen mangrove leaves.

Instead prawns within the creeks are perhaps digesting microorganisms that are either utilizing particulate or dissolved organic carbon that is originating from the mangroves. Such microbial intermediaries are likely to be most abundant in the tidal creeks where the mangrove detritus is most concentrated and hence are responsible for the strong mangrove stable isotope signal in the juvenile P. merguiensis.

Our results indicate that the abundant stocks of benthic microalgae on these intertidal mud flats (Thong et al. 1993). make a more important contribution to coastal production than detrital inputs from adjacent mangrove forests. We conclude that the intertidal mudflats along the edges of mangrove forests are essential to the ecology of the region and commercial important prawn fisheries. Thus, the role of mangrove forests in altering the hydrographic environment of coastal waters (Wolanski et al 1980, Wattayakorn et al. 1990) and perhaps enhancing the development of intertidal mud flats must be considered in future management plans of these ecosystems.

8: Future Work

Our results demonstrating the high contribution that benthic microflora makes to providing food for prawns both inshore and offshore of Malaysia was unexpected. This suggests that these intertidal mudflats where the microalgae grow are much more productive, and these algae more important, than previous studies have recognized. We believe that future studies should concentrate on determining the productivity of these microalgae and understanding how this algal carbon is grazed and passed to the offshore carnivores, such as prawns. Because we have shown that benthic diatom production on these intertidal mudflats is so essential to commercial fisheries it is important to understand the physical factors that lead to the development of these mudflats. Although mangrove forests are essential in trapping sediments it is unclear how the extent of these mudflats is affected by destruction of the mangrove forests.

9: Literature Cited

Calder , J.A. and P.L. Parker. 1968. Stable carbon isotope ratios as indices of petrochemical pollution of aquatic systems. Environ. Sci. Technol. 2:535-539.

Chong, V.C. and A. Sasekumar. 1981. Food and feeding habits of the white prawn Penaeus merguiensis. Mar. Ecol. Prog. Ser. 5:185-191.

Chong, V.C., A. Sasekumar, M.U.C. Leh and R. D'Cruz. 1990. The fish and prawn communities of a Malaysian coastal mangrove system, with comparisons to adjacent mud flats and inshore waters. Estuar. Coast. Shelf. Sci. 31:703-722

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochem. Cosmochim. Acta. 12:133-149.

Crosby, M.P. 1985. The use of a rapid radiolabeling method for measuring ingestion rates of detritivores. J. Exp. Mar. Biol. Ecol. 93:273-283.

Crosby, M.P., C.J. Langdon, and R.I.E. Newell. 1989. Importance of refractory plant material to the carbon budget of the oyster Crassostrea virginica. Mar. Biol. 100:343-352.

Dall, W. 1968. Food and feeding of some Australian penaeid shrimps. Fish. Rep. FAO. 57:251-258.

Dall, W. and D.J.W. Moriarty. 1983. Functional aspects of nutrition and digestion. pages 215-261 IN: The Biology of the Crustacea. Volume 5. L.H.Mantel, ed. Academic Press, New York, NY.

Ferguson, R.L. and R.B. Williams. 1974. A growth chamber for production of ^{14}C-labelled salt marsh plants and its application to smooth cordgrass Spartina alterniflora. J. Exp. Mar. Biol. Ecol. 14:251-259.

Fritz, P. and J.C. Fontes. 1980. Handbook of Environmental Isotope Geochemistry. Vol. 1. Elsevier Scientific Publ. Amsterdam, Netherlands.

Fritz, P. and J.C. Fontes. 1986. Handbook of Environmental Isotope Geochemistry. Vol. 2. Elsevier Scientific Publ. Amsterdam, Netherlands.

Fry, B. 1983. Fish and shrimp migrations in the northern Gulf of Mexico analyzed using stable C, N, and S isotope ratios. Fish. Bull. 81:789-801.

Fry, B., R.S. Scanlan, J.K. Winters and P.L. Parker. 1982. Sulphur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. Geochimica et Cosmochimica Acta 46:1121-1124.

Gleason, D.F. and G.M. Wellington. 1988. Food resources of postlarval brown shrimp (Penaeus aztecus) in a Texas salt marsh. Mar. Biol. 97:329-337.

Hatcher, B.G. R.E. Johannes and A.I. Robertson. 1989. Review of research relevant to the conservation of shallow tropical marine ecosystems. Oceanogr. Mar. Biol. Ann. Rev. 27:337-414.

Haines, E.B. 1976. Stable carbon isotope ratios in the biota, soils and tidal water of a Georgia salt marsh. Est. Coast. Shelf. Sci. 4:609-616.

Haines, E.B. and C.L. Montague. 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{14}C$ ratios. Ecology 60(1):48-56.

Hughes, E.H. and E.B. Sherr. 1983. Subtidal food webs in a Georgia estuary: $\delta^{13}C$ analysis. J. Exp. Mar. Biol. Ecol. 67:227-242.

Langdon, C.J. and R.I.E. Newell. 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster Crassostrea virginica and the mussel Geukensia demissa. Mar. Ecol. Prog. Ser. 58:299-310.

Leh, M.U. and A. Sasekumar. 1984. Feeding ecology of prawns in shallow waters adjoining mangrove shores. Pages 331-353 in E. Soepadmo et al. (eds.), Proc. Asian Symp. Mangrove. Environment - Res and Management. Universiti Malaya, Kuala Lumpur.

Marshall, N. M. 1994. Mangrove conservation in relation to overall environemntal considerations. Hydrobiologia. In Press.

Montague, C.L., S.M. Bunker, E.B. Haines, M.L. Pace, and R.L. Wetzel. 1981. Aquatic macroconsumers. Pages 69-85 In: L.R. Pomeroy and R.G. Wiegert (eds.). The ecology of a salt marsh. Springer.

Moriarty, D.J.W. 1976. Quantitative studies on bacteria and algae in the food of the mullet Mugil cephalus L. and the prawn Metapenaeus bennettae (Racek & Dall) J. exp. mar. biol. ecol. 22:131-143.

Nixon, S.W., B.N. Furnas, V. Lee, N. Marshall, J.E. Ong, C.H. Wong, W.K. Gong and A. Sasekumar. 1984. pages 534 - 544. The role of mangroves in the carbon and nutrient dynamics of Malaysian estuaries. Proc. Asian Symp. Mangr. Environment - Res and Management. Publisher?

Newell, R.I.E. and C.J. Langdon. 1986. Digestion and absorption of refractory carbon from Spartina alterniflora (Loisel.) by the oyster, Crassostrea virginica (Gmelin). Mar. Ecol. Prog. Ser. 34:105-115.

Stribling, J. 1994. Thesis Title, Doctoral dissertation. University of Maryland, College Park. ???pp.

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18:293-320.

Peterson, B.J. and R.W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. 32:1195-1213.

Peterson, B.J., R.W. Howarth and R.H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science 227:1361-1363.

Peterson, B.J., R.W. Howarth and R.H. Garritt. 1986. Sulfur and carbon isotopes as tracers of salt-marsh organic matter flow.

Pomeroy, L.R. and R.G. Wiegert (Eds.). 1981. The ecology of a salt marsh. Springer

Ribelin, B.W. and A.C. Collier 1979. Ecological considerations of detrital aggregates in the salt marsh. Pages 47-68 In: Ecological Processes in Coastal and Marine Systems. R.J. Livingston (ed.) Marine Science 10.Plenum Press, New York.

Robertson, A.I. 1988. Abundance, diet and predators of juvenile Banana prawns Penaeus merguiensis, in a tropical mangrove estuary. Aust. J. Mar. Freshwater Res. 39:467-478.

Robertson , A.I., D. M. Alongi, P.A. Daniel, and K.G. Boto 1991. Pages ?? - ?? In: H. Choat et al (eds.) Proc. Sixth Int. Coral Reef Symp. James Cook University, Townsville.

Rodelli, M.R., J.N. Gearing, P.J. Gearing, N. Marshall, and A. Sasekumar. 1984. Stable isotope ratios as a tracer of mangrove carbon in Malaysian ecosystems. Oecologia (Berl.) 61:326-333.

Sasekumar, A. and V.C. Chong. 1987. Mangroves and prawns: Further perspective. Pages 10-20 In Proc. 10th Annual seminar of the Malaysian Soc. of Marine Sciences.

Sasekumar, A., V.C. Chong, M.U. Leh and R. D'Cruz. 1992. Mangroves as a habitat for fish and prawns. Hydrobiologia 247:195-207.

Schleser, G.H. 1992. $\delta^{13}C$ pattern in a forest tree is an indicator of carbon transfer in trees. Ecology 73:1922-1925.

Stephenson, R.L., F.C. Tan and K.H. Mann. 1984. Stable carbon isotope variability in marine macrophytes and its implications for food web studies. Marine Biology. 81:223-230.

Stoner, A.W. and R. J. Zimmerman 1988. Food pathways associated with penaeid shrimps in a mangrove-fringed estuary. Fish. Bull. 86:543-551.

Strickland, J.D.H., and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Bulletin Fisheries Research Board Canada 167, 310 pp.

Sullivan, M.J. 1975. Diatom communities from a Delaware saltmarsh. J. Phycol. 11:384-390.

Sullivan, M.J. and C.A. Moncreiff 1988. Primary production of edaphic algal communities in a Mississippi salt marsh. J. Phycol. 24:49-58.

Sullivan, M.J. and C.A. Moncreiff 1990. Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analyses. Mar. Ecol. Prog. Ser. 62:149-159.

Thong, K.L. A. Sasekumar and N. Marshall. 1993. Nitrogen concentrations in a mangrove creek with a large tidal range, Peninsular Malaysia. Hydrobiologia 254:125-132.

Vance, D.J., M.D.E. Haywood and D.J. Staples. 1990. Use of a mangrove estuary as a nursery area by postlarval and juvenile Banana prawns, Penaeus merguiensis de Man, in northern Australia. Estuar. Coastal. Mar. Sci. 31:689-701.

Wattayakorn, G., E. Wolanski and B. Kjerfve 1990. Mixing, trapping and outwelling in the Klong Ngao mangrove swamp, Thailand. Est. Coast. Shelf. Sci. 31667-688.

Wolanski, E. M. Jones and J.S. Blunt. 1980. Hydrodynamics of a tidal creek-mangrove system. Aust. J. Mar. Fresh. Res. 31431-450.

Zieman, J.C., S.A. Macko, and A.L. Mills. 1984. Role of seagrasses and mangroves in estuarine food webs: Temporal and spatial changes in stable isotope composition and amino acid content during decomposition. Bull. Mar. Sci. 35:380-392.

Table 1. Carbon, nitrogen and sulfur stable isotope ratios for specimens collected either various distances off-shore (Fig. 1; Sites A, B, and C) or along the creeks draining the mangrove forests (Fig. 1; Sites D and E). Isotope ratios were obtained from samples consisting of the number of individuals (n) listed. Data on carbon stable isotope ratios obtained by Rodelli et al. (1984) in a previous study conducted in the same area are provided for comparison. Their values for prawns are grand means (± SE) of n separate samples (note that they did not separate the co-occurring P. sculptilis into juveniles and adults).

Sample Number	Specimen	Collection Site (see Fig. 1)	$\delta^{13}C$	$\delta^{15}N$ Ratios	$\delta^{34}S$	$\delta^{13}C$ (Rodelli et al.)
Prawns						
1.	P. merguiensis juv. n=10	Creek (D)	-22.37	+8.81	+5.4	-20.7 +1.72 (n = 4)
2.	P. merguiensis juv. n=10	Creek (E)	-19.90	+9.69	+9.4	
3.	P. merguiensis adults n=5	3.5 km (C)	-17.75	+11.08	+10.1	
4.	P. merguiensis adults n=10	7.5 km (B)	-16.06	+10.82		-16.9 (n = 2)
5.	P. merguiensis adults n=10	16 km (A)	-15.16	+10.47	+11.1	
6.	P. sculptilis juv. n=20	3.7 km (C)	-15.32	+12.08	+12.4	
7.	P. sculptilis juv. n=10	7.5 km (B)	-15.35	+11.33	+13.5	
8.	P. sculptilis juv. n=10	16 km (A)	-14.37	+10.59	+13.6	
9.	P. sculptilis adults n=10	7.5 km (B)	-16.12	+11.97	+14.0	-16.3 ±0.54 (n = 5)
10.	P. sculptilis adults n=10	16 km (A)	-13.98	+10.64	+13.6	
Mean (± S.E.) del values for offshore prawns			-15.51 ±0.41	+11.12 ±0.22	+12.61 ±0.56	-16.45 ±0.53
Mangrove Leaves						
11.	Avicennia alba n=10	Forest (D)	-26.66	+5.03	+5.1	-27.1
12.	Sonneratia alba n=10	Forest (E)	-27.06	+4.72	+0.8	-26.2
13.	Rhizophora macronulta n=5	Forest (E)	-28.52	+2.24	-1.4	-27.2
14.	Bruguiera parviflora n=5	Forest (E)	-29.81	+6.25	+1.85	-28.4
15.	mangrove leaves from trawl	3.7 km (C)	-27.55	+8.14	+4.25	
16.	mangrove leaves from trawl	3.7 km (C)	-29.15	+7.42	+7.4	
17.	mangrove leaves from trawl	7.5 km (B)	-28.61	+4.10	+6.8	
18.	mangrove leaves from trawl	16 km (A)	-29.36	+5.33	+11.8	
Mean (± S.E.) del values			-28.34 ±0.40	+5.40 ±0.66	+4.57 ±1.48	
Other Specimens						
19.	Sediment Sample	Creek (E)	-25.92	+4.11	-11.6	-26.2
20.	Sediment sample	16 km (A)	-21.87	+2.12	-8.85	-24.8

21.	Seston	Creek (E)	-22.68	+10.42	+16.1	
22.	Seston	16 km (A)	-18.58	7.99	+17.9	
23.	Stomach contents of Boleopthalmus boddaerti, mud skipper	Creek (E)	-19.57	+6.80	+6.5	
24.	Catenella nipae, macroalgae	Creek (E) bank	-22.04	+11.90	+15.0	
25.	Gracilaria sp., macroalgae	Creek (D) bank	-36.45	+11.43		
26.	Gracilaria blogettii, macroalgae	Creek (E) bank	-29.51	+11.08	+17.4	
27.	Dictyota dicotonia, macroalgae	Creek (D) bank	-19.94	+10.36	+16.4	
28.	Sesarma versicolor, Mangrove crab	Creek (E) bank	-24.97	+7.56	+5.25	-24.0
29.	Polymesoda erosa, bivalve n=4	Creek (E) bank	-26.01	+7.75	-4.3	-23.6
30.	Telescopium mauritsi, Gastropod on mangrove roots n=10	Creek (E) bank	-21.38	+9.11	+5.5 +5.2	-22/-26
31.	Littorina melanostoma, Gastropod on sediment n=20	Creek (D) bank	-17.78	+5.12	+10.0	-24.6
32.	Carcinoscopius rotundicauda, horseshoe crab n=6	7.5 km (B)	-15.93	+11.48		-16.2
33.	Sardinella fimbriata, fish n=2	7.5 km (B)	-16.71	+13.48	+16.55	-17.7
34.	Phyllophorus sp., sea cucumber n=3	16 km (A)	-12.97	+8.86	+18.4	
35.	Molpodia sp., sea cucumber n=2	16 km (A)	-14.49	+13.77		
36.	Benthic worms, mixed species	16 km (A)	-16.59	11.2		17.3

Table 2. The mean (± 1 S.E.) efficiency with which juvenile and adult P. merguiensis and P. sculptilis assimilated chemically defined lignocellulosic crude fibre prepared from radiolabeled (^{14}C) mangrove leaves. n = number of individual prawns tested.

Prawn Species	Life Stage	Collection Site	Mean (± S.E.) Assimilation Efficiency.
P. merguiensis	Juvenile	Tidal Creek	13.50 ± 2.60 n = 18
P. merguiensis	Adult	Offshore	2.82 ± 0.84 n = 17
P. sculptilis	Juvenile	Offshore	10.02 ± 1.03 n = 13
P. sculptilis	Adult	Offshore	10.94 ± 1.35 n = 16

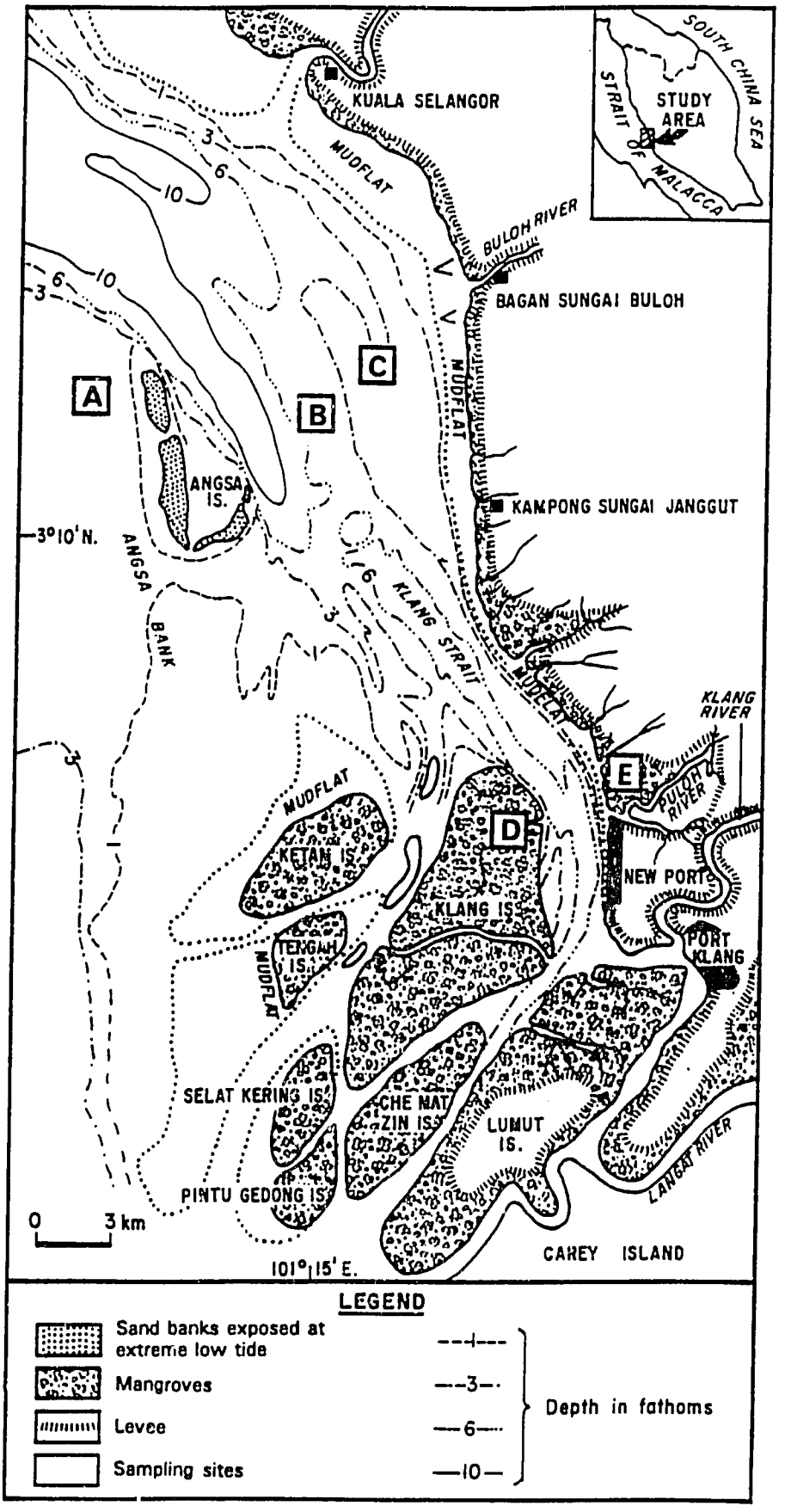

Figure 1. Section of the west coast of peninsular Malaysia where samples for stable isotope analyses were collected. Site A was 16 km offshore, Site B was 7.5 km offshore, and Site C was 3.7 km offshore. Site E was along the Sementa Besar tidal creek and Site D was along the Sungai Nyiris tidal creek.

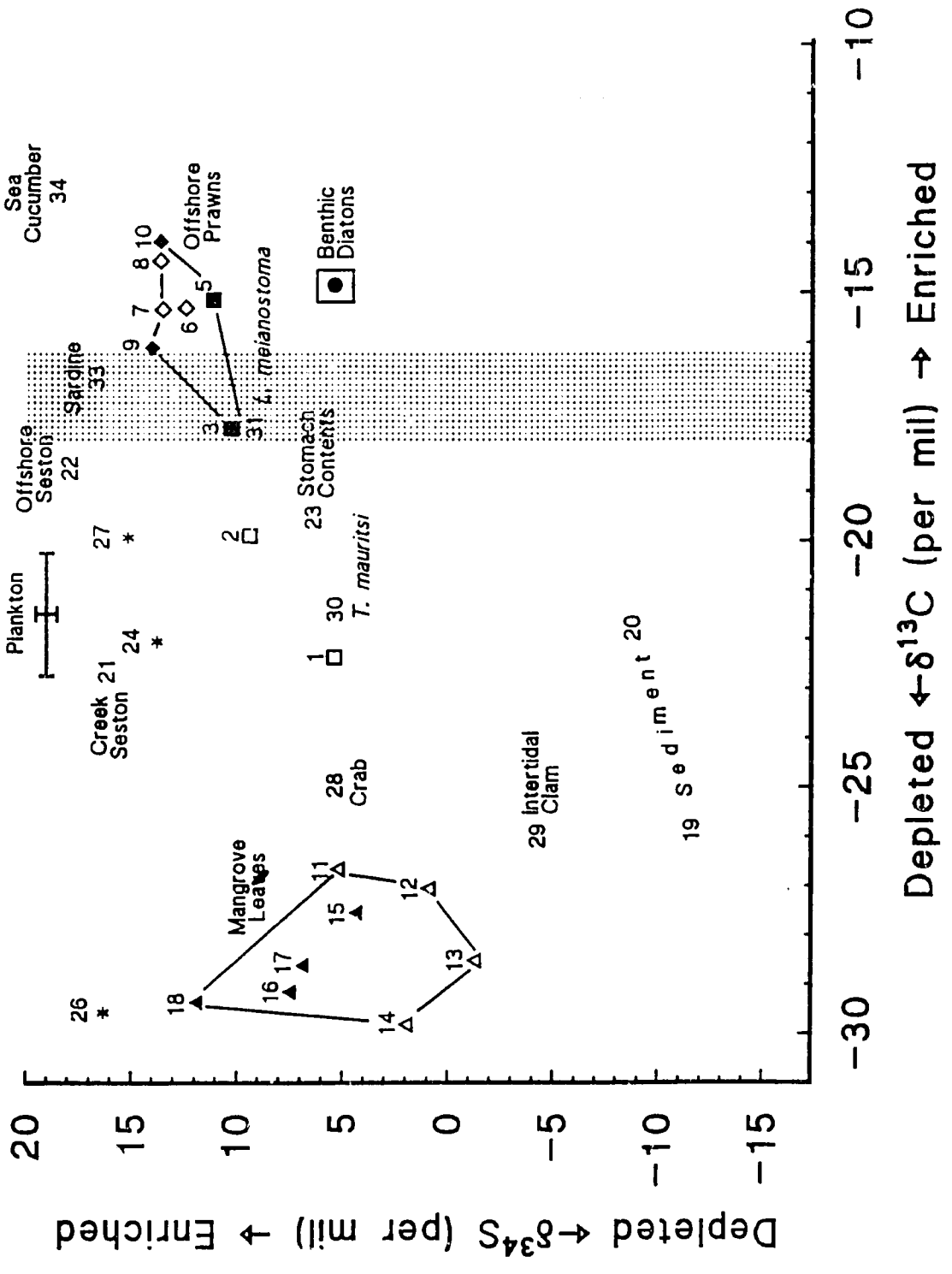

Figure 2. Relationship between del sulfur and del carbon values for samples collected within mangrove forests (open symbols) and at distances offshore (filled symbols). Numbers without symbols represent the actual value and correspond to the sample numbers listed in Table 1. Numbered square symbols represent banana prawns, Penaeus merguiensis, diamonds represent the rainbow prawns, Parapeneopsis sculptilis, and triangles represent mangrove material. Phytoplankton values are literature values for marine phytoplankton summarized by Peterson and Howarth (1987) and represent mean ± 1 s.d. (n = 56 for carbon and n = 4 for sulfur). The benthic diatom value (circle within open box) is a single sample of saltmarsh microalgae from Chesapeake Bay, Maryland obtained by Stribling (Univ of Maryland, pers. comm.). The stippled histogram is an mean (± S.E. n = 5) carbon value for cultured diatoms from Rodelli et al. (1984).

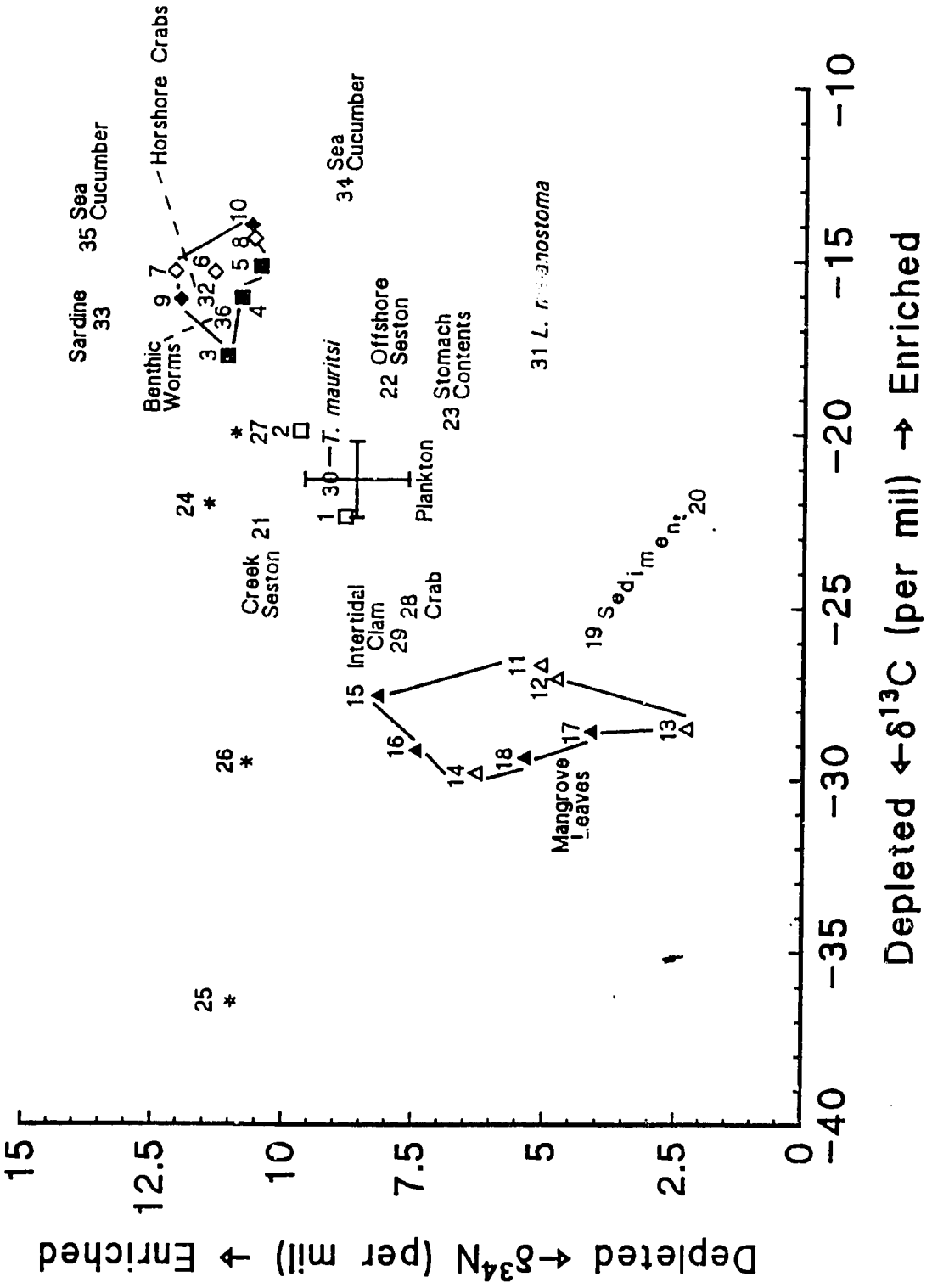

Figure 3. Relationship between del nitrogen and del carbon values for samples collected within mangrove forests (open symbols) and at distances offshore (filled symbols). Numbers without symbols represent the actual value and correspond to the sample numbers listed in Table 1. Numbered square symbols represent banana prawns, Penaeus merguiensis, diamonds represent the rainbow prawns, Parapeneopsis sculptilis, and triangles represent mangrove material. Data for marine plankton are literature values summarized by Peterson and Howarth (1987) and represent means ± 1 s.d. (n = 4 for nitrogen and n = 4 for sulfur)

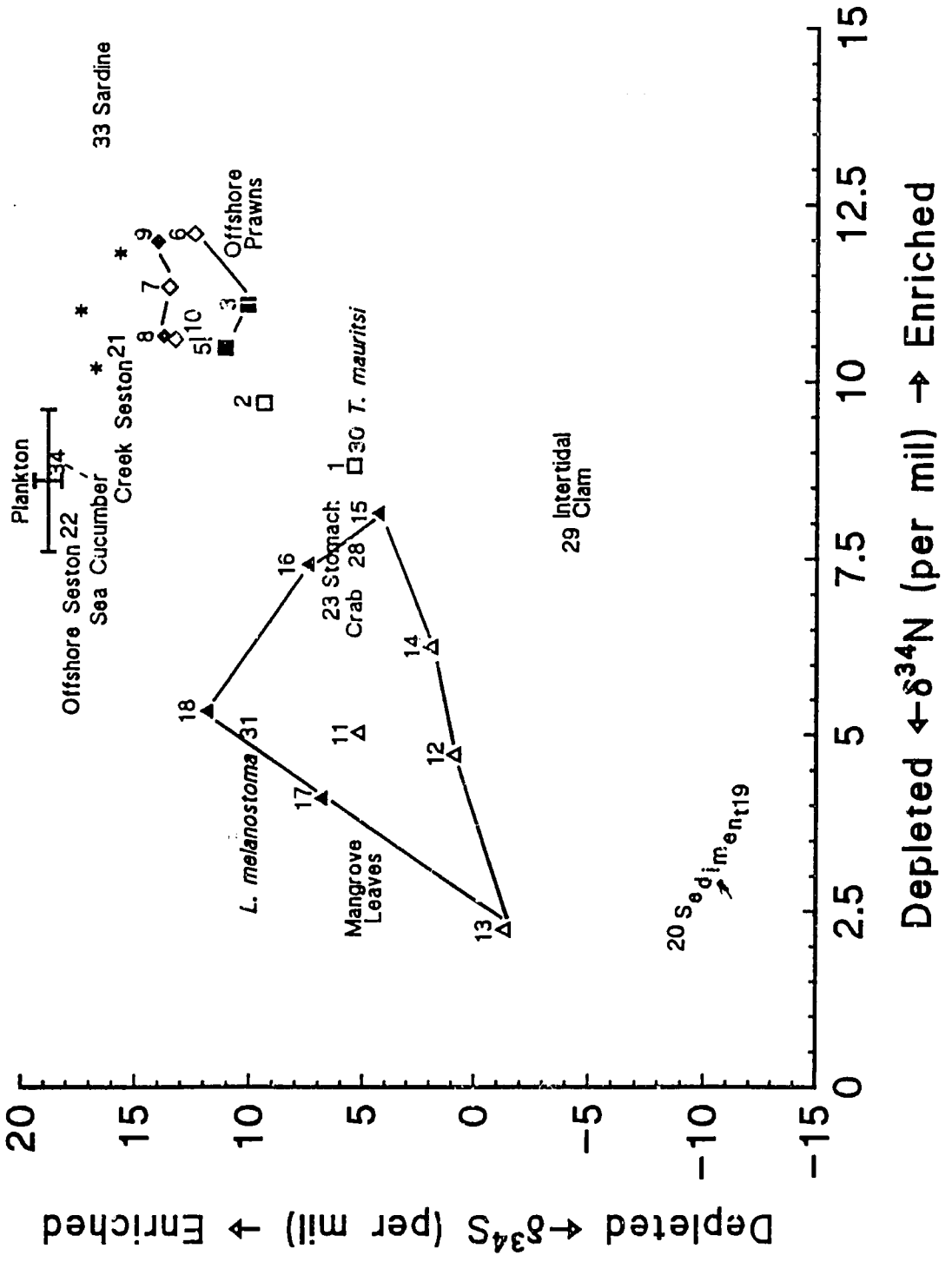

Figure 4. Relationship between del sulfur and del nitrogen values for samples collected within mangrove forests (open symbols) and at distances offshore (filled symbols). Numbers without symbols represent the actual value and correspond to the sample numbers listed in Table 1. Numbered square symbols represent banana prawns, Penaeus merguiensis, diamonds represent the rainbow prawns, Parapeneopsis sculptilis, and triangles represent mangrove material. Data for marine plankton are literature values summarized by Peterson and Howarth (1987) and represent means ± 1 s.d. (n = 56 for carbon and n = 4 for nitrogen).