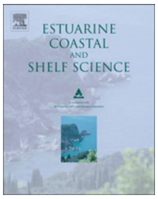
Estuarine, Coastal and Shelf Science 87 (2010) 583–590

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss



Food partitioning of leaf-eating mangrove crabs (*Sesarminae*): Experimental and stable isotope (^{13}C and ^{15}N) evidence

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ARTICLE INFO

Article history:
Received 18 October 2009
Accepted 19 February 2010
Available online 1 March 2010

Keywords:
Sesarmid crab
mangrove
food source
stable isotopes
mixing model

ABSTRACT

The feasibility of mangrove leaves as a full diet for sesarmid crabs has been questioned for decades. Since these leaves are nitrogen-poor, sesarmids probably obtain nitrogen from other sources to sustain growth. The aim of this study was to assess the food partitioning of the sesarmid species *Neoepisesarma versicolor* with emphasis on nitrogen allocation. The preference for animal tissue when crabs were pre-fed diets of different nitrogen content was determined in the laboratory. Furthermore, the possible in situ diet composition of *N. versicolor* was established from carbon and nitrogen stable isotope signature (δ^{13}C and δ^{15}N) of freshly caught individuals and their potential food sources, using a concentration-dependent mixing model. *N. versicolor* showed significantly higher feeding preferences for fish meat when pre-fed leaf material without than with access to meat, indicating that this crab species can meet its nitrogen demand by ingesting animal tissue. The stable isotope mixing model based on in situ materials suggests that the diet of *N. versicolor* consists of ~60% leaves in terms of biomass, leaving ~40% for other sources such as animal tissue and benthic microorganisms. The biomass contribution from animal tissues, in form of e.g. other crustaceans and fish carcasses, was found to account for ~15%. Despite the relative low biomass fraction, animal food sources may contribute with up to half of the nitrogen in the diet of *N. versicolor*. The quantity of ingested sediment most likely exceeds that of animal tissues. However, due to the low concentration of assimilable microalgae and other microorganism, we propose that sediment associated sources are less important as a nitrogen source for *N. versicolor* than hitherto presumed.

1. Introduction

Leaf-eating sesarmid crabs are known to have a high impact on the mangrove ecosystem in the Indo-West Pacific due to their distinctive behavior and feeding ecology (for review, see Lee, 1998 or Kristensen, 2007). Several studies have shown that these crabs consume mangrove leaves as their major food source (e.g. Robertson, 1986; Steinke et al., 1993; Thongtham and Kristensen, 2005), and stomach analyses reveal that leaf fragments account for 55–95% of the total content (Malley, 1978; Dahdouh-Guebas et al., 1999; Thongtham et al., 2008). Sesarmid crabs primarily consume leaves at the sediment floor or pull them into their burrows for later ingestion. Hence, they are capable of removing 30–90% of the annual litter production (Robertson, 1986; Micheli, 1993b; Slim et al., 1997), reducing tidal exports of mangrove derived organic matter considerably (Slim et al., 1996; Olafsson et al., 2002). In addition, the crabs facilitate decomposition of mangrove detritus and recycling of nutrients by macerating and fragmenting the litter through ingestion and gut passage. The crab fecal material consists of macerated and incompletely digested leaf litter, which provides easier access for colonization by bacteria and other organisms of the detritus food chain (Kristensen and Pilgaard, 2001).

Fresh mangrove leaves are unpalatable to most herbivores because of their high concentrations of refractory and indigestible polyphenolic compounds, such as tannins. Moreover, due to a substantial content of cellulose and lignin, the C:N ratio is high compared to plant materials of marine origin. The C:N ratio varies among mangrove species but is in general about 50–100 (e.g. Robertson, 1988; Kristensen et al., 1995). This far exceeds the value of 17, which is suggested as the maximum for sustainable animal nutrition (Russell-Hunter, 1970). It has been proposed that the observed preference of sesarmid crabs for partly decomposed leaves is caused by loss of tannins and structural carbon combined with enhanced nutritional (i.e. nitrogen) value by microbial colonization during aging (e.g. Lee, 1989; Robertson and Daniel, 1989;

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doi:10.1016/j.ecss.2010.02.016

Micheli, 1993a). However, this hypothesis has been questioned (e.g. Skov and Hartnoll, 2002; Thongtham and Kristensen, 2005), and even partly degraded mangrove leaves are probably insufficient to maintain crab growth. This is further corroborated by stable isotope analyses, which show that sesarmid crabs are more enriched in ^{13}C than mangrove leaves (Bouillon et al., 2002, 2004). The logical consequence is that leaf-eating crabs most likely supplement their leaf diet with nitrogen-rich food sources to meet the nitrogen demand. But the origin of these alternative nitrogen sources has been and still is under debate.

It is a common opinion in the literature that the majority of leaf-eating sesarmid crabs augment their nitrogen supply by ingesting sediment and assimilating the associated microalgae and bacteria (e.g. Robertson, 1986; Bouillon et al., 2002; Skov and Hartnoll, 2002). This is based on the observation that many sesarmid crabs spend a considerable amount of time feeding on sediment (e.g. Micheli, 1993b; Skov and Hartnoll, 2002; Thongtham et al., 2008). In support of this contention, Bouillon et al. (2002) demonstrated that stable carbon isotope ratios of sesarmids and sediment correlate. However, Thongtham and Kristensen (2005) argued that the large sesarmid species, *Neoepisesarma versicolor*, is physically unable to consume enough sediment to cover its nitrogen demand. Along this line of evidence, it has been suggested that some sesarmid crabs primarily achieve their nitrogen from occasional consumption of animal tissue, such as carcasses of fish, dead and live crustaceans (Thongtham and Kristensen, 2005; Thongtham et al., 2008), and small invertebrates associated with the sediment floor and leaf litter (Kwok and Lee, 1995). The reliance on animal tissue is supported by findings of crustacean exoskeleton remains and fish scales in the stomach of *N. versicolor* (Thongtham et al., 2008) and *Neosarmatium smithii* (Giddins et al., 1986).

The subfamily *Sesarminae* is very diverse regarding morphology and habitats, though most of the members are semi-terrestrial and associated with mangrove ecosystems. This study focused on the species *N. versicolor*, which plays an important ecological as well as a socio-economical role in mangrove areas of Thailand (Thongtham et al., 2008). The aim was to investigate the food partitioning of *N. versicolor*, with emphasis on nitrogen sources. The hypotheses tested were: 1. *N. versicolor* is capable of supplementing its diet with animal tissue; and 2. animal tissue contribute substantially as a nitrogen source for *N. versicolor*. These hypotheses were tested in laboratory feeding experiments supplemented with stable isotope composition analyses of wild caught crabs and their possible food sources. The most likely elemental composition and contribution of animal tissue in the diet were determined by applying a concentration-dependent stable isotope mixing model.

2. Materials and methods

2.1. Study area

Individuals of *N. versicolor* and samples of their potential food sources were collected in the Bangrong mangrove forest (8° 03′ N, 98° 25′E) located on the north east coast of Phuket Island, Thailand (Fig. 1). Bangrong is a 2.5 km^2 fringe forest receiving no river discharges and with the only freshwater supply originating from direct precipitation and run-off from land. The creek area covers 0.4 km^2 and consists primarily of a 3 km longitudinal main tidal channel. Tidal range in the area varies from 1 m at neap tide to 3 m at spring tide. The climate is monsoonal with a wet season from May to November and a dry season from December to April. The annual precipitation is about 2300 mm and the average

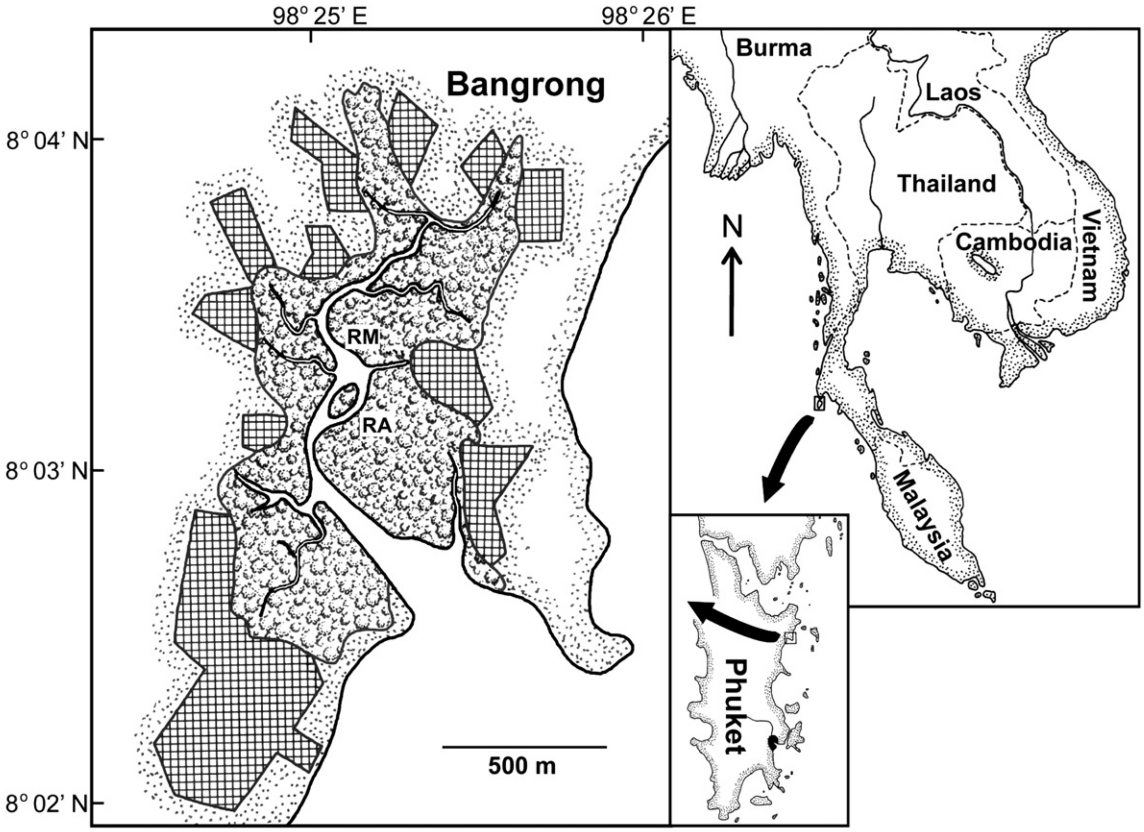

Fig. 1. Map of the Bangrong mangrove forest, Thailand. The two sampling sites are indicated.

temperature is 28 C. The dominant mangrove species are *Rhizophora apiculata*, *Rhizophora mucronata*, *Ceriops tagal*, and *Xylocarpus granatum*. The dominant benthic species are fiddler crabs (familie: Ocypodidae) and sesarmid crabs (family: Grapsidae).

2.2. *Neoepisesarma versicolor feeding experiment*

The selectivity and satiation of *N. versicolor* to animal tissue (i.e. herring meat) when pre-fed food sources of different nitrogen content was examined in a laboratory experiment carried out at Phuket Marine Biological Center in September and October 2007. The experimental set up consisted of three types of microcosms in which crabs initially had access to: 1) leaves only, 2) leaves and sediment, and 3) leaves and fish meat. Each microcosm was constructed by a glass aquarium (29 × 29 × 33 cm) angled so that 2/3 of the bottom was covered with seawater to a maximum depth of 2 cm. Full strength seawater was diluted with freshwater to a salinity of about 20 as a precaution to prevent high salinities due to evaporation. No tidal rhythm was simulated to avoid disturbing the crabs. The water was changed every five days to maintain an acceptable water quality. Mesocosms were maintained at air temperature (28–31 °C) and exposed to subdued sunlight. The humidity was constantly around 98%. A total of nine crabs were used in the three types of microcosms, providing three replicates of each. All crabs were intact non-moulting adult males with a carapace width of 3–4 cm.

Crabs were kept on the different pre-diets for extended time periods prior to the final meat-feeding observations. All crabs were offered fresh leaves of *R. apiculata*, which is a dominant mangrove species at their habitat. New leaves were given and uneaten remains were removed every second day during the pre-feeding period. Crabs in type 1 and type 2 mesocosms were pre-fed leaves for 17 days, while this treatment in the type 3 mesocosm only lasted 5 days. The dry part of the bottom in the type 2 mesocosm was covered initially with 3 cm deep layer of freshly collected mangrove surface sediment. The sediment was not renewed during the entire experiment. Crabs in the type 3 mesocosm were offered fish meat (~20 g herring) 4 days prior to the final observations. All remains of fish meat were removed one day later, leaving these crabs with leaves as the only food source for 3 days.

The response variable of the final observations was crab feeding preference for animal tissue. Each crab was offered a piece of a fresh herring at dusk, which is within their natural feeding period, and the total time spent feeding was observed for 40 min. The registration time did not include the first 10 min habituation time of the crabs after transferring the fish. Feeding was defined as the time where crabs had physical contact with the fish. Observations were carried out by two quiescent persons wearing head torches. The light beam did not seem to disturb the crabs. The response variable was expressed as percent eating time during the final 30 min registration period.

2.3. *In situ stable isotope signatures*

Carbon and nitrogen stable isotope signatures of *N. versicolor* and its possible food sources were used to identify the partitioning of these food items in its diet. Fauna, vegetation, and sediment samples for stable isotope analysis were collected at two sites along the main creek in the Bangrong mangrove forest in December 2008. Site RM was located within a monostand of *R. mucronata* close to an open creek bank, while site RA was selected about 200 m away within a monostand of *R. apiculata* that covered the creek bank completely (Fig. 1). Six individuals of *N. versicolor* were caught by hand at each site together with all visible smaller crabs of various species. Green and yellow leaves were handpicked from the trees, while brown leaves were collected from the forest floor between trees. Surface sediment (<1 mm) was obtained by careful scraping with a spatula. All samples were transported to the laboratory and immediately frozen (−18°C). Brown leaves were sorted into two groups before freezing; one was gently rinsed in tap water to remove surface debris and the other was not rinsed. Within a few days, the samples were thawed and oven dried for at least 24 h at 80°C (fauna and leaves) or 105°C (sediment). Subsequently the samples were grinded to fine powder in an agate mortar. Only muscle tissue from the chelae of crabs was used. The sample preparation for ^{13}C and elemental (C:N) analysis were completed by soaking the subsamples in diluted HCl to remove possible carbonates and redried. As this treatment is reported to affect ^{15}N-values (Bunn et al., 1995), subsamples for ^{15}N analysis were not acidified.

Organic carbon and nitrogen content as well as stable isotope composition (^{12}C, ^{13}C, ^{14}N and ^{15}N) of fauna, leaf and sediment samples were determined by combusting pre-weighed subsamples in a Thermo Scientific Flash1112 elemental analyzer coupled to a Thermo Scientific Delta V via a conflo III interface. Stable isotope analyses of carbon and nitrogen were analyzed separately to avoid acidification errors and to obtain more accurate measurements for sediment and mangrove leaves, which usually exhibit high C:N ratios. Stable isotopes ratios are expressed as δ values (‰) relative to the conventional standards (VPDB limestone for C and atmospheric N_2 for N) according to:

$$\delta X = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}\right] \times 10^3 [‰]$$

where X represents ^{13}C or ^{15}N and R is ^{13}C/^{12}C in the case of carbon and ^{15}N/^{14}N in the case of nitrogen.

2.4. *Statistical analysis*

Means within the feeding experiment were compared by two-sample *t*-tests. However, the group, which had a pre-diet of leaves and fish, did not have any variation due to a response variable of zero in all replicates. Consequently, the comparisons with this group were done by one-sample *t*-tests for means against zero. The significance level was set at 5%. An *a priori* Sequential Bonferroni correction was made on the succeeding *t*-tests to avoid the risk of type II errors.

3. Results

3.1. *Feeding experiment*

Despite the simple design and low sample size ($n = 3$) of the feeding experiment, the results are very informative. All specimens of *N. versicolor* behaved well with continuous ingestion of leaves during the experiment and survival was 100%. The two groups of crabs with a pre-diet of leaves only, and leaves and sediment spent most of the final registration period handling and ingesting fish meat (Table 1). There was no significant difference in fish-eating time between these two groups, but only the group on sole leaf pre-diet was significantly different from zero. Similarly, the individuals that were offered fish meat for 24 h three days prior to the final observations all consumed this food source willingly (on average ~0.5 g). However, none of these touched the fish they were offered during the final 30 min registration period. Access to sediment in the microcosms did not affect the crabs' willingness to feed on fish meat.

Table 1
Percentage total eating time of *Neoepisesarma versicolor* when offered a piece of fresh fish for 30 min (mean ± standard deviation, $n = 3$). Crabs were kept on different diets for various time periods prior to the observations; 17 days for leaves only or leaves and sediment and 5 days for leaves and fish. Values with the same letter are not significantly different ($p > 0.05$).

Pre-diet	Time on diet (days)	Total feeding time (%)
Leaves	17	92 ± 11 a
Leaves and sediment	17	74 ± 39 ab
Leaves and fish	5	0 ± 0 b

3.2. Stable isotope signatures

Leaves of the two *Rhizophora* species from the two sites showed comparable average δ^{13}C values irrespective of degree of senescence, decay and rinsing (Fig. 2). However, the favorite food of *N. versicolor*, partly degraded brown leaves covered with debris, was about 2 ‰ more depleted in ^{15}N than green leaves of both *R. mucronata* and *R. apiculata*. Surface sediment was slightly enriched in both ^{13}C and ^{15}N compared with mangrove leaves (about −26 ‰ for C and 5 ‰ for N; Fig. 3). Unfortunately, all our efforts in extracting benthic microalgae without sediment contamination failed.

The ocypodid crabs *Uca paradussumieri* and *Uca forcipata*, and the varunid crab *Metaplax elegans* were the most abundant potential prey items for *N. versicolor* at the two sampling sites. They were, however, much more common at the open site RM than the densely vegetated site RA. The few individuals caught at site RA were unfortunately too small to obtain sufficient muscle samples for analysis. The stable isotope signature of these three species from site RM were similar with δ^{13}C ranging from −16.6 to −18.0 ‰ and δ^{15}N ranging from 7.5 to 8.9 ‰ (Fig. 3). Due to the lack of specimens from site RA, we assume similar signatures here in the subsequent data analysis.

The δ^{13}C signature of *N. versicolor* was similar at site RM and RA (−24.3 ‰), which is roughly 5‰ enrichment compared to *Rhizophora* leaves (Fig. 3). Conversely, δ^{15}N of *N. versicolor* was 3–4‰ higher than mangrove leaves and notably 1‰ higher at site RM than site RA (7.6 and 6.7‰, respectively), suggesting a trophic difference between the two sites.

4. Discussion

4.1. Food preference and nitrogen source of N. versicolor

Fish meat is potentially a nitrogen-rich food source for the crabs and the fact that they willingly ingested it in the feeding experiment supports our hypothesis that animal tissue can be a supplementary food source for *N. versicolor*. The difference in feeding behavior among the three experimental groups clearly demonstrates that the preference of crabs for animal tissue depends on the pre-diet. Crabs can apparently be satiated with regard to nitrogen after a meal of meat and our experiment showed that such satiation lasts for at least three days. A reasonable explanation for this behavior is that *N. versicolor* can store excess nitrogen intracellularly, as suggested by Thongtham and Kristensen (2005). A similar mechanism is known from several species of land crabs, which store nitrogen intracellularly as uric acid in spongy connective tissue cells throughout the body (Gifford, 1968; Wolcott and O'Connor, 1992; Linton and Greenaway, 1997). The idea of nitrogen storage is consistent with our observations of sesarmid crabs in the wild, where their tendency to act as predators is unpredictable (personal observation).

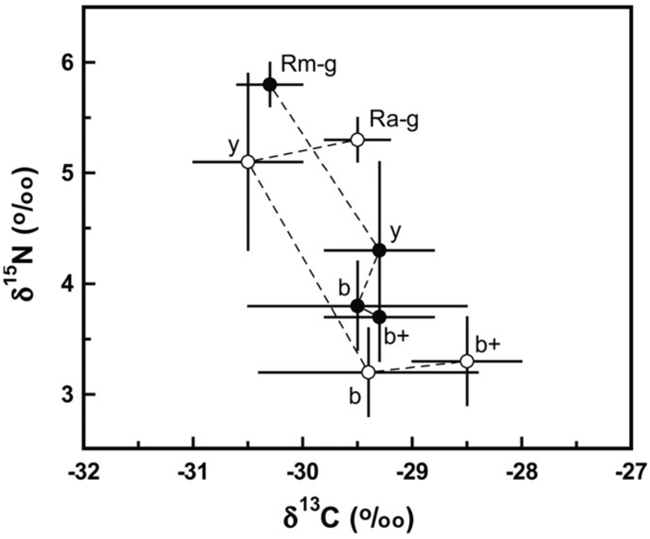

Fig. 2. δ^{13}C and δ^{15}N signatures of mangrove leaves at various stages of aging and decomposition. Rm-g and Ra-g indicates green leaves of *Rhizophora mucronata* and *R. apiculata*, respectively. The letters *y*, *b* and *b* + represent yellow leaves, brown leaves and brown leaves with debris, respectively. The broken lines trace the change in isotope signature during aging and decay for each species. Error bars indicate standard deviation (n = 4–6).

Both groups, with pre-diets of leaves only and leaves and sediment, respectively, were undoubtedly hungry for meat after 17 days on nitrogen-deprived diets. Thus, the microorganisms of the mangrove sediment did in this case not contribute significantly to the crabs nitrogen demand. Although the results indicate that *N. versicolor* does not rely on bacteria as a main nitrogen source, the role of benthic microalgae as a food source cannot be interpreted from this experiment because it was maintained under dim light conditions.

Animal tissue is not expected to be a frequent food source for sesarmid crabs in the wild, and they are presumably able to cope

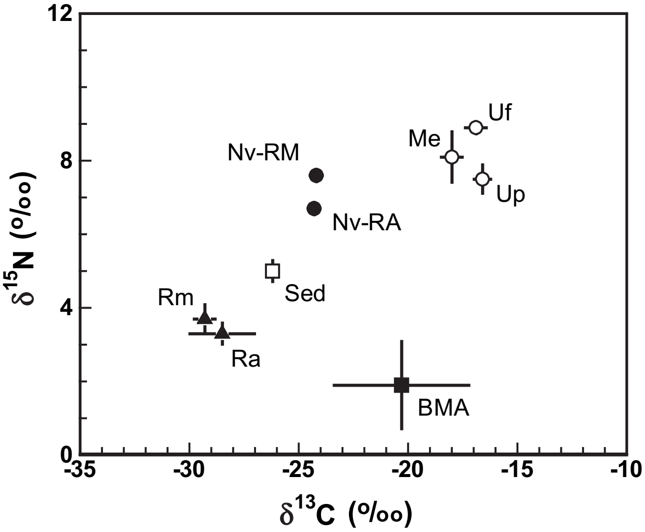

Fig. 3. δ^{13}C and δ^{15}N signatures of brown leaves with debris (Rm – *Rhizophora mucronata* and Ra – *R. apiculata*, $n = 5$), sediment (Sed, $n = 7$)), benthic microalgae (BMA, $n = 7$ (^{15}N) or 15 (^{13}C), potential crab prey for *Neoepisesarma versicolor* (Up – *Uca paradussumieri*, $n = 10$; Uf – *Uca forcipata*, $n = 4$; and Me – *Metaplax elegans*, $n = 3$) and *N. versicolor* (Nv-RM – individuals from site RM and Nv-RA – individuals from site RA, $N = 6$). Error bars indicate standard deviation.

with long periods without ingestion of meat. This is supported by the feeding experiment, since there were no visible signs that the crabs were suffering after 17 days on a restricted diet. On the other hand, we did not assess changes in crab biomass during the experiment and it cannot be ruled out that the animals lost weight. However, a long term study on two sesarmid species (*Chiromanthes bidens* and *Parasesarma plicata*) showed that these crabs are actually able to grow, but not reproduce, on a diet of pure mangrove leaves (Kwok and Lee, 1995). Further experimental studies are needed to confirm the necessity of animal tissue as a food source for sesarmids and their capacity to store excess nitrogen. In any case, it appears that *N. versicolor* obtains much of its nitrogen requirement from animal sources. This species may to some degree also supplement its nitrogen intake with benthic microalgae or small invertebrates associated with the sediment. This could explain why *N. versicolor* spends considerable time foraging on sediment (Thongtham unpublished). Taken the diversity of feeding habitats and habits of various sesarmid crabs (Lee, 1998) into consideration it seems feasible that they may have different strategies for obtaining nitrogen.

4.2. Stable isotope signatures of N. versicolor and its potential food sources

The stable carbon isotope signatures found for leaves (mean −30.1 ‰, Fig. 2) are within the range reported for mangroves leaves around the world, −35 to −22 ‰ (Bouillon et al., 2008), and are consistent with other measurements on the genus *Rhizophora* (Bouillon et al., 2004). The differences in δ^{15}N among the various leaf categories suggest that the leaf ^{15}N pool is diluted during leaching, aging and decomposition (Fry and Smith, 2002; Werry and Lee, 2005). Bacteria colonizing the leaves during decomposition may thus assimilate ^{15}N depleted inorganic nitrogen from the surroundings or through nitrogen fixation (Gu, 2009).

Stable isotope signatures of *N. versicolor* from Bangrong are consistent with measurements of the sister sesarmid species, *Episesarma versicolor*, from India (Bouillon et al., 2002, 2004). The roughly 5‰ enriched δ^{13}C signature of *N. versicolor* compared to *Rhizophora* leaves, confirms that this sesarmid species does not feed solely on mangrove litter (Fig. 3).

Although surface sediment was slightly enriched in both ^{13}C and ^{15}N compared with mangrove leaves, it is not realistic to consider bulk sediment as a food source for *N. versicolor*. It is known that other deposit-feeders ingesting bulk sediment primarily assimilate benthic microalgae (BMA) and to some degree bacteria, while the detrital fraction is largely undigestable (Andresen and Kristensen, 2002). Since our effort in extracting BMA without sediment contamination failed, we instead gathered a global average signature of various benthic microalgae from the literature for use in our modeling considerations (Table 2; Fig. 3).

The observed δ^{13}C values of the potential prey, *U. paradussumieri*, *U. forcipata*, and *M. elegans*, are comparable to those found for the same genera in Kenya and India, while δ^{15}N is higher than in Kenya and similar to those from India (Bouillon et al., 2004). Both *Uca* species and *M. elegans* are sediment surface grazers with BMA as their primary food source (France, 1998; Weis and Weis, 2004; Kon et al., 2007). However, ^{13}C and ^{15}N of these crabs are enriched 2–3‰ and 5–6‰, respectively, compared with BMA, which exceeds the global mean fractionation of 0.4‰ and 2.3‰ per trophic level for a large range of aquatic animals (McCutchan et al., 2003). These crabs may therefore also consume BMA grazing micro- and meiofauna as suggested for *Uca* spp. by Reinsel (2004).

Table 2

Stable carbon and nitrogen isotope signatures of benthic microalgae (BMA) from various mangrove locations. The global average (±standard deviation) used in the mixing model is shown.

Location	δ^{13}C (‰)	δ^{15}N (‰)	Reference
Molokai, Hawaii	−20.5		Demopoulos et al. (2007)
Jobos Bay, Puerto Rico	−21.5		Demopoulos et al. (2007)
Laguna Joyuda, Puerto Rico	−19.9	2.2	France (1998)
Estero Morales, Costa Rica	−21.0	3.9	Dittel et al. (1997)
Gazi, Kenya	−24.5	1.4	P. Mangion, unpubl
Ras Dege, Tanzania	−21.2	1.3	P. Mangion, unpubl
Zanzibar	−12.4		M. skov, unpubl
Coringa Sanctuary, India	−17.3	1.7	Bouillon et al. (2002)
Coringa Sanctuary, India	−18.5	1.7	Bouillon et al. (2004)
Pearl River Estuary, Hong Kong	−19.3		Lee (2000)
Sikao Creek, Thailand	−17.9	1.2	Kon et al. (2007)
Tanshui Estuary, Taiwan	−19.4		Hsieh et al. (2002)
Tanshui Estuary, Taiwan	−19.3		Hsieh et al. (2002)
Moreton Bay, Australia	−24.4		Guest et al. (2004)
Moreton Bay, Australia	−20.4	3.8	Connolly (2003)
Moreton Bay, Australia	−23.7		Guest and Connoly (2004)
Average	−20.1 ± 3.0	2.2 ± 1.1	

4.3. Dietary composition of N. versicolor

Stomach content analysis (Thongtham et al., 2008) and stable isotope surveys (this study, Bouillon et al., 2002) both suggest that *N. versicolor* has a heterogeneous and mixed diet composed of mangrove leaves, animal tissue in the form of invertebrates and fish carrions, and benthic microorganisms associated with sediment. The relative importance of these food sources can be assessed using stable isotope signatures and mixing models. In general, the proportion of $n+1$ different food sources can be found uniquely by n different elemental isotopic signatures with linear mixing models based on Euclidean-distances (e.g. Ben-David et al., 1997) or mass balance equations (e.g. Phillips and Koch, 2001). Other models that cope with unlimited number of sources have been developed as well. Among these, the IsoSource model (Phillips and Gregg, 2003) determines boundaries for the contributions of each source by examining all combinations of food sources that sum to the observed mixture of isotopic signatures. MixSir (More and Semmens, 2008; but see Jackson et al., 2009) and SIAR (Panell et al., 2008) are new Bayesian-mixing models based on IsoSource, but with the great advantage of incorporating uncertainty and prior information.

It is important, however, that the general assumptions of mixing models do not violate the biological reality when evaluating the food sources of an omnivore such as *N. versicolor*. Especially the model assumption of equal proportions of C and N assimilated from each food source is critical when dealing with omnivores (also noted by Stenroth et al., 2006). As pointed out by Gannes et al. (1997) meat is likely the main source of proteins and lipids for an omnivore, whereas plant material is the main source of carbohydrates. The same pattern is expected for *N. versicolor* as mangrove leaves are extremely poor in nitrogen, having C:N ratios around 30 and 15 times higher than potential animal sources and microalgae, respectively. Hence, bulk nitrogen is expected to originate from digested animal tissue and to some extend microorganisms.

Only one mixing model deals with unequal assimilation of C and N, namely the concentration-dependent IsoConc model developed by Phillips and Koch (2001). The model assumes that “for each element, a source’s contribution is proportional to the contributed mass times the elemental concentration in that source”. This model is recommended whenever the elemental composition varies substantially among food sources. However, the model has been criticized for incorrectly using C and N concentrations of the food itself rather than the fraction assimilated (Koch and Phillips, 2002; Robbins et al., 2002). Bearing this limitation in mind, we have chosen to use an adjusted version of IsoConc model to estimate the dietary composition of *N. versicolor* at the two mangrove habitats. Fractionation corrections of carbon and nitrogen were set at 0.4‰ and 2.3‰ per trophic level, respectively, as these are global means for a large range of aquatic animals (McCutchan et al., 2003).

Our choice of food source data (Table 3) was based on the knowledge of feeding behavior by *N. versicolor*. The leaf diet was represented by brown leaves only, as *N. versicolor* (Thongtham et al., 2008) and other sesarmids (e.g. Giddins et al., 1986; Lee, 1989) generally prefer leaves at this stage. Green and yellow leaves are inferior due to lower nutritional value and high levels of inhibitory compounds (e.g. tannins, Hernes et al., 2001; Kristensen et al., 2008). In addition, we selected brown leaves with intact debris layer to imitate leaves as they are ingested by the crabs. Sediment as a whole was not included as a food source, since inorganic particles and refractory detritus are not assimilated by the crabs. Instead benthic microalgae (BMA) were included as they are expected to make up the bulk of the microorganisms assimilated from the sediment. Their stable isotope signature was derived from the above mentioned literature survey (Table 2). Lastly, animal tissue was included as a food source. For this purpose we used the three abundant crab species mentioned above (i.e. *U. paradussumieri*, *U. forcipata* and *M. elegans*). As the model only handles three food sources, we used the average of the three species isotope signatures and C:N ratios.

The elemental C and N concentrations applied in the IsoConc model were, if possible, corrected for digestibility. C and N in the analyzed animal tissue are assumed to be 100% digestible (Koch and Phillips, 2002), which is a robust assumption as only muscle tissues was used. In contrast, the mass of consumed BMA is not fully digested as these mainly consist of diatoms with indigestible silica frustules (Thongtham et al., 2008), which may account for up to 50% of diatom biomass (Canfield et al., 2005). Accordingly, we assumed that the digestible organic tissue of diatoms contains 45% C, as commonly found for microalgae without thick inorganic envelopes (e.g. Kristensen, 1990). The corresponding N content was estimated to 6.8% from a Redfield C:N weight ratio of 6.6:1. Digestibility of vascular plant tissues varies among herbivores. Thongtham and Kristensen (2005) reported assimilation efficiencies for *N. versicolor* ingesting brown leaves of 6.5% for the dry matter and around 40% for C and N. Although these results are inconsistent, they indicate a high assimilation of C and N in proportions similar to the bulk C:N ratio of the ingested leaf material. However, since the assimilated concentrations are not known, we were forced to use the uncorrected C and N concentrations of brown leaves in the model.

The IsoConc model showed that brown leaves, crab tissue, and BMA provide a realistic combination of food sources for *N. versicolor* (Fig. 4). Brown leaves are the main food in terms of biomass and carbon with a contribution of slightly less than 60% at both site RM and RA (Table 4). This proportion may, however, be overestimated considering the uncorrected elemental concentrations of leaves. Nevertheless, the result agrees very well with Thongtham et al. (2008), who found that the stomach content of *N. versicolor* contained 62% higher plant material, while the remaining 38% were composed of detritus and mineral particles. These two independent observations certify the importance of leaves in the diet of *N. versicolor*. With respect to nitrogen, the situation is more complicated. The proportion of microalgae and animal tissues in the diet is dependent on the habitat of *N. versicolor*. Our results indicate that *N. versicolor* is capable of feeding on the crab fauna present, but also that it can switch between nitrogen-rich food sources depending on their availability. At site RM, where the prey crab species are abundant, microalgae and animal tissues account for 27% and 16%, respectively, of the assimilated biomass compared with 32% and 10% at site RA. As a consequence, the nitrogen budgets differ between the two sampling sites. *N. versicolor* obtains 50% nitrogen from animal tissue at site RM, while the proportion only is 35% at

Table 3
Input data used in the stable isotope mixing model. The* indicates that the values are estimated (see text for details).

	n	δ^{13}C	δ^{15}N	[C] (%)	[N] (%)	C:N
Study animal						
N.versicolor (RA.)	6	−24.3	6.7	40.8	13.0	3.1
N.versicolor (RM)	6	−24.2	7.6	40.3	12.8	3.1
Food sources						
Brown leaves (RA)	5	−28.5	3.3	44.1	0.5	91.6
Brown leaves (RM.)	5	−29.3	3.7	38.6	0.4	96.3
Benthic microalgae	16/8	−20.1	2.2	45.0*	6.8*	6.6
U. paradussumieri	10	−16.6	7.5	40.4	12.5	3.2
U. forcipata	4	−18	8.1	39.4	12.5	3.2
M. elegans	3	−16.9	8.9	39.6	12.6	3.1
Average for crab prey	17	−17.2	8.2	39.8	12.5	3.2

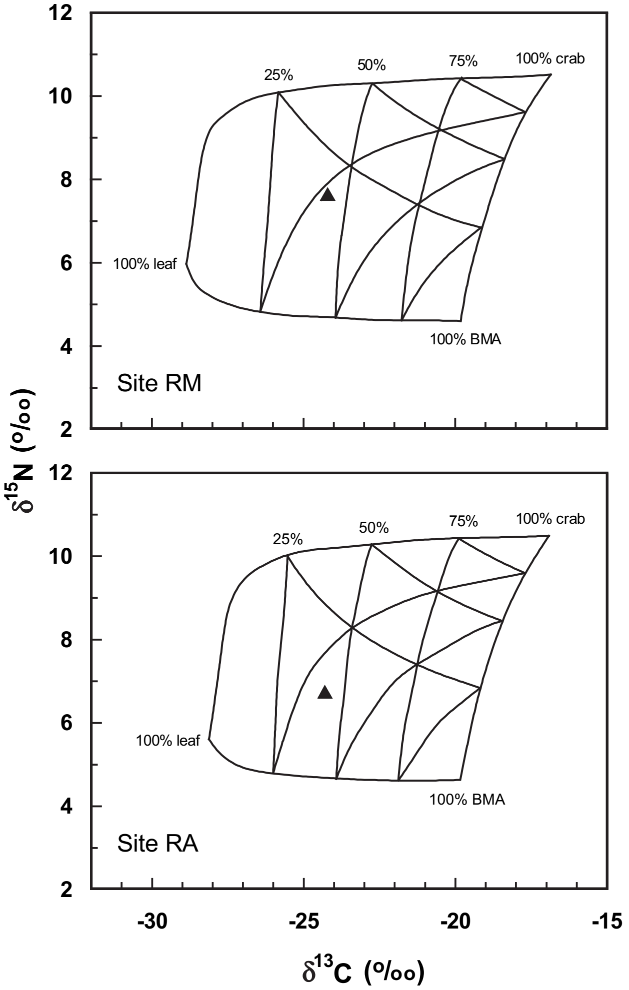

Fig. 4. Concentration-dependent mixing triangles for *Neoepisesarma versicolor* from sampling site RM (upper) and RA (lower). Isotope signatures for pure diets at the vertices of the triangles have been corrected for trophic fractionation (0.4‰ for C and 2.3‰ for N). The filled symbols indicate the isotope signature of the consumer (*N. versicolor*).

Table 4
Food source partitioning at the two sampling sites RM and RA as estimated by the concentration-dependent IsoConc mixing model. f indicates fractional contribution of biomass, carbon and nitrogen.

	N. versicolor (RM)	*N. versicolor* (RA)
f_{leaves}		
Biomass	0.57	0.58
Carbon	0.54	0.58
Nitrogen	0.06	0.08
f_{BMA}		
Biomass	0.27	0.32
Carbon	0.30	0.33
Nitrogen	0.44	0.57
f_{animal}		
Biomass	0.16	0.10
Carbon	0.16	0.09
Nitrogen	0.50	0.35

site RA (Table 4). Most of the remaining nitrogen is derived from BMA (44% at site RM and 57% at site RA) with only limited contribution from leaf material. It appears that the lack of animal prey at site RA forces *N. versicolor* to feed more on organisms from a lower trophic level (e.g. BMA) than at site RM. The modeling results support our hypothesis that animal tissues contribute substantially as a nitrogen source for *N. versicolor*, although the consumed mass is relative small. The food partitioning obtained by the mixing model agrees well with stomach analyses of *N. versicolor*. Thongtham et al. (2008) found that 11% of the examined crabs contained crustacean remains while 7% had fish scales in their stomach. Furthermore, benthic diatoms and filamentous algae occurred in 86% and 47% of the crabs, respectively. It can therefore be concluded that animal tissue is not likely an every-day-meal for *N. versicolor*, but even so it functions as an essential supplementary nitrogen source.

The estimated nitrogen contribution of microalgae was higher than expected. In order to obtain sufficient amounts of algae, *N. versicolor* must non-selectively ingest a large amount of sediment because it has chelae designed for grasping and not sorting (personal observation). Many specialized mangrove crabs (e.g. fiddler crabs) are adapted for the latter method. It has been estimated that *N. versicolor* must ingest 7-23 cm^3 sediment (g ww) crab^{-1} d^{-1} to fully cover its nitrogen demand from BMA and other sediment-based nitrogen-rich items (Thongtham and Kristensen, 2005). Although this species, according to the IsoConc model, only obtains about 40–50% of the nitrogen from BMA, the volume of sediment still seems unrealistically high compared to the size of the crab ($\sim$25 g ww). If *N. versicolor* is as inefficient in exploiting BMA from the sediment as we assume, it probably obtains more nitrogen from animal tissues or other easy accessible sources than predicted by the model. The model results may be misleading due to the lack of stable isotope signatures and elemental concentrations of microalgae from Bangrong. Furthermore, *N. versicolor* may rely on other food sources than those included in the model, such as macroalgae, bacteria or small invertebrates (e.g. meiofauna).

In conclusion, our results verify the hypotheses that *N. versicolor* can feed on animal tissue and by doing so supplement its dietary need for nitrogen. While the feeding experiment illustrates that *N. versicolor* adjusts the intake of animal tissue according to its satiation level, the applied mixing model suggests that animal-based food sources may cover up to 50% of the nitrogen demand of this mangrove sesarmid crab when prey is abundant. However, the modeling results should be considered as rough estimates because of several potential errors. Firstly, BMA isotope signatures and elemental composition are not available from our location. Secondly, key information is missing regarding the assimilation efficiency of different food sources. Thirdly, we used global average fractionation values as no information is available for mangrove crustaceans. Fourthly, we did not account for internal isotopic routing (for review, see Gannes et al., 1997), where stable isotopes are routed differently to various tissues and body compartments. Finally, stochasticity of stable isotope ratios of consumer and food sources were eliminated, as the model is deterministic and only provides one solution.

Acknowledgements

We thank N. Thongtham and the staff at Phuket Marine Biological Center, Thailand, for hospitality and logistical support during field samplings. This study was supported by grant # 09-071369 from the Danish Research Council.

References

Andresen, M., Kristensen, E., 2002. The importance of bacteria and microalgae in the diet of the deposit-feeding polychaete *Arenicola marina*. Ophelia 56, 179–196.

Ben-David, M., Flynn, R.W., Schell, D.M., 1997. Annual and seasonal changes in diet of martens: evidence from stable isotope analysis. Oecologia 111, 280–291.

Bouillon, S., Koedam, N., Raman, A.V., Dehairs, F., 2002. Primary producers sustaining macroinvertebrate communities in intertidal mangrove forests. Oecologia 130, 441–448.

Bouillon, S., Moens, T., Overmeer, I., Koedam, N., Dehairs, F., 2004. Resource utilization patterns of epifauna from mangrove forests with contrasting inputs of local versus imported organic matter. Marine Ecology Progress Series 278, 77–88.

Bouillon, S., Connolly, R.M., Lee, S.Y., 2008. Organic matter exchange and cycling in mangrove ecosystems: recent insights from stable isotope studies. Journal of Sea Research 59, 44–58.

Bunn, S.E., Loneragan, N.R., Kempster, M.A., 1995. Effects of acid washing samples on stable isotope ratios of C and N in penaeid shrimps and seagrass: implications for food web studies using stable isotopes. Limnology and Oceanography 40, 622–625.

Canfield, D.E., Kristensen, E., Thamdrup, B., 2005. Aquatic Geomicrobiology. Elsevier Academic Press, Amsterdam, p. 636.

Connolly, R.M., 2003. Differences in trophodynamics of commercially important fish between artificial waterways and natural coastal wetlands. Estuarine and Coastal Shelf Science 58, 929–936.

Dahdouh-Guebas, F., Giugglioli, M., Oluoch, A., Vannini, M., Cannicci, S., 1999. Feeding habits of non-ocypodid crabs from two mangrove forests in Kenya. Bulletin of Marine Science 64, 291–297.

Demopoulos, A.W.J., Fry, B., Smith, C.R., 2007. Food web structure in exotic and native mangroves: a Hawaii-Puerto Rico comparison. Oecologia 153, 675–686.

Dittel, A.I., Epifanio, C.E., Cifuentes, L.A., Kirchman, D.L., 1997. Carbon and nitrogen sources for shrimp postlarvae fed natural diets from a tropical mangrove system. Estuarine and Coastal Shelf Science 45, 629–637.

France, R., 1998. Estimating the assimilation of mangrove detritus by fiddler crabs in Laguna Joyuda, Puerto Rico, using dual stable isotopes. Journal of Tropical Ecology 14, 413–425.

Fry, B., Smith, T.J., 2002. Stable isotope studies of red mangroves and filter feeders from the Shark river Estuary, Florida. Bulletin of Marine Science 70, 871–890.

Gannes, L.Z., O'Brien, M.C., Marínez del Rio, C., 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78, 1271–1276.

Giddins, R.L., Lucas, J.S., Neilson, M.J., Richards, G.N., 1986. Feeding ecology of the mangrove crab *Neosarmatium smithii* (Crustacea: Decapoda: Sesarmidae). Marine Ecology Progress Series 33, 147–155.

Gifford, C.A., 1968. Accumulation of uric acid in the land crab, *Cardisoma guanhumi*. American Zoologist 8, 521–528.

Gu, B., 2009. Variations and controls of nitrogen stable isotopes in particulate organic matter of lakes. Oecologia 160, 421–431.

Guest, M.A., Connolly, R.M., 2004. Fine-scale movement and assimilation of carbon in saltmarsh and mangrove habitat by resident animals. Aquatic Ecology 38, 599–609.

Guest, M.A., Connolly, R.M., Loneragan, N.R., 2004. Carbon movement and assimilation by invertebrates in estuarine habitats at a scale of metres. Marine Ecology Progress Series 278, 27–34.

Hernes, P.J., Benner, R., Cowie, G.L., Goñi, M.A., Bergamaschi, B.A., Hedges, J.I., 2001. Tannin diagenesis in mangrove leaves from a tropical estuary: a novel molecular approach. Geochimica et Cosmochimica Acta 65, 3109–3122.

Hsieh, H.L., Chen, C.P., Chen, Y.G., Yang, H.H., 2002. Diversity of benthic organic matter flows through polychaetes and crabs in a mangrove estuary: delta C-13 and delta S-34 signals. Marine Ecology Progress Series 227, 145–155.

Jackson, A.L., Inger, R., Bearhop, S., Parnell, A., 2009. Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of More & Semmens (2008). Ecology Letters 12, E1–E5.

Koch, L.P., Phillips, D.L., 2002. Incorporating concentration dependence in stable isotope mixing models: a reply to Robbins, Hilderbrand, and Farley. Oecologia 133, 13–18.

Kon, K., Kurokura, H., Hayashizaki, K., 2007. Role of microhabitats in food webs of benthic communities in a mangrove forest. Marine Ecology Progress Series 340, 55–62.

Kristensen, E., 1990. Characterization of biogenic organic matter by stepwise thermogravimetry (STG). Biogeochemistry 9, 135–159.

Kristensen, E., 2007. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. Journal of Sea Research 59, 30–43.

Kristensen, E., Bouillon, S., Dittmar, T., Marchand, C., 2008. Organic carbon dynamics in mangrove ecosystems. Aquatic Botany 89, 201–219.

Kristensen, E., Holmer, M., Banta, G.T., Jensen, M.H., Hansen, K., 1995. Carbon, nitrogen and sulfur cycling in sediment of the Ao Nam Bor Mangrove forest, Phuket, Thailand: a review. Phuket Marine Biological Center Research Bulletin 60, 37–64.

Kristensen, E., Pilgaard, R., 2001. The role of fecal pellet deposition by leaf-eating sesarmid crabs on mineralization processes in a mangrove sediment (Phuket, Thailand). In: Aller, J.Y., Woodin, S.A., Aller, R.C. (Eds.), Organism-Sediment Interactions. University of South Carolina Press, Columbia, pp. 369–384.

Kwok, P.W., Lee, S.Y., 1995. The growth performances of two mangrove crabs, *Chiromanthes bidens* and *Parasesarma plicata* under different leaf litter diets. Hydrobiologia 295, 141–148.

Lee, S.Y., 1989. The importance of sesarminae crabs *Chiromanthes* spp. and inundation frequency on mangrove (*Kandelia candel* (L.) Druce) leaf litter turnover in a Hong Kong tidal shrimp pond. Journal of Experimental Marine Biology and Ecology 131, 23–43.

Lee, S.Y., 1998. Ecological role of grapsid crabs in mangrove ecosystems: a review. Marine and Freshwater Research 49, 335–343.

Lee, S.Y., 2000. Carbon dynamics of Deep Bay, eastern Pearl River estuary, China. II: Trophic relationship based on carbon- and nitrogen-stable isotopes. Marine Ecology Progress Series 205, 1–10.

Linton, S.M., Greenaway, P., 1997. Urate deposits in the Gecarcinid land crab *Gecarcoidea natalis* are synthesized de novo from excess dietary nitrogen. Journal of Experimental Biology 200, 2347–2354.

Malley, D.F., 1978. Degradation of mangrove leaf litter by the tropical Sesarmid crab *Chiromanthes onychophorum*. Marine Biology 49, 377–386.

McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102, 378–390.

Micheli, F., 1993a. Effect of mangrove litter species and availability on survival moulting, and reproduction of the mangrove crab *Sesarma messa*. Journal of Experimental Marine Biology and Ecology 171, 149–163.

Micheli, F., 1993b. Feeding ecology of mangrove crabs in North Eastern Australia: mangrove litter consumption by *Sesarma messa* and *Sesarma smithii*. Journal of Experimental Marine Biology and Ecology 171, 165–186.

More, J.W., Semmens, B.X., 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11, 470–480.

Olafsson, E., Buchmayer, S., Skov, M.W., 2002. The East African decapod crab *Neosarmatium meinerti* (de Man) sweeps mangrove floors clean of leaf litter. Ambio 31, 569–573.

Panell, A., Inger, R., Bearshop, S., Jackson, A.L., 2008. SIAR: stable isotope analysis in R. http://cran.r-project.org/web/pakages/siar/index.html.

Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136, 261–269.

Phillips, D.L., Koch, P.L., 2001. Incorporating concentration dependence in stable isotope mixing models. Oecologia 130, 114–125.

Reinsel, K.A., 2004. Impact of fiddler crab foraging and tidal inundation on an intertidal sandflat: season-dependent effects in one tidal cycle. Journal of Experimental Marine Biology and Ecology 313, 1–17.

Robbins, C.T., Hilderbrand, G.V., Farley, S.D., 2002. Incorporating concentration dependence in stable isotope mixing models: a response to Phillips and Koch (2002). Oecologia 133, 10–13.

Robertson, A.L., 1986. Leaf-burying crabs: their influence on energy flow and export from mixed mangrove forests (*Rhizophora* spp.) in northeastern Australia. Journal of Experimental Marine Biology and Ecology 102, 237–248.

Robertson, A.L., 1988. Decomposition of mangrove leaf litter in tropical Australia. Journal of Experimental Marine Biology and Ecology 116, 235–247.

Robertson, A.L., Daniel, P.A., 1989. The influence of crabs on litter processing in high intertidal mangrove forests in tropical Australia. Oecologia 78, 191–198.

Russell-Hunter, W.D., 1970. Aquatic Productivity: An Introduction to Some Basic Aspects of Biological Oceanography and Limnology. MacMillan, New York, 306 pp.

Skov, M.W., Hartnoll, G.R., 2002. Paradoxical selective feeding on a low-nutrien diet: why mangrove crabs eat leaves? Oecologia 131, 1–7.

Slim, F.J., Gwada, P.M., Kodjo, M., Hemminga, M.A., 1996. Biomass and litterfall of *Ceriops tagal* and *Rhizophora mucronata* in the mangrove forest of Gazi Bay, Kenya. Marine and Freshwater Research 47, 999–1007.

Slim, F.J., Hemminga, M.A., Ochieng, C., Jannink, N.T., Cocheret de la Moriniere, E., Van der Velde, G., 1997. Leaf litter removal by the snail *Terebralia palustris* (Linnaeus) and sesarmid crabs in an east African mangrove forest (Gazi Bay, Kenya). Journal of Experimental Marine Biology and Ecology 215, 35–48.

Steinke, T.D., Rajh, A., Holland, A.J., 1993. The feeding behavior of the red mangrove crab *Sesarma meinerti* De Man, 1887 (Crustacea: Decapoda: Grapsidae) and its effect on the degradation of mangrove leaf litter. South Africa Journal of Marine Science 13, 151–160.

Stenroth, P., Holmqvist, N., Nyström, P., Berglund, O., Larsson, P., Granéli, W., 2006. Stable isotopes as indicator of diet in omnivorous crayfish (*Pacifastacus leniusculus*): the influence of tissue, sample treatment, and season. Canadian Journal of Fisheries and Aquatic Science 63, 821–831.

Thongtham, N., Kristensen, E., 2005. Carbon and nitrogen balance of leaf-eating sesarmid crabs (*Neoepisesarma versicolor*) offered different food sources. Estuarine, Coastal and Shelf Science 65, 213–222.

Thongtham, N., Kristensen, E., Purangprasan, S.Y., 2008. Leaf removal by sesarmid crabs in Bangrong Mangrove forest, Phuket, Thailand: with emphasis on the feeding ecology of *Neoepisesarma versicolor*. Estuarine, Coastal and Shelf Science 80, 573–580.

Weis, J.S., Weis, P., 2004. Behavior of four species of fiddler crabs, genus *Uca*, in southeast Sulawesi, Indonesia. Hydrobiologia 523, 47–58.

Werry, J., Lee, S.Y., 2005. Grapsid crabs mediate link between mangrove litter production and estuarine planktonic food chains. Marine Ecology Progress Series 293, 165–176.

Wolcott, D.L., O'Connor, N.J., 1992. Herbivory in crabs: adaptations and ecological considerations. American Zoologist 32, 370–381.