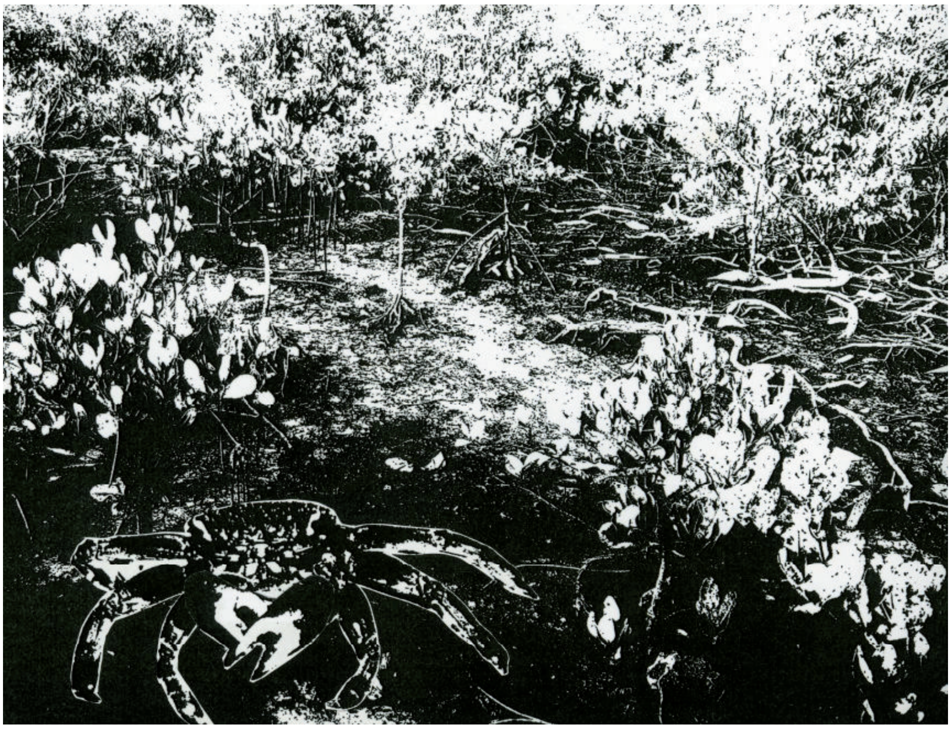
Spatial and temporal variability in benthic food webs of the mangrove fringed Segara Anakan Lagoon in Java, Indonesia



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Der Weg ist das Ziel.

The journey is the destination.

(Konfuzius)

List of content

1 Declaration

Corr. § 6(5) Nr. 1-3 PromO

I herewith declare that I

1) have completed this dissertation unassisted.
2) did not use more than the stated sources and aid.
3) have cited all references.

Gem. § 6(5) Nr. 1-3 PromO

Ich erkläre hiermit, dass ich

1) die Arbeit ohne fremde Hilfe angefertigt habe.
2) keine anderen, als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.
3) die den benutzen Werken wörtlich und inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremen,

2 Abstract

The overall aim of this study was to investigate the influence and importance of benthic communities on the carbon- and nitrogen- flow through the food web in the Segara Anakan Lagoon. I used the method of stable isotope analysis to resolve trophic structures of benthic food webs in several sampling stations within the lagoon (**Herbon et al. submitted: High spatial variability of $\delta^{13}C$ and $\delta^{15}N$ in intertidal benthic food webs in the mangrove fringed Segara Anakan Lagoon, Java, Indonesia**). Carbon isotopic compositions were used to trace carbon sources through the food web, whereas nitrogen isotopic compositions can help to distinguish between trophic levels. A high spatial variability was found on a small scale (in a range of few kilometers), mainly traced back to food availability and possibly also anthropogenic impacts, such as sewage wastes by the city and villages within the lagoon and pollution through effluents of the oil refinery. Certain herbivorous crabs (*Episesarma* spp.) and even a detritivorous snail (*Telescopium telescopium*) supplement their diet with ^{15}N enriched food sources, to satisfy their nitrogen needs. Two species of the same genus (*Episesarma*) occurred on different trophic levels based on different feeding habits.

Furthermore, seasonal variation in isotopic compositions of several abundant species was examined (**Herbon et al. submitted: Seasonal variations of $\delta^{13}C$ and $\delta^{15}N$ in mangrove benthic organisms in the Segara Anakan Lagoon, Java, Indonesia**). A seasonal area dependent variation was observed, which can be explained mainly by the impact of increased precipitation, and therewith an increased river runoff. In a global comparison, Indonesia was found to hold the highest nitrogen isotopic composition for oysters and *Telescopium telescopium* between the compared studies. The latter obviously is supplementing its diet with ^{15}N enriched food sources, in an amount as in no other comparable study found.

As underlying processes of isotopic fractionation are still unclear today, several experiments were conducted to estimate the importance of benthic decapod species on the turnover of carbon and nitrogen in the mangrove ecosystem of the SAL (**Herbon et al. in prep.: Consumption rates and fractionation of carbon and nitrogen isotopes by mangrove crabs, including long-term experiments with a *Rhizophora apiculata* diet**).

The most important outcomes are annotated in the following. Decapod species of the genus *Episesarma* can survive three months on a *Rhizophora apiculata* leaf diet only. A change in nitrogen isotopic signatures was only observed after 50 days, which results from starvation and internal recycling of remaining nitrogen. Furthermore it became clear that *Episesarma* spp. feed preferably on the shrub *Derris trifoliata*, apart from *Rhizophora apiculata*. This indicates that deforestation might not affect these species as much as previously presumed, as long as these areas are overgrown by *D. trifoliata*. The various mangrove species are consumed and assimilated. But clear dependencies on carbon or nitrogen contents were not observed. When comparing tissues, differences in isotopic compositions were expected, according to their role and status within the digestion and assimilation process. As expected, muscle had the significantly highest carbon isotopic compositions, as it is a long time storage tissue. Additionally it was examined whether ovigerous crab females have a lower isotopic composition in their muscle tissue than non-ovigerous, due to presumed differences in their metabolism, but there was no significant difference found. This can probably be explained due to a short reproduction time, or either due to their ability to balance the carbon and nitrogen budget during time of gestation.

To conclude, variation in isotopic signatures is high on a spatial and temporal scale, and should therefore be considered in the future when making generalizations and comparing research results to previous studies.

Decapods have a highly opportunistic feeding behavior. Species from the same genus can adapt differently to changes in food availability. *Episesarma* even preferably feed on shrub species overgrowing logged mangrove areas and can survive over a period of seven weeks on a nitrogen depleted diet without showing deficiency symptoms. Decapods therefore are highly adaptable to changes in food availability.

The method of isotope analysis is a sufficient tool to trace spatial and seasonal differences in invertebrates surrounding environments and their feeding habits. But carbon isotopic signatures can be questioned to sufficiently trace carbon sources of all invertebrates.

3 Zusammenfassung

Das übergeordnete Ziel dieser Studie war es, den Einfluss und die Bedeutung der benthischen Gemeinschaften auf den Kohlenstoff- und Stickstoff- Fluss durch das Nahrungsnetz in der Segara Anakan Lagune zu untersuchen. Ich nutzte die Methode der stabilen Isotopenanalyse zur Auflösung trophischer Strukturen des benthischen Nahrungsnetzes in mehreren Stationen innerhalb der Lagune (**Herbon et al. eingereicht: High spatial variability of δ^{13}C and δ^{15}N in intertidal benthic food webs in the mangrove fringed Segara Anakan Lagoon, Java, Indonesia**). Die Kohlenstoff-Isotopenzusammensetzung wurde verwendet, um Kohlenstoff- Quellen durch das Nahrungsnetz zu verfolgen, während die Stickstoff- Isotopenzusammensetzung helfen kann, zwischen trophischen Ebenen zu unterscheiden. Eine hohe räumliche Variabilität wurde bereits auf kleinem Maßstab (innerhalb weniger Kilometer) gefunden. Diese kann vor allem auf die Verfügbarkeit von Nahrung und möglicherweise auch auf anthropogene Einflüsse zurückgeführt werden, wie z.B. Verschmutzung durch Abwässer von der Stadt und den Dörfern innerhalb der Lagune und der Öl- Raffinerie. Bestimmte herbivore Krabben (*Episesarma* spp.) und auch eine detritivore Schnecke (*Telescopium Telescopium*) ergänzen ihre Ernährung mit ^{15}N angereicherten Nahrungsquellen, um ihre Stickstoff Bedürfnisse befriedigen zu können. Zwei Arten derselben Gattung (*Episesarma*) kamen auf verschiedenen trophischen Ebenen vor, basierend auf unterschiedlichen Ernährungsgewohnheiten.

Darüber hinaus wurden saisonale Schwankungen in der Isotopenzusammensetzung mehrerer abundanter Arten untersucht (**Herbon et al. eingereicht: Seasonal variations of δ^{13}C and δ^{15}N in mangrove benthic organisms in the Segara Anakan Lagoon, Java, Indonesia**). Saisonale gebietsabhängige Unterschiede wurden beobachtet, die vor allem durch die Auswirkungen der erhöhten Niederschläge und dadurch erhöhte Flußeinträge zu erklären sind. Im globalen Vergleich findet man in Indonesien die höchste Stickstoff Isotopenzusammensetzung für Austern und *Telescopium Telescopium.* Letztere ergänzt ihre Ernährung mit ^{15}N angereicherten Nahrungsquellen in einer Menge, wie in keiner anderen vergleichbaren Studie gefunden wurde.

Da zugrunde liegende Prozesse der Isotopenfraktionierung noch heute unklar sind, wurden mehrere Experimente durchgeführt, um die Bedeutung der benthischen decapoden Arten auf den Umsatz von Kohlenstoff und Stickstoff im Mangroven-Ökosystem der SAL einzuschätzen (**Herbon et al. in Vorbereitung: Consumption rates and fractionation of carbon and nitrogen isotopes by mangrove crabs, including long-term experiments with a *Rhizophora apiculata* diet**). Die wichtigsten Ergebnisse werden im folgenden erläutert. Decapode Arten der Gattung *Episesarma* können drei Monate mit einer puren *Rhizophora apiculata* Diät überleben. Eine Änderung der Stickstoff-Isotopensignaturen wurde erst nach 50 Tagen beobachtet, welche aus Aushungerung und internem Recycling von vorhandenem Stickstoff resultiert. Darüber hinaus wurde deutlich, dass *Episesarma* spp. abgesehen von den Mangrovenblättern der Art *Rhizophora apiculata* vorzugsweise die Unterwuchsarte *Derris trifoliata* frißt. Dies deutet darauf hin, dass Abholzung diese Arten möglicherweise nicht so stark beeinflußt, wie bisher angenommen, solange die abgeholzten Flächen mit *D. trifoliata* überwuchert werden. Verschiedene Mangrovenarten werden unterschiedlich konsumiert und assimiliert. Aber klare Abhängigkeiten vom Kohlenstoff- oder Stickstoff- Gehalt konnten nicht nachgewiesen werden. In der Isotopenzusammensetzung von verschiedenen Geweben wurden Unterschiede erwartet, entsprechend ihrer Rolle und ihrem Status innerhalb des Verdauungs- und Assimilations- Prozesses. Wie erwartet, wurde in Muskelmasse die signifikant höchste Kohlenstoff- Isotopenzusammensetzung gemessen, da es ein Langzeit-Speicher-Gewebe ist. Zusätzlich wurde untersucht, ob ovigere Krabben-Weibchen eine niedrigere Isotopenzusammensetzung in ihrem Muskelgewebe haben als nicht-ovigere, aufgrund angenommener Unterschiede im Metabolismus, aber es wurde kein signifikanter Unterschied gefunden. Dies ist vermutlich durch eine kurze Reproduktionszeit, oder durch ihre Fähigkeit, ihren Kohlenstoff- und Stickstoff- Haushalt während der Zeit der Trächtigkeit auszugleichen begründet.

Schlussfolgernd kann man sagen, dass die Variabilität auf räumlicher und zeitlicher Skala hoch ist und daher in Zukunft bei der Generalisierung und beim Vergleichen von Forschungsergebnissen mit anderen Studien bedacht werden sollte.

Decapoden haben eine hoch opportunistische Ernährungsweise. Arten von der selben Gattung können sich unterschiedlich an Veränderungen in der Nahrungsverfügbarkeit anpassen. Episesarma ernährt sich sogar bevorzugt von Unterwuchsarten, welche abgeholzte Flächen überwachsen, und können über einen Zeitraum von sieben Wochen nur auf Basis einer stickstoffarmen Nahrungsquelle überleben ohne Mangelerscheinungen zu zeigen. Decapoden sind demnach hoch anpassungsfähig an Veränderungen in der Nahrungsverfügbarkeit.

Die Methode der stabilen Isotopenanalyse ist ein hinreichendes Instrument um räumliche und zeitliche Unterschiede in den, die Invertebraten umgebenden, Umweltbedingungen und ihren Ernährungsweisen aufzuzeigen. Aber die Kohlenstoff-Isotopen-zusammensetzung kann als Indikator für Kohlenstoff- Quellen von allen Invertebraten angezweifelt werden.

4 List of Papers

In the following the first three publication outcomes of this dissertation are listed. The general concept of this study is part of the SPICE project (Science for the Protection of Indonesian Coastal Ecosystems). The specific implementation of the content of this dissertation was developed by me with advice and scientific guidance by Dr. Inga Nordhaus and Dr. Tim Jennerjahn from the Leibniz-Center for Tropical Marine Ecology, and evaluated additionally by Dr. Mark Lenz from IfM-GEOMAR in Kiel and Prof. Dr. Kai Bischof at the University of Bremen.

Laboratories for preparation of samples and analyses were provided by the Leibniz-Center for Tropical Marine Ecology in Bremen and the Department of Aquatic Biology at the Universitas Jenderal Soedirman in Purwokerto, Indonesia.

1) **Defining trophic levels using the stable isotope approach in intertidal benthic communities on Java, Indonesia**
   Authors: **Carolin M. Herbon**, Inga Nordhaus, Tim Jennerjahn, Andhi R. Suncoko
   I shared the field work and carbon and nitrogen analyses with A. R. Suncoko. The manuscript was written by me, with scientific and editorial advice by I. Nordhaus and T. Jennerjahn.
   This manuscript is submitted to *Hydrobiologia*.

2) **Seasonal variations of isotopic carbon and nitrogen signatures in mangrove intertidal benthic organisms in the Segara Anakan Lagoon, Java, Indonesia**
   Authors: **Carolin M. Herbon**, Steven Bouillon, Inga Nordhaus
   I conducted all the field work and carbon and nitrogen analyses. The manuscript was written by me, with scientific and editorial input by S. Bouillon and I. Nordhaus.
   This manuscript is submitted to *Marine Biology*.

3) **Consumption rates and fractionation of carbon and nitrogen isotopes by mangrove crabs, including long-term experiments with a *Rhizophora apiculata* diet**
   Authors: **Carolin M. Herbon**, Inga Nordhaus
   I conducted all laboratory experiments and carbon and nitrogen analyses. The manuscript was written by me, with scientific and editorial input by I. Nordhaus.
   This manuscript will be submitted to *Journal of Experimental Marine Biology and Ecology*.

5 General Introduction

5.1 Distribution of mangrove forests in Southeast Asia

The most species diverse areas on earth are located within the subtropical and tropical belt 40° north to 40° south of the equator. Mangroves are one of the manifold ecosystems that can be found in this region, forming the transitional zone between terrestrial and aquatic ecosystems. Such habitats are extremely sensitive regarding human impacts. Mangrove areas decreased very fast in the last decades due to deforestation and the conversion of mangroves into other land uses, such as shrimp ponds, aquaculture areas and rice paddies (Valiela et al. 2001). Between the years 1980 and 2005 mangroves declined from 18.8 million to 15.2 million hectares worldwide, which means a decrease of 19% in 25 years. These kinds of impacts lead to a threat to habitat diversity and consequently a decrease in species numbers (FAO 2007).

One of the global hotspots of biodiversity is the so called “golden triangle” between Malaysia, the Philippines and New Guinea including Indonesia in the south. With nearly 5.9 million ha, 39% of the worlds remaining mangroves (state 2005), can be found in South East Asia. Indonesia is home to the largest mangrove stands worldwide with 19% of the world mangroves. It also holds the highest mangrove tree species diversity in the world with 43 true mangrove species (FAO 2007).

5.2 Socioeconomic relevance and functioning of mangrove ecosystems

The overexploitation of mangrove trees mainly logged for the use of wood (Sukardjo 1993; FAO 2007) reduces mangrove density and growth. Several management programs have already successfully recovered mangrove forests in South East Asia, such as in Bangladesh, Malaysia, Thailand, Singapore and Vietnam, creating reserve forests, national parks and conservation centers (FAO 2007). In Indonesia and India e.g. protection plantation activities take place to conserve this ecosystem. These are managed by the government and NGO´s, as the importance of green mangrove belts protecting coastlines against natural hazards such as cyclones or tsunamis (Alongi 2008) became very clear within the last years (FAO 2007). Furthermore important natural resources of mangroves are utilized by humans living in coastal regions, such as timber, fuel, medical aid or food (Saenger 2002 and references therein). As food sources serve fish, which are spawning in the mangrove creeks, decapod crabs, such as *Scylla serrata* and *Portunus* spp. and bivalves.

Despite the importance of conservation of mangrove species itself, also its related faunal species diversity has to be concerned. Mangroves provide habitats themselves as well as spawning grounds, nurseries and nutrients for a variety of species, including fish, invertebrates and vertebrates (Robertson & Duke 1987; Little et al. 1988; Sasekumar et al. 1992; Krumme 2003). Benthic invertebrates play a major role in the processing of organic matter and nutrients in mangroves and therefore have a great impact on the energy flow within this ecosystem.

The cycling and reutilization of nutrients is mainly affected by the benthic community, which break down leaf litter and make nutrients available for microorganisms through their faeces (Robertson & Daniel 1989; Camilleri 1992; Twilley et al. 1997; Nordhaus et al. 2006). These are decomposed by benthic microorganisms and bacteria (Benner & Hodson 1985; Bosire et al. 2005). Detritus feeders, grazers and litter-consuming crabs are the most important functionaries in the nutrient cycling process (Nordhaus et al. 2009). Also by burying mangrove leaves and consuming leaf litter, they reduce the tidal export of organic matter and preserve the energy in form of carbon as well as nutrients in this usually nutrient poor ecosystem ‘mangrove’ (Lee 1989; Robertson & Daniel 1989; Emmerson & McGwyne 1992; Steinke et al. 1993; Nordhaus et al. 2006). These communities also contribute to bioturbation of the sediments, by worms and small crabs.

Of the worlds coastline 13.8% of barrier coasts are found in Asia which are usually backed by lagoons (Barnes 1980). Coastal lagoons are inland water bodies, permanently or temporary connected to the ocean by one or more restricted inlets. They are of variable volume and have variable salinities as they can be subject to tidal mixing with high turbidity and irregular topographic characteristics (Kjerfve 1994; Yánez-Arancibia et al. 1994). They have a high productivity (Oliveira & Kjerfve 1993; Kjerfve 1994), which is frequently interrupted by ecological disasters of either natural or anthropogenic origin (Mee 1978; Sikora & Kjerfve 1985). The respond of coastal lagoons e.g. to river input, wind stress, tides or precipitation differs (Kjerfve 1994). In ecological terms tropical lagoons are of great importance as spawning grounds and also in terms of energy supply especially to fish populations (Yánez-Arancibia et al. 1994).

'The understanding of physical, chemical, geological and ecological dynamics of lagoons is important for planning and implementation of coastal management strategies in coastal lagoons' (Kjerfve 1994).

5.3 The Segara Anakan Lagoon mangrove ecosystem

On the island of Java mangroves make up 1.2% (49900ha) of the total mangrove area in Indonesia, whereof 13600ha of mangroves are remaining in central Java (Choong et al. 1990). About half of the Indonesian population lives on this island and largely depends on its natural resources in the coastal regions (Yuwono et al. 2007).

The largest remaining mangrove ecosystem on Java with 9238ha is fringing the 1002ha large brackish water ecosystem, the Segara Anakan Lagoon (SAL, Figure 5.1), at the South Central Coast (Ardli & Wolff 2009). It is neighboring the city Cilacap (240.325 inhabitants in 2008; www.cilacapkab.go.id) and harbors several small villages.

It is delimited from the Indian Ocean by the 121km² large rocky mountainous island Nusa Kambangan (Lukas, pers. comm.). Therefore only two entrances to the lagoon in the east and west are left, allowing marine water masses to enter the lagoon at high tide. Freshwater input is given by the fifth largest river on Java, which is issued into the SAL near the western entrance to the Indian Ocean.

In the hinterland of the western part of the SAL there are mainly rice fields, whereas in the eastern part dry land agriculture and shrimp ponds as well as aquaculture dominate the landscape. Furthermore, two industrial companies of the city Cilacap face the lagoon, the cement factory and the oil refinery, the latter being the largest in Indonesia. All these parties contribute effluents to the lagoons water.

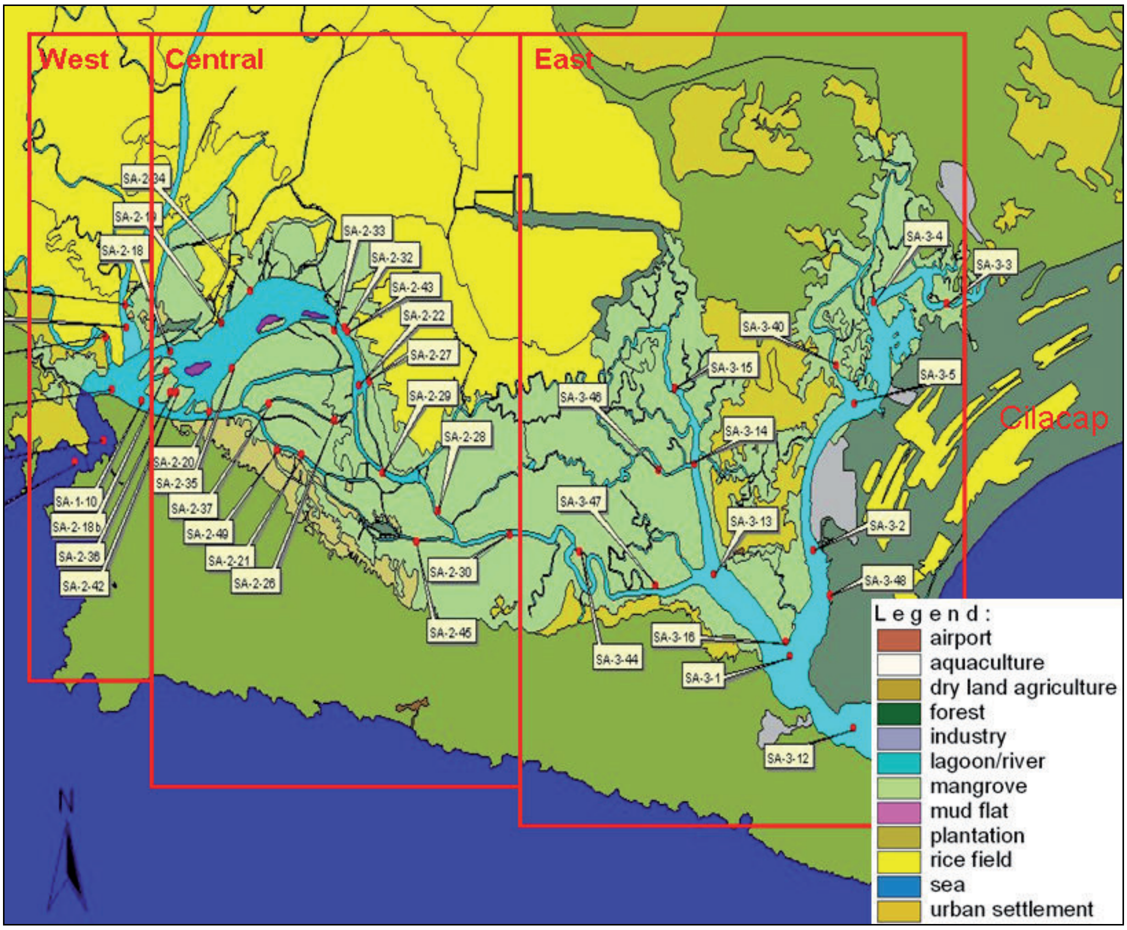

Figure 5.1: Map of the Segara Anakan Lagoon, with the city Cilacap in the east. Red boxes indicate the areas “West”, “Central” and “East” lagoon determined within the SPICE project.

Today the actual species number in the SAL is still not completely described, as there are areas in the lagoon, which were not included in diversity studies yet. Compared to other mangrove ecosystems in the Indo-Pacific region, species richness in the SAL is high with regard to gastropods and brachyuran crabs (Nordhaus et al. 2009). The Indo-West-Pacific holds the highest species richness worldwide (Ellison 2008). The SAL thus is amongst the highest in the world.

The economically most important species in the SAL are the fish families Mugilidae, Lutjanidae, Periophthalmidae and Anguillidae as well as decapod species of the genus

Scylla spp. and shrimps (Dudley 2000; Yuwono et al. 2007). Due to deforestation and thus destroying nursery grounds, these species are highly endangered. Also more than half of the original mangrove area has already been converted to agricultural land uses, such as rice fields and shrimp ponds (Ardli 2007).

The commercially most important mangrove species in this area are *Rhizophora apiculata* and *R. mucronata* as well as *Bruguiera gymnorrhiza* (Sastranegara et al. 2007). The mangrove area in the SAL has decreased by 1.4% each year within the last decade (Ardli 2007).

A recent study on species richness of the intertidal crab fauna of the SAL discussed whether the community composition and dominance structure within the group of decapods can be used as an indicator for the actual state of a mangrove (Geist et al. submitted). They found a high contribution of 2-3 species to the total abundance and biomass and higher species number in younger forests in the central lagoon. This suggests an indicator function of community attributes to define the age of a mangrove forest.

The outcome of a study on food choices and stomach contents of abundant crab species was the suggestion to further conduct one-choice experiments (Nordhaus et al. submitted), to estimate the impact of further logging on decapod crabs. This would be especially important for commercially valuable species such as *Rhizophora* spp., which is preferably ingested by decapods (Nordhaus et al. submitted). The consequences of the massive deforestation in the SAL on the benthic community in its full extend, particularly on the crab species, could not be valuated yet.

Since the 1950s the water body of the Segara Anakan was much larger than it is today. It shrank about one quarter of its original size (Lukas in prep.), mainly resulting from a high sedimentation load of the Citanduy River (Holtermann et al. 2009). In case of high Citanduy River discharges and neap tides a complete flushing of the whole Segara Anakan Lagoon can take place. This can also lead to a high sedimentation load into the lagoon (Holtermann et al. 2009).

5.4 Food web structures in mangrove ecosystems

Studying food webs is important to understand the complexity of interactions in mangrove ecosystems. Trophic levels can provide information about the hierarchy and the transfer of carbon and nitrogen within the benthic communities. Benthic organisms play a major role in recycling process of nutrients and preserving the energy in form of carbon and nitrogen mainly through breaking down leaf litter and their faeces (Nordhaus et al. 2009). Food webs have already been studied widely in several mangrove ecosystems, with respect to biodiversity, community composition and dynamics, food chain length or predator-prey interactions (e.g. Vander Zanden et al. 1999; Lepoint et al. 2000; Abrantes & Sheaves 2009). Food chain length is determined by several factors i.e. ecosystem size and species richness, and can help to quantify trophic structure (Vander Vander Zanden et al. 1999; Post et al. 2000; Vander Zanden & Fetzer 2007). In mangrove intertidal benthic communities usually three to four trophic levels were distinguished (e.g. Bouillon et al. 2002a; Abrantes & Sheaves 2009).

The stable isotope method proofed to be a very useful tool to investigate food web connections during the last three decades (Peterson & Fry 1987; Dauby 1990; Riera et al. 1999; Yoshii 1999; Lepoint et al. 2000). The most appropriate elements to compare species positions in an ecosystem are carbon and nitrogen, as they occur in every living organism. The stable isotope method is based on the fact that the predators carbon isotopic composition is approaching to its preys` with time. Carbon isotopic composition therefore differs between predator and prey by maximal 0.8‰ (e.g. Sheaves & Molony 2000; Vander Zanden & Rasmussen 2001), which was confirmed by a reviewing modeling approach (Caut et al. 2009).

Nitrogen is a sufficient tracer for trophic levels, as heavy nitrogen (^{15}N) is accumulated with each trophic level and the isotopically light ^{14}N is easier decomposed and excreted as faeces or in form of urate. The enrichment from one trophic level to the next is about 2.8‰ (Caut et al. 2009). This approach does not only allow to distinguish between full trophic levels (Bouillon et al. 2002a; Thimdee et al. 2004). Also steps between two trophic levels can be defined, which occur depending on the diet composition.

The isotopic composition is calculated by $\delta R= [(X_{sample}/X_{standard}) - 1] \times 1000$ ‰ with R= ^{13}C or ^{15}N and X= $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

It is important, however, to choose the appropriate tissue to achieve a convincing conclusion. Tissues differ from each other in isotopic compositions, depending on the speed of assimilation, but also on the amount of the heavy isotope fraction and time of storage needed. Muscle tissue is thus a reliable tissue for the investigation of trophic relationships (Tieszen et al. 1983), as it is a long-time-storage of heavy isotopic fractions, which do not underlie metabolic processes, due to strong chemical bonds.

5.5 Research hypotheses

Numerous food web studies using the stable isotope method were conducted over the last three decades. But only few investigated different locations within one study site or temporal differences such as between two seasons. Most studies are restricted to one study site within one area only at one certain point in time of a year. These factors are seldom considered in the discussion of publications and make global and habitat comparison difficult. As stable isotope compositions are influenced by a large number of factors, depending on the habitat, environmental conditions and anthropogenic impacts, possible coefficients should always be considered when interpreting data. The fractionation of isotopes e.g. in mangroves or invertebrates is not completely understood yet. The ability of individual organisms to 'choose' nutrients or food sources with lighter isotopes and the biochemical processes of fractionation are still unknown today.

This study shall aid to close the gaps of knowledge of spatial and temporal variation and contribute to the knowledge of stable isotope fractionation. Furthermore the question shall be answered, if stable isotopes are appropriate to resolve trophic structures by distinguishing trophic levels and tracing carbon sources.

Food web structures in intertidal benthic mangrove ecosystems have been examined in many countries. Variations have been detected comparing larger areas e.g. comparing pristine and human impacted mangroves (e.g. Abuodha & Kairo 2001; Alongi 2002), but small scale investigations within a study area have hardly been conducted. The first listed publication is therefore based on the following hypothesis:

1. **Food web structures vary already on a small spatial scale as different anthropogenic impacts result in varying food availability and quality. This can be differentiated by considering isotopic compositions of stable isotopes in muscle tissues of involved intertidal benthic species.**

Stable carbon and nitrogen isotopes do not only depend on geographical regions or food availability, but also on the time of sampling, as a food web investigated at a certain time of the year does not reflect the structure during any other time of the year (Thompson & Townsend 1999). The season does, as well as the study site, have an effect on several community related factors, e.g. species richness and predator-prey ratios (Whitlatch 1977; Alongi 1987; Winemiller & Jepsen 1998; Thompson & Townsend 1999; Poon et al. 2010). In the Southeast Asian tropics, seasonality is distinguished by the monsoon rains, which is an important factor, when capturing food webs. Seasonality is an umbrella term for i.e. changing salinities, nutrient availability, air and water temperatures or solar radiation (e.g. Cowan & Boynton 1996; Badran 2001; Graham et al. 2003; Holtermann et al. 2009). The second listed publication is therefore based on the following hypothesis:

2. **Seasonal changes in environmental factors lead to a divergent food availability and consequently to changes in the food web structures. These can be traced by stable isotope compositions in muscle tissues of intertidal benthic organisms.**

To understand the changes observed in muscle tissue isotopic compositions, it is important to understand the underlying biochemical metabolic processes in benthic organisms. Animals can adapt to food sources of different qualities with varying several physiological and behavioral characteristics, i.e. the selection of food items, the amount of food ingested and the mechanical fragmentation of food (Linton & Greenaway 2007). If e.g. leaves are mechanically incompletely broken down, the digestion and assimilation will be low and faeces will have similar concentrations of neutral detergent soluble material (e.g. nitrogen, carbon, cellulose, lignin, calcium) as leaves, implying that the leaves were not completely utilized (Greenaway & Linton 1995; Nordhaus & Wolff 2007).

Starving animals have increasing $\delta^{15}N$ values in their muscle tissue, which are a result of the internal recycling of nitrogen and the maladjusted excretion of ^{14}N (Hobson 1993). Isotopic fractionation occurs primarily during anabolic processes and production of new tissues (Yokoyama et al. 2005). Until today only little attention has been paid to physiological processes and biochemical mechanisms that have an impact on isotopic compositions (Adams & Sterner 2000). Therefore, in the third listed publication the following hypotheses are investigated:

3 a With only a one-choice diet given, benthic crabs show a response in the isotopic composition in their muscle tissue within a time period of three months.

b Several intertidal crab species consume different mangrove leaves and assimilate and excrete carbon and nitrogen differently, depending on their feeding habits.

c Isotopic compositions will substantially differ between hepatopancreas, muscle tissue and stomach content according to their role and status within the assimilation process.

d Ovigerous females have lower isotopic compositions than non ovigerous. The former invest more carbon and nitrogen into the development of the eggs than in their own metabolism.

With the knowledge of quantitative nutrient utilization by the benthic community in the SAL, the overall impact of benthic species on the recycling processes can be estimated and quantified.

The present work was conducted within the project SPICE (Science for the Protection of Indonesian Coastal Ecosystems) in the group of “benthic ecology”, with the overall aim to investigate the importance of benthic organisms for the cycling of carbon and nitrogen in the SAL.

The results and conclusions of these investigations will be presented in the following three chapters, followed by a general discussion.

6 High spatial variability of $\delta^{13}C$ and $\delta^{15}N$ in intertidal benthic food webs in the mangrove fringed Segara Anakan Lagoon, Java, Indonesia

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Abstract

Segara Anakan, a mangrove fringed lagoon in South Central Java, is strongly influenced by anthropogenic activities and related environmental changes such as deforestation and a related decrease in food availability. In order to investigate the benthic food web, 14 abundant species of four systematic groups, leaves of four mangrove tree species and sediment were sampled in the intertidal area of four stations in the lagoon. Samples were analysed for organic carbon and total nitrogen content as well as stable carbon and nitrogen isotope composition ($\delta^{13}C$, $\delta^{15}N$). Furthermore, the stomach contents of four crab species and one mud skipper were identified. Sediment $\delta^{13}C$ and $\delta^{15}N$ were similar throughout, but those of animal tissues and leaves varied highly between stations. Average leaf signatures ranged between -4.8‰ (*Aegiceras corniculatum*) and 4.3‰ (*Avicennia alba*) for $\delta^{15}N$ and between -29.9‰ (*Sonneratia alba*) and -26.7‰ (*A. alba*) for $\delta^{13}C$ over all stations. Average $\delta^{15}N$ of invertebrate muscle tissue was between 5.1‰ and 17.6‰ and between -25.2‰ and -15.6‰ for $\delta^{13}C$. The crab species *Episesarma versicolor*, *Epixanthus dentatus* and *Scylla serrata* ranged in the highest trophic levels as inferred from the high $\delta^{15}N$ of their muscle tissue. The gastropod *Telescopium telescopium* showed high $\delta^{15}N$ in one station and there most likely nourishes on carrion of decapods and fish. *Epixanthus dentatus* and *Perisesarma darwinense* are opportunistic feeders depending on the carbon and nitrogen sources available at the respective location. The examined species could be divided into three to four trophic levels depending on the station. Food web structure varied between stations depending on food availability and quality. Due to the opportunistic feeding habit of several macrobenthic species they seem to be highly adaptable to changes in vegetation.

Key words: *benthic food web, carbon, mangroves, nitrogen, stable isotopes, trophic level*

1 Introduction

During the last three decades, the natural occurrence of stable isotopes has been used to develop an adequate tool to answer geological, biogeochemical and ecological questions (Peterson & Fry 1987, Hobson 1999, Post 2002). This method has been used widely to investigate estuarine and coastal benthic communities, including food webs (Dauby 1990, Riera et al. 1999, Yoshii 1999, Lepoint et al. 2000), nutrient cycles within food webs (Parker 1964, Peterson & Howarth 1987, Machás & Santos 1999) and the growth of organisms (Tieszen et al. 1983). The concept of the stable isotope approach is based on the assumption that heavy ^{15}N in animal tissue increases significantly (3-5‰) with each trophic level (TL; Vander Zanden & Rasmussen 2001, McCutchan et al. 2003, Caut et al. 2009). Previous studies have shown that nitrogen isotopic compositions are helpful indicators of trophic levels (TLs) as variance in measured tissues is low (Bouillon et al. 2002a). On the other hand carbon isotopic compositions are due to a high variability and a discrepancy between two TLs of close to zero not accurate enough to resolve trophic structures. The fraction of ^{13}C of consumers is 0-2‰ higher than their diet (Peterson & Fry 1987). Carbon and nitrogen stable isotopes therefore reflect the assimilated diet of an animal (Hill et al. 1993). Choosing the appropriate tissue to investigate the time frame of interest is essential in trophic studies (Tieszen & Boutton 1989, Hobson 1993). Tissues such as muscle with low turnover rates, integrate diet isotopic signatures on a longer time period than those with higher turnover rates, e.g. hepatopancreas (Hesslein et al. 1993, Lorrain et al. 2002, Watanabe et al. 2005).

The use of naturally stable isotope compositions of both carbon and nitrogen has improved the understanding of food web structures and functions, as well as related dietary patterns in a variety of ecosystems (Michener & Schell 1994). Many studies have investigated benthic food web structure in mangroves, but spatial comparisons within one research area, e.g. along a coastline, have only been done on a large scale (e.g. Sheaves & Molony 2000). Small scale comparisons within a few kilometres range, e.g. within one lagoon or estuary, especially regarding food web studies based on the isotope approach (e.g. Cifuentes et al. 1996, Bouillon et al. 2002a, Guest & Connolly 2005, Le Loc`h et al. 2008), are rare. As variances in isotopic signatures in macrobenthic organisms were already found on a large scale (Wooller et al. 2003, Thimdee et al. 2004, Deling et al. 2005), further research on a smaller scale is needed to understand the factors influencing the specific feeding habits of benthic species (Ehleringer et al. 1986). This is the first study to compare food webs, also on a small scale within a few kilometres range, in Indonesia.

The Segara Anakan Lagoon (SAL), located at the south central coast of Java, Indonesia, is a 1002ha (in 2006; Ardli & Wolff 2009) mangrove fringed brackish water ecosystem. The 9238ha of mangrove forests (in 2006; Ardli & Wolff 2009) surrounding the lagoon are located close to the largest oil refinery of Indonesia in the city of Cilacap. Urban and industrial sewage of this city and several adjacent villages within the lagoon area are released into the lagoons water. Mangrove trees are logged at a high rate in the lagoon (Ardli &Wolff 2009). These factors imply a high anthropogenic influence on the mangrove ecosystem, referring especially to the significant increase of rice field areas, aquaculture and rural areas during the last two decades (Ardli &Wolff 2009). Previous studies have shown a decreasing effect on the nitrogen isotopic composition as well as an increase on carbon isotopic composition in phytoplankton, algae, animal tissue and mangrove leaves, triggered by industrial sewage (Rau et al. 1981, Gearing et al. 1991, Tucker et al. 1999, Costanzo et al. 2001, 2003, Gartner et al. 2002). Another factor influencing carbon isotopic signatures in sediments (Jackson et al. 1996, Lichfouse et al. 1997, McRae et al. 2000) and consequently in detritus feeding animals are polycyclic aromatic compounds (PAH), pollutants found in fossil oils and coil.

The SAL plays an important role for benthic organisms and as a nursery ground also for pelagic fish (Romimohtarto et al. 1991, Yuniar et al. 2007, Nordhaus et al. 2009). Economically important species, especially for local people in the lagoon, are the mud crabs *Portunus* sp. and *Scylla spp.* as well as the bivalves *Polymesoda erosa* and *Saccostrea* cf. *cucculata* (personal observation). The ecological importance of benthic species in the SAL is especially the recycling of nutrients e.g. by consumption of leaf litter (Nordhaus et al. 2009), and also the ventilation of soils by bioturbation.

The overall aim of this study is to investigate spatial variability in food web structure on a small scale in the SAL. We hypothesise that food web structures at the chosen locations differ substantially from each other, as it is expected that intertidal macro-invertebrates respond differently to varying ambient conditions. Therefore it is expected that food availability and quality are reflected in isotopic compositions of invertebrate muscle tissues. As sewage pollution can cause lower $\delta^{15}N$ and higher $\delta^{13}C$ and PAH pollution can result in decreased $\delta^{13}C$, differences between stations are expected to be reflected in the isotopic composition in muscle tissues.

Our specific objectives were (1) to define the number of TLs, including the most abundant species of the intertidal benthic community by using the stable isotope approach, (2) to examine the feeding habits of the above analysed species using stomach content and stable isotope analysis, and (3) to generate a food web based on the results of isotope and stomach content analyses.

2 Material and Methods

2.1 Study site

Organisms were collected during rainy season between February and April 2008 (mean annual precipitation in rainy season: 152mm per month; http://climate.usurf.usu.edu) in the mangrove fringed Segara Anakan Lagoon (SAL), Java, Indonesia (108°50’-109°00’E, 07°39’- 07°43’S; Fig. 6.1). The lagoon is separated from the Indian Ocean by the rocky mountainous island Nusa Kambangan. Exchange with saline water masses is provided by two entrances to the lagoon, east and west of the island. The hydrology of the SAL is governed by semidiurnal tides, ranging between 0.3m at neap tides and 1.9m at spring tides. Freshwater is mainly provided by the Citanduy River, the fifth largest river of Java, and the Cibereum in the west (Holtermann et al. 2009).

The SAL has a high commercial and ecological value due to its high diversity of marine macrobenthic and fish species (White et al. 1989, Naamin et al. 1991, Yuwono et al. 2007). It is strongly influenced by human activities through aquaculture, agriculture (rice fields) and villages within the lagoon and the city of Cilacap (240.325 inhabitants in 2008; www.cilacapkab.go.id) with the biggest oil refinery of Indonesia. By overfishing, deforestation and over-exploitation of the natural resources (also regarding benthic economically important species, such as *Polymesoda erosa* and *Scylla serrata*) the lagoons resources, serving as food for local people, are depleted. Additionally sedimentation through rivers, mainly due to deforestation in the hinterland, reduces the water volume of the lagoon (Ardli 2007).

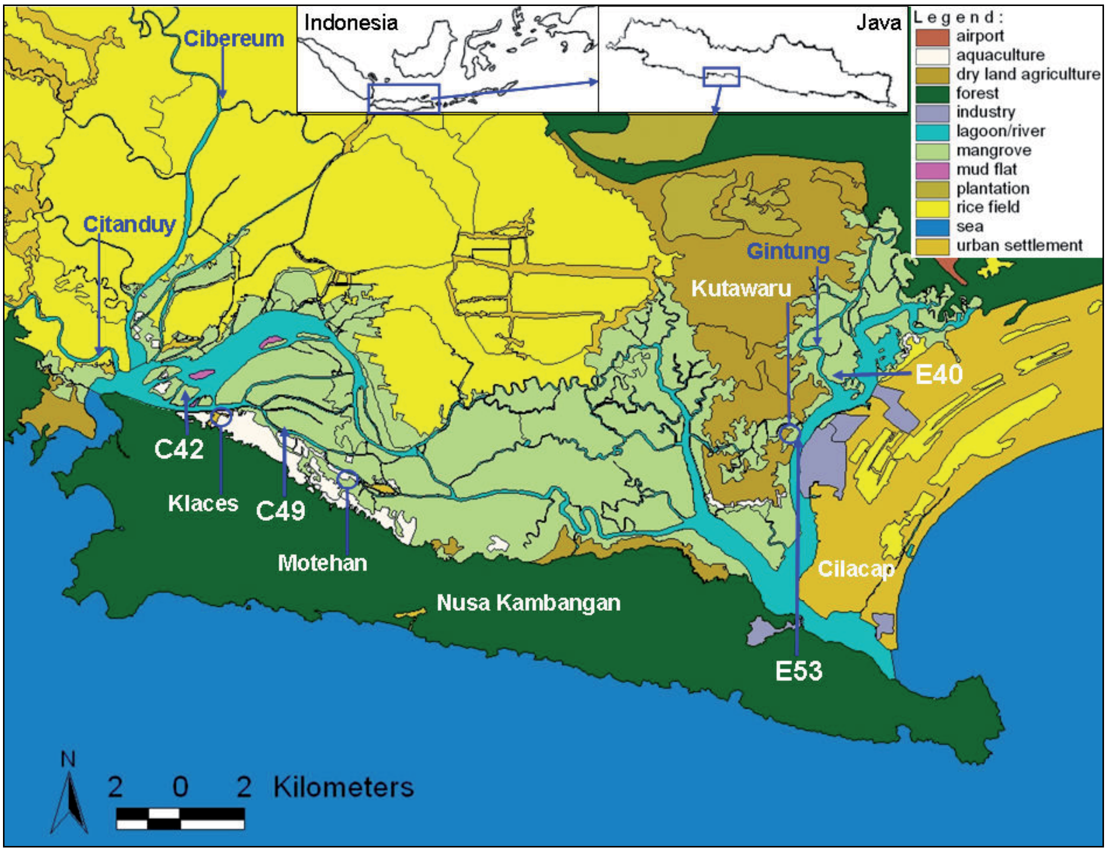

Figure 6.1: Map of Segara Anakan with four sampling stations (C42, C49, E53, E40), the rivers Citanduy, Cibereum and Gintung, the villages Klaces, Motehan, Kutawaru and the city Cilacap, modified from Ardli (unpublished), http://istgeography.wikispaces.com/1Bindonesia and http://www.gamelannetwork.co.uk/assets/pics/Java_map.gif.

Species richness of mangrove trees and macrobenthos is high in the SAL (Hinrichs et al. 2009, Nordhaus et al. 2009). Of the so far described total macrobenthic species number of 186 in the lagoon and its fringing mangroves, the contribution of brachyuran crabs (57 taxa), and gastropods (55 taxa) were the highest (Nordhaus et al. 2009).

2.2 Sampling stations

Samples were taken from four stations (Fig. 6.1) within intertidal mangrove areas of approximately 40m x 40m each. Stations were chosen isochronous, two in the central lagoon, with higher natural (freshwater) influence and two in the eastern lagoon with higher anthropogenic influence due to the oil refinery and the city of Cilacap.

Station E40 is located in the eastern part of the lagoon (108°59.57'E, 07°40.28'S), in a northern creek, which provides freshwater during rainy season. This station is diagonally opposite the oil refinery and covered by a large swamp area. Dominant mangrove tree species are *Aegiceras corniculatum* and *Ceriops* spp. (Hinrichs et al. 2009). Station E53, is located opposite the oil refinery in the eastern lagoon area (108°59.33'E, 07°41.33'S). Anthropogenic influence by urban waste water and household wastes such as plastic from the city Cilacap is probably quite high. The station is neighboring the village Kutawaru, which might additionally pollute the area by household wastes. Predominant mangrove species are *Aegiceras* spp. and *Sonneratia* spp. (personal observation).

Station C42 is located on a small island (108°49.11'E, 07°40.76'S), one of four new islands in the central lagoon. Situated near the entrance to the Indian Ocean, this station is completely exposed to saline water masses at high tide (Holtermann et al. 2009). Occurring mangrove species are mainly *Avicennia* spp. and *Sonneratia* spp. (Hinrichs et al. 2009). Station C49 is located in the central lagoon (108°50.76'E, 07°41.44'S). It is influenced by saline water masses during high tide (Holtermann et al. 2009). The predominant mangrove tree species here is *Sonneratia* spp. (Hinrichs et al. 2009). Due to its location between the villages Klaces and Motehan it is probably also under high anthropogenic influence through household wastes.

2.3 Sample collection and preparation

Thirteen abundant intertidal benthic species and pooled species of polychaetes of five systematic groups (Annelida: Polychaeta; Decapoda: *Epixanthus dentatus*, *Episesarma singaporense*, *E. versicolor*, *Metaplax elegans, Perisesarma darwinense*, *Scylla serrata*, *Uca forcipata*; Mollusca: *Cerithidea cingulata*, *Polymesoda erosa*, *Saccostrea* cf. *cucculata*, *Telescopium telescopium*; Sipunculida: *Phascolosoma arcuatum*, Teleostei: *Periophthalmus* sp.) were collected by hand at four intertidal mangrove stations. Animals of one species were of comparable size. The portunid crab *S. serrata* was bought from local fisherman in the central and eastern lagoon. Additionally three invertebrate feeding fish species (*Epinephelus areolatus*, *E. coioides*, *Scatophagus argus*; Heemstra & Randall (1993), Froese & Pauli (2010)) were bought from fishermen in the eastern lagoon.

Five individuals of each benthic species were collected at each station and frozen for a minimum time period of 12 hours in PVC sampling bottles before further treatment. Crabs and fish were dissected and muscle tissue from the chelae and white muscle tissue respectively was removed for analysis. *C. cingulata, Periophthalmus* sp.*, Phascolosoma arcuatum, P. erosa*, *Saccostrea* cf. *cucculata*, *T. telescopium* and polychaetes were analysed as a whole (only soft tissue, without shell). *P. arcuatum* and polychaetes were kept in lagoon water for 24 hours to make them empty their guts, before further treatment. Additionally, stomach contents of *E. singaporense*, *E. versicolor, P. darwinense* and *S. serrata* were sampled for analysis. Also muscle tissue of three abundant benthic feeding fish species (*Epinephelus areolatus*, *E. coioides* and *Scatophagus argus*) was sampled in the eastern part of the lagoon (see Table 6.1) to include possible higher trophic levels into the benthic food web. Samples were homogenized by hand with an agate mortal after drying at 40°C for a minimum of two weeks.

Of every mangrove tree species (*Avicennia alba*, *Aegiceras corniculatum*, *Rhizophora apiculata* and *Sonneratia alba*) three yellow and brown leaves each (replicates) were collected. Brown leaves were collected by hand from the sediment surface. Senescent yellow leaves were picked directly from mangrove trees. Surface sediment was sampled within a distance of 20m from the water edge for isotope analysis, including three replicates at each station.

Sediment samples were dried at 40°C, treated with 200µl 1M HCl to remove carbonates and then redried. Subsamples were analysed for organic carbon (C_{org}) and nitrogen (N) and also stable isotope composition of organic carbon ($^{13}C_{org}/^{12}C_{org}$) and nitrogen ($^{15}N/^{14}N$). No significant differences were found between tissue samples treated with HCl and samples without HCl during preliminary analysis (t-test: F=1.11, p=0.89, with n=8), therefore no HCl was applied for tissue samples.

At each station 50ml of water was sampled from water places trapped between mangroves while the tide was going out, in order to measure salinity.

2.4 Stable isotope analysis

Samples were combusted in the Carlo Erba NA 2100 Elemental analyzer for C_{org} and N measurements. Stable isotope ratios were determined using the coupled EA-IRMS gas isotope ratio mass spectrometer (ConFlo III) and expressed relative to conventional standards $\delta R = [(X_{sample}/X_{standard}) - 1] \times 1000$ ‰ with R= ^{13}C or ^{15}N and X= $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Ammonium sulfate (IAEA-N1, IAEA-N2) was used as standard for $\delta^{15}N$, and graphite (USGS-24) and mineral oil (NBS-22) for $\delta^{13}C$. Analytical precision was ±0.2‰ for both nitrogen and carbon, as estimated from standards analyzed together with the samples.

2.5 Trophic level determination

Caut et al. (2009) reviewed 66 isotope studies with a large number of estimated animal diets (n= 290), and found an overall discrepancy of $\Delta\delta^{15}N$=2.8±0.1‰ and $\Delta\delta^{13}C$=0.8±0.1‰ between two trophic levels. We use the steps of fractionation from their study as they are based on the largest dataset available at present.

If we assume a discrepancy between two trophic levels (TLs) of $\Delta^{15}N$ =2.8±0.1‰ we can set up a range system from 0‰ to 2.8‰ to distinguish the several steps between two TLs, as intertidal benthic organisms are widely distributed over the $\delta^{15}N$-scale. Therefore we introduce five ranges to define two organisms on the same TL around the critical values of 0 (0-0.34‰), with a discrepancy of 0.25 TL (0.35-1.04‰), 0.5 TL (1.05-1.74‰) and 0.75 TL (1.75-2.44‰), as well as on two different TLs (2.45-2.8‰), assuming that the invertebrates with the lowest $\delta^{15}N$ are setting the first TL. For the calculation of trophic levels, mean values of the species within one station were used. Species with larger standard deviations are assumed to have slightly divergent alimentation and therefore differ less than one TL from the mean in the majority of cases.

This method is used to sufficiently define nuances between two trophic levels.

2.6 Stomach content analysis

From station C49 four to five individuals of *Episesarma versicolor*, *E. singaporense*, *Perisesarma darwinense, Scylla serrata* and *Uca forcipata* were collected and frozen directly in PVC sampling bottles for at least 12 hours before dissection. Stomachs were removed extending from oesophagus to pylorus. Stomach fullness was estimated and contents were defined by estimating the contribution (%) of each food category to the total volume of a sample in a Bogorov tray. Alimentary categories which could be distinguished were animal, bark, detritus, leaves, polychaetes, roots, sediment and miscellaneous.

Due to limitations of time, stomach content analysis was only conducted for abundant species and only at one station. Station C49 was chosen because it had the overall highest values of $\delta^{13}C$ and lowest of $\delta^{15}N$ in invertebrate muscle tissue.

Stomach content was also analyzed for 15 individuals of *Periophthalmus* sp. The frequency of occurrence of distinguished categories (polychaetes, fish and unidentified insects) was examined.

2.7 Statistical analysis

Isotopic signatures of organisms were tested for significant differences within and between the stations, separately for $\delta^{13}C$ and $\delta^{15}N$. Data was tested for normality, Log-transformed and tested for homogeneity of variances. A non-parametric Kruskal-Wallis-ANOVA was used followed by a Mann-Whitney-U post hoc test.

C/N ratios and isotopic signatures of sediments and leaves were normally distributed and homogeneous, therefore an ANOVA followed by a Tukey HSD post hoc test were used to identify significant differences.

3 Results

3.1 Primary carbon sources

The stable isotope composition of sediments was similar for all stations (ANOVA: $p>0.05$), with an average of -26.5±0.4‰ for $\delta^{13}C$ and 4.2±0.1‰ for $\delta^{15}N$ (Fig. 6.2, Table

6.1). C/N ratios for sediments were 14.4±1.3 and similar at all stations (Tukey´s HSD: $p > 0.05$).

Mangrove leaves had low isotope compositions at all stations, ranging between -26.7‰ and -29.9‰ for $\delta^{13}C$ and from -4.8‰ to 4.3‰ for $\delta^{15}N$. C/N ratios of mangrove leaves ranged between 22.6 (min.) and 218.2 (max.) in the Segara Anakan Lagoon.

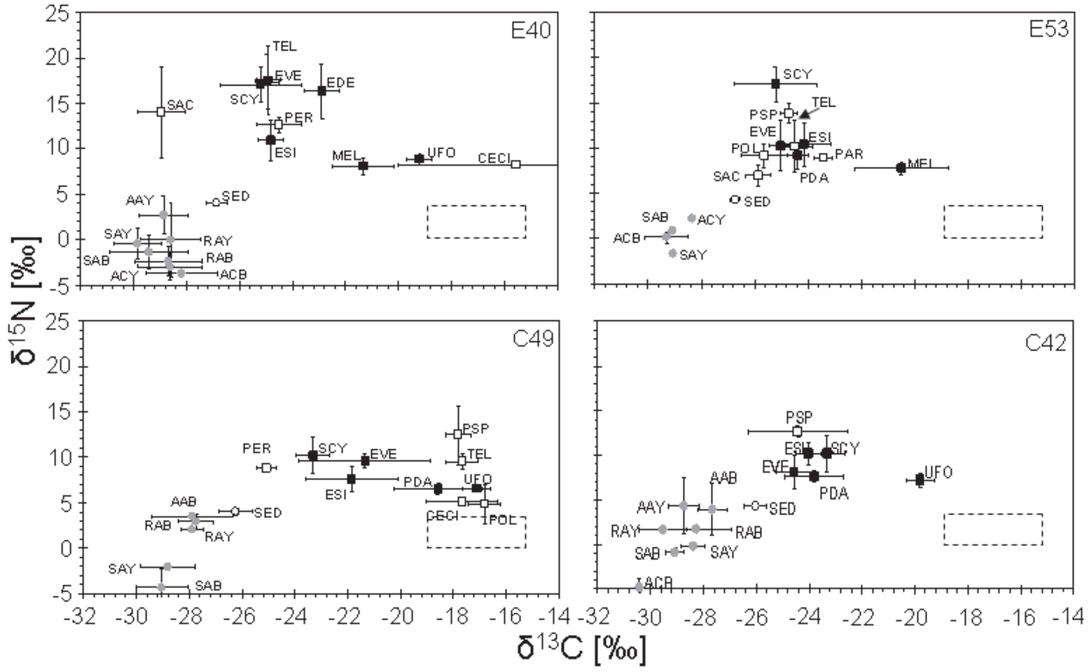

Figure 6.2: Biplots of four stations (C49, C42, E53, E40) in the lagoon, based on carbon and nitrogen isotopic signatures of muscle tissue (of *Episesarma singaporense* (ESI), *Episesarma versicolor* (EVE), *Epixanthus dentatus* (EDE), *Metaplax elegans* (MEL), *Perisesarma darwinense* (PDA), *Polymesoda erosa* (PER), *Saccostrea* cf. *cucculata* (SAC), *Scylla serrata* (SCY), *Uca forcipata* (UFO)) of the whole organism (*Cerithidea cingulata* (CECI), *Periophthalmus* sp. (PSP), *Phascolosoma arcuatum* (PAR), Polychaetes (POL), *Telescopium telescopium* (TEL)) and of primary carbon sources (AAx: *Avicennia alba*, ACx: *Aegiceras corniculatum*, SAx: *Sonneratia alba*, RAx: *Rhizophora alba*, xxB: brown leaves, xxY: yellow leaves (grey circles); SED: inorganic sediment (white circles)) with indicated standard deviation for $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Black squares: Brachyuran crabs, white squares: other invertebrates (gastropods, molluscs and polychaetes), dashed square: approximate position of benthic microalgae (after Bouillon et al. 2002b).

3.2 Invertebrates

Overall we found higher $\delta^{15}N$ values in the eastern compared to the central lagoon (Fig. 6.2, Table 6.1; ANOVA: $p < 0.01$, Tukey´s HSD: $p < 0.01$). Invertebrate tissues had significantly lower $\delta^{15}N$ at C49 compared to the other stations (M-W-U: $p < 0.05$). Lowest values were analyzed for *Cerithidea cingulata* and Polychaetes ($\delta^{15}N \leq 5.1$‰) at this station. The overall highest $\delta^{15}N$ for invertebrates were measured at station E40 with

17.6‰ for *Telescopium telescopium* (M-W-U: $p<0.05$). Within this station *Episesarma versicolor*, *Epixanthus dentatus* and *T. telescopium* were at the top end of the analyzed species ($\delta^{15}N \geq 16.4$‰). *Scylla serrata* had higher $\delta^{15}N$ values in the eastern area and higher $\delta^{13}C$ values in the central lagoon (ANOVA: $p=0.00$, Tukey´s HSD: $p<0.05$.

In general carbon isotopic signatures did not show any change or trend regarding areas. $\delta^{13}C$ were highest at C49 (M-W-U: $p<0.05$) with exception of *Cerithidea cingulata* and *Polymesoda erosa*, which were higher in E40 (M-W-U: $p<0.05$).

Table 6.1: Isotopic signatures [‰] of carbon and nitrogen of leaves (AAx: *Avicennia alba*, ACx: *Aegiceras corniculatum*, SAx: *Sonneratia alba*, RAx: *Rhizophora alba*, xxB: brown leaves, xxY: yellow leaves), muscle tissue of macrobenthic species (*Episesarma singaporense* (ESI), *Episesarma versicolor* (EVE), *Epixanthus dentatus* (EDE), *Metaplax elegans* (MEL), *Perisesarma darwinense* (PDA), *Polymesoda erosa* (PER), *Saccostrea* cf. *cucculata* (SAC), *Scylla serrata* (SCY), *Uca forcipata* (UFO)) and the whole organism (*Cerithidea cingulata* (CECI), *Periophthalmus* sp. (PSP), *Phascolosoma arcuatum* (PAR), Polychaetes (POL), *Telescopium telescopium* (TEL)), fish (*Epinephelus coioides* (Eco), *Epinephelus areolatus* (Ear), *Scatophagus argus* (Sar)) and sediment (SED) at four stations with indicated standard deviation.

	central	$\delta^{13}C$ [‰]	east		central	$\delta^{15}N$ [‰]	east	
	C42	C49	E40	E53	C42	C49	E40	E53
AAB	-27.7 ± 0.5	-27.9 ± 1.5			3.9 ± 2.9	3.5 ± 0.2		
AAY	-28.8 ± 0.6		-28.9 ± 0.9		4.3 ± 3.2		2.8 ± 2.1	
ACB	-30.4		-28.2 ± 1.3	-29.3 ± 0.8	-4.8 ± 1.0		-3.7 ± 0.4	0.1 ± 0.6
ACY			-28.6 ± 1.2	-28.4			-3.0 ± 1.5	2.2
RAB	-28.3 ± 1.4	-27.8 ± 0.7	-28.7 ± 1.3		1.7 ± 0.1	2.9 ± 0.8	-2.4 ± 1.7	
RAY	-29.5 ± 0.9	-27.9 ± 0.4	-28.6 ± 1.1		1.7 ± 0.2	2.1 ± 0.3	-0.0 ± 4.1	
SAB	-29.1 ± 0.4	-29.0 ± 1.0	-29.4 ± 1.5	-29.1	-0.9 ± 0.5	-4.4 ± 2.1	-1.4 ± 1.8	0.9
SAY	-28.4 ± 0.5	-28.8 ± 1.0	-29.9 ± 0.9	-29.1	-0.2 ± 0.1	-2.1 ± 0.3	-0.4 ± 1.7	-1.6
SED	-26.1 ± 0.4	-26.2 ± 0.6	-26.9 ± 0.4	-26.7 ± 0.2	4.3 ± 0.4	4.0 ± 0.4	4.1 ± 0.5	4.3 ± 0.2
CECI		-17.7 ± 1.3	-15.6 ± 4.5			5.1 ± 0.4	8.3 ± 0.4	
PER		-25.2 ± 0.4	-24.5 ± 0.9			8.8 ± 0.3	12.7 ± 0.8	
SAC			-29.0 ± 0.9	-25.9 ± 0.5			14.0 ± 5.0	7.0 ± 1.1
TEL		-17.7 ± 0.6	-24.9 ± 0.5	-24.5 ± 0.7		9.5 ± 0.9	17.6 ± 3.8	10.2 ± 2.9
EDE			-22.9 ± 0.7				16.4 ± 3.0	
ESI	-24.0 ± 0.6	-21.8 ± 1.8	-24.8 ± 0.5	-24.2 ± 1.0	10.2 ± 1.3	7.6 ± 1.4	11.0 ± 2.3	10.4 ± 2.4
EVE	-24.6 ± 0.7	-21.3 ± 2.5	-25.0 ± 0.5	-25.0 ± 0.5	8.1 ± 1.9	9.6 ± 0.8	17.4 ± 3.0	10.2 ± 2.8
MEL			-21.3 ± 1.2	-20.5 ± 1.8			8.1 ± 1.0	7.7 ± 0.7
PDA	-23.8 ± 1. 1	-18.6 ± 1. 7		-24.4 ± 0.4	7.6 ± 0.6	6.5 ± 0.6		9.2 ± 1.5
SCY	-23.3 ± 0.6		-25.2 ± 1.6		10.2 ± 2.0		17.1 ± 2.0	
UFO	-19.8 ± 0.5	-17.1 ± 0.5	-19.2 ± 0.5		7.2 ± 0.8	6.6 ± 0.4	8.9 ± 0.7	
PAR				-23.4 ± 0.4				9.0 ± 0.0
PSP	-24.4 ± 1.9	-17.8 ± 0.5		-24.7 ± 0.3	12.7 ± 0.6	12.5 ± 3.2		13.8 ± 1.2
POL		-16.8 ± 0.6		-25.7 ± 0.9		4.8 ± 2.2		9.2 ± 1.3
Eco			-20.9 ± 0.5				16.0 ± 2.5	
Ear			-16.1 ± 0.6				15.9 ± 0.5	
Sar			-23.9 ± 1.5				11.1 ± 0.6	

3.3 Fish

For *Epinephelus areolatus* and *E. coioides* similar $\delta^{15}N$ were measured (average 16.0±0.1 ‰; Table 1; M-W-U: p>0.05). *Scatophagus argus* muscle tissue had significantly lower $\delta^{15}N$ than the genus *Epinephelus* (11.1±0.6‰; ANOVA: p<0.01, M-W-U: p<0.01). ^{13}C was most enriched in *E. areolatus* (-16.1±0.6‰), and *S. argus* had lowest $\delta^{13}C$ of the three examined fish species (-23.9±1.5‰). $\delta^{13}C$ in all three species was significantly different from each other (ANOVA: p<0.01, M-W-U: p<0.01).

3.4 Trophic levels

From our calculation based on the discrepancy of $\delta^{15}N$ after Caut et al. (2009) we determined four trophic levels (TLs). Species occurred in different TLs depending on the location (Fig. 6.3, Table 6.2).

In station C42 $\delta^{15}N$ values only indicated three TLs (Table 6.2), due to a higher baseline (=TL 1) of $\delta^{15}N$. Species that were defined as TL=1 in other stations were not found here, therefore only three levels could be defined (Vander Zanden et al. 1999).

The species *Scylla serrata*, *Telescopium telescopium*, *Episesarma versicolor* and *Saccostrea* cf. *cucculata* occurred in different trophic levels throughout the lagoon (Table 6.2), whereas *Episesarma singaporense*, *Uca forcipata*, *Metaplax elegans* and *Cerithidea cingulata* occurred on the same trophic level throughout.

Table 6.2: Trophic levels at four stations based on means of nitrogen isotopic signatures, excluding primary carbon sources; 1: low, 2: moderate, 3: high, 4: top level.

	C42	C49	E40	E53
Scylla serrata	2	3	4	4
Epixanthus dentatus			3.75	
Periophthalmus sp.	3	3.75		3
Telescopium telescopium		2.75	4.25	2
Episesarma versicolor	1.5	2.75	4.25	2
Polymesoda erosa		2.5	2.5	
Episesarma singaporense	2	2	2	2
Saccostrea cf. *cucculata*			3	1
Polychaeta		1		1.75
Perisesarma darwinense	1.5	1.5		1.75
Phascomosoma arcuatum				1.5
Uca forcipata	1	1.5	1.25	
Metaplax elegans			1	1.25
Cerithidea cingulata		1	1	

3.5 Stomach content analysis

The diet composition analysis of *Scylla serrata* showed that this species feeds exclusively on animal tissue (100%), including its own species (10% of total stomach content volume), other Decapoda (75%), fish (10%) and Gastropoda (5%). For *Episesarma versicolor* the most important food category besides decomposed leaves (58%) was animal tissue (18%) (Fig. 6.3). For *E. singaporense* animals only had a minor contribution to the food composition (2%), this species mainly fed on bark, leaves and roots (together 70%). *Perisesarma darwinense* almost exclusively fed on leaves (97%). A large part of the diet of *Uca forcipata* consisted of polychaetes (30%), but no other animals could be identified in the stomachs. Sediment contributed 21% to the stomach contents.

$\delta^{13}C$ of stomach contents were very similar for all observed species within one station (Table 6.3), only *Epixanthus dentatus* stomach contents had different $\delta^{13}C$ ($\delta^{13}C$ = -23.8±0.9‰) compared to the other species (average $\delta^{13}C$ = 27.8±0.2‰) at station E40. For $\delta^{15}N$ no such similarities were observed, values differ largely within species between stations and also between species within the stations.

Table 6.3: Isotopic signatures [‰] of carbon and nitrogen of stomach contents of four macrobenthic species at four stations.

$\delta^{13}C$ [‰]	C42	C49	E40	E53
Episesarma singaporense	-27.2 ± 1.2	-24.9 ± 2.2	-27.9 ± 0.7	-27.5 ± 0.9
Episesarma versicolor	-27.2 ± 0.6	-24.5 ± 0.9	-27.6 ± 0.6	
Perisesarma darwinense	-27.2 ± 0.1			-27.2 ± 1.0
Epixanthus dentatus			-23.8 ± 0.9	

$\delta^{15}N$ [‰]	C42	C49	E40	E53
Episesarma singaporense	7.9 ± 1.0	6.2 ± 1.3	10.7 ± 2.1	9.8 ± 0.9
Episesarma versicolor	5.5 ± 2.1	5.8 ± 1.8	11.4 ± 0.8	
Perisesarma darwinense	7.1 ± 1.8			10.3 ± 1.9
Epixanthus dentatus			10.7 ± 0.9	

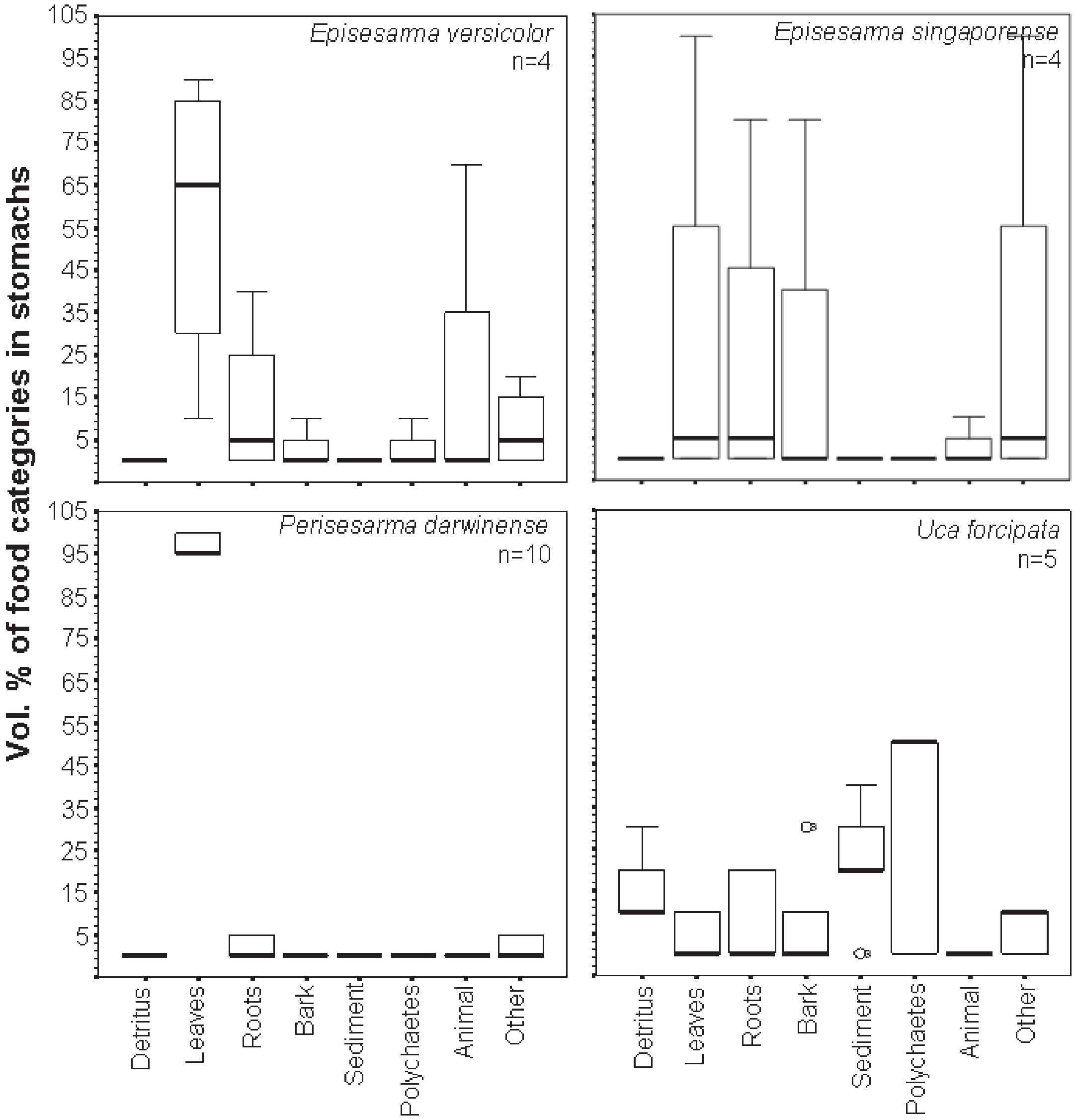

Figure 6.3: Volume % of food categories detritus, leaves, roots, bark, inorganic sediment (surface), polychaetes, unidentified animal material and other for *Episesarma singaporense* (other= unidentified material), *E. versicolor* (other= own or fish eggs), *Perisesarma darwinense* (other= unidentified material) and *Uca forcipata* (other= plastic pieces and wires). Data are plotted as medians (line) with confidence intervals from 25% to 75% (box), standard deviation (error bars) and outliers (circles).

The category "other" in *U. forcipata* stomachs refers to plastic pieces and plastic wires, and for *E. versicolor* to eggs, probably either own or fish eggs. The stomach content analysis of *Periophthalmus* sp. showed a frequency of occurrence of 45% for fish, 40% for polychaetes and 15% for unidentified insects.

4 Discussion

4.1 Primary food sources

Compared to previous studies (e.g. Bouillon et al. 2008) the $\delta^{13}C$ of mangrove leaves and sediments in the Segara Anakan Lagoon (SAL) are very low ($\delta^{13}C$ average: -28.6±0.8‰ and -26.5±0.4‰ respectively). McKee et al. (2002) suggested that $\delta^{13}C$ in mangrove leaves can be decreased by decreasing stomatal conductance and intercellular CO_2 by elevated or fluctuating salinities. In the SAL, fluctuating salinities are present depending on the station and the tidal cycle (Holtermann et al. 2009), and could therefore be an explanation for the low $\delta^{13}C$ in mangrove leaves in this ecosystem. Recent studies discussed whether mangrove leaves can be the primary carbon source for a benthic community when ^{13}C is strongly depleted in leaves compared to their assumed consumers (Imgraben & Dittmann 2009, Mazumder & Saintilan 2009). Nevertheless intertidal mangrove crabs in the SAL feed on leaves as shown by isotopic analysis of stomach contents in this study and previous feeding experiments (Nordhaus et al., submitted).

Total suspended matter (TSM) in lagoon water near the examined stations had mean $\delta^{13}C$ values of -27.3±1.0‰ for the central and -27.1±1.6‰ for the eastern area of the lagoon (Moll, unpublished data). The throughout similar values of TSM and sediment can have two explanations: First, that sedimentary organic matter originates from microalgae in the water column as also suggested by Bouillon et al. (2002a, 2003) or second, that organic matter in the water column originates from sediment through resuspension, as shown by Wainright & Hopkinson (1997). But also mangrove detritus or terrestrial sediments washed in from the hinterland during rainy season could contribute organic matter to the sediments (Jennerjahn et al. 2009).

We found C/N ratios between 3.5 to 16.7 for invertebrates and 14.4, 79.1 and 8.4 on average for sediment, leaves (Table 6.4) and TSM (Moll, unpublished data). Bouillon et al. (2002b) suggested that a C/N ratio in animal tissues >12 indicates a large contribution of terrestrial carbon whereas a low C/N ratio combined with a high $\delta^{13}C$ indicates that mangrove derived matter is not the principal food source. In such cases imported carbon from the creeks, such as microalgae, contributed to the sediment organic pool (Bouillon et al. 2002b). In the Segara Anakan lagoon both the inflow of the Indian Ocean as well as the mangroves and in situ primary production therein (Jennerjahn et al. 2009) are important nutrient and primary carbon sources for the macrobenthic community.

Table 6.4: C/N ratios of animal muscle tissue, leaves and sediment at four stations, showing -with a C_{org}/N >12- terrestrial (bold numbers), and -with a low C_{org}/N and a high $\delta^{13}C$- marine carbon sources (*italic* numbers) (based on findings of Bouillon et al. 2002a, 2003).

	C42	C49	E40	E53
Leaves and sediment				
Avicennia alba brown	**23.2**	**29.3**	**31.3**	
Avicennia alba yellow	**22.6**		**33.4**	
Aegiceras corniculatum brown	**29.5**		**105.2**	**262.0**
Aegiceras corniculatum yellow			**103.8**	**161.4**
Rizophora apiculata brown	**155.5**	**96.5**	**83.0**	
Rhizophora apiculata yellow	**138.0**	**90.7**		
Sonneratia alba brown	**68.8**	**57.0**	**127.0**	**160.0**
Sonneratia alba yellow	**69.0**	**57.0**	**127.0**	**65.4**
Sediment	**15.1**	**12.2**	**15.6**	**14.5**
Invertebrates				
Epixanthus dentatus			4.7	
Scylla serrata	9.1		10.1	
Episesarma versicolor	3.5	*3.6*	5.1	
Episesarma singaporense	4.4	7.0	6.0	4.1
Metaplax elegans			*4.2*	*3.6*
Uca forcipata	*3.6*	*3.9*	*3.8*	
Perisesarma darwinense	3.7	*3.7*		3.7
Telescopium telescopium		7.8	7.2	7.1
Cerithidea cingulata		9.7	**13.6**	5.6
Saccostrea cf. *cucculata*			5.9	4.6
Polymesoda erosa		4.5		
Polychaetes		**16.7**		4.9
Periophthalmus sp.	4.6	4.4	4.1	4.4
Phascolosoma arcuatum				3.6

4.2 Benthic food web

4.2.1 Invertebrates feeding strategy

Epixanthus dentatus was only found at station E40. The low $\delta^{13}C$ of the stomach contents of *E. dentatus* indicate a partly mangrove derived diet (Table 6.3). Previous studies have shown that apart from mangrove leaves, this species also feeds on crustaceans and molluscs in a mangrove ecosystem in Kenya (Dahdouh-Guebas et al. 1999). As it occurred within the highest trophic level (TL) in this study, we conclude that it is an omnivorous species, feeding on a heterogeneous diet, most probably including leaves, gastropods and small decapod species.

Scylla serrata had similar $\delta^{13}C$ values in the eastern and central lagoon. $\delta^{15}N$ were much higher in the eastern part. This can probably be explained by the fact that in the eastern lagoon only females were caught, and in the central part 80% of analyzed individuals were males. Analyses of muscle tissue of males and females showed a significant difference in $\delta^{15}N$ (female: 13.7±0.0‰, male: 9.3±0.7‰; unpublished data). Previous studies showed that females move to offshore areas for spawning (Hill 1994). This might explain why in the eastern part, where the seabed shows steep incline within a short distance (Holtermann et al. 2009), only females were found. *S. serrata* occurred in the highest TLs throughout. In the central lagoon *S. serrata* was found in the second TL, which is probably a result of a more carnivorous diet in the central lagoon, compared to an omnivorous diet in the eastern part. Females seem to be more selective in food choice, probably due to their high nitrogen needs in times of spawning.

In contrast, the genus *Episesarma* mainly feeds on leaves and roots. Stomach content analysis showed that both species had a certain contribution of animal material in their stomachs at station C49. In case of *E. versicolor* stomachs also contained polychaetes. The main food category found in the stomachs was leaves. $\delta^{13}C$ was likewise low for both species, indicating mangrove leaves to be the main carbon source. This was also found for *E. versicolor* in a mangrove forest in Thailand (Thongtham & Kristensen 2005, Thongtham et al. 2008). However, *E. versicolor* occurred in different TLs depending on the station and showed significantly higher $\delta^{13}C$ values at C49 (M-W-U: $p<0.05$). This opportunistic feeding habit was also observed in laboratory experiments (unpublished data), in which *E. versicolor* survived three months on an exclusively *Rhizophora apiculata* leave diet. In other consumption rate experiments we recorded that *E. versicolor* consumed preferably leaves of *Derris trifoliata* apart from *R. apiculata*. Brown *D. trifoliata* leaves had higher $\delta^{13}C$ values (-27.0±0.2‰) than mangrove species (ANOVA: $p<0.01$, Tukey´s HSD: $p<0.01$), except *Avicennia alba* (Tukey´s HSD: $p>0.05$; unpublished data). In the central area of the SAL the shrub species *Acanthus ilicifolius* and *D. trifoliata* colonize deforested mangrove sites. Nevertheless, the $\delta^{15}N$ of *E. versicolor* are >6‰ enriched in ^{15}N compared to the highest $\delta^{15}N$ of occurring leaves, indicating a supplementing ^{15}N enriched nitrogen source. Previous studies found that *E. versicolor* can supplement its mangrove leaf diet with animal tissue (Thongtham & Kristensen 2005, Kristensen et al. 2010). An exception in this study is station C42 where *E. versicolor* is found in the first TL (4‰ enrichment of δ^{15}N compared to leaves). In contrast to the other stations there is a dense and diverse mangrove tree cover with only a few shrubs at C42 (Hinrichs et al. 2009). A mainly mangrove derived diet could explain the low TL. Nordhaus et al. (submitted) found that up to 67% of stomach contents of *E. versicolor* in the SAL were detritus (including leave pieces), followed by cortex (19%) and leaves (2%). Due to its moderate δ^{13}C and a high contribution of detritus to the stomach content (at station C49), a heterogeneous diet, including a large mangrove leaf contribution, is suggested, which agrees with previous findings (Nordhaus et al. submitted).

E. singaporense was consistently found in the second TL, and variation in δ^{15}N of stomach contents over all stations were significantly lower than for *E. versicolor* (Table 6.2), suggesting a consistent diet baseline. Different species of the same genus can therefore adapt differently to spatial differences in food availability.

Uca forcipata and *Metaplax elegans* were found at the first TL throughout. Stomach contents of *U. forcipata* at station C49 had a very high variability, as sample size was small. Apart from a large amount of polychaetes, mainly plant components were found. Plastic pieces and sediment in the stomachs indicate a detritivorous diet. These plastic pieces probably mainly originate from the two villages Klaces and Motehan, which account for a large amount of waste in the mangroves especially in the central lagoon (personal observation). It was stated in earlier studies that *Uca* spp. feeds on bacteria and microalgae (Rodelli et al. 1984, Guest et al. 2004). In this study such carbon sources were indicated by high values of δ^{13}C (-18.7±1.1‰). Detritus derived from mangrove leaves are not the main carbon source for *U. forcipata*. In this study muscle tissue of *M. elegans* had low δ^{15}N, indicating a mainly herbivorous feeding habit. In the stomachs of *M. elegans* a high contribution of detritus was found by Salewski (2007) in the SAL, with only a small amount of bark and sediment. We therefore conclude that this species is mainly feeding on detritus.

The muscle tissue of *P. darwinense* had moderate $\delta^{13}C$ values (average -22.3±2.1‰) in the first TL. Stomachs of the animals collected at station C49 indicate a mainly mangrove leave derived diet, which agrees with findings of previous studies (Skov & Hartnoll 2002, Poon et al. 2010). *P. darwinense*, a species occurring in high densities in the SAL, is also an ecologically important species due to its leaf litter processing, as previously shown in mangroves of Kenya (Gillikin & Schubart 2004). An exception in our study is station C49, where tissue of *P. darwinense* had comparatively high $\delta^{13}C$, which indicates a diet enriched by microalgae. These might originate from the western outlet of the lagoon, which allows a regular inflow of marine water masses from the Indian Ocean at high tide. Nutrients and detritus enriched sediments washed in from the Citanduy River, providing a large input of freshwater into the central lagoon especially during rainy season (Holtermann et al. 2009) could also contribute to the high $\delta^{13}C$.

The gastropod *Telescopium telescopium* had unexpected high $\delta^{15}N$ values at C49 and E40, as it was previously reported to be a surface grazing gastropod ranging in the lower TLs (Houbrick 1991, Bouillon et al. 2002b). Our results indicate that *T. telescopium* feeds on a highly ^{15}N-enriched diet at these stations. Apart from microalgae, our data suggests that this species is feeding on carrion of fish or invertebrates, making this species an opportunistic feeding, facultative scavenger. Comparing the $\delta^{15}N$ of *T. telescopium* ($\delta^{15}N$ =17.6±3.8‰ at station E40) and fish in this study (average $\delta^{15}N$ =16.0±0.1‰), it can be concluded that -amongst other fish species- the carrion of *Epinephelus* probably serves as a supplementing diet for *T. telescopium*. The remarkable higher $\delta^{13}C$ at station C49 suggest a high contribution of microalgae to the diet, originating from the nearby Indian Ocean inflow as well as from the Citanduy River.

Very low $\delta^{13}C$ values in *Polymesoda erosa* and *Saccostrea* cf. *cucculata* muscle tissues and in case of *P. erosa* the occurrence in the moderate TL (=2.5) indicate a diet consisting mainly of suspended matter of the water column, including terrestrial carbon, e.g. originating from mangrove leaves, which agrees with findings of previous studies (Rodelli et al. 1984, Bayne 2002). The assimilation of organic material washed in from the hinterland during rainy season, originating in agriculture and depleted soils (Jennerjahn et al. 2009) can most probably explain the occurrence of *S.* cf. *cucculata* in the third TL (=3) at station E40.

The snail *Cerithidea cingulata* is clearly feeding on a ^{13}C enriched diet. Being found at the first TL it is most probably feeding on microbenthic organisms such as algae, therefore being an obligate deposit feeder. Other studies found average $\delta^{13}C$ for benthic microalgae between -17.3‰ (Bouillon et al. 2002b) and -19.3‰ (Hsieh et al. 2002), which are similar to those of *C. cingulata* in SAL ($\delta^{13}C$ =-17.7‰ at C49 and -15.6‰ at E40).

The peanut worm *Phascolosoma arcuatum* is occurring mainly in the eastern lagoon, where a more sandy sediment texture is apparent. At E53 it is located between the first and second TL (=1.5), and with a moderate $\delta^{13}C$ it is possibly only partly dependent on mangrove carbon sources, but also feeds on bacteria and microalgae. The moderate $\delta^{15}N$ suggests a partly omnivorous diet, including e.g. polychaetes. This agrees with findings of Rodelli et al. (1984) in a Malaysian mangrove ecosystem.

Polychaetes in this study were pooled for analysis, as their dry weight was very low. Detrivorous, omnivorous and carnivorous species were therefore not separately measured. Hence polychaetes occur in different TLs, as the contribution of different species was not equal at all stations. With our data we cannot clearly define the feeding habits of polychaetes, but it became clear that they serve as a carbon and nitrogen source for miscellaneous intertidal benthic organisms. This systematic group had high $\delta^{13}C$ at station C49, indicating a largely microalgae dependent carbon source. At station E53 a mainly detritivorous diet is suggested by very low $\delta^{13}C$, which were close to those of the sediment $\delta^{13}C$. But their occurrence in a higher trophic level at this station implies a supplemented ^{15}N enriched diet.

4.2.2 Feeding habits of fish

$\delta^{15}N$ of the mud skipper *Periophthalmus* sp. were equally high throughout all stations, which could be explained by a homogeneous and carnivorous diet. Stomach contents showed that this is an omnivorous species feeding mainly on polychaetes and fish. These results agree with earlier studies showing that the mud skipper *Periophthalmus sobrinus* is an active carnivorous predator (Blaunstein et al. 1996). An exception at SAL is station C42, where a high variability in $\delta^{13}C$ indicates a heterogenous diet. The mud skippers diet changes during growth, but they never appear to be in competition with other zoobenthivores, because of their access to abundant food sources, such as other fish, which are inaccessible for crab species (Kruitwagen et al. 2007).

Scatophagus argus occurs on the second TL (=2) with moderate $\delta^{13}C$ (-23.9±1.5‰), suggesting a mainly detritivorous diet supplemented by ^{15}N enriched nitrogen sources. Previous studies showed that *S. argus* is feeding amongst others on phytoplankton, filamentous algae, detritus, bivalves, crustaceans, protozoa and copepods (Ghandi 2002, Thimdee et al. 2004, Lin et al. 2007). In the SAL this species is the basis of carbon source for higher TLs, and as carrion serves as an additional nitrogen source, e.g. for crabs of higher TLs.

The genus *Epinephelus* occurred in the fourth TL (=4) of the benthic food web, indicating that these species feed on invertebrates in the SAL. It was shown in previous studies, that *E. coioides* in Australia is mainly feeding on leaf eating crabs, including *Neosesarma* sp. and *Perisesarma* spp. (Sheaves & Molony 2000). As *Epinephelus* occur in the same TL as *Scylla serrata* and *Epixanthus dentatus*, it can be assumed that this genus has an omnivorous feeding habit in the SAL also.

4.3 Affiliation to trophic levels

Our results showed that single species occurred at different TLs depending on the location (Fig. 6.4), and thus a site-specific food web structure exists.

Significant differences were found while comparing single species between stations. Also comparing the whole benthic community between stations showed significant differences (t-tests, $p<0.05$). Especially decapod species can be assumed to use a range of food sources. Previous studies suggested that some animals have the capacity to change their feeding habits according to the availability of food at different locations, and as a result individuals of the same species can occur in different trophic levels (Thimdee et al. 2004). Due to the opportunistic behavior we conclude that most macrobenthic organisms are highly adaptable to changing food availability. Our spatial variability of $\delta^{13}C$ of invertebrate muscle tissues is high due to station dependent food sources. This is in agreement with small-scale investigations showing that $\delta^{13}C$ of muscle tissue is very location dependent (Guest et al. 2004, 2006, Benstead et al. 2006). The variability within the same species at one station is in general higher in higher TLs, which can be explained by an increase in food choices with increasing trophic levels.

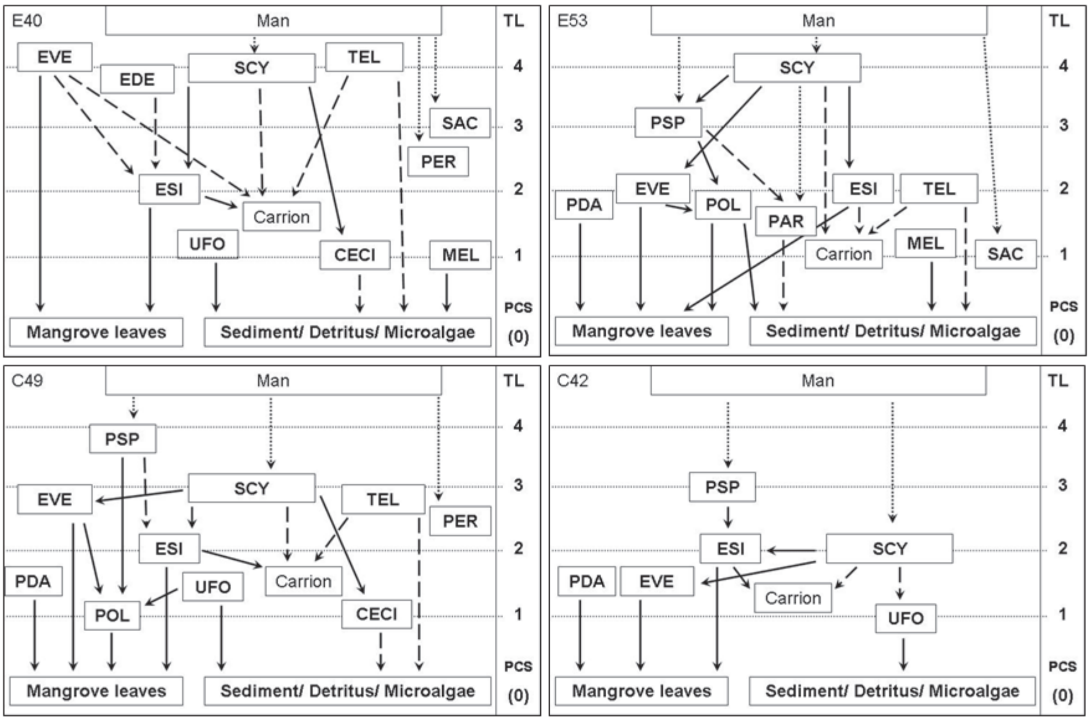

Figure 6.4: Trophic food web structure in the Segara Anakan lagoon based on abundant species (*Cerithidea cingulata* (CECI), *Episesarma singaporense* (ESI), *Episesarma versicolor* (EVE), *Epixanthus dentatus* (EDE), *Metaplax elegans* (MEL), *Periophthalmus* sp. (PSP), *Perisesarma darwinense* (PDA), *Phascolosoma arcuatum* (PAR), Polychaetes (POL), *Polymesoda erosa* (PER), *Saccostrea* cf. *cucculata* (SAC), *Scylla serrata* (SCY), *Telescopium telescopium* (TEL), *Uca forcipata* (UFO)); TL= trophic level: 4= top level, 3= high, 2= moderate, 1= low, PCS (0)= primary carbon sources; continuous arrows: knowledge gained by isotopic signatures of carbon and nitrogen and stomach content analysis of benthic organisms in this study, dashed arrows: assumed connections based on isotopic signatures of carbon and nitrogen of benthic organisms in this study, dotted arrows: intervention of humans on the benthic food web (personal observation).

4.4 Anthropogenic impact

Previous studies showed that sewage input affects isotopic composition in benthic organisms (Rau et al. 1981; Gearing et al. 1991; Tucker et al. 1999), mangrove leaves, macro algae and phytoplankton (Costanzo et al. 2001, 2003; Gartner et al. 2002). $\delta^{15}N$ is thereafter lower whereas $\delta^{13}C$ is higher in sewage polluted areas. It was therefore expected to find higher $\delta^{13}C$ and lower $\delta^{15}N$ at station C49, located between the two villages, and E53, directly opposite the city Cilacap. At E49 the expected values were measured, in comparison we found the highest $\delta^{13}C$ and the lowest $\delta^{15}N$ at this station, suggesting an accumulation of household sewages in invertebrate tissues at this station. Isotopic compositions at station E53, in contrast, do not meet our expectations, as in comparison to the other stations values are moderate. The opposite, with low $\delta^{13}C$ and high $\delta^{15}N$, was found at station E40 indicating a possible pollution with PAH´s due to the proximity to the oil refinery in the city of Cilacap. It was already shown in previous studies that PAH´s and therefore PAH polluted areas hold sediments with extreme low $\delta^{13}C$ (Jackson et al. 1996; Lichtfouse et al. 1997; McRae et al. 2000). Dsikowitzky et al. (submitted) found a higher number of PAHs in invertebrate tissues and sediments in the eastern lagoon compared to the central, which supports our findings.

It cannot be ruled out that the impact of PAH and sewage pollution of the City Cilacap on the carbon isotopic signatures in invertebrates neutralize each other especially at station E53. But clearly the number and concentration of organic pollutants in sediments and animal tissues was much higher in the eastern area compared to the central part (Dsikowitzky et al. submitted).

4.5 Summary and Conclusions

Within the overall four trophic levels (TL) found in the intertidal benthic food web of the Segara Anakan Lagoon, excluding the primary carbon sources, most species, especially several decapods, can be assumed to use a range of food sources opportunistically. These species supplement their mainly mangrove derived diet by feasible scavenging e.g. decapod crabs in form of carrion. Single species occurred in different TLs depending on the location, as $\delta^{13}C$ and $\delta^{15}N$ of their tissues had a high spatial variability already on a small scale. Different species from the genus *Episesarma* can adapt differently to changing food availability.

Due to the opportunistic diet of several decapod species we conclude that they are highly adaptable to changing food availability. The conversion of mangroves into other land uses, e.g. for agriculture, will reduce the habitat of the intertidal macrobenthic community within a short time, lead to more uniform sites and less microhabitats. This will most probably lead to a decrease of mangrove tree species and therefore a decrease of food heterogeneity for the inhabitants of the lagoon.

Overfishing could have an effect on the communities in the SAL, as fish serves as a supplementing diet in form of carrion for the invertebrates' nitrogen needs. Anthropogenic impacts reflected as sewage inputs and PAH pollution might have an influence on isotopic fractionation in invertebrate tissues in the eastern lagoon. A better resolution of study sites for PAH measurements and further analysis of sewage impacts on sediments are necessary to be able to make a reliable statement on the actual anthropogenic impact in the SAL.

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7 Seasonal variations of $\delta^{13}C$ and $\delta^{15}N$ in mangrove benthic organisms in the Segara Anakan Lagoon, Java, Indonesia

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Abstract

Food web studies conducted at a certain time of the year only reflect the actual status, and do not cover seasonal variability. We aimed to examine the isotopic compositions in benthic invertebrates, reflecting seasonal dependent environmental conditions in the Segara Anakan Lagoon (SAL). Eight abundant intertidal species were collected at ten study sites in the SAL during rainy and dry season. Also, leaves of four mangrove tree species and sediment were collected to examine seasonal differences in food sources. Muscle tissues and food sources were analyzed for total carbon and nitrogen content as well as $\delta^{13}C$ and $\delta^{15}N$. Seasonal discrepancies of $\delta^{13}C$ and $\delta^{15}N$ were dependent on species and location in the SAL and did not show clear patterns. Spatial variability was much higher during rainy season, probably due to an increased nutrient discharge by the Citanduy river. Benthic invertebrates are highly adaptable to temporal and spatial changes in food availability and quality. Differences in food web structures with different numbers of trophic levels can be assumed for the two seasons. $\delta^{15}N$ were highest in the SAL for *Saccostrea* cf. *cuccullata* and *Telescopium telescopium* on a global scale. In case of *S.* cf. *cucculata*, this is probably resulting from highly ^{15}N enriched suspended matter in the water originating from agricultural effluents. *T. telescopium* seems to feed on ^{15}N enriched food sources, such as carrion. Isotopic compositions of the other species were found in a similar range in the SAL and other mangrove forests.

Key words: benthic intertidal communities, food availability, mangroves, seasonality, stable isotope analysis

1 Introduction

Stable isotopes can be used as tracers to examine food web structures, which has already been demonstrated in a variety of ecosystems (e.g. Peterson & Fry 1987; Hobson 1999; Post 2002). The method is based on the assumption that consumers have a $\delta^{13}C$ enrichment of about 0.8‰ relative to their diet, and two trophic levels are distinguished by a $\delta^{15}N$ discrepancy of about 2.8‰ (Caut et al. 2009). It became clear that spatial variability in the structure of food webs in mangroves and salt marshes is high already on a small scale (Guest et al. 2004, 2006; Herbon et al. submitted). This can result from different impacts, e.g. anthropogenic or environmental (McClelland & Valiela 1998). A food web gathered at a certain time in the year does not reflect the food-web structure during any other time of the year or the whole year (Thompson & Townsend 1999). In fact, study site and season do have a significant effect on species richness, number of food web links, main chain length, predator-prey ratios or invertebrate diets in tropical food webs (Whitlatch 1977; Alongi 1987; Winemiller & Jepsen 1998; Thompson & Townsend 1999; Poon et al. 2010).

The aspect of seasonality is a very important factor in tropical ecosystems, as in most regions two very distinct seasons are apparent. Seasons in the tropics are predominantly defined by the monsoons. Environmental parameters, such as salinity or nutrient availability in water and sediments differ substantially between seasons (Cowan & Boynton 1996; Francoeur et al. 1999; Badran 2001; Holtermann et al. 2009; Eyre & Fergusen 2005). Lower average air and water temperatures, lower conductivity and solar radiation and increasing water depths prevail during rainy season (Camargo & Esteves 1995; Graham et al. 2003). During dry season a very hot and dry climate is predominant, along with high evaporation rates.

Macrobenthic intertidal organisms show different compositions of naturally occurring stable carbon and nitrogen isotopes, depending on the feeding habits and therefore the trophic level (Minagawa & Wada 1984; Post 2002; Herbon et al. submitted). Macrobenthic food webs in mangrove ecosystems were investigated in several countries, such as Kenya and Thailand (e.g. Bouillon et al. 2002a, b; Thimdee et al. 2004; Alongi 2009). However, comparisons regarding seasonality within mangrove ecosystems especially regarding isotopic compositions in food webs are rare (e.g. Michener & Schell 1994; Barletta et al. 2003). In this study, seasonal variations in carbon and nitrogen isotopic compositions of abundant benthic invertebrates were investigated during rainy and dry season in the mangrove fringed Segara Anakan Lagoon (SAL; Fig. 1).

The 1002ha (in 2006; Ardli & Wolff 2009) large brackish water lagoon on the south central coast of Java is located in one of the hotspots of biodiversity (Roberts et al. 2002) and is surrounded by 9238ha of mangrove forest. It is neighbouring the city Cilacap with the largest oil refinery of Indonesia. A high anthropogenic influence on the mangrove ecosystem is assumed, as urban and industrial wastes of the city and several villages are released into the lagoons water. The lagoon, its mangroves and tidal flats play an important role as a nursery ground for the macrobenthic communities and for pelagic fish (Nordhaus et al. 2009, Yuniar et al. 2007). So far, 186 macrobenthic species were described in the lagoon and its fringing mangroves (Nordhaus et al. 2009), including economically important invertebrates, in particular *Polymesoda erosa and Saccostrea* cf. *cuccullata*.

Herbon et al. (submitted) found a high spatial variability in isotopic carbon and nitrogen compositions in muscle tissues of intertidal benthic communities in the SAL during rainy season 2008 on a small scale. In the present study, feeding habits of eight selected abundant species were compared spatially in a more elaborate set of sampling sites within the SAL and between seasons. This is the first study to investigate seasonal induced shifts of trophic levels for mangrove invertebrates in Indonesia with regard to feeding habits.

Our hypothesis is that isotopic signatures in muscle tissue of the proposed species differ substantially between seasons. It is expected that seasonal induced changes in nutrient and food availability have an impact on trophic shifts.

The aims of this study were (1) to examine seasonal variations in the appearance in trophic levels of eight abundant intertidal macrobenthic species by using the stable isotope method and (2) to investigate spatial variability in the SAL and to additionally compare our data globally with other mangrove forests.

2 Material and Methods

2.1 Study site

The Segara Anakan Lagoon (SAL), Java, Indonesia (108°50’- 109°00’E, 07°39’- 07°43’S) is separated from the Indian Ocean by the rocky mountainous island Nusa Kambangan (Fig. 7.1).

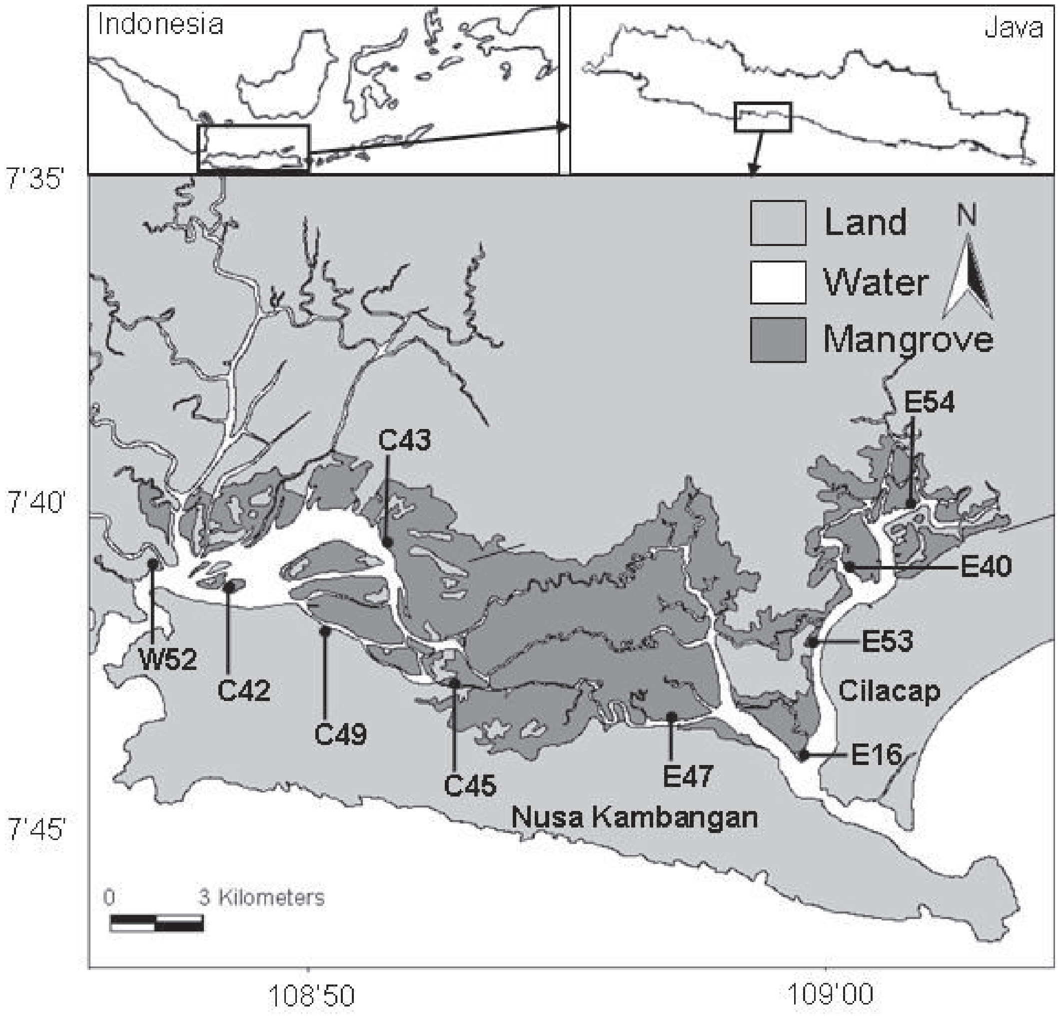

Figure 7.1: Map of Segara Anakan with ten sampling stations in west (W), central (C) and eastern (E) lagoon and the city Cilacap, modified from Ardli (unpublished), http://istgeography.wikispaces.com and http://www.gamelannetwork.co.uk/assets/pics/Java_map.gif.

An exchange with the Indian Ocean water masses is provided by two entrances to the lagoon in the east and in the west. The hydrology of the SAL is governed by semidiurnal tides, 0.4m at neap tide and 1.4m at spring tide (Holtermann et al. 2009). The Citanduy River, the fifth largest river of Java, and the Cibereum River in the west are the main freshwater sources. Salinity is higher in the eastern part than in the western part of the lagoon during rainy (east: 27, west: 10) and during dry season (east: 32, west: 21; Holtermann et al. 2009). Precipitation is 152mm/ month during rainy and 39mm/ month during dry season (http://climate.usurf.usu.edu). In rainy season the sediment load through the Citanduy River is increased (Holtermann et al. 2009).

The SAL has a high commercial and ecological value due to its high diversity of intertidal macrobenthic and fish species (Yuwono et al. 2007). By agriculture (rice fields), aquaculture, villages within the lagoon and the city of Cilacap (240.325 inhabitants in 2008; www.cilacapkab.go.id) with several industries, the lagoon is strongly influenced by human activities. An over-exploitation of the natural resources is caused e.g. by overfishing and deforestation (Ardli 2007). The latter, conducted in the hinterland, leads to a reduction of the water volume of the lagoon by fostering sedimentation through the rivers (Ardli 2007).

2.2 Sample collection and preparation

Eight intertidal abundant benthic species (Decapoda: *Episesarma singaporense*, *Metaplax elegans, Perisesarma darwinense*, *Uca forcipata*; Gastropoda: *Cerithidea cingulata*, *Telescopium telescopium*; Bivalvia: *Saccostrea* cf. *cuccullata*; Teleostei: *Periophthalmus* sp.) were collected by hand at ten intertidal mangrove stations (Fig. 7.1) during rainy (February-April 2008) and during dry season (August-October 2008).

Five individuals of each species were collected at each station and frozen for at least 12 h in PVC sampling bottles before further treatment. Crabs were dissected and muscle tissue from the chelae was removed for analysis. The shells of *C. cingulata, Saccostrea* cf. *cuccullata* and *T. telescopium* were removed and the whole soft tissue was used for analyses. *Periophthalmus* sp. was used completely for analysis. Samples were homogenized by hand with an agate mortal after drying at 40°C.

No significant differences were found between tissue samples treated with HCl and samples without HCl during preliminary analysis (t-test: F=1.11, p=0.89), therefore no HCl was applied for tissue samples.

Samples were combusted in the Carlo Erba NA 2100 Elemental analyzer for carbon and nitrogen content measurements. Separately stable isotope ratios were determined with a coupled EA-IRMS gas isotope ratio mass spectrometer (ConFlo III) and expressed relative to conventional standards $\delta R = [(X_{sample}/X_{standard}) - 1] \times 1000$ ‰ with R = ^{13}C or ^{15}N and X = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Ammonium sulfate (IAEA-N1, IAEA-N2) were used as standard for $\delta^{15}N$, and graphite (USGS-24) and mineral oil (NBS-22) for $\delta^{13}C$. Analytical precision was ±0.2‰ for both nitrogen and carbon, as estimated from standards analyzed together with the samples.

Of four mangrove tree species (*Aegiceras corniculatum*, *Avicennia alba, Rhizophora apiculata* and *Sonneratia alba*) three brown leaves each (replicates) were sampled at four stations (C42, C49, E53, E40), as preliminary results showed that these stations cover the whole range of $\delta^{15}N$ and $\delta^{13}C$ values. Leaves were collected by hand from the sediment surface. Surface sediment was sampled with a distance of 20m from the water edge for analyses, including three replicates at each of the four stations. Sediment samples were dried at 40°C, treated with 200µl 1M HCl to remove carbonates and then redried. Subsamples were analysed for organic carbon (C_{org}) and nitrogen (N) content and also stable isotope composition of organic carbon ($^{13}C_{org}/^{12}C_{org}$) and nitrogen ($^{15}N/^{14}N$).

2.3 Trophic level determination

To distinguish between trophic levels, we used the fractionation steps from Caut et al. (2009) as these are based on the largest dataset available, including 66 isotope studies with a large number of estimated animal diets (n= 290). An overall discrepancy of $\Delta\delta^{15}N=2.8\pm0.1$‰ and $\Delta\delta^{13}C=0.8\pm0.1$‰ was determined between prey and consumer. Based on this we determined the discrepancies of trophic levels between seasons. Trophic levels and food web structures were discussed in Herbon et al. (submitted) and are therefore not presented in this paper.

2.4 Statistical analysis

Data were tested for normality, not normal distributed data were analyzed non-parametrically, as transformation did not lead to normal distribution. Homogeneity of variances was tested. To avoid a high error by a high number of significance tests regarding seasonality of each invertebrate at each station, a general linear model was run with “station” as the random factor, as differences between stations are known.

For leaves and sediment an ANOVA followed by a Tukey´s HSD Post-hoc analysis was used. All analyses were run separately for $\delta^{13}C$ and $\delta^{15}N$ with Statistica®.

3 Results

3.1 Mangrove leaves and sediment

$\delta^{13}C$ were, due to their high variability within replicates, very similar between seasons for all species (Tukey´s HSD: $p>0.05$). A significant change between seasons was only found for *Sonneratia alba* at station C49, increasing in dry season (Tukey´s HSD: $p<0.05$).

Regarding nitrogen, the examined mangrove species showed a significant increase (Tukey´s HSD: $p<0.05$) of $\delta^{15}N$ in dry season with exception of *Avicennia alba* in C42 (Table 7.1).

Table 7.1: Carbon and nitrogen isotopic compositions ($\delta^{13}C$, $\delta^{15}N$) and C_{org}/N ratios of brown mangrove leaves (AA: *Avicennia alba*, AC: *Aegiceras corniculatum*, SA: *Sonneratia alba*, RA: *Rhizophora alba*) and surface sediment (SED) for four stations (central: C42, C49, east: E40, E53) in rainy and dry season. Bold values indicate significantly higher values comparing seasons.

		Rainy season				**Dry season**			
		C42	C49	E40	E53	C42	C49	E40	E53
$\delta^{13}C$	AA	-27.7±0.5	-27.9±1.5			-26.3±0.0			-28.3±0.5
	AC	-30.4±0.0		-28.7±1.3	-29.3±0.8	-30.9±0.9		-29.6±0.7	-29.5±0.6
	RA	-28.3±1.4	-27.8±0.7	-28.7±1.3		-27.5±1.3	-28.4±1.4	-29.1±0.2	-27.8±0.6
	SA	-29.1±0.4	-29.0±1.0	-29,4±1.5	-29.1±0.0		**-26.6±0.7**	-28.9±0.5	-28.9±0.1
	SED	-26.0±0.5	-26.2±1.0	-26.9±0.9	-26.8±0.2	-26.0±0.4	-26.1±0.1	-27.4±0.3	-27.0±0.7
$\delta^{15}N$	AA	**3.9±2.9**	3.5±0.2			-1.1±0.0			3.8±0.7
	AC	-4.8±1.0		0.7±0.4	0.1±0.6	**1.1±1.3**		**4.4±0.5**	**3.8±3.0**
	RA	1.7±0.1	2.9±0.8	-2.4±1.7		**4.8±2.6**	**6.2±0.7**	**4.2±0.9**	4.4±0.5
	SA	-0.9±0.5	-4.4±2.1	-1.4±1.8	0.9±0.0		**2.1±1.6**	**3.2±0.9**	**3.1±0.9**
	SED	4.3±0.4	4.0±0.4	4.1±0.5	4.3±0.2	4.2±1.0	4.4±0.2	4.3±1.3	3.9±0.2
C_{org}/N	AA	23.2±1.4	29.3±4.5	31.3±1.6		35.6			59.2±6.4
	AC	29.5±0.0		28.0±0.0	262.0±0.5	70.0±20.3		108.0	53.4±16.8
	RA	155.5±27.8	96.5±15.8	83.0±0.0		50.0±0.6	108.1±15.1	103.2	96.6±1.7
	SA	68.8±9.7	57.0±9.6	127.0±0.9	160.0±0.0	65.8	49.9±1.0	20.1±2.7	83.7±15.9
	SED	15.1±1.1	12.2±1.3	15.6±0.8	14.5±2.2	10.7±0.4	10.1±0.2	14.7±1.7	16.5±2.2

Table 7.2: Isotopic carbon and nitrogen signatures [‰] of eight intertidal benthic organisms (*Cerithidea cingulata* (CECI), *Episesarma singaporense* (ESI), *Metaplax elegans* (MEL), *Periophthalmus* sp. (PSP), *Perisesarma darwinense* (PDA), *Saccostrea* cf. *cuccullata* (SAC), *Telescopium telescopium* (TEL), *Uca forcipata* (UFO)) at ten stations in the lagoon (5 in west/central and 5 in east) in rainy and dry season, bold values indicate significantly higher signatures comparing seasons ($p < 0.05$).

Rainy season

δ^{13}C	W52	C42	C43	C45	C49	E16	E40	E47	E53	E54
ESI	-23.0±0.5	-24.0±0.6		**-23.9±0.7**	-21.8±1.8		-24.8±0.5	-24.5±2.3	-24.2±1.0	
PDA		-23.8±1.1	-24.4±0.6		**-18.6±1.7**			-24.8±1.6	-24.4±0.4	-23.4±1.6
UFO		-19.8±0.5		-23.2±2.6	-17.1±0.5	-15.2±0.4	-19.2±0.5	-18.7±1.0		
CECI			-25.0±1.5	-22.6±1.1	-17.7±1.3		-15.6±4.5	-24.5±0.8		-21.1±2.0
SAC				-32.7±0.4			-29.0±0.9		-25.9±0.5	-29.1±1.1
TEL		-24.6±0.3	-23.9±1.3		**-17.7±0.6**		-24.9±0.5	-25.8±0.8	-24.5±0.7	-23.2±0.8
PSP		-24.4±1.9	-24.6±1.2	-24.4±0.5	**-17.8±0.5**	-19.1±1.8	-24.5±0.9	-23.3±1.0	-24.7±0.3	-25.1±1.5
MEL				-22.6			-21.3±1.2	-21.3±0.8	-20.5±1.8	-21.0±1.0
δ^{15}N										
ESI	6.9±0.2	10.2±1.3		9.3±1.1	7.6±1.4		11.0±2.3	9.4±1.2	10.4±2.4	
PDA		7.6±0.6	8.1±0.8		6.5±0.6			8.5±0.9	**9.2±1.5**	**12.2±3.0**
UFO		7.2±0.8		6.9±1.0	6.6±0.4	6.6±0.6	8.9±0.7	17.9±4.4		
CECI			6.3±0.6	4.1±0.6	5.1±0.4		**8.3±0.4**	5.4±1.8		6.4±0.4
SAC				5.7±0.2			**14.0±5.0**		7.0±1.1	8.9±2.2
TEL		7.2±0.8	6.1±1.4		9.5±0.9		**17.6±3.8**	17.1±4.2	10.2±2.9	13.2±2.0
PSP		12.7±0.6	10.2±0.9	12.3±0.2	12.5±3.2	10.5±1.4	12.7±0.8	15.0±2.2	**13.8±1.2**	**18.2±3.7**
MEL				6.9			8.1±1.0	**8.3±0.5**	**7.7±0.7**	7.8±0.4

Dry season

δ^{13}C	W52	C42	C43	C45	C49	E16	E40	E47	E53	E54
ESI	-24.5±0.1	-24.6±0.5		-25.3±0.4	-23.1±1.3		-24.3±0.6	-23.4±0.5	-24.3±0.5	
PDA		-23.2±1.0	-23.3±1.9		-24.1±2.3			-22.0±1.3	-23.9±0.2	-24.4±0.2
UFO		**-17.6±0.3**		-21.3±1.5	-17.6±0.3	-14.8±0.8	**-19.1±0.3**	-19.1±0.0		
CECI			-21.8±0.7	**-15.6±1.6**	-18.4±1.2		-17.9±1.7	-25.7±2.7		-18.0±2.9
SAC				**-25.5±0.7**			**-25.6±0.6**		-22.2±1.0	-29.8±0.2
TEL		-22.3±0.8	-24.9±0.7		-21.6±1.4		-25.2±1.4	-25.2±0.5	-23.8±0.8	**-19.4±1.6**
PSP		-22.1±0.7	**-22.5±0.9**	-25.6±1.4	-21.7±1.2	-19.5±1.2	**-21.3±1.0**	-23.8±1.1	**-22.6±1.7**	**-22.0±1.8**
MEL				-21.5±0.5			**-18.5±0.8**	**-18.3±0.8**	**-17.9±0.5**	**-19.0±0.2**
δ^{15}N										
ESI	9.7±0.8	7.8±1.3		**16.6±5.7**	7.8±1.1		8.2±0.5	8.1±1.9	7.8±1.2	
PDA		7.5±0.9	8.5±2.0		7.6±1.1			8.5±1.1	7.1±0.5	9.0±0.6
UFO		7.5±1.7		6.5±0.3	7.5±1.7	6.2±0.5	7.1±1.0	6.5±0.1		
CECI			7.4±3.8	7.0±3.7	**7.1±1.5**		7.5±0.7	4.0±2.6		7.2±0.6
SAC				**12.2±4.7**			4.7±1.0		2.8±0.6	7.2±1.6
TEL		7.9±2.4	**14.9±5.0**		8.4±2.2		4.3±0.4	17.2±3.9	9.4±3.2	16.6±4.0
PSP		12.0±1.3	11.9±1.0	14.6±3.3	11.8±1.6	14.5±2.8	12.4±1.2	11.8±2.5	10.0±0.5	11.8±1.7
MEL				9.8±2.9			7.8±0.5	6.5±0.5	6.6±0.4	7.8±0.4

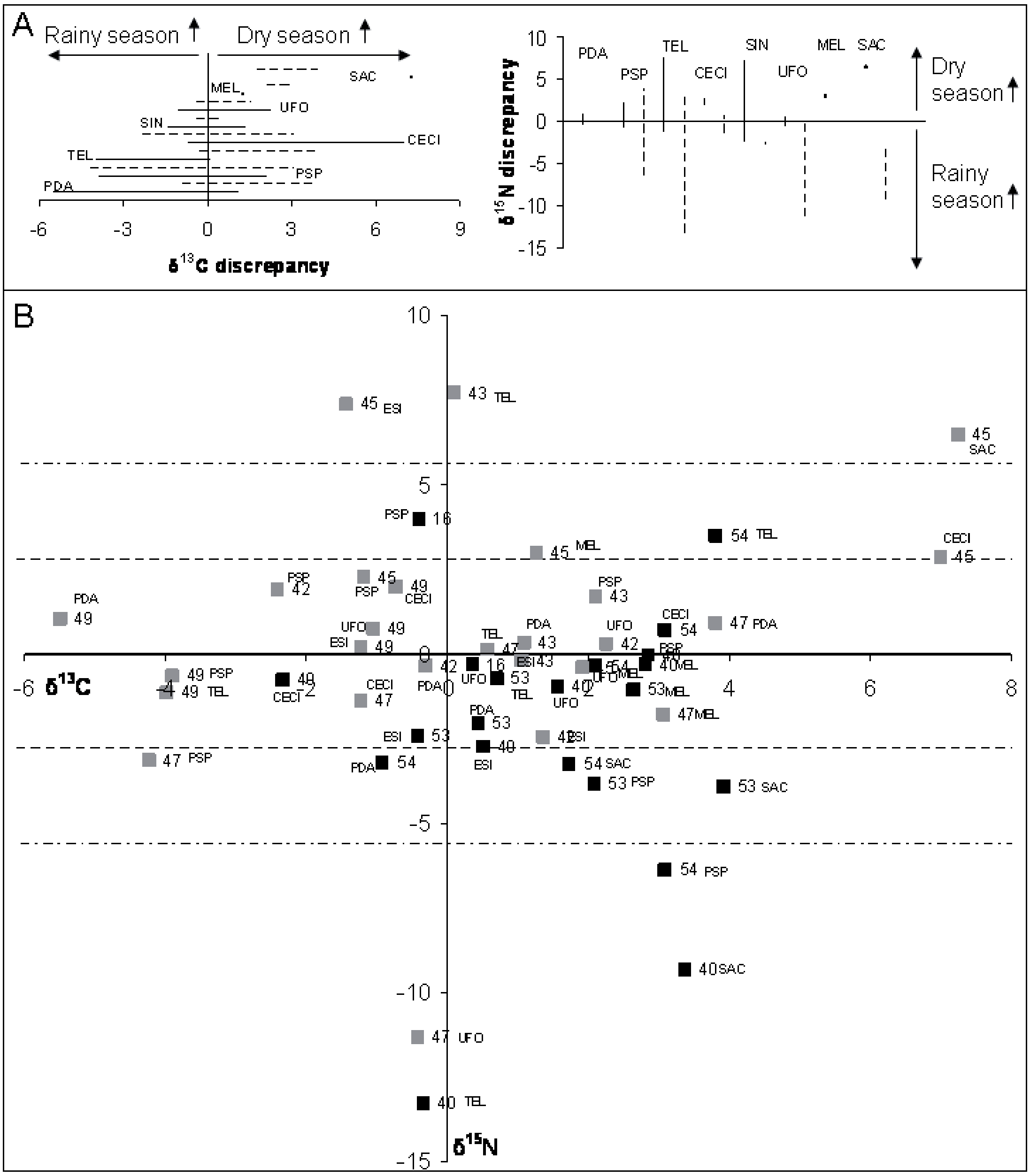

Figure 7.2: (**A**) Discrepancy ranges for differences between seasons for each species for δ^{13}C and δ^{15}N (below zero indicating higher values in rainy season, above zero higher values in dry season). Dashed lines= eastern lagoon, continuous lines= central lagoon. (**B**) Discrepancies between seasons of δ^{13}C and δ^{15}N for eight species (*Episesarma singaporense* (ESI), *Metaplax elegans* (MEL), *Perisesarma darwinense* (PDA), *Uca forcipata* (UFO), *Cerithidea cingulata* (CECI), *Saccostrea* cf. *cuccullata* (SAC), *Telescopium telescopium* (TEL), *Periophthalmus* sp. (PSP)) at ten stations calculated by subtracting δ^{13}C and δ^{15}N values of rainy season from dry season values based on means. Stations: black squares: eastern lagoon, grey squares: central lagoon. Lines: border for discrepancy between seasons up to 1 trophic level (TL) (dashed line = 1 TL difference: ±2.8‰) and up to 2 TLs (dashed line = 2 TL difference: ±5.6‰).

$\delta^{15}N$, $\delta^{13}C$ and C/N ratios of sediments were very similar at all stations compared between seasons (ANOVA: $p>0.05$; Table 1). No significant differences were found between areas within one season for leaves or sediments (Multiple Comparison: $p<0.05$).

3.2 Seasonal differences in invertebrate isotopic compositions

The discrepancy between seasons calculated from Table 7.2, revealed that $\delta^{13}C$ values were varying in positive and negative directions from rainy to dry season without following any consistent patterns depending on species or stations. Only for *Saccostrea* cf. *cuccullata* and *Metaplax elegans* an increase of $\delta^{13}C$ towards dry season was found.

3.3 Spatial differences in invertebrate isotopic compositions

Significant spatial differences in carbon and nitrogen isotopic composition of species were found between the central and eastern lagoon (K-W-ANOVA: $p<0.05$; Fig. 7.2). Especially in rainy season $\delta^{15}N$ of all species but *Metaplax elegans* were significantly higher in the eastern compared to the central lagoon. $\delta^{13}C$ only differed between areas for *Perisesarma darwinense* ($p<0.05$, higher in central), *Saccostrea* cf. *cuccullata* ($p<0.001$, higher in eastern) and *Telescopium telescopium* ($p<0.01$, higher in central). During dry season only few significant differences between areas were found. *M. elegans* had significantly higher $\delta^{15}N$ and lower $\delta^{13}C$ in the central lagoon ($p<0.001$). Furthermore $\delta^{15}N$ of *S.* cf. *cuccullata* and *E. singaporense* were significantly higher in the central part ($p<0.05$).

4 Discussion

4.1 Seasonal and spatial differences of $\delta^{13}C$ and $\delta^{15}N$ of primary carbon sources

Except for *Avicennia alba* at C42 the $\delta^{15}N$ values of mangrove leaves were between 2-6‰ higher in dry season. This could be explained by a lower concentration of nutrients in sediments in dry season. In rainy season an increased discharge of the Citanduy River into the central lagoon due to a higher precipitation leads to a higher nutrient availability (Moll et al. submitted) and results in selective uptake of isotopically lighter nitrogen and therefore a stronger fractionation. Previous studies showed that rainfall is crucial for the transport of terrestrial energy into aquatic systems (Junk et al. 1989; Douglas et al. 2005). Sediment $\delta^{13}C$ values were similar at stations within one area. In the eastern lagoon higher C_{org} and lower $\delta^{13}C$ values were found than in the central part in both seasons (Fig. 7.3; ANOVA: $p < 0.05$). These high C_{org} values most probably resulted from the Citanduy River discharge, which brings in a high amount of C_{org} from the rice fields in the hinterland during rainy season (Jennerjahn et al. 2009). Organic carbon and nitrogen contents were not correlated with grain size. All stations had mainly clay and silt contributions, except station E40 where a slightly higher contribution of sand was examined (unpublished data). Sediment $\delta^{15}N$ values did neither change significantly between stations nor seasons (ANOVA: $p > 0.05$), indicating a sufficient supply of nitrogen organic matter throughout, e.g. by benthic microalgae, carrion or originating from ^{14}N depleted soils in the hinterland.

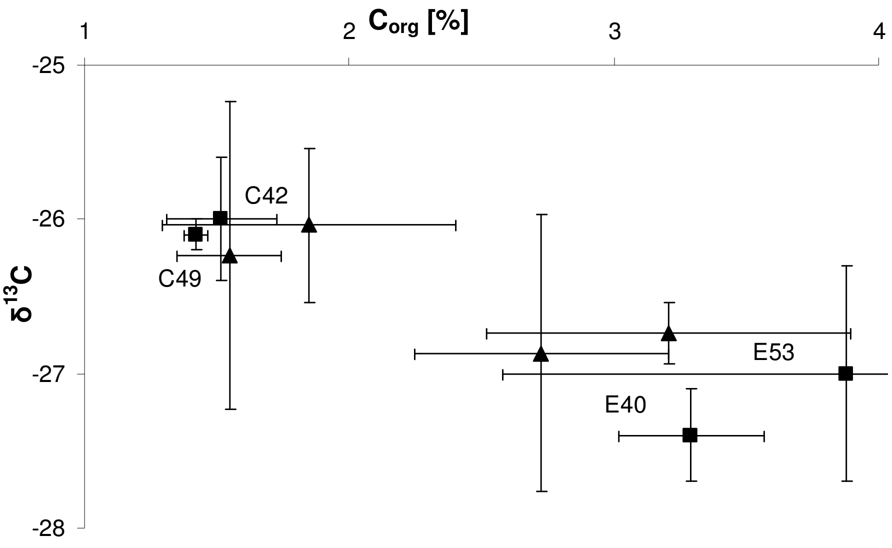

Figure 7.3: Relation between C_{org} [%] and $\delta^{13}C$ for sediment of four stations in the central (C42, C49) and eastern (E40, E53) lagoon during dry (squares) and rainy (triangles) season.

4.2 Trophic shifts of invertebrates

4.2.1 Seasonal variability

From figure 7.2 it becomes clear that a shift of trophic level from one season to the other was only occurring for a few species at certain stations, even though a different feeding habit seemed to be apparent for many of the species, indicated by large discrepancies in

$\delta^{13}C$ between seasons. It can be assumed that most consumers in the first TL have a sufficient availability of food throughout the year and a coexistence of several trophic pathways exists, as also suggested in previous studies (Schaal et al. 2010). Seasonal differences of more than two TLs were only observed for five different species at five distinct stations. It is assumed that these shifts of more than one trophic level could have consequences for the food web structure and also the number of trophic levels.

Cerithidea cingulata, *Metaplax elegans* and *Perisesarma darwinense* did not have large discrepancies between the two seasons in their $\delta^{15}N$ values; seasonal differences in nitrogen isotopic compositions were hardly more than one trophic level. These differences can probably be explained by high inner-species variation. But $\delta^{13}C$ discrepancies were high for *P. darwinense* and *C. cingulata*, suggesting a significant change in their diet with the changing season. $\delta^{13}C$ values of *M. elegans* had discrepancies between the seasons of 2.4±0.7‰, showing higher $\delta^{13}C$ in dry season. As it is a deposit feeder, this probably results from an increase of organic carbon (%C) in the sediments in the eastern lagoon during dry season (ANOVA: $p<0.05$) as also indicated by sediment C/N ratios.

Episesarma singaporense had, with one exception, trophic shifts between seasons by maximal one trophic level, with $\delta^{15}N$ tending to increase in dry season. This species is known to be an opportunistic feeder mainly subsisting on mangrove leaves, and is usually occurring in the second trophic level within the benthic food web in the SAL (Herbon et al. submitted). As most nitrogen isotope discrepancies in leaves between seasons indicated a shift of 1 to 2.5 TLs, it is therefore likely that *E. singaporense* switched to other diets such as microbenthic algae. Furthermore, mangrove leaves were probably less available during rainy season as they are presumed to be increasingly washed out due to increased precipitation. It was observed that during rainy season flooding periods are longer and partly sampling stations did not even fall dry at all. Therefore it can be assumed that intertidal decapods probably had less time for foraging outside burrows.

The mud skipper *Periophthalmus* sp. and the ocypodid crab *Uca forcipata* had trophic shifts of 2.5 and 4 TLs between seasons respectively. A substantially different diet between seasons was suggested, which was confirmed by large discrepancies in $\delta^{13}C$

values, especially in *Periophthalmus* sp. France (1998) stated that the isotopic composition of fiddler crabs can probably not be used to define its carbon sources, as variability in carbon isotopic composition is high. It can also be assumed that the main food source of *Uca* spp. is not only sediment organic matter, such as benthic microalgae and bacteria, but includes e.g. polychaetes as a main supplementary nitrogen source, as also found in stomach content analysis previously (Herbon et al. submitted).

The largest seasonal discrepancy of δ^{15}N was 13.3‰ for the species *Telescopium telescopium*, equal to a trophic shift of 4.75 TLs, indicating a switch in its diet to a more ^{15}N enriched food source, such as carrion of other invertebrates in dry season. But spatial variability in δ^{15}N for *T. telescopium* was high within one season also. Seasonal differences could therefore also be due to an opportunistical scavenging feeding behavior.

In this study we did not find any clear consistent patterns in the discrepancy between seasons, which is in agreement with other studies (e.g. Needoba et al. 2003; Grey et al. 2004; Baeta et al. 2009; Schaal et al. 2010). Many factors can lead to seasonal differences in isotopic compositions, such as omnivorous feeding behavior depending on availability of food sources, changes in biogeochemical processes like the turnover of species internal nitrogen pools, and changes in e.g. flood pulse and water level (Mariotti et al. 1984; Wantzen et al. 2002; Vizzini & Mazzola 2003; Soreide et al. 2006; Baeta et al. 2009; Cabanellas-Reboredo et al. 2009). However, trophic shifts for the species found in this study could also be due to inter-annual variation.

Zeng et al. (2008) stated that many studies assume a large seasonal variation in food sources, but only a small trophic shift in consumers. He found that this result from a higher isotopic fractionation by the selective uptake of isotopically lighter carbon and nitrogen by primary producers. A large variation for primary producers and a small shift for the majority of consumers was confirmed in this study.

4.2.2 Spatial variability

Spatial variability was high during rainy season, with higher δ^{15}N in the eastern part of the lagoon. This could be explained by an increased nutrient contribution during high precipitation periods, especially by discharges of the Citanduy river (Moll et al.

submitted). Due to a high nutrient offer in the central part, primary producers such as mangroves thus can select isotopically lighter nitrogen, which can be used more easily for metabolic processes and growth.

During dry season in contrast, variances between areas were low. In general nutrient availability is minor (Moll et al. submitted) and internal recycling is a more important process. Higher $\delta^{15}N$ in the central area were found for *M. elegans*, *S.* cf. *cuccullata* and *E. singaporense*, due to high values at C45 (Multiple comparison: $p<0.05$). Carbon isotopic compositions were significantly lowest at C45 (Multiple comparison: $p<0.05$) for *Episesarma singaporense*, *Uca forcipata* and *M. elegans*. As this station is located in the middle of the lagoon many factors can have an influence on the isotopic composition of benthic invertebrates. From the central part freshwater and agricultural inputs, as well as sewage from the villages Klaces and Motehan could have had an impact. From the eastern part of the lagoon saline water masses and pollutants from the oil refinery could affect isotopic compositions at this location. We cannot determine which factors resulted in these substantially different isotopic compositions of these invertebrates, which also have different feeding habits.

4.4 Global comparison

14 studies which also investigated species addressed in this study or at least the same genera were compared (Table 3).

Cerithidea cingulata is a grazer in the SAL, as average $\delta^{13}C$ values indicate mixed carbon sources of terrestrial and marine origin. This is also supported by studies in other countries, where a dependency of *Cerithidea* spp. on benthic microalgae (BMA) was suggested (Table 7.3). Only in Japan $\delta^{13}C$ for *C. cingulata* were lower (Table 7.3), indicating an exclusively BMA derived diet, which themselves had a highly ^{13}C enriched carbon source. $\delta^{15}N$ values of *C. cingulata* were in general 3-4‰ enriched to those of BMA, indicating a supplementation of ^{15}N enriched food sources, such as carrion on the sediment surface.

In the SAL carrion is a large part of the diet of *Telescopium telescopium*, resulting in higher $\delta^{15}N$ in muscle tissue (Herbon et al. submitted). $\delta^{15}N$ here were much higher compared to other studies. In Malaysia, where comparably moderate $\delta^{15}N$ were found, the authors suggested a diet mainly on microalgae and phytoplankton, and more general on organic matter on the sediment surface (Table 7.3). But the diet probably also includes carrion of other invertebrates. Apart from Australia where low $\delta^{13}C$ of -18.8‰ were found (Table 7.3), indicating probably less opportunities of scavenging, $\delta^{13}C$ values ranged between -26.6‰ and -21.4‰ including this study (Table 3).

$\delta^{13}C$ values of *Crassostrea/ Saccostrea* spp. were lowest in this study. As plankton $\delta^{13}C$ varied only little between countries (-21.0‰ to -22.8‰; Table 3), additional carbon sources of rather terrestrial origin could be assumed. Suspended matter probably was also an important carbon source for these species (Table 7.3). $\delta^{15}N$ are high in Indonesia, Japan and Thailand. All these study areas are highly impacted by humans, e.g. by agriculture (rice fields, shrimp ponds, oyster farming) and are located in the proximity to high populated cities (Table 7.3). It is therefore suggested that the high $\delta^{15}N$ in these filter feeders resulted from highly ^{15}N enriched suspended matter in the water especially from effluents of agricultural origin. The highest $\delta^{15}N$ values for oysters were measured in Indonesia in combination with the lowest $\delta^{13}C$, indicating a high anthropogenic impact. Previous studies showed that extremely low $\delta^{13}C$ in sediments can result from PAH pollution (Jackson et al. 1996; Lichtfouse et al. 1997; McRae et al. 2000). High PAH concentrations were measured near the oil refinery of the city Cilacap in the SAL (Dsikowitzky et al. accepted).

$\delta^{13}C$ for *Metaplax elegans* varied highly between Malaysia, India, Thailand and this study (Table 7.3). Authors suggest a mainly microphytobenthos derived diet. Sediment $\delta^{13}C$ data, if available, indicated an organic matter content of mainly terrestrial origin, being far below those of *Metaplax* $\delta^{13}C$. Therefore a mixed diet for *Metaplax* consisting of benthic microalgae and terrestrial organic matter could be assumed (Table 7.4).

In Indonesia *Uca forcipata* feeds on polychaetes and most probably microphytobenthos (Herbon et al. submitted). *Uca* spp. outside the Indo-Pacific had high variances in $\delta^{13}C$ (Table 3). Sediment organic matter, presumed to be the main carbon source for *Uca* spp., had $\delta^{13}C$ values below -21.0‰ in all studies, which was the lowest value for microphytobenthos measured. This indicates a high contribution of terrestrial carbon sources to the sediment carbon pool. $\delta^{15}N$ in tissue of *Uca* spp. varied highly between the countries. This could be due to the investigation of different *Uca* spp. Also a different contribution of polychaetes or other animal material to the diet could be assumed.

$\delta^{13}C$ of *Perisesarma* in Sri Lanka (Table 3) were similar to sediment values and about 3‰ enriched to mangrove leaves. At all other study sites $\delta^{13}C$ were much higher, indicating a bacteria or microphytobenthos derived carbon source. $\delta^{15}N$ of this species had a discrepancy of 0.5‰ to 1.4‰ to those of mangrove leaves in India and Sri Lanka (Table 3). Therefore the main carbon source for Perisesarma were microphytobenthos and bacteria and not mangrove leaves. In Sri Lanka a more ^{15}N enriched diet was indicated with discrepancies of $\delta^{15}N$ between 3.6‰ and 4.7‰. This indicated a higher contribution of carrion to the diet.

The genus *Episesarma* had similar $\delta^{13}C$ in India, Thailand and the SAL (Table 3). The main food source of *Episesarma* spp. are fresh mangrove leaves, which are supplemented by carrion e.g. in case of *E. versicolor* (Table 3). Variations in $\delta^{15}N$ are species specific, as different species of this genus have different feeding habits and ways of adaptation to food availability (Herbon et al. submitted).

Periophthalmus sp. is carnivore in the SAL, having a homogenous diet at all sampling sites. It mainly feeds on polychaetes and fish, and therefore occurs in the third trophic level (Herbon et al. submitted). Rodelli et al. (1984) found average $\delta^{13}C$ values of -22.9‰

in a Malaysian mangrove swamp, similar to values found in the SAL. They also suggested a predatory feeding behavior.

Table 7.3: Geographical comparison of isotopic compositions of eight mangrove invertebrate species, values in permill with standard deviation. In Kenya and Sri Lanka numbers refer to different study sites. RS= rainy season, DS= dry season, UNK= unknown sampling time. Values from Indonesia were averaged over all stations.

Source	Study area	Species	$\delta^{15}N$	$\delta^{13}C$	RS	DS
This study	Indonesia	*Cerithidea cingulata*	5.9±1.4	-21.1±3.8	X	
Bouillon et al. 2004	Kenya (1)	*Cerithidea decollata*	4.7±1.2	-21.6±0.8	X	
Bouillon et al. 2002	India	*Cerithidea obtusa*	4.8±0.2	-19.3±0.7	X	
This study	Indonesia	*Cerithidea cingulata*	6.7±1.3	-19.6±3.6		X
Abrantes & Sheaves 2009	Australia	*Cerithidea cingulata*	6.9	-20.2		X
Bouillon et al. 2004	Sri Lanka (1)	*Cerithidea cingulata*	6.3±0.1	-21.2±0.2		X
Kruitwagen et al. 2010	Tanzania	*Cerithidea decollata*	9.5±0.1	-17.8±0.2		X
Rodelli et al. 1984	Malaysia	*Cerithidea obtusa*		-23.1±1.3	UNK	
Ishihi & Yokoyama 2009	Japan	*Cerithidea cingulata*	9.3±0.1	-12.1±0.1	UNK	
This study	Indonesia	*Telescopium telescopium*	11.6±4.6	-23.5±2.7	X	
Bouillon et al. 2002	India	*Telescopium telescopium*	6.8±1.0	-22.0±1.5	X	
Abrantes & Sheaves 2008	Australia	*Telescopium telescopium*	6.3	-18.8	X	
Bouillon et al. 2004	Kenya (1)	*Terebralia palustris*	3.5±1.1	-26.1±0.9	X	
Bouillon et al. 2004	Kenya (2)	*Terebralia palustris*	4.5±0.5	-22.9±0.8	X	
This study	Indonesia	*Telescopium telescopium*	11.2±5.0	-23.2±2.2		X
Newell et al. 1995	Malaysia	*Telescopium telescopium*	9.1	-21.4		X
Bouillon et al. 2004	Sri Lanka (2)	*Terebralia palustris*	4.8±1.5	-24.8±1.7		X
Rodelli et al. 1984	Malaysia	*Telescopium telescopium*		-26.6	UNK	
This study	Indonesia	*Saccostrea cf. cuccullata*	8.9±3.7	-29.2±2.8	X	
Bouillon et al. 2004	Kenya (2)	*Crassostrea cuccullata*	5.1±0.6	-18.4±0.6	X	
Bouillon et al. 2004	Kenya (1)	*Saccostrea cuccullata*	4.8	-23.7	X	
This study	Indonesia	*Saccostrea cf. cuccullata*	6.7±4.1	-25.8±3.1		X
Thimdee et al. 2004	Thailand	*Crassostrea commercialis*	9.7±0.3	-20.0±0.5		X
Abrantes & Sheaves 2009	Australia	*Sacostrea echinata*	6.6±0.2	-24.3±0.5		X
Ishihi & Yokoyama 2009	Japan	*Crassostrea gigas*	9.9±0.4	-19.4±0.4	UNK	
Schwamborn et al. 2002	Brazil	*Crassostrea rhizophorae*		-23.2	UNK	
Rodelli et al. 1984	Malaysia	*Crassostrea* sp.		-18.8±0.9	UNK	
This study	Indonesia	*Metaplax elegans*	7.8±0.5	-21.3±0.8	X	
Bouillon et al. 2004	India	*Metaplax distinctus*	7.0±0.0	-22.7±0.2	X	
Bouillon et al. 2002	India	*Metaplax elegans*	6.4±1.0	-18.4±1.1	X	
This study	Indonesia	*Metaplax elegans*	7.7±1.3	-19.0±1.4		X
Kristensen et al. 2010	Thailand	*Metaplax elegans*	8.9	-16.9		X
Rodelli et al. 1984	Malaysia	*Metaplax elegans*		-21.9±1.5	UNK	
This study	Indonesia	*Uca forcipata*	9.0±4.4	-18.9±2.7	X	
Bouillon et al. 2002	India	*Uca rosea*	6.5±0.8	-21.1±0.5	X	
Bouillon et al. 2004	Kenya (1)	*Uca* spp. (average)	3.7±0.5	-21.0±0.2	X	

Table 7.3: Continued

Source	Study area	Species	δ^{15}N	δ^{13}C	RS	DS
Bouillon et al. 2004	Kenya (2)	*Uca* spp. (average)	2.6±0.4	-16.5±0.1	X	
Bouillon et al. 2004	India	*Uca* spp. (average)	7.5±0.4	-20.4±2.0	X	
France 1998	Puerto Rico	*Uca* spp. (average)	3.9±0.5	-21.0±0.5	X	
This study	Indonesia	*Uca forcipata*	6.9±0.6	-18.3±2.1		X
Kruitwagen et al. 2010	Tanzania	*Uca* spp. (average)	9.8±2.2	-19.6±1.3		X
Kristensen et al. 2010	Thailand	*Uca forcipata*	8.1	-18.0		X
Thimdee et al. 2004	Thailand	*Uca vocans*	7.2±0.3	-19.2±0.7		X
Abrantes & Sheaves 2009	Australia	*Uca vomeris*	5.6	-16.3		X
Rodelli et al. 1984	Malaysia	*Uca forcipata*		-20.0	UNK	
Demopoulos et al. 2007	Puerto Rico	*Uca* sp.	6.0±1.3	-25.0±0.7	UNK	
Schwamborn et al. 2002	Brazil	*Uca* spp. (average)	5.7±0.1	-15.0±1.4	UNK	
This study	Indonesia	*Perisesarma darwinense*	8.7±2.0	-23.2±2.3	X	
Bouillon et al. 2002	India	*Parasesarma asperum*	5.5±0.7	-25.5±0.6	X	
Bouillon et al. 2004	India	*Perisesarma bengalensis*	7.6±1.1	-25.4±0.5	X	
Bouillon et al. 2004	Kenya (1)	*Perisesarma guttatum*	4.2±0.7	-23.3±0.9	X	
This study	Indonesia	*Perisesarma darwinense*	8.0±0.7	-23.5±0.9		X
Bouillon et al. 2004	Sri Lanka (1)	*Perisesarma dussumieri*	4.2±1.2	-27.3±0.8		X
This study	Indonesia	*Episesarma singaporense*	9.3±1.5	-23.7±1.0	X	
Bouillon et al. 2002	India	*Episesarma versicolor*	6.6±1.0	-24.2±1.0	X	
Bouillon et al. 2004	India	*Episesarma versicolor*	6.6±2.1	-25.4±0.5	X	
This study	Indonesia	*Episesarma singaporense*	9.4±3.2	-24.2±0.7		X
Bouillon et al. 2004	Sri Lanka (1)	*Episesarma tetragonum*	3.2	-25.2		X
Newell et al. 1995	Malaysia	*Episesarma versiolor*	7.6	-25.0		X
Thimdee et al. 2004	Thailand	*Episesarma mederi*	7.9±0.4	-26.0±0.6		X
Kristensen et al. 2010	Thailand	*Episesarma versicolor*	7.2±0.4	-24.3±0.1		X
Rodelli et al. 1984	Malaysia	*Episesarma singaporense*		-26.0	UNK	
This study	Indonesia	*Periophthalmus* sp.	13.1±2.4	-23.1±2.7	X	
This study	Indonesia	*Periophthalmus* sp.	12.3±1.4	-22.3±1.7		X
Rodelli et al. 1984	Malaysia	*Periophthalmus* sp.		-22.9	UNK	

Table 7.4: Geographical comparison of baseline component isotopic compositions, values in permill with standard deviation. Numbers in brackets for Kenya and Sri Lanka refer to different study sites. MPB= microphytobenthos, BMA= benthic microalgae, MA= macro algae, SGR= seagrass, FCH= filamentous chlorophytes, PL= plankton, ML= mangrove leaves, DET= detritus, SED= sediment, SSED= surface sediment, POM= particulate organic matter as presented in the respective studies. RS= rainy season, DS= dry season, UNK= unknown sampling time. Values from Indonesia were averaged over all stations.

Source	Study area	Baseline component	δ^{15}N	δ^{13}C	Season
This study	Indonesia	SED	4.2±0.1	-26.5±0.4	RS
		ML (average)	0.0±2.9	-28.8±0.8	
This study	Indonesia	SED	4.2±0.2	-26.6±0.7	DS
		ML (average)	3.3±1.9	-28.5±1.3	
Abrantes & Sheaves 2009	Australia	SGR (average)	2.4±0.2	-18.4±0.2	DS

Table 7.4: Continued

Source	Study area	Baseline component	δ^{15}N	δ^{13}C	Season
Abrantes & Sheaves 2009	Australia	ML (average)	2.7±0.3	-28.8±0.7	
		MPB (average)	2.9±1.2	-22.8±0.3	
Abrantes & Sheaves 2008	Australia	MPB	2.9±0.3	-16.5±0.3	RS
Schwamborn et al. 2002	Brazil	ML (average)	4.4	-26.8±0.4	UNK
		SED		-25.1±0.5	
		POM	3.8±1.7	-24.9±0.4	
Ishihi & Yokoyama 2009	Japan	BMA	5.6±1.4	-15.8±1.8	UNK
		PL	6.1±1.6	-21.7±1.2	
Newell et al. 1995	Malaysia	ML (average)	5.4±0.7	-28.3±0.4	DS
		SED (BMA & PL)	6.8	-19.6	
Rodelli et al. 1984	Malaysia	ML (average)		-27.1±1.4	UNK
		SED		-24.8	
		FCH		-20.1±1.5	
		POC (offshore)		-21.4	
		PL (offshore)		-21.0±0.5	
Thimdee et al. 2004	Thailand	MA	3.5±0.9	-16.8±1.5	DS
		ML (average)	5.0±0.5	-28.9±0.4	
		SSED	5.1±0.2	-26.3±1.0	
		PL	5.7	-21.8	
Bouillon et al. 2002	India	BMA	1.7±1.7	-17.3±1.7	RS
		ML(average)	4.6±1.1	-28.6±1.4	
		SED	3.5±0.5	-21.8±1.0	
Bouillon et al. 2004	Kenya (1)	ML(average)	0.6±1.6	-29.4±1.8	RS
		MPB	1.9±0.1	-22.1±0.0	
		SSED	2.1±1.2	-25.2±0.0	
	Kenya (2)	ML(average)	1.7±2.0	-29.1±1.8	RS
		SSED	1.7±0.8	-23.0±0.9	
	India	ML(average)	7.1±3.1	-28.6±1.8	RS
		SSED	5.6	-24.9	
	Sri Lanka (1)	ML (average)	2.8±2.0	-30.4±1.0	DS
		SSED	-0.1±0.3	-27.5±0.9	
	Sri Lanka (2)	ML (average)	1.2±1.1	-31.4±2.6	DS
		SSED	1.8±1.7	-27.8±1.2	
Kristensen et al. 2010	Thailand	ML (average)	3.5±0.2	-28.9±0.4	DS
		BMA	2.2	-20.1	
Kruitwagen et al. 2010	Tanzania	MA	8.8±0.2	-17.6±0.7	DS
		MPB	5.6±0.4	-21.8±0.3	
		DET	3.2±0.9	-24.7±0.5	
France 1998	Puerto Rico	SED	-1.4±1.8	-26.8±1.5	RS
		ML (average)	-3.8±1.2	-29.5±1.3	
		BMA	2.3±1.0	-19.9±1.6	
		MA	6.3±1.3	-19.9±1.6	
		PL	6.3±0.5	-25.9±1.3	
Demopoulos et al. 2007	Puerto Rico	ML (average)	4.5±3.0	-29.5±1.6	UNK

Table 7.4: Continued

Source	Study area	Baseline component	$\delta^{15}N$	$\delta^{13}C$	Season
Demopoulos et al. 2007	Puerto Rico	POM/BMA	1.0±1.0	-21.5±0.5	
		DET	5.6±0.5		

4.4 Summary and conclusion

Stable isotopes seem to be a sufficient tool to trace changes in nutrient concentrations and therewith food quality induced by changes of environmental conditions, such as precipitation, in a short time period. But distinguishing factors influencing isotopic compositions in benthic invertebrates is rather difficult.

Seasonal differences in isotopic compositions of invertebrates muscle tissue did not follow any clear patterns. Discrepancy ranges between seasons depended on the species and location within the lagoon. Factors that had an impact on seasonal differences include precipitation and therewith nutrient availability, food availability and quality and carbon and nitrogen contents in sediments. Spatial variability was much higher during rainy season. This can be traced back to an increased amount of nutrients in the central part from the Citanduy washed in from the hinterland (Moll et al. submitted). Benthic communities are thus highly adaptable to large spatial and temporal environmental changes and therewith changes in food availability and quality.

In a global comparison, $\delta^{15}N$ of *Telescopium* were highest in the SAL. These are resulting from supplementing ^{15}N enriched food sources, most likely carrion, in an amount as found in no comparable study.

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8 Consumption rates and fractionation of carbon and nitrogen isotopes by mangrove crabs, including long-term experiments with a Rhizophora apiculata diet

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Abstract

In this study the underlying processes leading to differences in isotopic compositions were studied, which were used as the basis to resolve food web structures in the last three decades in several studies. With experimental designs the aim of this study was to increase the knowledge of assimilation processes, by studying consumption and excretion rates and the differences in isotopic compositions between leaves fed and faeces excreted. A response in the isotopic composition of muscle tissue after a one-choice-diet given was observed after 50 days. Only then an increase in nitrogen isotopic composition occurred due to internal recycling of nitrogen and excretion of ^{14}N. Intertidal crabs are therefore able to balance their nitrogen budget over 50 days and survive three month on a one-choice mangrove-diet only. The species *Derris trifoliata* and *Rhizophora apiculata* were preferably consumed, indicating a high nutritional value. As expected, muscle tissue, which is a long-time storage had highest isotopic composition values, compared to hepatopancreas and stomach content with the lowest values. There were no significant differences found comparing ovigerous and non-ovigerous females, both groups have similar isotopic compositions. Crabs are probably able to balance the discharge, which they provide their eggs. Their reproduction time is probably shorter than the muscle tissue turnover time. The overall outcome of these experiments is that carbon isotopic signatures do not seem to be a sufficient tool to examine recent carbon diets from consumers. Furthermore the presumed discrepancy between prey and consumer of 0 to 0.8‰ does not seem to apply for all invertebrates in a benthic food web.

Key words: benthic food web, carbon, mangroves, nitrogen, nutrient-cycling, stable isotopes

1 Introduction

Food web structures have already been investigated for several mangrove habitats all over the world (e.g. Rodelli et al. 1984; Bouillon et al. 2004; Mazumder & Saintilan 2009). It is known that the heavy ^{15}N isotope fraction is accumulated in consumers and therefore increasing with each trophic level (Vander Zanden & Rasmussen 2001, McCutchan et al. 2003, Caut et al. 2009), whereas the carbon isotopic composition does not change more than 0.8‰ from diet to consumer (Peterson & Fry 1987). The excretion of isotopically light ammonium is one of the outcomes of isotopic fractionation of nitrogen in animal tissue (Minagawa & Wada 1984; Checkley & Miller 1989). Physiologically, this means that amine groups containing isotopically light nitrogen such as glutamate are favored during transamination and deamination, which results in the excretion of these (Gannes et al. 1998). Isotopic fractionation occurs primarily during anabolic processes and the production of new tissues (Yokoyama et al. 2005a). Large isotopic discrepancies associated with low quality diet can result from internal recycling of nitrogen which is also found in starving animals (Hobson et al. 1993; McCutchan et al. 2003). The assimilation of dietary nitrogen needed for the growth of animals is increased when growth and longevity are slow (Nordhaus & Wolff 2006; Linton & Greenaway 2007; Nordhaus et al. submitted). Large consumers have longer turnover rates, so that their diet is reflected in isotopic compositions over a long time period (Post 2002).

High population densities bear intra- and inter- specific competition for food (Linton & Greenaway 2007); many crab species especially feed on leaves (Emmerson & McGwyne 1992). The ingestion of leaf litter includes microorganisms coating it, which are potentially of higher nutritional value and also easier to digest than the leaves itself. Bacterial cover on fresh fallen leaves is low, but increase fast within a few days after falling (Linton & Greenaway 2007). Tree climbing mangrove crabs do not eat whole leaves, but scrape of tissue from the upper or lower surfaces of growing leaves (Cannicci et a. 1996; Dadouh-Guebas et al. 1999). Sesarmidae e.g. prefer plant detritus over microalgae in the sediment, and the diet is a heterogeneous mixture of detrital organic compounds of sediments and mangrove litter, encrusting algae from mangrove roots and trunks as well as invertebrates (Linton & Greenaway 2007).

Animals can adapt to food sources of different qualities, with varying physiological and behavioral characteristics, which include the selection of food items, food ingestion, mechanical fragmentation of food, complementation of digestive enzymes produced, retention time of digesta in the gut and the anatomy of the alimentary tract (Linton & Greenaway 2007). If leaves are incompletely broken down mechanically and residence time in the gut is short, digestion and assimilation will be low and faeces have similar concentrations of neutral detergent soluble material (e.g. nitrogen, carbon, cellulose, lignin, calcium) as leaves, so that the leaves can not be utilized wholly (Greenaway & Linton 1995). An increase in residence time of food in the gut increases digestion of hardly digestible substances such as cellulose and leads to higher assimilation coefficients (Wilde et al. 2004).

For *Sesarma* sp. e.g. the largest sink for the assimilated energy of leaves is probably respiration, a small part is lost by urine or exuviae, leaving the balance for somatic production and reproduction (Emmerson & McGwyne 1992). Crabs have the ability to store excess nitrogen as urate (Wolcott & Wolcott 1984, 1987). In *Sesarma* sp. the faecal production increases with body size (Emmerson & McGwyne 1992). In general the isotopic composition of excretion products and that assimilated equals that of what is consumed. Consumers' nitrogen is typically depleted in ^{15}N in the faeces compared to the tissue (DeNiro & Epstein 1981a; Checkley & Miller 1989). Heat and water stress can lead to an increased urea concentration and amount excreted, which is probably increasing the discrepancy between the consumers tissue and its diet (Ambrose 1991, 2000; Ambrose & DeNiro 1986; Hobson et al. 1993).

Until today only little attention has been paid to physiological processes and biochemical mechanisms that have an impact on isotopic compositions (Adams & Sterner 2000).

In this study several experiments were conducted to understand the turnover of carbon and nitrogen and their fractionation in the crabs´ body. This is the first study examining the change in carbon and nitrogen isotopic composition in muscle tissues of benthic invertebrates over a time period of three months.

We hypothesize the following: (1) Benthic crab species show a response in their isotopic composition of their muscle tissue within a time period of three months, given a one-choice diet only. (2) Leaves of different mangrove species are consumed and fractionated differently by various crab species. (3) Carbon and nitrogen isotopic compositions will substantially differ between tissues, according to their status within the digestion and assimilation process. (4) Ovigerous females have lower carbon and nitrogen isotopic compositions in their muscle tissue than non-ovigerous, as they invest more carbon and nitrogen into the development of their eggs, leaving less for their own metabolism.

2 Materials and methods

2.1 Study site

The Segara Anakan lagoon (SAL; 108°50’- 109°00’E, 07°39’- 07°43’S) is a 1002ha large brackish water ecosystem surrounded by 9238ha of mangrove forest (Ardli & Wolff 2009). The lagoon is separated from the Indian Ocean by the rocky mountainous island Nusa Kambangan, leaving only two entrances to the lagoon, east and west of the island, allowing an exchange with saline water masses. The hydrology of the SAL is governed by semidiurnal tides, ranging between 0.3 m at neap tides and 1.9 m at spring tides (Holtermann et al. 2009). Freshwater is mainly provided by the Citanduy River, the fifth largest river of Java, and the Cibereum River in the west (Holtermann et al. 2009). Sedimentation through these rivers, mainly due to deforestation in the hinterland, reduces the water volume of the lagoon consistently (Ardli 2007).

Dominant benthic invertebrates in the lagoon are from the genera *Episesarma*, *Parasesarma*, *Perisesarma*, *Sesarma*, *Uca* and *Scylla* (Nordhaus et al. 2009), from which the former four at least partly feed on mangrove leaves. In the central lagoon dominant tree species are *Aegiceras corniculatum*, *Nypa fructicans* and *Rhizophora apiculata*, the latter two being indicators for a mature forest. Whereas in the central lagoon pioneer species such as *Avicennia alba*, *Aegiceras corniculatum* and *Sonneratia caseolaris* dominate the vegetation (Hinrichs et al. 2008).

Station E40 in a northern creek of the eastern lagoon (108°59.57’E, 07°40.28’S), is provided with freshwater during rainy season. This station is diagonally opposite the oil refinery and covered by a large swamp area. Dominant mangrove tree species are *Aegiceras corniculatum* and *Ceriops* spp. (Hinrichs et al. 2009).

Station C49 in the central lagoon (108°50.76’E, 07°41.44’S) is influenced by saline water masses during high tide (Holtermann et al. 2009). The predominant mangrove tree species here is *Sonneratia* spp. (Hinrichs et al. 2009). It is located between the villages Klaces and Motehan and therefore likely provided with additional nutrients from household wastes.

2.2 Feeding habits of decapods

The main carbon sources of the genus *Episesarma* in the SAL are mangrove leaves. *E. versicolor* additionally supplements its diet with animal material such as polychaetes, to satisfy its nitrogen needs. That makes this species an opportunistic feeder, occuring on different trophic levels at different locations in the lagoon, depending on food availability and quality (Herbon et al. submitted). *E. singaporense*, on the other hand seems to feed rather heterogeneous on a consistent diet baseline, as it was througout found in the second trophic level in the SAL. It mainly feeds herbivorous on detritus, roots and bark, with only a small amount (<5%) of animal material. Even though these two species are of the same genus, they show different feeding habits in the field (Herbon et al. submitted).

Metaplax elegans is a deposit feeder whereas *Uca forcipata* besides deposit feeding supplements its diet with animal material such as polychaetes (Herbon et al. submitted). The genus *Perisesarma* subsists herbivorous, exclusively on mangrove leaves, whereas *Epixanthus dentatus* and *Scylla serrata* are carnivorous species (Herbon et al. submitted).

2.2 Long-time experiment

The change in isotopic signatures in muscle tissue of the crab species based on a given one-choice-diet was observed over three months. 64 aquaria were constructed of 40cm x 40cm x 40cm each. If possible 15 individuals of *Episesarma singaporense* and *E. versicolor* each were collected from stations E40 and C49 each and kept separately in the aquaria in 2L of lagoon water (water depth about 2cm) with a blue colored tumbler to hide, a saucer placed in the aquaria upside down for the crabs to be able to move out of the water and provided with sufficient brown *Rhizophora apiculata* leaves as food. Aquaria were cleaned every fourth day and water was exchanged with fresh lagoon water.

Over a period of 90 days at five sampling times (T_0, $T_{20/30}$, T_{50}, T_{70}, T_{90}; Fig. 8.1) 4-5 individuals of *E. singaporense* of each station were taken out, frozen for about 24 hours and dissected to remove muscle tissue from the chelae and legs. For *E. versicolor* the same procedure was conducted for animals of station 40, but here only three individuals were taken out and proceeded as explained above, as this species was generally difficult to find/catch. For the same reason also at station C49 a limited number of individuals of *E. versicolor* were caught, which led to a setup with only two sampling times (T_0, T_{90}) with only three animals each.

Muscle tissues were dried in the oven at 40°C, and grained afterwards, to homogenize the sample. Samples were afterwards stored at -20°C until analysis (see 2.6).

2.3 Consumption rate experiment

Six individuals of *Episesarma singaporense* and *E. versicolor* each were kept separatly in glass aquaria with about 2cm depth of lagoon water and the possibility to move out of the water on a saucer. Before the first experiment was run, crabs were kept without food for about four days, to let them empty their gut. Between each experiment also four days were given without providing any food.

Five experiments were conducted with a duration of 24 hours, with leaves of one of the five mangrove and shrub species each. The offered species were *Acanthus ilicifolius*, *Aegiceras corniculatum, Avicennia alba, Derris trifoliata* and *Rhizophora apiculata* (see Table 8.1 for species attributes). Within each experiment each crab was supplied with about 3g of brown leaves in the beginning. When the offered food became scarce, more leaves were provided. Faeces were removed every hour throughout the experiment, to limit leaching of carbon and nitrogen. The aquaria were regularly cleaned and the water was exchanged, to avoid toxic ammonium concentrations in the water through excretion.

Wet weight of remaining leaves and produced faeces within the 24h of the experiment were determined. Consumption rates were calculated from the difference of leaf mass given and leaf mass remaining, considering the crab weight. As leaves soak water within the 24 hours of the experiment, a correction value was needed for the leaves provided, as they were relatively dry. Therefore the weight of the provided leaves were corrected with a factor, experimentally determined by the discrepancy of the weight of whole leaves before and after incubated in lagoon water for 24 hours (Corrected value= [Leaf weight provided] x [% wws/100], weight gained by soaking water (wws) for each species: Rhizophora apiculata= 100%, Acanthus= 58%, Derris= 43%, Avicennia= 29%, Aegiceras= 94%).

The consumption rate experiment was also prepared for *Perisesarma darwinense*, which hardly survived alone in an aquarium, and therefore were kept in a group of three. To calculate consumption rates, values were divided by three, as crabs had similar body sizes.

Table 8.1: %C, %N, C/N and carbon and nitrogen isotopic compositions of leaves in the order offered in consumption rates experiment.

Species	%C	%N	C/N	δ^{13}C [‰]	δ^{15}N [‰]
Rhizophora apiculata	44.4±1.4	0.6±0.2	83.7±21.1	-27.6±0.3	2.6±0.5
Acanthus ilicifolius	39.5±1.4	1.0±0.1	39.8±4.2	-28.5±1.3	3.3±0.6
Derris trifoliata	37.8±5.1	0.9±0.1	42.5±5.2	-26.9±0.6	0.6±0.5
Avicennia alba	42.0±1.0	0.7±0.2	58.8±16.0	-27.5±0.7	4.6±1.7
Aegiceras corniculatum	49.0±1.0	0.7±0.3	76.6±29.4	-29.5±0.5	4.2±0.3

2.4 Differences between tissues

Five individuals of eight decapod species (*Episesarma singaporense*, *E. versicolor*, *Epixanthus dentaus*, *Perisesarma darwinense, P. semperi*, *Metaplax elegans*, *Scylla serrata*, *Uca forcipata*) each were collected by hand and frozen for 24h. After defrosting, the crabs were dissected and muscle tissue, hepatopancreas and if available stomach content were removed. Tissues and stomach contents were separately stored in glass vials, dried in an oven at 40°C, grained to homogenize the samples and stored at -20°C. Isotopic compositions of tissues were then analysed (see 2.6). Individuals sampled for this comparison were collected at another sampling time than those for the long-time experiment.

2.5 Ovigerous and non-ovigerous females

To compare isotopic compositions in muscle tissues of ovigerous and non-ovigerous females two to five individuals of each status of *E. versicolor* and *E. singaporense* were collected. The animals were frozen for 24h and muscle tissue was dissected from the chelae after defrosting. Tissues were dried at 40°C in an oven, grained afterwards and seperately stored in glass vials. Samples were stored at -20°C until analysis. Isotopic compositions of carbon and nitrogen were then analysed (see 2.6).

2.6 Sample analyses

Samples were combusted in the Carlo Erba NA 2100 Elemental analyzer for total carbon and nitrogen content measurements without previous HCl treatment, as previous test showed no significant differences between samples treated with and without HCl. Stable isotope ratios were seperately determined with the coupled EA-IRMS gas isotope ratio mass spectrometer (ConFlo III) and expressed relative to conventional standards $\delta R = [(X_{sample}/X_{standard}) - 1] \times 1000$ ‰ with R= ^{13}C or ^{15}N and X= $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Ammonium sulfate (IAEA-N1, IAEA-N2) was used as standard for $\delta^{15}N$, and graphite (USGS-24) and mineral oil (NBS-22) for $\delta^{13}C$. Analytical precision was ±0.2‰ for both nitrogen and carbon, as estimated from standards analyzed together with the samples.

2.7 Statistics

Significant differences over time in the long-time experiment were calculated in Statistica© with an ANOVA followed by a Tukey´s Post Hoc test for parametric data, and with an Kruskal-Wallis-ANOVA followed by a Mann-Whitney-U test for nonparametric data. Not normally distributed data were log-transformed and in case of achieving normality data were tested for homogeneity of variances, otherwise non parametrical proceedings were conducted.

To find significant differences between tissues, an ANOVA followed by a Tukey´s Post Hoc test was used, as data were normally distributed and homogeneous. To statistically analyze the difference between ovigerous and non-ovigerous females and consumption preferences the same statistical analysis as for tissues was used.

3 Results

3.1 Long-time experiment

Nitrogen isotopic compositions reflected a response to the one-choice diet given, only after 50 days (Fig. 1). A significant increase in $\delta^{15}N$ was recorded from day 50 to 70 and

70 to 90 for *E. singaporense* (station 49) (ANOVA: $p<0.001$, Tukey´s HSD: $p<0.05$), as well as for *E. versicolor* (station 40) between days 0 and 90 as well as 50 and 90 (ANOVA: $p<0.05$, Tukey´s HSD: $p<0.05$). No significant differences were found from day 0 to 90 for *E. singaporense* and from day 0 to 50 for *E. versicolor* (ANOVA: $p<0.05$, Tukey´s HSD: $p>0.05$). Carbon isotopic compositions remained the same over the whole duration of the experiment (K-W-ANOVA and ANOVA: $p>0.05$; Fig. 2) except for *E. singaporense* (station 40) between the first two samplings (ANOVA: $p<0.01$, Tukey´s HSD: $p<0.01$). There was no approximation to the isotopic compositions of *Rhizophora apiculata* leaves.

3.2 Consumption rates

Preferred leaves of the genus *Episesarma* are from *Rhizophora apiculata* and the shrub *Derris trifoliata*. The shrub species *Acanthus ilicifolius* and the mangrove *Aegiceras corniculatum* were only consumed in small amounts (*E. versicolor*: *Acanthus*: 10.4±25.6, *Aegiceras*: 7.7±6.4; *E. singaporense*: *Acanthus*: 8.2±21.5, *Aegiceras*: 6.7±11.5; in mg leaf/ g crab weight/ day; Fig. 8.3).

Perisesarma darwinense only consumed *D. trifoliata* (124.9±0.0 mg leaf/ g crab weight/ day) and did not feed on the other offered leaves.

Nitrogen (%N) did not change at all between leaves offered and faeces excreted (average discrepancy “leaf-faeces” over all leaf species: *E. versicolor*: 0.0±0.2, *E. singaporense*: 0.2±0.1 mg leaf/ g crab weight/ day).

During the experiment we observed that *Rhizophora apiculata* was digested fast, and hard and compact faeces were excreted after one hour already. The midrib was predominantly left over. *Derris trifoliata* was consumed willingly, but lead to fine easily soluble faeces in 50% of cases. Overall only few faeces were excreted within the 24hours. The midrib was consumed almost completely. *Acanthus ilicifolius* also led to fine and easily soluble faeces and primarily was slowly eaten. Only few faeces were excreted, especially from the larger crabs. The midrib was not consumed. *Avicennia alba* was eaten willingly and faeces were relatively compact but could disaggregate very fast. When *Aegiceras corniculatum* was consumed, faeces were compact and stable. The midrib was not consumed.

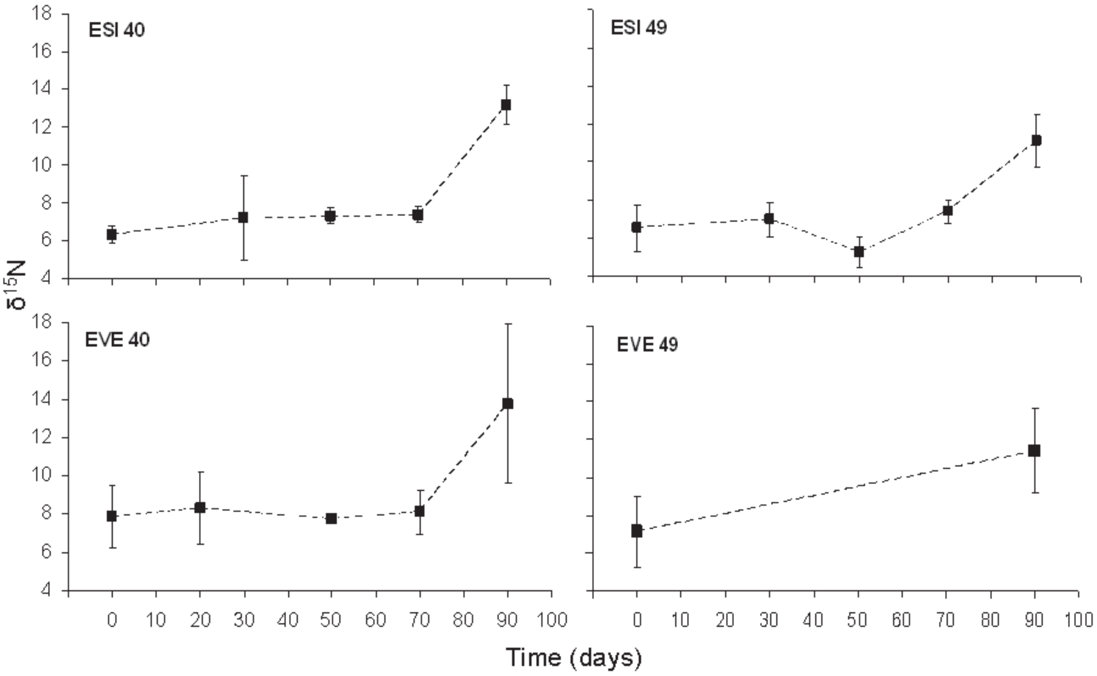

Figure 8.1: Change in nitrogen isotopic composition of muscle tissue over time in *Episesarma singaporense* (ESI) and *E. versicolor* (EVE) from station 40 and 49 fed on *Rhizophora apiculata* leaves.

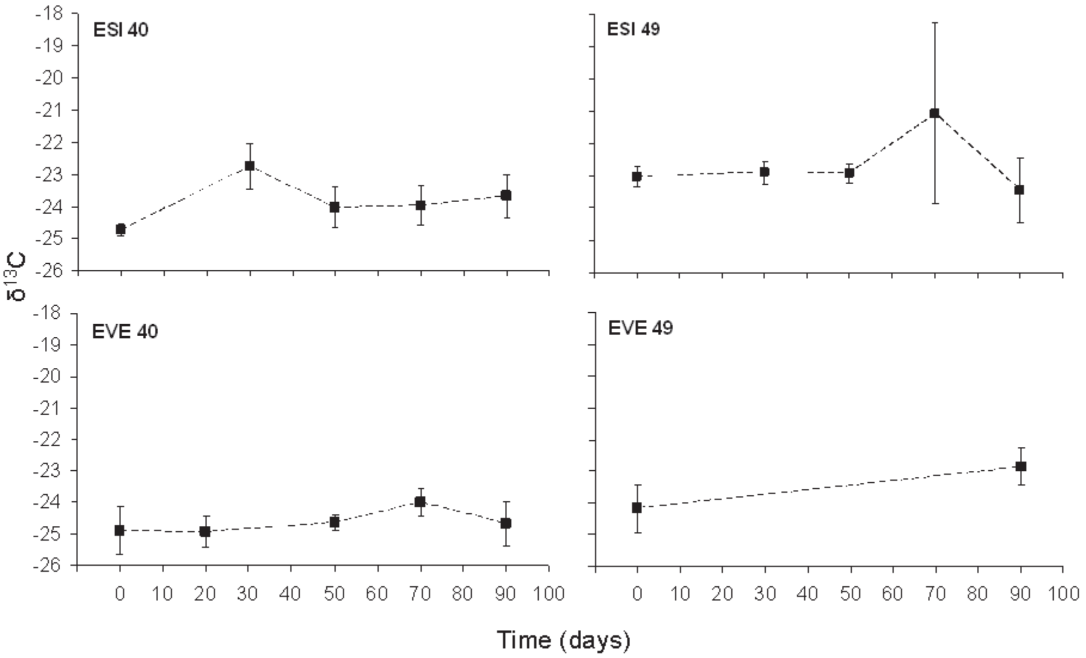

Figure 8.2: Change in carbon isotopic composition of muscle tissue over time in *Episesarma singaporense* (ESI) and *E. versicolor* (EVE) from station 40 and 49 fed on *Rhizophora apiculata* leaves.

In average two fecal pellets per hour were produced, which were mainly found under the crabs body or on the leaves remaining. Crabs rested between 10pm and 11pm, but continued producing faeces during that period.

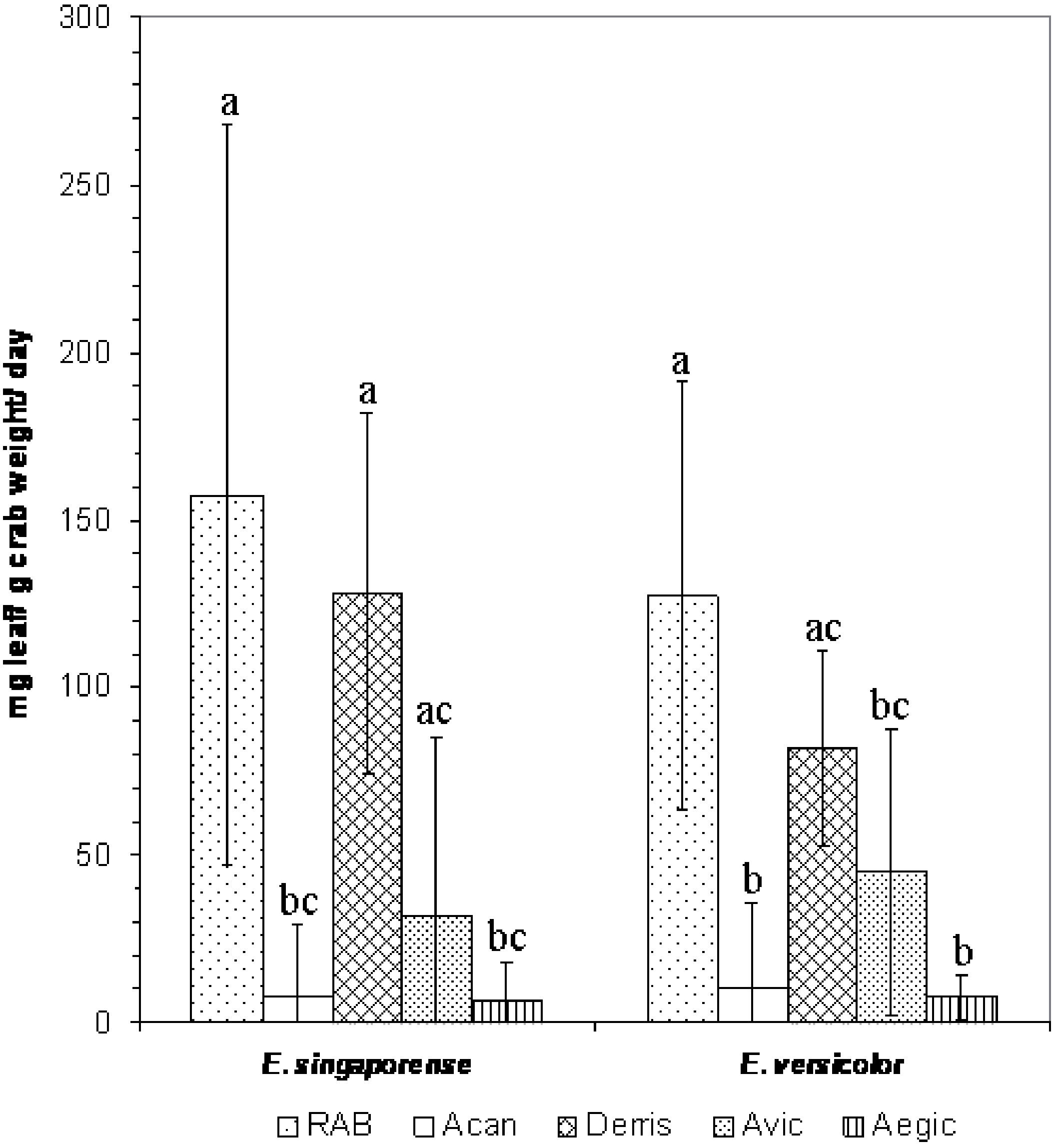

Figure 8.3: Consumption rates of two abundant crab species (n=6 for each species) fed with five mangrove and shrub species (in order offered: *Rhizophora apiculata* (RAB), *Acanthus ilicifolius* (Acan), *Derris trifoliata* (Derris), *Avicennia alba* (Avic), and *Aegiceras corniculatum* (Aegic)), values are given in mg leaf/ g crab weight/ day, with indicated standard deviation. Letters over bars indicate significant differences (same letter= not significant different).

3.3 Fractionation

The discrepancy of $\delta^{13}C$ and $\delta^{15}N$ respectively of leaves provided and faeces excreted is negative if the heavy isotope (^{13}C, ^{15}N) is enriched or the light one (^{12}C, ^{14}N) is depleted in faeces, positive values result from the opposite. Differences in the turnover of offered leaves were found (Fig. 8.3). *Aegiceras corniculatum*, *Derris trifoliata* and *Rhizophora apiculata* are similarly metabolized by the two crab species. The discrepancy of $\delta^{15}N$ is positive in case of *A. corniculatum* and *R. apiculata* and negative for *D. trifoliata*, whereas the discrepancy for $\delta^{13}C$ is negative for all three species.

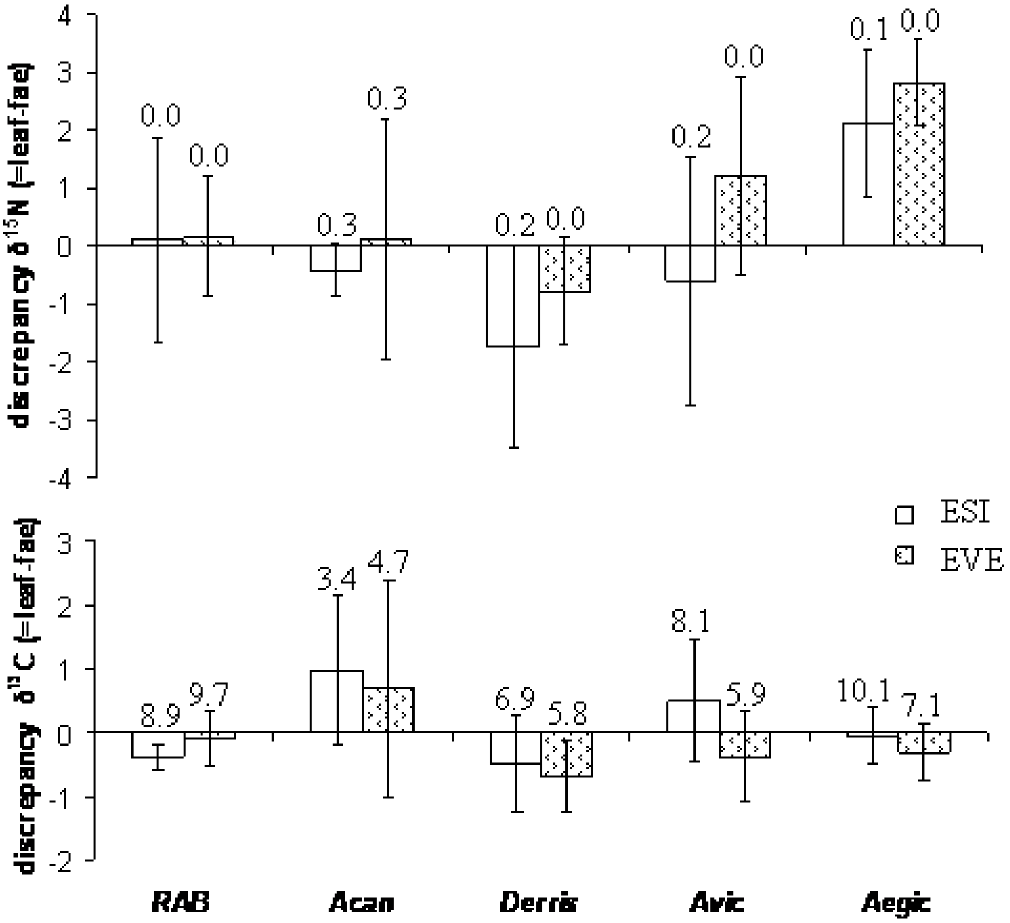

Figure 8.4: Discrimination of $\delta^{15}N$ and $\delta^{13}C$ for two crab species (ESI= *Episesarma singaporense*, EVE= *E. versicolor*) calculated by Δ δX=leaf-faeces with X= ^{15}N and ^{13}C respectively, with indicated standard deviation. Numbers over bars indicate mean discrepancy of carbon (%C) and nitrogen (%N) respectively between leaves eaten and faeces excreted. For leaf abbreviations see figure 8.2.

Table 8.2: Differences between tissues (stomach content (StoCon^1), hepatopancreas (Haepat.^{2}) and muscle tissue (Muscle^3); superscript numbers related to significant levels) in eight decapod species of %C, %N, C/N ratios and carbon and nitrogen isotopic composition [‰] with indicated standard deviation. Stars indicate significant differences comparing two tissues (*** p<0.001, ** p<0.01, * p<0.05).

Species (station catched)	Parameter	StoCon^1	Haepat.^{2}	Muscle^3	1/2	2/3	1/3
Episesarma singaporense	%C	42.6±2.6	44.6±8.2	38.9±3.3			
(40)	%N	5.6±2.5	5.1±0.8	7.0±2.1			
	C/N	8.4±2.5	9.0±2.7	6.0±2.0			
ANOVA: p<0.05	δ^{13}C [‰]	-27.9±0.7	-29.2±1.2	-24.8±0.5		***	**
	δ^{15}N [‰]	10.7±2.1	10.3±0.7	11.0±2.2			
Episesarma versicolor	%C	39.7±2.3	42.6±6.0	38.1±5.2			
(40)	%N	4.6±0.3	6.2±0.7	7.6±1.1			**
	C/N	8.6±0.7	6.9±1.3	5.0±0.3		*	**
ANOVA: p<0.01	δ^{13}C [‰]	-27.6±0.6	-27.9±1.1	-24.9±0.5		**	**
	δ^{15}N [‰]	11.4±0.8	10.6±1.9	17.4±3.0		**	*
Epixanthus dentatus	%C	41.7±5.4	32.1±10.2	37.9±6.3			
(40)	%N	9.0±1.6	4.6±0.6	8.1±1.7	*		
	C/N	4.7±0.7	6.9±1.6	4.7±0.4			
ANOVA: p<0.01	δ^{13}C [‰]	-23.8±0.9	-26.5±0.8	-22.9±0.6	*	**	
	δ^{15}N [‰]	10.7±0.9	12.7±0.5	16.4±3.0	*		
Perisesarma semperi	%C	43.8	50.3±4.5	40.5±2.6		**	
(40)	%N	13.0	7.6±4.9	7.3±2.6			
	C/N	3.4	11.2±10.5	6.0±1.4			
ANOVA: p<0.01	δ^{13}C [‰]	-25.7	-30.0±1.9	-23.0±1.9		**	
	δ^{15}N [‰]	7.1	9.4±0.7	8.4±0.9			
Perisesarma darwinense	%C	39.5±4.2	38.3±7.5	39.0±5.8			
(47)	%N	3.6±0.7	6.8±2.6	9.3±1.9			**
	C/N	11.7±4.1	6.2±2.4	4.4±1.6	*		**
ANOVA: p>0.05	δ^{13}C [‰]	-27.5±1.2	-26.2±3.5	-24.8±1.6			
	δ^{15}N [‰]	9.8±0.9	8.2±1.5	8.5±0.9			
Uca forcipata	%C	37.5±18.3	53.0±3.6	46.7±5.9			
(47)	%N	2.0±0.3	2.2±0.8	3.1±3.2			
	C/N	18.0±8.3	25.9±9.3	25.7±14.7			
ANOVA: p<0.05	δ^{13}C [‰]	-22.1±0.6	-23.6±0.8	-18.6±0.9		***	***
	δ^{15}N [‰]	10.1±1.2	9.6±0.7	16.0±5.7			
Metaplax elegans	%C	-	60.1±0.6	41.3±1.8		***	
(53)	%N	-	4.3±0.4	11.6±1.1		***	
	C/N	-	14.1±1.2	3.6±0.2		***	
ANOVA: p<0.001	δ^{13}C [‰]	-	-23.7±1.0	-20.5±1.8		*	
	δ^{15}N [‰]	-	10.9±0.9	7.7±0.7		**	
Scylla serrata	%C	-	51.0±2.7	37.3±5.0		***	
(Area 3)	%N	-	6.2±3.2	4.4±2.2			
	C/N	-	10.5±6.4	10.1±4.1			
ANOVA: p>0.05	δ^{13}C [‰]	-	-28.3±2.7	-25.2±1.5			
	δ^{15}N [‰]	-	13.4±2.1	17.1±1.9		*	

Acanthus ilicifolius and *Avicennia alba* were differently metabolized by the two crab species. There is no correlation between the discrepancy of %C and δ^{13}C of leaves and faeces.

3.4 Differences between tissues

Muscle tissue had significantly highest δ^{13}C values (statistics see table 8.2), except for *P. darwinense* and *S. serrata*, whose δ^{13}C were similar in all tissues. δ^{15}N was significantly highest in muscle tissue in *Episesarma versicolor, M. elegans* and *Scylla serrata* (table 8.2). It became clear that a large inter-specific variation is apparent, as nitrogen isotopic compositions of individuals collected for the long-time experiment are much lower, than for those used in this comparison.

3.5 Ovigerous and non-ovigerous females

There were no significant differences between ovigerous and non-ovigerous females (ANOVA: $p>0.05$ for both species). Standard deviation was high for all treatments in δ^{15}N; for δ^{13}C only in non-ovigerous females (Table 8.3).

Table 8.3: Comparison of carbon and nitrogen isotopic compositions of ovigerous and non-ovigerous females of two *Episesarma* spp. with indicated standard deviation and number of individuals (n).

Species	ovigerous	δ^{15}N [‰]	δ^{13}C [‰]	n
E. versicolor	+	6.7±1.5	-22.9±0.4	2
	-	6.2±1.5	-22.0±1.6	4
E. singaporense	+	6.2±1.2	-22.6±0.6	5
	-	6.8±1.3	-21.3±3.9	5

4 Discussion

4.1 Long-time experiment

During the long time experiment $\delta^{15}N$ started to increase in muscle tissue of the decapods after about 50 days fed on a *Rhizophora apiculata* leaf diet only. Previous studies showed that animals fed on a nitrogen low quality diet start recycling their internal nitrogen to further keep up their metabolism, and excrete the isotopically light nitrogen which leads to an increase in ^{15}N (Hobson et al. 1993; McCutchan et al. 2003). This also occurs in starving unfed animals, as lean body mass is lost without replacement of excreted ^{14}N (Adams & Sterner 2000). Linton & Greenaway (1997a) showed that crabs fed on leaves had similar concentrations of non-urate nitrogen, urate and total nitrogen after six weeks. Urate accumulation is a vehicle for storage of access dietary nitrogen and functions as a nitrogen reserve (Linton & Greenaway 1997a). Synthesized urate is stored as a solid in spongy connective tissue cells throughout the body (Linton & Greenaway 1997b), which is common for numerous crustaceans (Greenaway 1991; O´Donnell & Wright 1995). Crabs were able to keep the nitrogen balanced over the time period of six weeks (Linton & Greenaway 1997a). Apparently, as shown in this study, *Episesarma* spp. are able to keep the nitrogen budget balanced, up to a minimum of seven weeks. Furthermore the crabs were even molting during the experiment.

In the field, *Episesarma* spp. are used to supplement their mainly mangrove leaf derived diet with animal material (Herbon et al. submitted; Nordhaus et al. submitted), which is most likely necessary to fulfill the nitrogen needs of this genus. Therefore it can be assumed that after 50 days, nitrogen starvation occurred and internal nitrogen recycling was conducted. *E. versicolor* can adapt to different food availability and is an opportunistic omnivore (Bouillon et al. 2002; Thongtham et al. 2008; Herbon et al. submitted), whereas *E. singaporense* feeds on a heterogeneous herbivorous diet (Herbon et al. submitted). But the isotopic compositions of the two species with different feeding habits changed similarly over time. Thus probably the interplay of different physiological characteristics, such as the food intake, the mechanical fragmentation of food, the assimilation efficiency, internal nitrogen recycling, the complementation of digestive enzymes produced and the retention time of digesta in the gut (McCutchan et al. 2003;

Linton & Greenaway 2007) resulted in similar isotopic compositions at the end of the experiment. A mechanically incomplete break down of leaves and a short residence time in the gut can lead to a low digestion and assimilation, visible in similar values of faeces excreted and leaf ingested (Greenaway & Linton 1995). It can be assumed that this was the case with the crabs in our experiment, due to a nitrogen deficit from N depleted diet and possibly resulting fast ingestion, leading to an incomplete utilization of the leaves´ carbon and nitrogen. Nitrogen usually is excreted mainly as urinary urea, whose $\delta^{15}N$ is significantly lower than the diet consumed (Ambrose 2000). The excretion of urinary urea might have been increased by laboratory condition induced stress. This can, as shown previously, increase the discrepancy between consumers and its diets tissue additionally (Ambrose & DeNiro 1986; Hobson et al. 1993).

Carbon isotopic compositions did not change significantly from the first to the last day of the experiment. *E. singaporense* was consistently found on the second trophic level in the SAL (Herbon et al. submitted), even though it mainly feeds on mangrove leaves. This was explained by occasional supplementation of the diet by animal material, i.e. carrion. But, as it was shown in this experiment, that even after 90 days no change or approximation of carbon isotopic signatures occurs, it could be argued whether carbon isotopic signatures are sufficient tracers for carbon sources for this species. A time frame of 90 days, however, was not sufficient to observe any changes in carbon isotopic signatures after a change in food supply (from field to laboratory). If no approximation of carbon isotopic signatures in muscle tissue to that of mangrove leaves occurs, it can be assumed, that this species is fractionating *Rhizophora* leaves differently. Therefore a suggested diet-consumer discrepancy of 0.8‰ does not seem to apply for all species. This becomes even more evident for *E. versicolor*, which was found on various trophic levels in the SAL (Herbon et al. submitted) and was therefore suggested to be highly adaptable to changing food availability. Carbon isotopic signatures of consumers therefore do not reflect their recent diet. This is supported by findings of previous studies (McCutchan et al. 2003).

The factor station and therefore a different background in food quality do not seem to have an impact in dealing with a one-choice-diet only, or at least not in this time frame. It could also be that a high inter-species variation results in similar isotopic compositions, such as appeared for nitrogen in this study. This also becomes obvious when comparing nitrogen isotopic signatures in muscle tissues from *Episesarma* spp. used in this experiment and those used to examine tissue differences, as they were collected within a different sampling time.

Yokoyama et al. (2005b) also found a significant increase in $\delta^{15}N$ in starvation experiments with bivalves but no change in $\delta^{13}C$ values. But an overall rule for the fractionation of $\delta^{13}C$ between diet and tissue is not applicable for all animals in all ecological systems (Yokoyama et al. 2005b).

Previous studies (Adams & Sterner 2000) already found a high variability in organismal $\delta^{15}N$ when fed a mono-diet. This was confirmed in our study.

4.2 Consumption rate experiment

Besides *Rhizophora apiculata* the genus *Episesarma* consumed preferably the shrub species *Derris trifoliata* over other mangrove and shrub species offered (M-W-U test: $p<0.05$). This is in agreement with Salewski (2007), who found that more than 50% of stomach contents of *E. versicolor* were *R. apiculata* and *D. trifoliata*, followed by *Avicennia alba*. *Perisesarma darwinense* even solely consumed *D. trifoliata*. Carbon and nitrogen of preferred leaf species were not assimilated noticeable differently from the others. There is no correlation between the nitrogen and carbon content and C/N of leaves and the consumption rate. This is in agreement with Nordhaus et al. (submitted), who found that mainly the nitrogen compound composition is important. Logged mangrove areas which are overgrown by *Derris* therefore still have a sufficient food supply for *Episesarma* spp. Deforestation might, under these conditions, do not have a species richness reducing effect as previously assumed. Thongtham & Kristensen (2005) found a consumption rate of *Rhizophora apiculata* leaves by *E. versicolor* of 510 mg dry weight leaf/ crab/ day. This is much less than we found in this study (127±64 mg leaf/ **g** crab weight/ day). Salewski (2007) found consumption rates of *Rhizophora apiculata* for *E. versicolor* of 15mg leaf/ g crab weight/ day, which is less then 10% of the consumption rates found in this study. Factors that could have an effect on the consumption rates in this study are (1) the previous starvation period of four days before each experiment, (2) the calculation of the consumption rates including correction coefficients for leaves, (3)

stress induced by laboratory conditions and possibly also (4) the previous diet given in the experiments. The five tree species were provided successively (order of feeding experiments: *R. apiculata, A. ilicifolius, D. trifoliata, A. alba, A. corniculatum*) with four days of starvation in between two approaches. Also a longer implementation of this experiment could have displayed different results.
Discrepancies between leaves´ and faeces´ nitrogen isotopic compositions revealed partly higher isotopic compositions in faeces. Usually ^{15}N typically is depleted in faeces compared to a consumers´ diet or tissue (DeNiro & Epstein 1981a; Checkley & Miller 1989; Ambrose 2000). Therefore it can be assumed that in this case some metabolic processes, such as the buildup of muscle tissue, in the decapods led to an excretion of faeces with a higher $^{15}N/^{14}N$ ratio than usual. Also a high impact of bacteria in the decapods guts, assimilating the isotopically lighter nitrogen, could have an impact. Previous studies found that degraded leaf material passing digestion processes provides an ideal basis for bacterial growth in the hid gut of crabs (Plante et al. 1990; Nordhaus et al. 2007), especially as digestive enzymes are few (Brunet et al. 1994). In case of *Uca* spp. these bacteria are taken up with old fecal material on the sediment surface (Micheli 1993), which have a high bacterial density (Nordhaus et al. 2007; Werry & Lee 2005) and are of high nutritional value (Hall et al. 2006). However, Thongtham & Kristensen (2005) did not find evidence for nitrogen fixation by bacteria in *E. versicolor*.

Herbivorous crabs have generally low assimilation efficiencies of nutrients compared to omnivorous and carnivorous species (Linton & Greenaway 2007). Thongtham & Kristensen (2005) calculated an assimilation efficiency of 41% for carbon and nitrogen respectively for *E. versicolor*. They found that *E. versicolor* is using 77% of assimilated carbon for growth when fed on brown *Rhizophora apiculata*, whereas 17% is lost by respiration and 6% by leaching.

The discrepancy between the nitrogen content (%N) in leaves and faeces is zero for all mangrove species fed. Thongtham & Kristensen (2005) found that assimilated nitrogen from brown *R. apiculata* leaves is balanced by excretion and therefore no nitrogen for growth is left. This balance seems to be apparent for crabs in this study also, which were starved before every experiment.

4.3 Differences between tissues

In *E. versicolor*, *E. dentatus* and *S. serrata* significantly higher δ^{15}N were found in muscle tissue compared to hepatopancreas. Muscle tissue is a long time storage tissue. Therefore the heavy nitrogen isotopic fraction is accumulated in this tissue and mirrors the diet over a long time period, in arthropods e.g. for the last six to eight weeks (Gorokhova & Hansson 1999). Large consumers such as fish have tissue turnover rates ranging from months to years and their isotopic signature is representative of their diet over long periods of time (Post 2002 and references therein). Pinnegar & Polunin (1999) found a significant difference in isotopic compositions between red and white muscle tissue of fish. Previous studies already found higher δ^{15}N values for bivalves' muscle and gills (Cabanellas-Reboredo et al. 2009).

Our data showed that δ^{13}C of muscle tissue is significantly higher compared to hepatopancreas in most of the species. This is in agreement with previous studies suggesting that tissues with low lipid content, such as muscle tissue, have higher δ^{13}C values (McCutchan et al. 2003; Cabanellas-Reboredo & Blanco 2009), as tissue turnover rate is lower here.

4.4 Ovigerous and non-ovigerous females

There was no difference found in isotopic compositions between ovigerous and non-ovigerous females of the two *Episesarma* species. There are two possible explanation approaches: (1) The reproduction time is shorter than the muscle tissue turnover time, and therefore possible differences in metabolism are not reflected in isotopic composition. Diele (2000) showed that *Uca* spp. e.g. take 3.5-4 weeks for egg incubation. Or, (2) the ovigerous crabs are balancing the discharge to their eggs, e.g. by assimilating more, by selection of specific food items such as carrion or controlling ingestion and mechanical fragmentation of food. It is suggested to examine several tissues when comparing ovigerous and non-ovigerous females and also include analysis of eggs in different stadia of development.

4.5 Summary and conclusion

Intertidal mangrove crabs of the genus *Episesarma* are able to balance their nitrogen budget over 50 days and survive three months on a one-choice mangrove-diet only. The species *Derris trifoliata* and *Rhizophora apiculata* were preferably consumed, indicating a high nutritional value of these. Deforested areas, overgrown by *D. trifoliata* therefore still provide sufficient food for *Episesarma* spp. to avoid the expulsion of these species from logged areas. As expected muscle tissue, which is a long-time storage had highest isotopic compositions, compared to hepatopancreas and stomach, as muscle tissue turnover rates are low. There were no significant differences in isotopic compositions between ovigerous and non-ovigerous females. Crabs are probably able to balance the discharge they provide their eggs or their reproduction time is shorter than the muscle tissue turnover time.

The results of this study question the validity of carbon isotopic signatures as tracers for carbon sources within a food web. A previously assumed discrepancy between prey and consumer of 0 to 0.8‰ does not seem to apply for all species in a benthic food web. In case of *Episesarma* spp. the discrepancy might be 2‰ to their main food source, mangroves leaves.

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9 General Discussion

9.1 The benthic food web in the Segara Anakan Lagoon

The total species number of the benthic macrofauna in the SAL discovered until today sums up to 163, from which 127 species are found only in the mangrove forest and 33 species only in the subtidal, 29 species are home in both habitats (Nordhaus et al. 2009). Species diversity of the subtidal zone is three times higher in the east compared to the western area. The 163 species found can systematically mainly be classified into 55 gastropod, 16 bivalves, 57 crustacean and 23 polychaetes taxa. Other groups occurred in low species numbers, such as sipunculids, oligochaets, nematods or chordata. In the subtidal, gastropods (60.7%) and polychaetes (48.8%) contribute largest species numbers in the central and eastern part respectively, whereas in the mangrove forest crustaceans (43.3%) and gastropods (32.3%) predominate (Nordhaus et al. 2009). These numbers are much higher than previously reported by Yuwono et al. (2007) as the resolution of the above quoted study is much higher. Until today the actual species number of the SAL is still unknown, as occasionally new species are found on every expedition (personal observation).

In this study the feeding habits of several intertidal benthic invertebrates were examined in the SAL (Chapter 6). It was observed, that brachyuran crabs can have very diverse food sources. Carnivore and omnivore species, such as *Scylla serrata* (Hill 1976), *Portunus* sp. (Wu & Shin 1998) and *Epixanthus dentatus* (Dahdouh-Guebas et al. 1999), respectively, are the top predators of the intertidal benthic food web in the SAL. *Episesarma singaporense* and *E. versicolor* mainly feed on mangrove leaves (Thongtham & Kristensen 2005; Thongtham et al. 2008), but in the SAL they also supply their diet with animal tissue to satisfy their nitrogen needs. These two species, even though from the same genus, showed differences in feeding habits and also in the occurrence of trophic levels. While *E. singaporense* was consistently found in the same trophic level, *E. versicolor* seemed to be a very opportunistic feeder and showed trophic variability, depending on the sampling site, of more than two trophic levels. *Uca forcipata* and *Metaplax elegans* feed on sediment and detritus. Within this study also for *U. forcipata* a supplementation of nitrogen by animal tissue, such as polychaetes, was observed.

Gastropods in the SAL include detritivore grazers such as *Cerithidea cingulata* and *Telescopium telescopium*. In this study the latter was found to be a facultative scavenger, supplementing its diet occasionally with animal material, i.e. carrion. A further facultative scavenger is *Nassarius* sp. (Scheltemar 1964).

Filter feeders are presented by e.g. *Polymesoda erosa* and *Saccostrea* sp. (Rodelli et al. 1984; Bayne 2002), both economically important species in the SAL. The study of food webs in the SAL indicates a very diverse food web structure spatially different already on a small scale, including complex interactions within the benthic community. The understanding of nutrient flows through this system is therefore very essential to clarify the complex cross-linkages.

This is the first study to define the number of trophic levels in an Indonesian mangrove ecosystem. Depending on the sampling site three to four trophic levels were determined within the SAL. In a comparison between seasons even more trophic levels were indicated during dry season (Chapter 7). Therefore we can conclude that intertidal mangrove benthic food webs are highly dynamic systems and communities are highly adaptable to changing environmental conditions, as long as their habitat is maintained. Invertebrates, especially decapod species are even able to survive a certain time period on nitrogen depleted food sources, if food availability is scarce (Chapter 8).

In a study reporting the status of the SAL, 21 mangrove tree species of 11 botanical families were found, whereof all occurred in the eastern area but only 10 thereof in the central (Hinrichs et al. 2009). The highest total density with 24.8% of all trees has *Aegiceras corniculatum*, followed by *Nypa fructicans*, *Rhizophora apiculata* and *Avicennia alba*. In the eastern lagoon the overall tree density and stem diameter are higher compared to the central, whereas the understorey species percentage cover was higher in the central (Hinrichs et al. 2009). *Derris* sp. and *Acanthus* sp. are shrub species which overgrow logged areas, indicating heavy degradation of mangroves (Whitten et al. 2000). In the SAL their abundance is, probably due to high deforestation activities, higher in the central lagoon (Pribadi 2007). During this study one of the sampling sites was logged completely and was not overgrown with any shrub species. A strong decline of invertebrate species richness and an increase in biomass of especially small gastropod species, such as *Cerithidea* spp. was observed (personal observation). Particularly mainly herbivorous species such as the genus *Episesarma* vanished completely. This showed imposingly what consequences further deforestation activities could have, if mangrove habitats are destroyed continuously in this way. But in this study it was proofed that the genus *Episesarma* preferably feeds on the shrub *Derris trifoliata* (Chapter 8). This indicates that the maintenance of these decapod species will not be endangered as long as this shrub is overgrowing logged areas.

Benthic food webs in the SAL are subject to diverse influences changing seasonally. Especially food availability and quality is altering due to a higher precipitation in rainy season, going along with different water residence times, higher water levels and nutrient availability. During rainy season agricultural derivation from the hinterland is contributing to the nutrient composition in the lagoon. If primary producers such as mangrove trees are affected by these, a location-dependent isotopic shift in consumers' isotopic composition can be observed (Chapter 7).

Several studies quoted that experimental investigations on carbon and nitrogen turnover by organisms are lacking and are urgently required to better understand isotopic fractionation and correctly interpret isotopic compositions in food webs (Boesch & Turner 1984; Adams & Sterner 2000). The experiments conducted in this study contribute to a broader knowledge of isotopic fractionation of *Rhizophora apiculata* in two decapods and their consumption and turnover rates of different mangrove leaves offered. But further experiments on longer time scales with further species need to be conducted under various laboratory conditions, as isotopic fractionation apparently is highly influenced by various factors (Chapter 8).

9.2 Implications for structure, function and dynamics in the Segara Anakan ecosystem

Nowadays, the lagoons water depths in the central part measures only between 0.3m in dry and 1.3m in rainy season. In the eastern area the lagoon has maximum water depths of 13.4m during rainy season at the eastern outlet to the Indian Ocean (Moll, personal communication). If a continuous development of sedimentation is given, it could be assumed that the lagoon is filled up and therefore vanishing soon. But more likely is that, with tidal channels already showing signs of deepening, with rising sea levels and declining trapping efficiencies, a small and stable residual lagoon will always remain (Turner 1985).

The factors affecting the western/ central part of the lagoon are in equal parts the exchange with the Indian Ocean water masses through the western outlet as well as the Citanduy River and the east-west exchange fluxes; whereas the eastern area is mainly affected by the tidal exchange with saline water masses from the eastern outlet (Holtermann et al. 2009).

Food webs within the lagoon are mainly supplied with energy through allochthonous depositions (Fig. 9.1). Only at the entrances to the Indian Ocean outlets, autochthonous contributions, such as phytoplankton and particulate organic matter, can be assumed. Residence times of water masses differ between seasons. In the central lagoon water residence time is 3-6 days in dry and 0-3 days in rainy season. In the east during dry season water residence time is 6-9 days and in rainy season 3-6 days (Holtermann et al. 2009). This indicates a larger export of allochthonous material in the central lagoon, where water is exchanged more often.

In the SAL spatial differences in isotopic compositions of invertebrates are high already within a few kilometers range (Fig. 9.1). Food availability is an important factor when examining food web structures. From Figure 9.1 it is clearly evident that organic material in sediments and particulate organic matter in lagoon water originate mainly from terrestrial vegetation, i.e. mangrove leaves. Invertebrates included in this plot are apparently mainly feeding on leaf litter in the sediment as well as on polychaetes and benthic microalgae, according to the approximation of consumers' isotopic composition to their diets'.

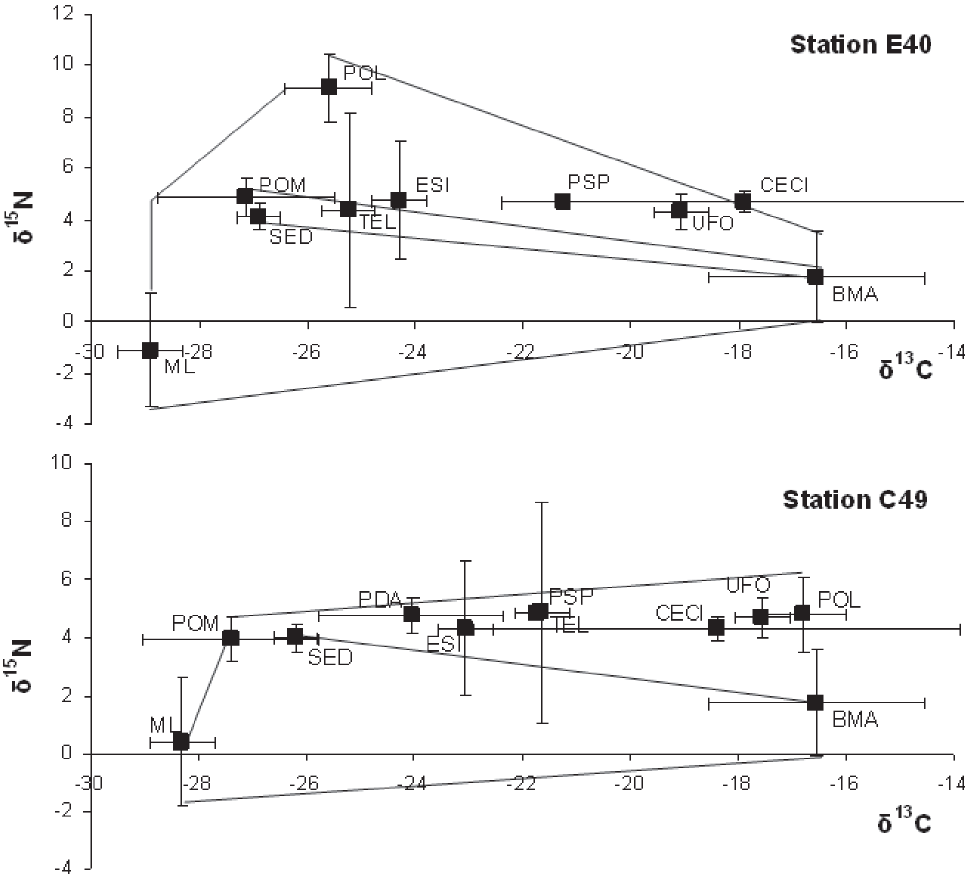

Figure 9.1: Diet-field mixing polygons for $\delta^{15}N$ versus $\delta^{13}C$ of benthic invertebrates (CECI= *Cerithidea cingulata*, ESI= *Episesarma singaporense*, PDA= *Perisesarma darwinense*, PSP= *Periophthalmus* sp., UFO= *Uca forcipata*, TEL= *Telescopium telescopium*) corrected for trophic fractionation (subtracting 2.8‰ per trophic level) of two stations in the east (E40) and central (C49) lagoon. Food sources: BMA= benthic microalgae, ML= mangrove leaves, POL= polychaetes, POM= particulate organic matter, SED= sediment. POM data from Moll et al. (submitted); BMA data from Bouillon et al. (2002b). Lines indicate the areas where isotopic values of consumers (corrected for trophic fractionation) are expected when feeding on a mixed diet (connection points of polygons).

The isotopic fractionation depends highly on the location and the season (Fig. 9.2) and thus on food availability. The spatial and seasonal variation of isotopic compositions of the food web baselines (primary producers) should be aligned to those of the consumers. It is important for future studies to consider these factors (location, season, baselines) regarding food webs and organism interactions, to avoid misinterpretation of data and wrong generalizations.

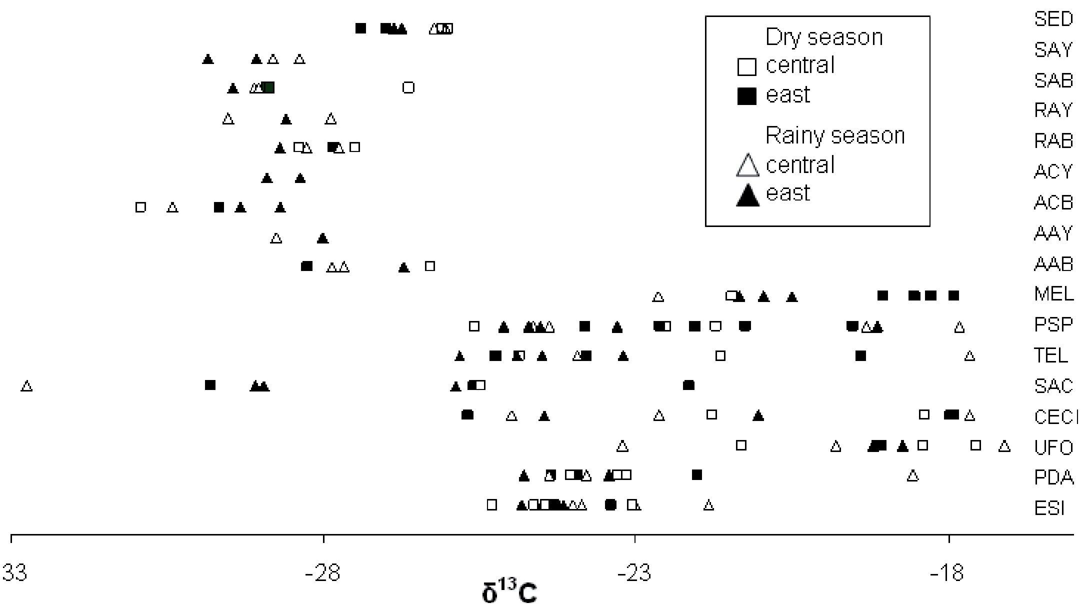

Figure 9.2: $\delta^{13}C$ from sediment (SED), mangrove leaves (AAX= *Avicennia alba*, ACX= *Aegiceras corniculatum*, SAX= *Sonneratia alba*, RAX= *Rhizophora apiculata*, XXB= brown leaves, XXY= yellow leaves) and invertebrates (CECI= *Cerithidea cingulata*, ESI= *Episesarma singaporense*, MEL= *Metaplax elegans*, PDA= *Perisesarma darwinense*, PSP= *Periophthalmus* sp., SAC= *Saccostrea* cf. *cucculata*, TEL= *Telescopium telescopium*, UFO= *Uca forcipata*) in the SAL. Data points are species averages at the single stations.

9.3 Is the SAL a good representative for other tropical lagoon systems?

9.3.1 Mangrove ecosystems in the tropics

In the SAL salinity shows spatio-temporal variations. It increases from the Citanduy, with salinities always near zero, to western and central, where the salinity reflects a mixture of Indian Ocean and Citanduy water. Most saline water masses are found in the eastern lagoon (Jennerjahn et al. 2009). Seasonal variations due to a higher precipitation explain the salinity decrease during rainy season, with a higher impact in the central lagoon resulting from a higher freshwater runoff from the Citanduy (Holtermann et al. 2009). The Citanduy River is rated as moderate on a scale of “pristine” to “heavily impacted” considering nutrient pollution. Nitrogen and phosphorus contribution to the surrounding waters, confining to Java, makes the SAL a high-yield and low-load region compared on a global scale. Considering the SAL as a lower marine trophic state in terms of nutrient concentrations, it ranges between oligotrophic to mesotrophic conditions regarding dissolved inorganic nitrogen and phosphate. Compared to other heavy polluted lagoons, the SAL has low nutrient concentrations (Jennerjahn et al. 2009).

Overall the Segara Anakan seems to be an appropriate representative for tropical mangrove coastal lagoons. Mangrove cover in the SAL is among the highest compared to other study sites in mangrove ecology studies (Table 9.1). Salinities cover the whole range from riverine freshwater discharges to marine inputs. Tidal range is low to moderate in the SAL, as overall maximum tidal ranges are up to five meters (Table 9.1).

Table 9.1: Global comparison of characteristics of shallow mangrove ecosystem coastal lagoons. RS= rainy season.

Source	**Site**	**Area size**	**Mangrove Cover**	**Salinity**	**Tidal range**	**River discharge**	**Precipitation**
		[km²]	**[km²]**		**[m]**		**[mm/year]**
This study	Indonesia	102.4	92.4	0-35	0.3-1.9	x	1824
Nordhaus et al. 2006	Brazil	13800	180	5-37			2508
Rivera-Monroy & Twilley 1996	Mexico	1800			0.7	x	
Bouillon et al. 2000	India	150			2.3-4.5	x	
Conde & Diaz 1989	Venezuela	140		0.5-40		x	
Slim et al. 1997	Kenya	18	6.6		3	x	
Szefer et al. 1998	Mexico	16		26-38	1.5		
Thimdee et al. 2004	Thailand	12	2.6				
Tam et al. 1998	China	3	1.1		2.8		1927
Morell & Corredor 1993	Puerto Rico	1.4				x	
France 1998	Puerto Rico	1.2		20-30	<0.15		
Rao et al. 1994	East Africa		6.6			x	
Holmer et al. 1999	Thailand			35	1-3		2300
Barletta et al. 2003	Northeast Brazil			6-35	4-5		2545
Dham et al. 2002	West India					x	3000 (in RS)
Twilley et al. 1997	Ecuador			1-22		x	885

9.3.2 Comparison with other soft sediment habitats

9.3.2.1 Salt marsh ecosystems

Halophytes in salt marsh ecosystems are nurseries for fish and invertebrates (Boesch & Turner 1984), as are also mangroves. A dominant marsh grass is *Spartina alterniflora* (in the following referred to as *Spartina*). It has the C3 pathway of photosynthesis and has relatively high isotopic compositions compared to mangroves (δ^{15}N= 5.2±0.5‰, δ^{13}C= -12.3 to -13.6‰; Haines & Montague 1979; Sullivan & Moncreiff 1990; Creach et al. 1997). *Spartina* detritus is an important food source for all marsh and estuarine macro fauna. It dominates the carbon and therewith energy flows in the marsh ecosystem by carbon fixation, but equals algae in terms of being assimilated by consumers (Peterson & Howarth 1987). Salt marsh vascular plants are not as digestible as algal organic matter and therefore first have to be mineralized by bacteria (Deegan & Garritt 1997). Marsh halophytes and benthic diatoms are the main carbon source of bacteria (Creach et al. 1997).

It was widely discussed what the actual carbon sources of consumers are in this ecosystem, as for marsh and estuarine consumers a variety of food sources were indicated by a broad spectrum of δ^{13}C values (Peterson & Howarth 1987). The contribution of vascular plants appeared to be minor. Consumers mainly feed on edaphic algae and zooplankton (Sullivan & Moncreiff 1990). *Spartina* only serves as a carbon source indirectly, as its detritus is decomposed by bacteria in sediments, which are assimilated by plankton, which in turn can be assimilated by invertebrates (Peterson et al. 1980; Boesch & Turner 1984). Benthic diatoms are the principal source of organic matter assimilated by macro consumers, but over 50% of food intake by macro invertebrates is of higher plant origin (Creach et al. 1997). Salt marsh food webs therefore depend on a mix of algae and salt marsh organic matter (Deegan & Garritt 1997). When *Spartina* is absent from a salt marsh ecosystem, the base of the food web is formed by macro- and microalgae (Kwak & Zedler 1997).

Variations in nutrient availability in littoral marshes are indicated by animals' δ^{15}N. Depending on the location, phytoplankton and benthic microalgae can be relatively more important food sources than *Spartina* (Peterson & Howarth 1987). In salt marshes the contribution of vascular plants and benthic microalgae respectively changes along the Atlantic coast. These changes are possibly mainly influenced by hydrological factors, i.e. the tidal range, the regularity of flooding as well as freshwater and terrestrial inputs (Sullivan & Moncreiff 1990). Haines (1977) found that organic seston in salt marshes originates mainly from terrestrial carbon sources. Most of the vascular plant detritus exported from a marsh is consumed by detritivorous fish species; and only a small proportion of energy fixed by the marsh grasses is available for higher fish consumers (Boesch & Turner 1984).

Macro invertebrates also have seasonal preferences. Data should therefore be interpreted with caution (Creach et al. 1997).

The contribution of vascular plants within salt marshes is therefore not as important as mangroves are within their ecosystem. At least the contribution of salt marsh plants depends highly on spatial and hydrological factors. The number of trophic levels is similar in both ecosystems, but also varying spatially. In a Mississippi salt marsh ecosystem e.g., consumers were determined to have maximal 2.5 trophic levels (Sullivan & Moncreiff 1990), whereas in California 4 trophic levels for invertebrates were found (Kwak & Zedler 1997). No seasonal trophic shifts were found in $\delta^{13}C$ values but an increase of about 2‰ in $\delta^{15}N$ values from May to September in a Georgia salt marsh (Peterson & Howarth 1987).

A study conducted in a mangrove-salt marsh interface in Australia (Guest et al. 2006) found that burrowing crabs do not move between habitats. They even only forage in a range of 1m around their burrow, due to their territorial behavior. But particulate organic matter from both producers, mangroves and salt marshes, is assimilated by crabs. Carbon moves in the form of particulate material about 5-8m across the mangrove-salt marsh interface, with a slightly greater amount of mangrove carbon into the salt marsh habitat. Therefore carbon and not crab movement explains the patterns in isotopic compositions of crabs across a mangrove-salt marsh habitat boundary. The main food source for the examined crabs in that study was found to be detritus of both habitats. Only in the transition zone an alternative food source was suggested, as a small disparity was found between salt marsh detritus and mangrove crabs.

9.3.2.2 Seagrass ecosystems

Seagrass beds are extremely productive ecosystems in shallow coastal waters with a three dimensional structure (Moncreiff & Sullivan 2001). They provide a habitat for invertebrates and small marine vertebrates and substrate for epiphytic algae assemblages. The detrital food web has long been inferred to be the major trophic path of energy flow in systems dominated by vascular plants (Zieman et al. 1984; Kharlamenko et al. 2001). But the relative contribution of the seagrass itself and their epiphytes remains unclear (Loneragan et al. 1997). Epiphytic algae seems to be a more important carbon source in seagrass ecosystems contributing 75% in contrast to seagrass leaves or detritus with only 25% (Moncreiff & Sullivan 2001).

If seagrass is the dominant carbon source, organisms develop a carbon signature matching or being close to that of seagrass (Kitting et al. 1984). The assimilation of seagrass and salt marsh plants is discussed to be of limited importance as they contain noxious sulfated phenolic compounds that can inhibit bacterial degradation and animal grazing for the majority of consumers (McMillan et al. 1980). Invertebrate isotopic compositions respond rather to shifts in epiphytes than to seagrass isotopic compositions; $\delta^{13}C$ of animals is closer to those of epiphytes than to that of seagrasses (Kitting et al. 1984). In contrast, if mangroves are the dominant carbon source, consumer isotopic compositions approach that of the mangroves but do not overlap with these (Zieman et al. 1984). Small invertebrates e.g. were found to exclusively feed on epiphytic algae on seagrass leaves (Kitting et al. 1984). This can probably be explained by the fact that productivity in epiphytes can be as high or even higher as in seagrasses (Kitting et al. 1984). Food webs in seagrass habitats are therefore rather mainly based on epiphytic algae, sand micro flora and macroalgae (Moncreiff & Sullivan 2001). A study conducted in Puerto Rico comparing carbon sources of nearby habitats found that seagrass and algae were major carbon sources with only little contribution of mangroves in the food webs (Olsen et al. 2010).

When decaying, mangroves have an increase in $\delta^{15}N$ of more than 10‰, whereas seagrass remains about the same. Tropical seagrasses contain more %N (2-4% dry weight or higher) than mangroves (0.5-1.7%). The relative food values (%N, C/N) depend on location, season and age of the source material (Zieman et al. 1984).

During decay, amino acids and proteins leach from seagrasses, whereas in mangroves minimal leaching but high microbial activity occurs, contributing proteinaceous material to the detritus (Zieman et al. 1984). Organisms decaying and consuming seagrass derived material are obtaining more nutritive value from the seagrass substrate, than organisms consuming mangrove detritus. A similar difference between seagrass and mangroves is suggested (Zieman et al. 1984).

In a seagrass ecosystem in the Gulf of Mexico 3-4 trophic levels were found (Mendoza-Carranza et al. 2010), which is equal to the number determined in mangrove food webs.

9.3.2.3 Comparing habitat characteristics

In summary, all three habitats are highly productive and important as nursery grounds for diverse species (Table 9.2). The eponymous flora in the habitats is not compulsory the main carbon source for the respective food webs. The contribution of these decreases from mangroves over salt marshes to seagrasses and coheres with the digestibility of these. The number of trophic levels in invertebrate food webs is similar in all habitats, about two to four trophic levels.

Table 9.2: Comparison of habitat characteristics.

	Mangrove	**Salt marsh**	**Seagrass**
main carbon source	mangroves	plants detritus, algae	epiphytic algae
nursery	fish, invertebrates	fish, invertebrates	Invertebrates, small vertebrates
productivity	high	high	high
photosynthetic pathway	C3, C4, CAM	C3, C4	C3 (majority)
contribution to food web energy	high	50%	25%
δ^{15}N	-4.8 to 6.2‰	5.2±0.5‰	2 to 4‰
δ^{13}C	-30.9 to -26.0‰	-13.6 to -12.3‰	-13 to -9‰
number of trophic levels	3-4	2.5-4	3-4
%N	0.5-1.7%	0.6-1.9%	2-4%
digestibility	high	low	low
amino acid & protein leaching	low		high

9.3.3 Global comparison of species

A global comparison regarding isotopic compositions of eight in the SAL abundant invertebrates revealed that the SAL has two important distinctions compared to similar studies in mangrove ecosystems (Chapter 7). The snail *Telescopium telescopium* had the highest δ^{15}N values of all studies compared. The generally high values also compared to other invertebrates in the SAL are explained by facultative scavenging of this usually mainly detritivorous snail. Furthermore, the global comparison revealed that the oyster *Saccostrea* cf. *cucculata* has among the highest δ^{15}N values in combination with the lowest δ^{13}C values in the SAL during rainy season. The δ^{13}C measured in rainy season for this species is the overall lowest value measured for invertebrates in the compared studies. As *S.* cf. *cucculata* is a filter feeder, this can only be explained by extremely low δ^{13}C in particulate organic matter and phytoplankton in the water column. In case of a sufficient nitrogen supply it can probably also be traced back to selective isotopic fractionation, as oysters filter about 200L water per day (www.Auster.com). These sources are most probably washed in from the agricultural fields in the hinterland during rainy season, bringing along ^{13}C depleted sediments and ^{15}N enriched effluents from fertilizers.

The Segara Anakan therefore seems to be an exception among mangrove-fringed coastal lagoons with regard to the amount of effluent contribution and nitrogen supply.

9.4 Implications of management of the Segara Anakan mangrove ecosystem

Invertebrates in the SAL show a strong dependence on terrestrial carbon sources, such as mangroves. Agriculture and deforestation in the hinterland affect nitrogen supply and mangrove growth in this rather nitrogen depleted environment. As shown in this study mangroves are sensitive to even small changes in nitrogen supply between seasons (Chapter 7).

It is important that in the future the actual impact of humans on the benthic food web is examined carefully in terms of deforestation, pollution through household wastes and by effluents of the oil refinery within a fine spatial resolution. Species that are strong ecological interactors in this mangrove ecosystem, such as e.g. the leaf processing genus *Episesarma* or the top predator of the benthic food web *Scylla serrata*, should be sheltered to maintain the balance of the benthic community. In order to implement a management program, which ensures a sustainable use of the lagoons natural resources, possible consequences of the overexploitation of these until today should be valuated.

9.5 Future scenario for benthic communities in an era of global environmental change

9.5.1 Deforestation and sedimentation

Mangroves are used for wood products, housing and building bridges among others. Furthermore, deforested mangrove areas are converted to aquaculture ponds and agricultural land (Alongi 2002 and references therein). One main feature of mangroves is their function as nursery grounds especially for fish species (Yánez-Arancibia et al. 1994). If mangroves are continued to be logged in the current way, the maintenance of these species is not given anymore. As especially fish carrion serves as nitrogen supplementation for several invertebrate species, the benthic food web will most probably be affected too. Invertebrates would have to find other ^{15}N enriched food sources to rely on. Mangroves also serve as food sources themselves. Intertidal herbivorous decapods feed predominantly on mangrove leaves and leaf litter but also on bark as well as roots or pneumatophores (Salewski 2007). Furthermore, decapods climb mangrove trees to escape predators or night high tides (Hagen 1977; Sivasothi 2000) and hide between roots to avoid predators on the ground (personal observation). Thus deforestation destroys their habitats and regarding herbivores also their main carbon sources.

Mangroves also stabilize sediments and prevent large sedimentation loads into the water body, especially during rainy season. Without this shelter, the water body and therewith the habitat of aquatic species will be reduced in a short time.

9.5.2 Storms, sea-level rise, temperature, precipitation and CO_2

Most of the biodiversity hotspots on earth are tropical forests, and are less vulnerable to climate change in contrast to other biomes (Smith et al. 2001). In the following map from Alongi (2008) it is shown which mangrove forests are most vulnerable to climate change, considering current rates of deforestation and if a mangrove forest is protected in a marine reserve or not. Mangroves occupying low-relief islands or carbonate settings with a low sediment supply and available upland space, such as small islands in the pacific, are most vulnerable, as well as forests without rivers and subsiding landforms are (Alongi 2008). Mangroves on Java are partially in most vulnerable regions.

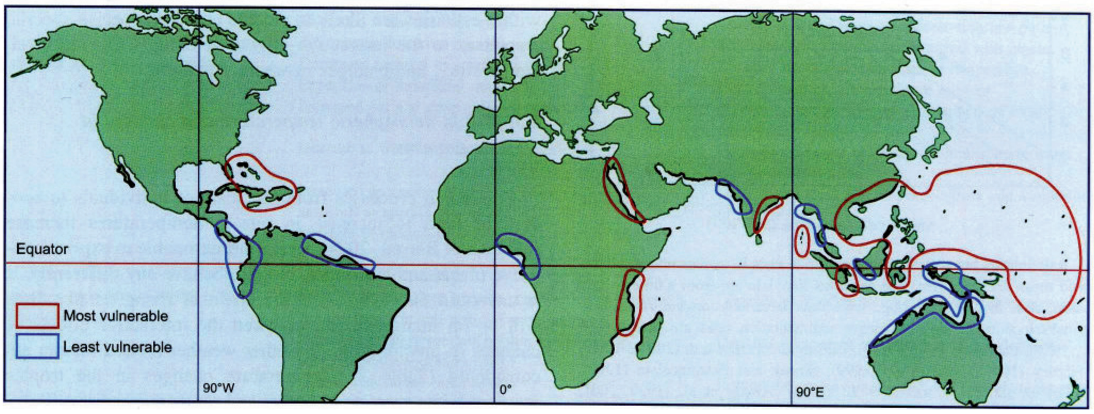

Figure 9.3: Least and most vulnerable regions to climate change of the world´s mangrove forests, from Alongi (2008).

In the following figure from Lovelock & Ellison (2007) factors resulting from climate change are shown which affect mangrove ecosystems in coastal zones.

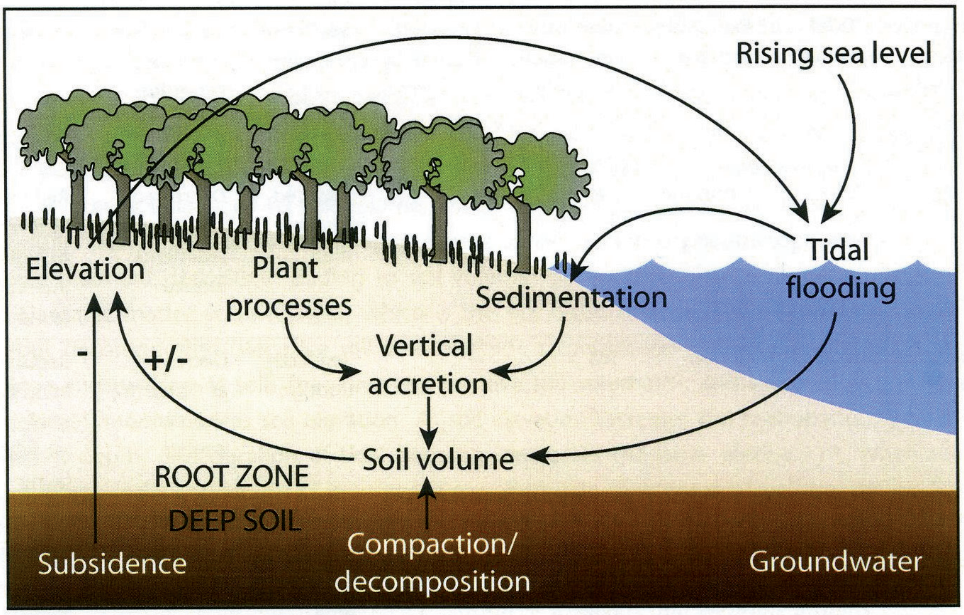

Figure 9.4: Model indicating the processes influencing vertical accretion in mangrove systems, from Lovelock & Ellison (2007).

The increased intensity and frequency of storms, as observed in the last years has the potential to increase damage to mangroves through defoliation and tree mortality (Gilman et al. 2008). And the intensity of storms is predicted to still increase (Solomon et al. 2007). The ability of a benthic population to recover from physical disturbance depends on the magnitude of the disturbance, the supply of larval recruits and the availability of substrate suitable for larval settlement especially in the future (Moran & Reaka-Kudla 1991, Fabricius et al. 2008).

Relative sea level rise could be a substantial cause of future reductions in regional mangrove areas, contributing about 10-20% of total estimated losses (Gilman et al. 2008). Global average sea level rise is 1.8±0.3 mm/year. Regional patterns occur, such as on the west coast of Malaysia and Sumatra, where seas level rise in maximal with values of 5mm/year between 1950 and 2000 (Church et al. 2004). Mangroves in Australia, where natural eroding occurs similar to the effect of sea level rise, are migrating landwards (Hughes 2003). But areas with higher predicted sea level rises will completely inundate the existing mangrove zones, as the mangroves will not be able to keep pace with the retreat (Semeniuk 1994).

In the longer term a sea level rise of 1-2m could lead to a return of swamp conditions with saline wetlands supporting mangrove communities (Woodroffe et al. 1986). As mangroves are particularly vulnerable to sea level rise, an increase in mangrove areas and mitigation of mangroves upslope can be expected (Lovelock & Ellison 2007). The actual amount of intertidal habitat lost with rising sea level will be determined by geomorphology and tidal amplitude (Lovelock & Ellison 2007). “It appears from the geological record that previous sea level fluctuations presented a series of crisis and opportunities for mangroves and that they tended to survive or even expand in several refuges, the most likely being continental coastlines with healthy sediment budgets” (Field 1995).

Rising sea levels can inundate obligate intertidal and shallow water invertebrate species in tropical regions. If the rate of sea level rise is slow, then benthic species and communities have the potential to expand landward (Przeslawski et al. 2008).

Between 1906 and 2005 the global average surface temperature has increased by 0.74±0.18°C (Solomon et al. 2007). Increased surface temperatures are expected to affect mangroves by changing species composition, phonological patterns (timing of flowering & fruiting), increasing mangrove productivity where temperature does not exceed an upper treshhold and expanding mangrove ranges to higher latitudes where range is limited by temperature (Field 1995, Ellison 2000). Mangroves reach a latitudinal limit at the 16°C isotherm for air temperature and not exceeding water temperatures of 24°C (Ellison 2000). The optimum mangrove leaf temperature for photosynthesis is 28-32°C, while photosynthesis ceases when leaf temperatures reach 38-40°C (Clough et al. 1982, Andrews et al. 1984).

It was suggested that some marine invertebrates in Australia will become locally extinct with an increase in water temperatures of 1-2°C (O´Hara 2002). Thermal tolerance is species specific as even closely related species can respond differently to heat stress (Przeslawski et al. 2008). In general, tropical species seem to have less tolerance to temperature variation than their temperate counterparts (Przeslawski et al. 2008). But there is no information on the lethal treshhold temperature for tropical benthic invertebrates. Warmer temperatures increase rates of development and growth across all life stages, depending on the magnitude and duration of temperature change, as well as the physiological and life history traits that determine exposure to thermal change (Przeslawski et al. 2008). Rising water temperatures in the tropics will almost certainly lead to changes in the reproductive phenology and fecundity for those species with spawning periods that are tightly regulated by exogenous cues such as temperature (Lawrence & Soame 2004), as the timing of reproduction for many marine invertebrates is affected (Olive 1995, Bates 2005). A direct effect of temperature and salinity on invertebrates may be changes in the abundance and distribution of suspension feeders, such as mussels, clams and oysters (Scavia et al. 2002).

Warming temperatures are associated with enhanced hydrological cycles which will likely result in increasing occurrences of extreme rainfall events, including drought and flooding which are associated with freshwater coastal runoff (Solomon et al. 2007).

Rainfall is predicted to increase by 25% by the year 2050 in response to climate change (Houghton et al. 2001). These changes in precipitation patterns are expected to affect mangrove growth and spatial distribution (Field 1995, Ellison 2000). Decreased rainfall and increased evaporation will increase salinity and lead to less freshwater input to mangrove ecosystems (Gilman et al. 2008). Also soil salinity will increase, leading to increasing salt levels in the tissues, and therefore decreasing the net assimilation rate per unit leaf area and therewith reducing growth (Field 1995). Reduced precipitation can also result in mangrove encroachment into salt marsh and freshwater wetlands (Saintilan & Wilton 2001, Rogers et al. 2005). In contrast, increased rainfall will result in increased growth rates, biodiversity and an increase in mangrove areas within the tidal wetland zone (Field 1995, Duke et al. 1998).

Changing precipitation patterns can also influence nutrient delivery, which can already be seen in seasonal variations of precipitation and therewith river discharges (Scavia et al. 2002). Salinity changes and coastal runoff due to rainfall can also cause decreased growth and reproduction rates in some invertebrates (Roberts et al. 2006). Responses of benthic invertebrates to stress associated with flooding and salinity changes will vary within and between groups (Przeslawski et al. 2008). But heavy rainfall and associated freshwater runoff will mainly affect invertebrate populations negatively if occurring during reproductive periods (Przeslawski et al. 2008).

The effect of CO_2 on mangroves depends on complex interactions between several physiological and environmental factors (Field 1995). A direct effect of elevated atmospheric CO_2 levels may be an increased productivity of some mangrove species (Field 1995, Ball et al. 1997, Komiyama et al. 2008). Photosynthesis in *Aegiceras corniculatum* and *Avicennia marina* e.g. would be enhanced if CO_2 concentrations would increase (Ball & Farquahar 1984a, b). As salinity increases, stomatal conductance declines with an accompanying decrease in transpiration rates as CO_2 diffusion into the leaf is inhibited and low assimilation rates are resulting (Field 1995). For *Bruguiera* spp. and *Rhizophora apiculata*, in contrast, photosynthesis performance will not be affected by increased CO_2 levels (Cheeseman et al. 1991). Climate change appears likely to produce a net increase in leaf-air vapour pressure difference in warmer-drier regions as well as increasing the extent of such areas (Yeo 1999). Whether elevated CO_2 will lead to a reduction in leaf salt concentrations in salinised plants is uncertain (Yeo 1999). Elevated CO_2 concentrations can be expected to enhance growth of mangroves when carbon supply is limited by evaporative demand at the leaves but not when it is limited by salinity at the roots (Ball et al. 1997). If mangroves were continually exposed to high salinities seawater, their production would likely decline (Snedaker 1995). Soil warming that will accompany any global temperature rise could escalate the increase of atmospheric CO_2 through stimulation of soil respiration (Field 1995). Metabolic responses in mangroves to increased atmospheric CO_2 levels are likely to be increased growth rates (Farnsworth et al. 1996).

Increased CO_2 in surface waters may lower the metabolic rate of some benthic invertebrates due to acidosis, potentially impacting populations by negatively affecting feeding, growth and reproduction (Michaelidis et al. 2005, Pörtner et al. 2005). Sensitivity to elevated CO_2 is highest in animals with high metabolic rates and pH sensitive blood oxygen transport systems (Pörtner et al. 2005). Ocean acidification furthermore may interfere with shell formation in the broad suite of benthic calcifiers (Przeslawski et al. 2008).

In the future high temperatures, low humidity and more severe storms could lead to reduced productivity, subsidence and erosion. A loss of biodiversity of flora and fauna is expected with reductions in salt marsh area and encroachment of mangroves into freshwater marshes. Of economical interest is that the reductions of mangrove areas will decrease the level of ecosystem services they provide (Lovelock & Ellison 2007).

9.6 The usefulness of stable isotopes

Mangrove forests are complex, intertidal, soft-substrate habitats that occur circum-tropically and comprise a substantial portion of protected coast lines and estuaries (Wilson 1989). But only little is known about food web interactions, and the process of passing carbon and nitrogen through the trophic steps of a food web. Macro invertebrate food webs are the main link between high primary production and top consumers in wetlands (Hart & Lovvorn 2002). As the quantification of natural food webs is a universal problem due to methodological and logistical limitations hampering simultaneous measurement of all flows (Oevelen et al. 2006), the method of stable isotope analysis became a helpful method to resolve trophic structures.

The chemical basis for the fractionation of nitrogen involves the lower vibrational frequency of the chemical bonds of ^{15}N compared to ^{14}N. Heavier isotopes form bonds of greater energy than their isotopically lighter counterparts and thus are less likely to undergo chemical reactions (Gannes et al. 1998). Physiologically this means that amine groups containing ^{14}N are favored during transamination and deamination which results in isotopically light excreted nitrogen and the enrichment of certain amino acids such as glutamate (Gannes et al. 1998). Isotopic fractionation is species- and tissue- specific and occurs primarily during the production of new tissues through anabolic processes (Yokoyama et al. 2005a).

In the following two plots from Peterson & Fry (1987), several sources of carbon and nitrogen are summarized with their isotopic compositions and their fractionation ranges. They provide a broad overview over coastal biochemical processes and therewith occurring isotopic fractionations.

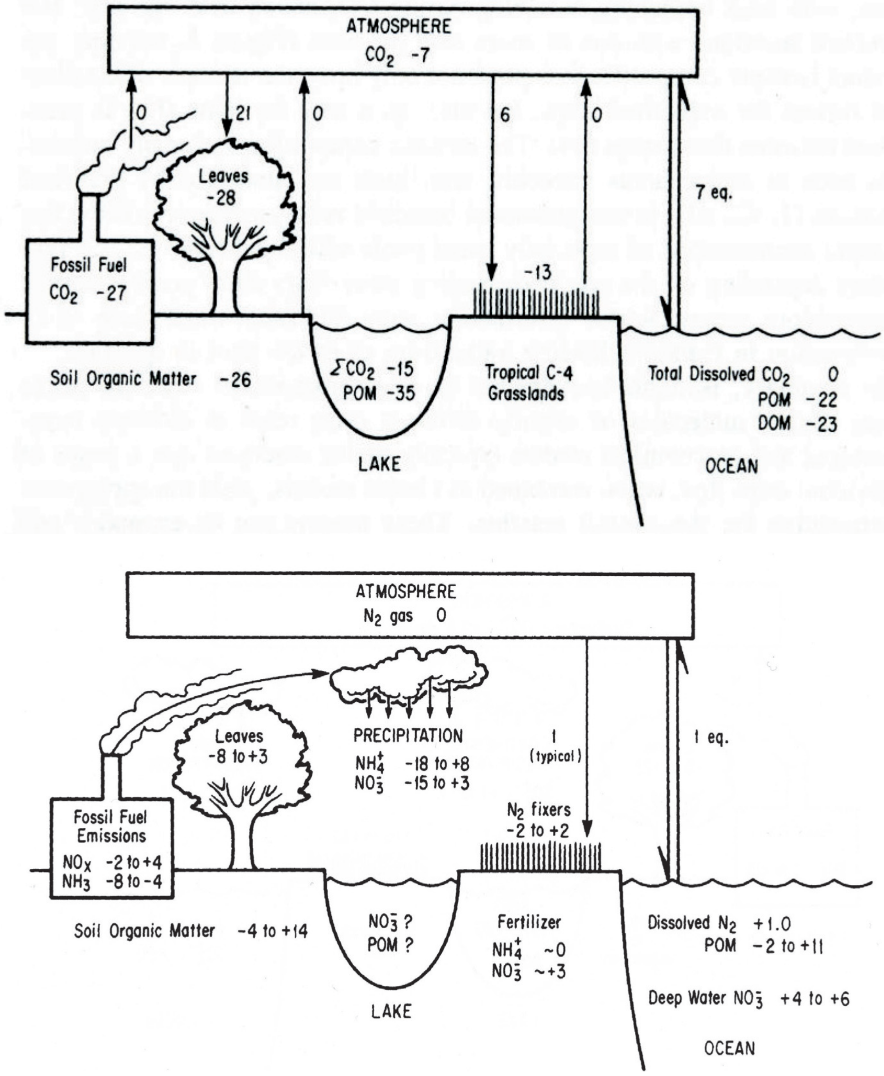

Figure 9.5: The distribution of $\delta^{13}C$ (upper figure) and $\delta^{15}N$ (lower figure) in coastal ecosystems, from Peterson & Fry (1987). Single arrows indicate CO_2 fluxes; double arrow signifies an equilibrium isotope fractionation. Numbers [‰] and arrows indicate fractionation occurring during transfers. POM= particulate organic matter, DOM= dissolved organic matter.

Peterson & Fry (1987) reviewed the use of stable isotopes as tracers in a variety of ecological studies. Nowadays, the stable isotope method is considered to be amongst the most powerful tools to study trophic relationships and animal diets in aquatic and terrestrial ecosystems (McCutchen et al. 2003, Caut et al. 2009). As there is less variability in carbon ($\Delta\delta^{13}C$) compared to nitrogen discrimination ($\Delta\delta^{15}N$), nitrogen seems to be a more specific tool to distinguish between trophic levels (TL) (Minagawa & Wada 1984, Vander Zanden & Rasmussen 2001, Post 2002, McCutchan et al. 2003). The actual discrepancy of $\delta^{15}N$ distinguishing two TL is widely discussed (e.g. Minagawa &Wada 1984, Caut et al. 2009). Adams & Sterner (2000) stated a $\Delta\delta^{15}N$ of 3.4‰±1.1‰ per TL for consumers. Vander Zanden & Rasmussen (2001) verified these findings and added a $\Delta\delta^{13}C$ value of 0.8‰ for tracing carbon sources, meaning that the prey can be identified within a +0.8‰ range from the predator's $\delta^{13}C$ value. They also stated that primary consumers should be used as the baseline TL of a food web, as they form the basic carbon and nitrogen source for higher trophic levels. DeNiro & Epstein (1978) also reported values of $\Delta\delta^{13}C$ from -1.5 to 2.7‰ and a mean of about 0.8‰. McCutchan et al. (2003) found much lower shifts in $\delta^{13}C$ and $\delta^{15}N$ between diet and consumers. They distinguished between measurements of whole organisms ($\Delta^{13}C=0.3\pm0.1$‰) and muscle tissue of organisms ($\Delta^{13}C=1.3\pm0.3$‰) for carbon. For $\delta^{15}N$ they found a discrepancy of $\Delta^{15}N = 2.3\pm0.2$‰ per trophic level. In this study we used the discrimination factors ($\Delta\delta^{15}N=2.8$‰±0.1‰; $\Delta\delta^{13}C=0.8$‰±0.1‰) suggested by Caut et al. (2009) to define the TLs within our food webs, as they reviewed the largest number of isotopes studies until today with an overall number of animal diets of 290.

Different models were set up by distinct authors to calculate TLs based on discrimination factors (Gannes et al. 1998; Post 2002; McCutchan et al. 2003; Tiunov 2007). Variability in these factors is high and the range of discrimination between two trophic levels remains unclear. Previous studies suggested that the discrimination between two TLs is highly dependent on the ecosystem observed and food web examined, such as littoral, pelagic or benthic food webs (Post 2002). It is also widely discussed which model explains best the dietary composition of a predator. Several studies have used different modelling approaches depending on the food web examined and the researchers' appraisement of how many diets should be included (Riera et al. 1999; Hart & Lovvorn 2002; McCutchan et al. 2003; Riera et al. 2004; Oevelen et al. 2006; Tiunov 2007). Also programs such as IsoSource rely on this approach (Benstead et al. 2006). But in fact these are only estimations and actually a reliable statement could only be made if all possible food sources of one predator in a specific ecosystem were sampled and considered. Food composition also depends on the age and the phase of life of the consumer. To obtain the isotopic baseline required to estimate trophic position is one of the most difficult problems facing the application of stable isotope techniques to multiple system food web studies (Peterson 1999; Post 2002). Although there will always be spatial and temporal variation between the baseline and the secondary consumer of interest, a good baseline will integrate isotopic changes at a time scale near that of the secondary consumer and capture the spatial variability that contributes to the isotopic signature of the secondary consumer of interest (Post 2002).

The experiments conducted within this study (Chapter 8) revealed that carbon isotopic compositions do not change if mainly herbivorous species of the genus *Episesarma* are fed on *Rhizophora apiculata* leaves only, over a time period of three month. As variation in $\delta^{13}C$ was very low (<1‰) with one exception, a reasonable doubt arises, that carbon isotopic composition can be a sufficient tracer for carbon sources for all species within a food web. France (1998) also raised these doubts for fiddler crabs in Puerto Rico.

Tiunov (2007) concluded in his review, that a detailed understanding of community composition and structure as well as time related changes in these are required to effectively utilize stable isotopes. He also suggested taking into account fractionation and biogeochemical processes of all levels, from organism to ecosystem level. Nevertheless, stable isotope based estimates of trophic position provide a powerful fusion of trophic level and food web paradigms to evaluate actual trophic structures of complex food webs (Post 2002).

9.7 Synthesis

9.7.1 Answering study questions

1 Do food web structures in the SAL vary on small spatial scales due to varying food availability and quality resulting from different anthropogenic impacts?

Overall four trophic levels (TL) of consumers were found in the intertidal benthic food web of the SAL. The majority of invertebrates, especially several decapods, can be assumed to use a range of food sources opportunistically. They supplement their mainly mangrove derived diet by feasible scavenging e.g. in form of carrion. Different species from the genus *Episesarma* can adapt differently to changing food availability and therefore occurred in different TLs comparing study sites already on a small scale. Due to the opportunistic diet of several decapod species it is concluded that they are highly adaptable to changing food availability.

2 Do seasonal changes in environmental factors lead to a divergent food availability and consequently to changes in the food web structures?

Seasonal differences in isotopic compositions of invertebrates muscle tissue were found, but did not follow any clear patterns. The discrepancy of isotopic compositions between seasons depends on the species and the location within the lagoon. Spatial variability was high during rainy season, with higher δ^{15}N in the eastern part of the lagoon. During dry season in contrast, variability between areas was small. Factors that have an impact on seasonal differences include precipitation and therewith nutrient availability, freshwater plankton input through rivers, marine plankton input through tides and carbon and nitrogen contents in sediments as well as food quality.

3 a Is a one-choice diet affecting isotopic compositions in muscle tissues of benthic intertidal crabs within a time period of three months?

Intertidal mangrove crabs of the genus *Episesarma* are able to balance their nitrogen budget over 50 days and survive three month on a one-choice-mangrove-diet only. After 50 days an increase in $\delta^{15}N$ was observed, probably resulting from starvation and internal nitrogen recycling. $\delta^{15}C$ values did not change within this time frame.

b Do intertidal crab species prefer certain mangrove species? How do they metabolise the leaves?

The species *Derris trifoliata* and *Rhizophora apiculata* were preferably consumed, over *Acanthus ilicifolius*, *Aegiceras corniculatum* and *Sonneratia alba*, indicating a high nutritional value of these two species. A difference in ingestion and egestion of the offered species by the two *Episesarma* species due to different feeding habits in the field were not observed.

c Do isotopic compositions differ substantially between hepatopancreas, muscle tissue and stomach content according to their role and status within the assimilation process?

As expected muscle tissue, which is a long-time storage had highest isotopic compositions, compared to hepatopancreas and stomach content with the lowest values.

d Do ovigerous females have lower isotopic compositions than non-ovigerous females?

There were no significant differences found comparing ovigerous and non-ovigerous females, both groups have similar isotopic compositions. This can probably either explained by the ability of crabs to balance the discharge they provide their eggs, or by a shorter reproduction than muscle tissue turnover time.

9.7.2 Conclusion

Mangrove ecosystems are highly dynamic systems, with spatially and temporally varying environmental conditions on small scales. Macrobenthic intertidal communities are perfectly adapted to these continuous changes in their surroundings such as e.g. food availability. However, deforestation constitutes a threat to these communities, which means an extreme habitat change. Pollution by household sewages and even oil refinery effluents do not seem to affect the invertebrates feeding habits in the SAL. But differences in isotopic compositions in invertebrates on a small spatial scale indicate that stable isotopes are highly sensitive indicators for surrounding environmental and anthropogenic impacts.

In ecology the method of stable isotope analysis is particularly helpful to study food webs if combined with further knowledge, such as on feeding habits, stomach contents, species interactions or experimental outcomes regarding e.g. food choices and assimilation. But it is questioned if carbon isotopic signatures are sufficient tracers for carbon sources within a food web.

For future studies it is suggested to consider the season in which samples were taken, as well as the representativeness of one location in a study area for the whole area. Experimental approaches to further clarify the processes of isotopic fractionation in numerous organisms are essential. A more elaborate set of food sources, i.e. primary producers, should be included to decipher mixed diets of consumers.

9.7.3 Outlook

In the future additional experimental approaches shall contribute to the clarification of biochemical material cycles through the benthic food web. Such experiments were not conducted until today but are suggested in recent food web studies as the necessary next step to better interpret and understand the results of stable isotope analysis.

Due to time limitation only abundant species were included in this study. For a complete picture of the benthic food web and a quantitative estimation of the turnover of carbon and nitrogen by benthic invertebrates in the lagoon, further species, especially of high biomasses such as *Sesarma* and *Uca* spp. and top predators such as *Portunus* spp., should be incorporated in a complementing project.

As it is presumed that intertidal benthic invertebrates in the SAL are highly adaptable to changing environmental conditions and therefore food availability, a location exchange experiment in field should be conducted. Different crabs from intact mangrove areas that are exposed to logged areas e.g. should cope differently with this drastic habitat change.

One choice feeding experiments over a time period of at least 4 months with diverse food sources should be conducted, to examine actual species specific turnover rates of different diets. Also two to three food sources could aid to understand the isotopic composition of consumers on mixed diets. This experiment conducted with shrub species as food sources could aid to estimate the consequences of deforestation.

Crabs that died within the experiments conducted in this study showed fast increases in $\delta^{15}N$ values after a few days only. This also occurs in starving crabs. It is known, that invertebrates supplement their diet with ^{15}N enriched sources, such as carrion of dead organisms. In an experiment it should be examined how fast the light nitrogen fraction (^{14}N) is degraded in dead invertebrates, so that they can serve as a valuable nitrogen source for other invertebrates.

Apart from the tissues that were analyzed for stable isotope composition in this study a more detailed analysis of all available tissues should be accomplished, including exuviae of decapod species. This would lead to a better understanding of metabolic processes of benthic decapods.

Stomach content analysis was conducted in this study for few selected species. As was discussed before, stomach content analysis is a very important complementation to stable isotope analysis. It should therefore be examined for several important benthic invertebrates. Variation in food preferences could be examined by including several different study sites in this approach. Also further consumption rates with different decapod species and food sources could aid to quantitatively estimate the turnover of benthic invertebrates in this ecosystem.

All these experiments could help to quantitatively estimate the turnover of carbon and nitrogen by benthic invertebrates in this ecosystem and to elucidate food web interactions previously only examined by stable isotope analysis mostly of muscle tissue.

In future investigations the actual contribution by benthic microalgae, such as benthic diatoms, as carbon and nitrogen sources to the benthic food web in the SAL, should be investigated. Benthic microalgae play an important role as primary producers and therefore as a food source especially for organisms of the first trophic level, such as deposit feeders, in the benthic food web. Also primary producers in the water column should be analyzed for stable isotopes and actual contributions of these as a food source to the benthic food web should be estimated.

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