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Decomposing mangrove litter supports a microbial biofilm with potential nutritive value to penaeid shrimp post larvae

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ABSTRACT

The use of fish meal in shrimp culture not only contributes to the decline of wild fish stocks, but also undermines its profitability and enhances ecosystem pollution. There is an urgent need for alternative natural food supply in shrimp cultures. The present study investigated the potential of mangrove litter from *Rhizophora mucronata* and the associated microbial biofilm as food for shrimp post larvae of *Penaeus indicus* and *Penaeus monodon* in a community-based ecological shrimp farm in Mtwapa creek, Kenya (3°57′S; 39°42′E). Senescent mangrove leaves were incubated together with shrimp post larvae, PL 15–25, for 6 weeks in shallow mangrove pools. Leaf litter degradation, carbon and nitrogen nutrient remineralization, bacterial community structure and algal biomass in the periphytic biofilm were investigated weekly for 6 weeks. Food uptake and assimilation was assessed by comparing fatty acid profiles and $\delta^{13}C$ isotope values in the shrimp tissue, litter and biofilm. Post larvae from the open creek were used as a control. Decomposing mangrove litter supported the growth of microalgae and bacteria in the form of periphytic biofilm with a maximum growth at the 3rd and 4th weeks when the litter was 43% decomposed. Bacterial community varied in structure with the progress of litter decomposition by declining in abundance after the 3rd week toward a minimum at the 6th week. The diversity of bacterial colonies also changed from a high dominance, at the early stages of litter decomposition, to evenly diverse colonies in the litter decomposed beyond 5 weeks. Shrimp stocked in mangrove forest had 1) highest levels of linoleic acid, linolenic acid and highly unsaturated fatty acids (HUFA) in the 3 to 4 weeks old litter, 2) lower fatty acid levels compared to the shrimp from the creek and 3) were isotopically close to seagrass and biofilm. In terms of nutritional value, mangrove litter supports penaeid shrimp post larvae with a periphytic biofilm during the early stages of decomposition (weeks 3–4). The results of this study suggest that optimal nutrient supply to ecological shrimp aquaculture in mangrove systems could be optimized by controlling residence time of mangrove litter in shrimp ponds and selecting sites linked to other ecosystems such as creeks and open sea.

1. Introduction

In the year 2007, a once lucrative semi-industrial shrimp trawling activity of the commercial species *Penaeus monodon* and *Penaeus indicus* along the Kenyan coast (Indian Ocean) was officially closed due to assumed depletion of shrimp stocks (GOK, 2006). The closure came just eight years after a once successful and viable alternative practice of shrimp culture in Ngomeni bay (Rasowo, 1992) collapsed under the management of a local government ministry (FID, 1999). Although a good alternative to the declining wild shrimp fishery, shrimp aquaculture industry has been criticized severely for causing environmental and socio-economic problems globally (Naylor et al., 2000; Primavera, 1998; Ronnback et al., 2002). However, with a better understanding of shrimp biology and ecology, shrimp aquaculture can be sustained by adopting ecologically healthy practices (Primavera, 2006; Rothlisberg, 1998).

Penaeidea, included in the decapod suborder Dendrobranchiata, inhabit shallow and inshore tropical and subtropical waters and comprise most of the total world catch of shrimp (Dall et al., 1990). The large *Penaeus* species are of the greatest value and includes, *P. monodon*, which may exceed 200 g and a total length of 336 mm has been recorded (Dall et al., 1990). Tropical penaeids spawn twice a year (Rothlisberg, 1998). Adult penaeid shrimp migrate into the open water to spawn, where fertilized eggs are shed free into the water where they hatch and develop into various stages ranging from nauplius, protozoea and mysis. The shrimp-like postlarvae reach inshore waters about two weeks after hatching where they become demersal and settle on various habitats among them the muddy banks of mangrove

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lined estuaries and seagrass beds (Dall et al., 1990). Understanding penaeid life cycle is of great importance in ecological shrimp aquaculture especially when the supply of wild postlarvae to seed shrimp culture ponds is required (Phillips et al., 1993). The ontogenetic shift in diet in the different larvae stages even within the progressing postlarvae stage (Rothlisberg, 1998) also necessitates a good biological knowledge in order to manage food inputs with the relevant nutrition. For instance, protozoea are generally herbivorous while mysis and postlarvae become increasingly carnivorous. The early stages are also opportunistic feeders, for instance if the diatoms dominate the environment they will dominate the diet (Preston et al., 1992). Fish and shrimp larvae are very sensitive to the deficiency of certain fatty acids (FA) such as the n−3 poly unsaturated fatty acids (PUFA) (Sorgeloos and Lavens, 2000; Watanabe et al., 1983). This essential nutrient is ultimately derived from the natural food sources such as the phytoplankton, zooplankton and macro-invertebrates (Parrish, 2009). Bacteria are also abundant in the natural food sources and are therefore a potential food source (Azim and Wahab, 2005; Burford et al., 2004; Keshavanath and Gangadhar, 2005). Nutrition biomarkers such as the fatty acids (Alfaro et al., 2006; Coutteau et al., 1999; Kelly and Scheibling, 2012; Lee and Meziane, 2006; Parrish, 2009) and stable isotopes (Boecklen et al., 2011; Bouillon and Boschker, 2006; Brito et al., 2006; Primavera, 1996) have widely been used to identify and nutritionally qualify potential food sources in both ecological and aquaculture studies.

Ecological shrimp aquaculture also referred to as extensive aquaculture differs from the intensive aquaculture in the sense that shrimp are stocked in low stocking densities and culture conditions are manipulated to enhance proliferation of natural food sources to feed the stocked shrimp (Primavera, 1998). Many reports have criticized intensive shrimp aquaculture due to its overreliance on fish meal despite the decreasing wild fish stocks (Naylor et al., 2000; Ronnback, 2001; Tacon, 1996), its potential to undermine profitability (Naylor et al., 2000) and its contribution to ecosystem pollution (Lin, 1989; Primavera, 2006; Primavera et al., 1993). Extensive shrimp aquaculture has also been blamed for its need for space leading to mangrove deforestation and ecosystem modification (Primavera, 2006; Spalding et al., 1997). However, there is a growing support for ecological shrimp aquaculture since it involves farming up the food chain and therefore sustaining a continuous supply of ecosystem services from the adjacent coastal habitats such as the mangrove wetlands and seagrass beds (Kautsky et al., 2000; Naylor et al., 2000; Primavera, 1998, 2006).

Sustainability of ecological shrimp culture can be supported by identifying the preferential natural food sources of the target shrimp species that could naturally stimulate the nutritional requirements of shrimp in culture conditions. Mangrove litter, mainly consisting of decaying leaves, in addition to other primary producers of natural coastal habitats leaches large amounts of soluble organic material which supports a microbial food web that ultimately may serve as food for (post) larvae (Benner and Hodson, 1985). However, microbial nutrition on the mangrove litter may not be optimal as such, since the microbiota may also require import of material from adjacent ecosystems. For instance, Bouillon and Boschker (2006) demonstrated a microbial preference of carbon from root exudates and microphytobenthos. Such an observation may contribute to identify the preferred ecological setting of shrimp culture activities, especially in subtidal zones where the exchange of nutrients between habitats or ecosystems likely optimizes the proliferation of natural food for shrimp post larvae. The present study identifies the growth of a microbial and microphytobenthic biofilm (or the periphyton) on decomposing mangrove litter and uses fatty acid and stable isotope biomarkers to estimate to what extent this periphyton contributes to the natural diet of shrimp post larvae. The outcome of the present study is important to support sustainable shrimp farming in mangrove forests worldwide in order to reduce the impact on the overall functioning in coastal ecosystems.

2. Materials and methods

2.1. Study site

The study was carried out in a mangrove forest and shrimp ponds at Majaoni Silvofishery and the mangrove conservation Farm located in Mtwapa creek, Northern coastal region of Kenya (3°57′S; 39°42′E). Mtwapa creek is characterized by a reforested mangrove forest dominated by *R. mucronata*. The penaeid shrimp species *P. indicus* and *P. monodon* are commonly fished within this creek.

2.2. Shrimp feeding experiment

Senescent mangrove leaves (hereafter referred to as mangrove litter) which had just turned yellow-brown and dropped from the trees were dried in the shade to a constant weight and incubated in shallow mangrove pools at a concentration of 10 g l^{-1}. The shallow mangrove pools (0.6 m deep during low tide) were enclosed with a netting cage (hapa) measuring 4 m^2 base area, 2 m high and covered at the top to prevent content overflow during high tide. Shrimp postlarvae (PL) of *P. indicus*, PL 25–35, were stocked in one set of hapa containing mangrove litter and another set without litter in triplicate at a density not exceeding 1 PL l^{-1} for a period of 6 weeks. Shrimp sampled from the open creek were used as control. Mangrove litter, the associated biofilm (the periphyton) and the shrimp were weekly sampled for fatty acid analysis and $\delta^{13}C$ stable isotope measurements by immediate storage at −20 °C and then transferred to −80 °C prior to analysis. The biofilm was also weekly sampled for algae biomass, chlorophyll a, bacterial abundance and diversity.

2.3. Litter degradation and nutrient analysis

Senescent mangrove leaves were dried in the shade to a constant weight placed in litter bags (30×30 cm, 2 mm mesh size) and incubated in shallow mangrove pools for 6 weeks. A batch of leaf samples each weighing the respective weights as in litter bags were retained and oven dried at 80 °C to a constant weight to get initial dry weight and allow for initial carbon and nitrogen concentration analysis. Litter bags were weekly sampled in triplicates. Biofilm on mangrove litter was scraped off and gently washed with distilled water and oven dried at 80 °C for 24 h and weighed. Litter degradation was recorded as percentage weight loss. Litter samples for nutrient analysis were finely ground, weighed and placed in tin capsules, and analyzed for carbon and nitrogen content using a Flash 2000 Organic Elemental Analyser (Thermo Scientific, Italy).

2.4. Algal biomass

Mangrove leaves were sampled, weekly, in triplicates by pooling 3 leaves per sample. The periphytic biofilm was gently scraped from the surface of the mangrove leaves with a known volume of filtered sea water and filtered over a glass fiber GF/F filter (0.45-μm mesh, 47-mm diameter). The surface area of both sides of the leaf was measured in order to convert the algal biomass from Chl a (μg l^{-1}) to μg cm^{-2} of leaf surface. Phytopigments were extracted from the collected biofilm after adding 10 ml 90% acetone to the lyophilized GF/F filters at 4 °C in the dark and the supernatant was analyzed for chlorophyll a according to a modified protocol of Granger and Lizumi (2001).

2.5. Fatty acid extraction and analysis

Samples of shrimp tissue, mangrove litter and biofilm were freeze-dried (lyophilized), weighed and fatty acids extracted and methylated to fatty acid methyl esters (FAMEs) by a modified one-step derivatization method after Abdulkadir and Tsuchiya (2008). The fatty acid

methylnonadecanoate C19:0 was added as an internal standard for later quantification (Fluka 74208). The boron trifluoride–methanol reagent was replaced by a 2.5% H_2SO_4–methanol solution since BF_3–methanol can cause artifacts or loss of PUFA (Eder 1995). FAMEs were dried of hexane using a Rapid Vap Machine: Labconco Corporation, USA; at a speed of 50, 30 °C, 240 mbar, then dissolved into 1 ml hexane and analyzed using a Hewlett Packard 6890N GC equipped with a mass spectrometer (HP 5973). The samples were run in splitless mode injecting 1 μl extract per run at an injector temperature of 250 °C using a HP88 column (60 m × 0.25 mm internal diameter × 0.20 μm film thickness) (Agilent J&W; Agilent Co., USA). Helium was used as carrier gas. The oven temperature was programmed at 50 °C for 2 min, followed by a ramp at 25 °C min^{-1} to 175 °C and then a final ramp at 2 °C min^{-1} to 230 °C with a 4 min hold. The FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and available spectra in mass spectral libraries (WILEY, NITS05), and analyzed with the software MSD ChemStation (Agilent Technologies). Quantification of individual FAMEs was accomplished by the use of external standards (Supelco # 47885, Sigma-Aldrich Inc., USA).

Fatty acid biomarkers which are essential to the physiological performance of shrimp postlarvae were identified (Kanazawa et al., 1977, 1978, 1979; Sorgeloos and Lavens, 2000) and included 18C-PUFA (linoleic acid; 18:2n6 and linolenic acid; 18:3ω3) and HUFA (EPA; 20:5ω3 and DHA; 22:6ω3). Fatty acid trophic markers (Alfaro et al., 2006) were used to identify potential food sources for shrimp in mangrove litter and the periphytic biofilm: (1) $\Sigma 16/\Sigma 18 > 2$ and 16:1/16:0 > 1 as proxy for microalgae; (2) 16:1/16:0 > 1.6, $\Sigma 16/\Sigma 18 > 2$ and 20:5ω3 (EPA) as proxy for diatoms; (3) 20:5ω3/22:6ω3 < 1 was used to identify food sources from dinoflagellate and planktonic algae (Parrish et al., 2000); (4) 20:1 + 22:1 refers to food sources from zooplankton (Falk-Petersen et al., 2002).

2.6. Stable isotope analysis

Stable isotope measurements ($\delta^{13}C$) were performed on biofilm, mangrove litter and shrimp tail muscle by oven drying samples at 60 °C for 24 h. For shrimp tissue, at least 6 individuals were pooled. Only muscle tissue (specifically from the tail) was used because its slow turnover rate reflects integrated diet effects over months and thus excludes short term variability effects (Gearing, 1991). Dry shrimp tissue and litter were ground to fine powder, triplicated and wrapped in tin capsules. Stable isotope signatures were measured with a continuous flow isotope ratio mass spectrometer (type Europa Integra) by the UC Davis Stable Isotope Facility (University of California, USA). Stable carbon ratios are presented as δ values where $\delta = [(R_{sample}/R_{standard} - 1)] \times 1000‰$; with $\delta = \delta^{13}C$ and $R = {}^{13}C/{}^{12}C$; $R_{standard}$ = Vienna Pee Dee Belemnite (VPDB = 0.01118) (Fry, 2008).

2.7. Bacterial abundance

The biofilm covering a uniform area of leaf surface (18 cm^2) was gently scraped off with a glass cover slip and washed with distilled water into a falcon tube. Scraped biofilm was thoroughly mixed in distilled water and a subsample of known volume was sampled, filtered through a 0.2-μm filter, oven dried at 60 °C for 24 h and weighed. The remaining biofilm was preserved in 2% formalin and stored at 4 °C until analysis. During analysis, total prokaryotic abundances (TPA) were determined using Acridine Orange (Danovaro et al., 2001). For all replicates and treatments, 0.5 g of biofilm equivalent volume was transferred into sterile vials and fixed with 4 ml of 2% formalin PSBF (0.2 μm pre-filtered and salt-buffered, $Na_2B_4O_7 \cdot 10H_2O$). Samples were sonicated three times (Branson Sonifier 2200; 60 W for 1 min) with intervals of 30 s during which they were manually shaken, diluted 10, 100 and 1000 times with PSBF, stained for 5 min with Acridine Orange (final concentration 0.01%) and then filtered onto black Nuclepore polycarbonate filters (0.2-μm pore size) at ≤100 mm Hg. Bacterial counts were performed under an epifluorescence microscope at 100× magnification with a valid count of 200 cells between 10 and 50 fields. Bacterial cells emitted bright green fluorescence while detritus and clay emitted red fluorescence (Bolter et al., 2002). Triplicate counts in No. cells/g were considered statistically valid at a coefficient of variance below 30%.

2.8. Bacterial community analysis by DGGE

Bacterial DNA from biofilm was extracted using FastDNA® SPIN KIT for soil according to the manufacturer's recommendations. From each DNA extract a fragment of the V3 region of the 16S rRNA gene was amplified using the primer set 357F and 518R (SigmaAldrich) (Van Hoorde et al., 2008) with a GC-clamp (5′CGCCCGCCGCGCGCGGCGGGCGGGGC-GGGGGCACGGGGG3′) (Temmerman et al., 2003) coupled to the forward primer. PCR mixtures were prepared according to De Troch et al. (2010). A touchdown PCR (De Mesel et al., 2004) with 10 cycles of decreasing annealing temperature (0.5 °C $cycle^{-1}$ decrement, from 61 to 56 °C) followed by 25 cycles of regular PCR was performed with a Bio-Rad DNA thermal cycler. Subsequent DGGE analysis using a 35–70% gradient and staining of the gel were done as described by Van Hoorde et al. (2008). Digitized DGGE gels were normalized and analyzed by means of the BioNumerics Software (version 4.61, Applied Maths, Sint-Martens-Latem, Belgium). Calculation of the Pearson correlation coefficient and application of Unweighted Pair Group Method with Arithmetic Mean (UPGMA) resulted in a dendrogram visualizing similarity between the bands' pattern of biofilm scraped from litter at different weeks of decomposition.

2.9. Water quality parameters

Water quality was recorded weekly by measuring temperature, dissolved oxygen, pH, salinity and total ammonium nitrogen.

2.10. Data analysis

Statistical analyses (ANOVA) were conducted with Statistica 7.0 software. All data were checked for normality and variance homogeneity requirements for parametric analysis. Data which did not meet normality requirements after being transformed were analyzed non-parametrically following Kruskal–Wallis ANOVA & Median Test. Multidimensional scaling (MDS) and analysis of similarity (ANOSIM) were used to compare similarity in the distribution of target parameters using Primer 6.0 software. A Bayesian stable isotopic mixing model (Parnell et al., 2010) in SIAR v4 (stable isotope analysis in R) was applied to estimate the likely contribution of each potential food source to the diets of shrimp. Data were mean isotopic signatures of all replicate samples of shrimp foraging in various estuarine habitats (open creek, bare mangrove, decomposing mangrove litter) and potential food sources (biofilm, leaf litter, seagrass). Mean trophic enrichment factors (fractionation/discrimination factors) of 1 ± 1.2‰ for $\delta^{13}C$ were considered for the food sources as suggested by Vander Zanden and Rasmussen (2001) and adopted by Ouisse et al. (2012).

3. Results

3.1. Leaf litter degradation and nutrients

Mangrove leaf litter decomposed rapidly within the first 3 weeks with a relative weight loss of 1.8–2.5% day^{-1} (Table 1). However the rate of decomposition declined to only 1.0% day^{-1} between the 5th and 6th weeks when they were already decomposed for 53 ± 3% and 61 ± 1%, N = 3, respectively. In a period of 6 weeks, nitrogen and carbon contents increased by 300% and 16.6% respectively in

Table 1
Nitrogen and carbon content in decomposing mangrove litter of *Rhizophora mucronata*. Values in brackets represents the standard error.

Weeks	0	1	3	4	5	6
Leaf weight loss %	0	17.5 (8)	43.4 (2)	42 (2)	53.1 (3)	60.8 (1.2)
Daily leaf weight loss %	0	2.5	1.8	0.6	0.3	1
Carbon (mg/g)	433.9 (9.8)	451.2 (2.8)	460.9 (4.4)	467.8 (4.6)	475 (7.5)	506 (9.4)
Nitrogen (mg/g)	1.4 (0.26)	1.56 (0.23)	2.2 (0.42)	2.97 (0.30)	2.51 (0.35)	3.51 (0.30)
C/N ratio	309.3 (37.9)	289 (12.1)	209.2 (10.4)	157.7 (15.4)	189.2 (21.5)	144.1 (30.4)

the decomposing mangrove leaf litter, while the C/N ratio dropped from 309.3 ± 37.9 to 144.1 ± 30.4, $N = 3$. The organic nitrogen content increased over the entire period of litter decomposition shifting from 1.40 ± 0.26 to 3.51 ± 0.30 mg g^{-1}, $N = 3$; within 6 weeks.

3.2. Bacterial abundance and micro algal biomass

The submerged mangrove litter supported the growth of bacteria as a constituent of the periphytic biofilm although with varying abundances at different stages of the decomposition process. The initial stage of the litter decomposition is marked by a rapid bacterial colonization occurring after 3 weeks coinciding with the highest rate of decomposition (2.5%) (Table 1). The maximum number of bacteria recorded at this stage reached an abundance of $3.99 \times 10^9 \pm 8.03 \times 10^8$ cells g^{-1}, $N = 9$, of the periphytic biofilm (Fig. 1). A similar fast growth of microalgae in the biofilm was found during the first 3 weeks of litter decomposition and maintained up to week 4 recording a maximum chlorophyll a of 3.33 ± 0.63 μg cm^{-2}, $N = 3$, of leaf litter surface. Both bacteria and microalgae seem to collapse after respectively 3 and 4 weeks of litter decomposition. A rapid decrease in bacterial abundance is observed after the 3rd week with a minimum bacterial abundance of $1.44 \times 10^9 \pm 1.21 \times 10^8$ cells g^{-1}, $N = 9$, in week 6. However, overall, no significant difference was observed in bacterial abundance between the weeks (Kruskal–Wallis test, $H_{(4,25)} = 8.459$, $p = 0.0761$). Microalgae, on the other hand, declined after the 4th week which statistically differed with the 6th week when the lowest biomass of 0.43 ± 0.009 μg cm^{-2}, $N = 3$, was recorded (Tukey Post Hoc: $p = 0.01090$). Apart from the 3rd week, which was not significantly different from the 4th week ($p > 0.05$), all other pairwise comparisons with the 4th week were significantly different ($p < 0.05$) (Fig. 1).

3.3. Bacterial community

The structure of the bacterial community in the periphytic biofilm varied at different stages of leaf litter decomposition (Fig. 2), indicating a strong temporal change in the composition of the microbial community. Pearson's correlation coefficient revealed 2 clusters of bacterial communities with a similarity level of >40% each. The first large cluster (top cluster in Fig. 2) consists of three sub-clusters. The bottom 2 sub-clusters, containing only samples from week 4 or beyond, are characterized by the absence of dominant bands, unlike in the other sub-clusters. These two sub-clusters are more homogenous in composition (higher similarity), indicating that microbial communities on older leaves tend to converge. On the contrary, microbial communities on younger leaves are scattered over different sub-clusters, indicating that the initial colonization is done by differential pioneering species with strong leaf-dependent dynamics. The microbial communities of less decomposed leaf litter tend to contain dominant bands and hence are less diverse.

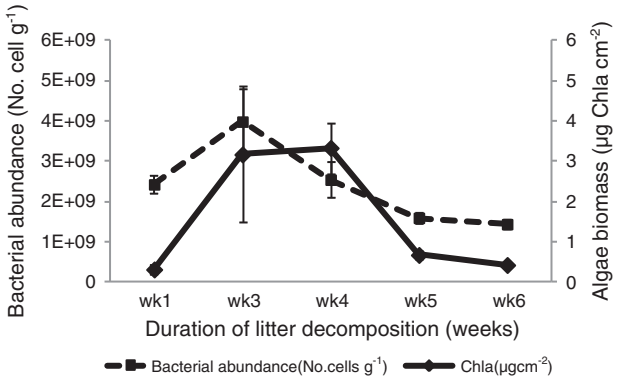

Fig. 1. Abundance of bacteria cells and microalgae in the biofilm developing on mangrove litter at different periods of decomposition.

3.4. Fatty acid biomarkers

3.4.1. Mangrove litter and biofilm

The composition of fatty acids in the decomposing mangrove litter was different compared to that in the periphytic biofilm at all stages of litter decomposition investigated (one-way ANOSIM; Global $R = 0.834$; $p = 0.001$) (Fig. 3). Polyunsaturated fatty acids (18C-PUFA: linoleic acid; 18:2ω6 and linolenic acid; 18:3ω3) and highly unsaturated fatty acids (HUFA: EPA; 20:5ω3 and DHA; 22:6ω3) varied significantly in mangrove litter and similarly in the periphytic biofilm at the different periods of litter decomposition with peaks between weeks 3 and 4 and lowest levels at weeks 5 and 6 (Fig. 4a–d). However, the concentration of linoleic acid in the biofilm did not differ significantly over the entire period of development (Kruskal–Wallis test, $H_{(4,15)} = 9.370$, $p = 0.0525$). Mangrove litter had significantly higher levels of 18C-PUFA and HUFA than the biofilm during the peak periods of decomposition, i.e. weeks 3 and 4: 18C-PUFA was ranging from 2.40 to 4.04 mg g^{-1} in mangrove litter and from 0.023 to 0.195 mg g^{-1} in the biofilm whereas HUFA ranged from 0.043 to 0.234 mg g^{-1} in mangrove litter and 0.013 to 0.117 mg g^{-1} in the biofilm.

3.5. Shrimp tissue

FAs like 18C-PUFA (linoleic; 18:2ω6 and linolenic; 18:3ω3) and HUFA (EPA; 20:5ω3 and DHA; 22:6ω3) varied in shrimp postlarvae foraging on mangrove litter, over the period of litter decomposition recording higher levels during the first 4 weeks (Fig. 4e,f). In these first 4 weeks, the FA concentration in shrimp tissue ranged from 0.3 to 0.4 mg g^{-1} linolenic acid, from 1.98 to 2.77 mg g^{-1} EPA and from 1.32 to 1.85 mg g^{-1} DHA. Lower concentrations of EPA ranging from 1.08 to 1.65 mg g^{-1} and of DHA, ranging from 0.71 to 1.18 mg g^{-1} were found in shrimp foraging on mangrove litter decomposed for 5 and 6 weeks, respectively. 18C-PUFA in shrimp tissue foraging on 5 to 6 week old mangrove litter declined significantly especially in terms of linolenic acid (ANOVA, $F_{(4,10)} = 7.40$, $p = 0.0048$). However, although there was a similar trend in the decline in HUFA, the decline was not significantly different.

Shrimp foraging in the open creek showed higher mean PUFA concentration (linoleic acid: 4.37 ± 2.50 mg g^{-1}, $N = 3$) compared to shrimp foraging in decomposing mangrove litter whose tissue concentrations of linoleic acids ranged from 0.11 mg g^{-1} to 0.68 mg g^{-1}. Likewise shrimp foraging in the open creek had significantly higher linolenic acid (0.18 ± 0.04 mg g^{-1}, $N = 3$), EPA (2.38 ± 0.15 mg g^{-1}, $N = 3$) and DHA (1.99 ± 0.40 mg g^{-1}, $N = 3$) than shrimp foraging on 5 and 6 weeks decomposed mangrove litter whose tissues had lower concentration of linolenic acid (0–0.05 mg g^{-1}), EPA (1.08–

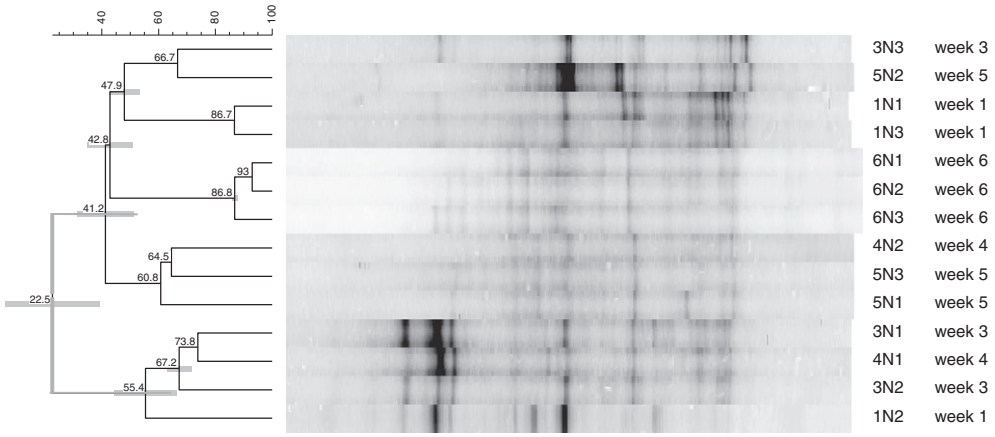

Fig. 2. Dendrogram of DNA bands derived from Pearson's correlation coefficient. Similarity levels are indicated per cluster. Bands represent bacterial community in periphytic biofilm developing on decomposing mangrove litter of *Rhizophora mucronata* at different periods of decomposition. Significant clusters are detected and are separated by a gray line.

1.65 mg g^{-1}) and DHA(0.71–1.18 mg g^{-1}) ($p<0.05$) (Fig. 4g,h). The temporal variation of FA in shrimp foraging on decomposing mangrove litter followed a similar trend to that of algae biomass and bacterial abundance, since both latter decreased significantly beyond 3 weeks. Based on comparison of fatty acid profile of food sources and shrimp, shrimp foraging in the open creek showed FAs that are characteristic for diatoms (20:5ω3) and zooplankton (20:1 + 22:1). Shrimp feeding on decomposing mangrove litter showed fatty acid ratios characteristic for phytobenthos, Σ16/Σ18 > 1, and for an overall non-planktonic feeding, 20:5ω3/22:6ω3 > 1 (Table 2).

3.6. Bacterial fatty acids

Analysis of odd carbon fatty acids conventionally used as bacterial biomarkers revealed a constant temporal increase in C15:0 and C17:0 fatty acids in the shrimp tissues foraging in the decomposing mangrove litter. The highest mean concentration of these fatty acids was recorded between weeks 3 and 4 (1.17 ± 0.33 mg g^{-1} for C17:0 and 1.84 ± 0.50 mg g^{-1} for C15:0, N = 3) (Fig. 5a). These bacterial fatty acid biomarkers declined rapidly in the tissues of shrimp foraging on mangrove litter decomposed beyond 4 weeks and a significantly lowest concentration was noted in weeks 5 and 6 (C15:0: Kruskal–Wallis test, $H_{(5,33)} = 13.27$, $p = 0.02$; C17:0: ANOVA, $F_{(5,27)} = 6.34$, $p = 0.0005$; Tukey Post Hoc: $p = 0.001$–0.03).

Shrimp foraging in the open creek recorded the lowest bacterial fatty acids in the tissue while shrimp foraging in decomposing mangrove litter showed a significantly higher concentration of C15:0 (Kruskal–Wallis test, $H_{(2,33)} = 9.16$, $p = 0.012$) (Fig. 5a). Concentration of bacterial fatty acids in shrimp foraging in the bare zones in the mangrove forest could not be clearly separated from the other two conditions (open creek, mangrove litter) especially in terms of C17:0 concentration which was not significantly different (ANOVA, $F_{(2,30)} = 1.22$, $p = 0.308$) (Fig. 5b).

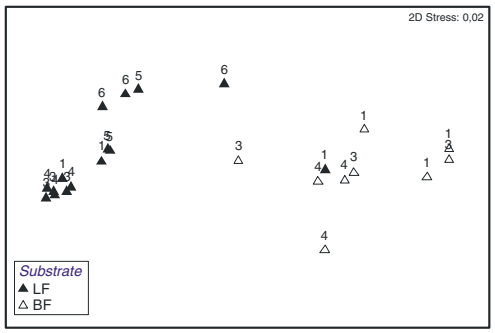

Fig. 3. MDS plot of similarity (Bray–Curtis) in fatty acids in LF (mangrove litter) and BF (periphytic biofilm developing on the decomposing mangrove litter). Numbers refer to the duration of the litter decomposition in weeks.

3.7. Isotope tracing

Mangrove litter was significantly more depleted in ^{13}C ($\delta^{13}C = -28.4 \pm 0.6$‰, N = 3) than biofilm ($\delta^{13}C = -25.2 \pm 0.2$‰, N = 3) and shrimp foraging on mangrove litter, in bare mangrove zones and in the open creek ($p<0.05$). Tissues extracted from shrimp stocked with decomposed mangrove litter were more ^{13}C depleted ($\delta^{13}C = -21.2 \pm 0.5$‰) compared to the shrimp from bare mangrove zones and control shrimp from the open creek that had slightly higher $\delta^{13}C$ values of -20.5 ± 0.5‰ and -19.30 ± 0.03‰, respectively (Table 3). Shrimp that were stocked in mangrove litter were therefore isotopically closer to biofilm and mangrove litter in comparison to shrimp from bare mangrove zones and open creek. However, they were not significantly different (ANOVA, $F_{(2,30)} = 1.307$, $p = 0.28$). The results of SIAR mixing model show that shrimp foraging in the open creek had high proportions of $\delta^{13}C$ derived from seagrass material, on average contributing 60% to their ^{13}C pool. Shrimp foraging in bare mangrove zones also had higher proportions of $\delta^{13}C$ derived from seagrasses (50%) and biofilm (40%) than from mangrove litter (20%). These three food sources contributed equally to the ^{13}C signature of shrimp foraging in mangrove litter (Fig. 6).

4. Discussion

4.1. Mangrove leaf litter degradation

The extent of leaf litter decomposition of *R. mucronata* has been found to range between 48% and 98% during the dry and rainy seasons after a period of 7 weeks (Woitchik et al., 1997). The present study observed a weight loss of 53 and 61% after 5 and 6 weeks which is similar to the 50% weight loss in *Rhizophora* leaf litter after 4 weeks of immersion in the bay of Panama (D'Croz et al., 1989). Bosire et al. (2005) observed a higher weight loss in mangrove leaf litter incubated in the intertidal zone of a reforested mangrove area, compared to the values in the present study (73% versus 61% in 6 weeks) based on continuously submerged samples. Also Woitchik et al. (1997) reported higher decomposition levels in *R. mucronata* leaf litter kept on the forest floor, than in leaf litter continuously submerged. The lower leaf litter weight loss in the present study seems therefore attributed to the continuously submerged state in which the mangrove leaf litter was incubated in the mangrove pools.

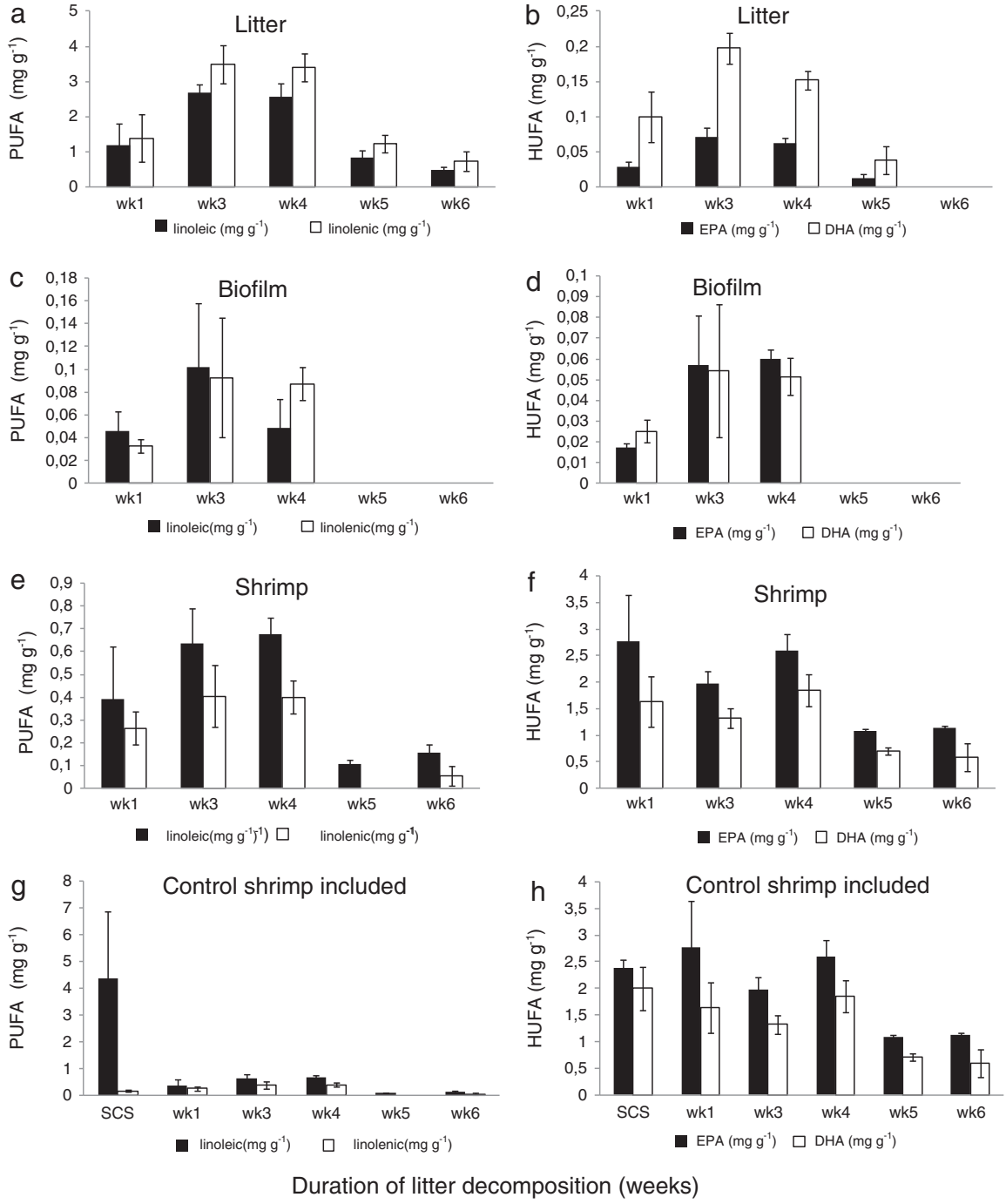

Fig. 4. Concentration (mean and standard error) of essential fatty acid biomarkers in potential food substrates; mangrove leaf litter (a–b) periphytic biofilm (c–d) and shrimp tissue (e–h) at different stages of decomposition of the mangrove leaf litter. SCS (control shrimp from the open creek).

In the present study, mangrove leaf litter decomposed rapidly within the first 3 weeks with a percentage weight loss of 2.5% and 1.8% day^{-1} which declined to 1.0% day^{-1} between the 5th and the 6th weeks. A similar observation was made by Bosire et al. (2005) in Gazi bay (Kenya) where weekly leaf litter weight loss was highest in the 2nd week while incubated in the intertidal zone of the mangrove forest. The fast rate of leaf litter decomposition may be due to rapid leaching of water soluble compounds such as tannins, amino acids and amino sugars and may also include heavy grazing by micro-invertebrates (Rajendran and Kathiresan, 2000). This is also supported by the study of Bosire et al. (2005) where a high rate of litter decomposition was observed in mangrove litter which was heavily colonized by amphipods, nematodes, turbellarians, isopods and polychaetes. Tremblay and Benner (2006) observed succession of the initial fast stage of litter decomposition by a longer and slow decomposition phase where microbial activity was predominantly responsible for nitrogen immobilization as a result of accumulation of microbial biomass and products of microbial activity, and their incorporation into humic compounds. However, Hernes et al. (2001) suggested that the interaction of immobilized nitrogen with phenolic compounds of mangrove leaf litter tissues such as lignin and tannin may produce complex compounds that are resistant to further decomposition. The diverse microbial community in the most decomposed litter (6 weeks) (Fig. 2) has more potential to metabolize the diverse complex compounds in the old litter.

Table 2
Ratios of fatty acid biomarkers indicating potential food organisms and feeding grounds for shrimp post larvae.

	Open sea (CS)	Mangrove litter (SBF)	Bare ground (SNBF)	Conclusion
Benthic phytoplankton				
Benthic Σ16/Σ18 > 1	0.8	1.1	1.1	Benthic primary trophism in SBF and SNBF
Phyto 16:1/16:0 > 1	0.3	0.3	0.3	Below limit
Diatom				
16:1/16:0 > 1.6	0.3	0.3	0.3	Below limit
Σ16/Σ18 > 2	0.8	1.1	1.1	Below limit
20:5ω3 (EPA)	7.5	2.3	2.6	Diatom foraging in CS
Dinoflagellates				
20:5ω3/22:6ω3 < 1	1.6	1.4	1.6	Below limit (non planktonic feeding)
Zooplanktons				
20:1 + 22:1	0.92	0.81	0.79	Secondary trophism in CS and SBF

Table 3
^{13}C stable isotope ratios of the tail tissue of shrimp post larvae and different potential food sources.

Shrimp tissue		$\delta^{13}C$ ‰
Control shrimp from open sea	CS	−19.28 ± 0.02
Shrimp not fed litter with biofilm	SNBF	−20.52 ± 0.49
Shrimp fed litter with biofilm	SBF	−21.15 ± 0.52
Seagrass	SG	−16.23 ± 0.16
Biofilm	BF	−25.16 ± 0.24
Decomposed mangrove Litter	LF	−28.43 ± 0.59

4.2. Bacterial abundance in biofilm on decomposing mangrove leaf litter

Bacterial colonies may develop on decomposing mangrove leaf litter under the influence of intrinsic biochemical processes such as diagenesis which leads to nutrient mineralization during leaching, autolysis of leaf tissue and utilization of organic compounds by microbes (Tremblay and Benner, 2006). The early stages of decomposition are characterized by few strong DGGE bands which are proof for the fact that some bacteria are dominating and hence there is less diversity or at least more uneven distribution in the bacterial community. On further decomposed leaf litter, weeks 5 and 6, not a single bacterial strain is dominating resulting in more even distribution of DGGE bands and hence more diversity in the bacterial community (Fig. 2). The change in the diversity of bacterial community might reflect a strong temporal change in the quality and quantity of organic substrates available. The dominating microflora is utilizing the wide range of organic and inorganic leachates such as amino sugars and amino acids available during the early stages of leaf litter decomposition (Tremblay and Benner, 2006). Once the nutritive constituent of the leachate is exhausted, further decomposition may produce complex compounds which may be preferentially utilized by more specialized microbiota. Decomposing mangrove leaf litter produces tannin (Rajendran and Kathiresan, 2006), a phenolic compound which is the fourth most abundant biochemical substance after cellulose, hemicelluloses and lignin, and which forms complexes with proteins influencing N release, but also with enzymes leading to antimicrobial and antiviral properties (Lin et al., 2007). However, leaching is an important mechanism in tannin removal from decomposing mangrove leaf litter accounting for up to 30% loss of measurable tannin (Hernes et al., 2001). Leaching may not necessarily lead to a loss in the nitrogen budget since bacteria may play a significant role in recovering it both from their biomass or fixing it from the surrounding environment. Tremblay and Benner (2006), observed that, whereas the amount of amino acids in decomposing mangrove leaf litter stabilized between 6 and 27 weeks, N-losses during leaching were recovered by the incorporation of exogenous N or N-immobilization through a microbial mediated degradation process during the whole process of decomposition. The differentiation of a bacterial community after 5 weeks of decomposition may mark the end of the 5 weeks leaching phase opening up to a longer and more extensive decomposition phase. This phase may be attributed to a microbial degradation process targeting a remnant substrate which is resistant to rapid mineralization. According to Benner and Hodson (1985), after leaching, decomposing mangrove leaf litter becomes relatively enriched in lignin derived carbon with time. This remaining fraction of organic matter is a plant structural polymer referred to as lignocellulose which is indigestible by most animals but which is degraded by certain fungi and bacteria. The progressive increase in nitrogen with leaf litter decomposition even after the leaching phase suggests an input of nitrogen by bacteria through nitrogen fixation and immobilization as has been observed by Tremblay and Benner (2006). Therefore the change in bacterial community in decomposing leaf litter after 6 weeks is possibly explained by microbial functional specialization such as nitrogen fixing or physiological tolerance to a new biochemical state of the substrate mangrove litter.

4.3. Nutritive quality of biofilm

Various studies have suggested bacteria as an important nutrition source for penaeid shrimp in promoting grazing ability, growth and survival when occurring as periphyton on structures in semi-intensive and extensive ponds (Azim and Wahab, 2005; Bratvold and Browdy, 2001; Keshavanath and Gangadhar, 2005; Nga et al., 2004). Juvenile penaeid

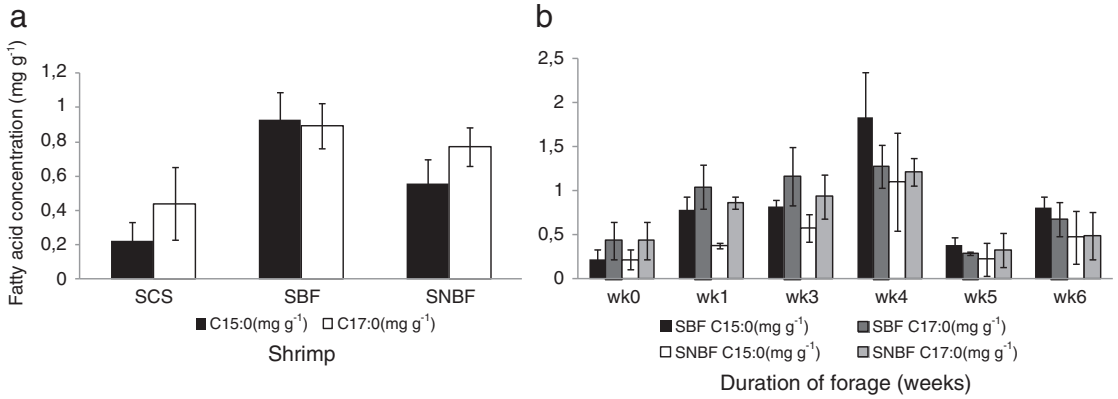

Fig. 5. Concentration of bacterial biomarkers in shrimp tissue at different zones and periods of forage; SCS, control shrimp from the open creek; SBF, shrimp stocked with decomposing mangrove litter; SNBF, shrimp stocked without mangrove litter.

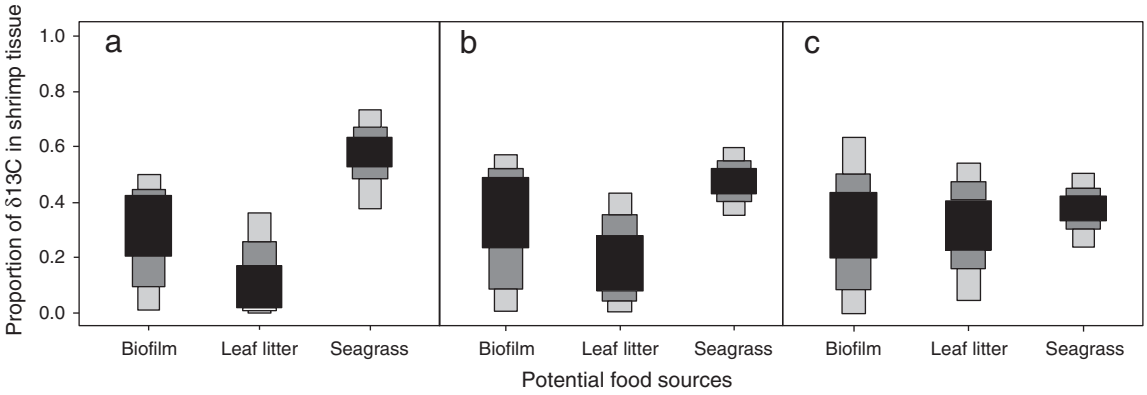

Fig. 6. Proportion of $\delta^{13}C$ in the shrimp post larvae foraging in various estuarine habitats; a) shrimp foraging in the open creek; b) shrimp foraging in the bare mangrove zones; c) shrimp foraging in the decomposing mangrove litter.

shrimp have been observed to aggregate around the mangrove litter colonized with bacteria (Rajendran and Kathiresan, 2004, 2006). On average 60–75% of the nitrogen and 20–40% of the carbon in highly decomposed mangrove leaf litter have been found to be derived from heterotrophic bacteria and not from the remaining plant tissues (Tremblay and Benner, 2006). The increase of odd-chain fatty acids (odd carbon atom numbered fatty acids) (C15:0, C17:0), which are conventionally used as bacterial biomarkers, over time in shrimp foraging in the decomposing mangrove leaf litter, underlines the importance of bacteria. The highest mean of these fatty acids in the shrimp tissue was recorded between weeks 3 and 4, coinciding with higher bacterial mean counts recorded in the mangrove leaf litter decomposed for 3 and 4 weeks (Fig. 1). The present observation differed in timing by almost 2 weeks from a study by Rajendran and Kathiresan (2004, 2006) where high counts of nitrogen fixing azotobacters and total heterotrophic bacteria were observed in mangrove leaf litter that have been decomposed for 6 weeks. This difference may be attributed to the high enumeration precision in the epifluorescence microscopy used in the present study compared to the total plate count used in the earlier study. In natural systems, bacteria may not occur as a separate functional or ecological entity but in combination with other micro-biota forming benthic and epiphytic biofilms or flocculated mass in suspension (Azim and Wahab, 2005; Burford et al., 2003). According to the present study, mangrove leaf litter decomposed for 3 and 4 weeks is important in supporting microbial biomass and fatty acid production which may be essential to shrimp postlarvae. Up to this stage of decomposition, mangrove leaf litter is still rich in nitrogen, amino sugars and amino acids which are beneficial to shrimp postlarvae directly or indirectly through nourishment of lower trophic levels of primary producers and consumers (Woitchik et al., 1997). Mangrove leaf litter decomposed for more than 4 weeks is characterized by low levels of reduced amino sugars and amino acids (Tremblay and Benner, 2006) and may not be nutritionally sufficient to support a climax community of microbiota.

4.4. Quality of potential natural food sources to shrimp postlarvae

The use of stable isotopes and fatty acids may further clarify the nutritional importance of mangrove litter and the associated biofilm for shrimp postlarvae. Primavera (2006) already used stable isotope analysis to show that *P. monodon* was feeding on phytoplankton and epiphytic microalgae in a riverine mangrove in Guimaras (Central Philippines). In our study, conclusions on fatty acid profiling were based on correlative relationships between the fatty acids in shrimp and their potential food sources. Due to their biological specificity, and the fact that they are (in most cases) transferred from primary producers to higher trophic levels without change, make fatty acids suitable for use as biomarkers (Parrish et al., 2000). For example, previous studies have used fatty acids as biomarkers for bacteria (Rajendran et al., 1995), diatoms (Parrish et al., 2000), dinoflagellates (Parrish et al., 2000) and zooplankton (Falk-Petersen et al., 2002). However one should be aware of the fact that the shrimp had fatty acid already in their tissues at the start of the experiment. Comparison with the control shrimp allowed to account for this. In the case of bacterial uptake, it was found that control shrimp had lower biomarker content. Therefore our conclusion on the higher bacterial consumption in shrimp feeding on biofilm was justified. Secondly, bioconversion (Kelly and Scheibling, 2012) of short chain fatty acids to PUFA and HUFA should not be neglected. Only a compound-specific stable isotope analysis would allow us to account for this. Unfortunately we don't have that information present in the current study. Comparison with control samples indicated reduced HUFA in shrimp feeding on litter and biofilm. This reduction could have been a result of (1) absence of HUFA in the food source (litter and biofilm) or (2) lack of bioconversion.

Shrimp have an essential nutritional requirement of lipids. Certain fatty acids such as polyunsaturated fatty acids (PUFA), highly unsaturated fatty acids (HUFA), phospholipids and sterols have been found to impact important physiological functions such as reproduction, growth, metamorphosis of crustacean larvae to juvenile, survival and resilience to stressful conditions (Bell et al., 1986; Read, 1981; Sorgeloos and Lavens, 2000). However, shrimp among other crustaceans have been found to lack the ability to biosynthesize these important fatty acids and therefore they have to obtain them from their food (Wouters et al., 2001). Read (1981) indicated that juvenile *P. indicus* has a limited capacity to chain elongate and desaturate PUFA to HUFA. Therefore an exogenous source of HUFA is necessary as part of the essential fatty acids (hereafter referred to as EFA). Past studies have specified two classes of PUFA, linoleic acid (18:2ω−6) and linolenic acid (18:3ω−3), and HUFA, eicosapentaenoic acid (EPA; 20:5ω−3) and docosahexaenoic acid (DHA; 22:6ω−3) as essential for the growth of the shrimp *P. japonicus*, (Guary et al., 1976; Kanazawa et al., 1977, 1978, 1979) and *P. monodon* (Meunpol et al., 2005). ω−6 FA, such as linoleic, are essential as energy sources while ω−3 FA, such as linolenic and HUFA, are utilized for the biosynthesis of longer chain polyunsaturated fatty acids for tissue incorporation (Sandifer and Joseph, 1976). Sorgeloos and Lavens (2000) documented in their review that feeding HUFA-enriched *Artemia* to postlarvae of *P. monodon* resulted in improved postlarvae quality by increasing their ability to survive exposure to salinity shocks.

Significant increases in weight and growth have been achieved with relatively small additions (e.g. 0.075%) of PUFA and HUFA to the diet of penaeid shrimp postlarvae (D' Abramo, 1989). In the present study, the concentration of biomarkers for essential fatty acids (PUFA and HUFA) in shrimp foraging within mangrove litter varied according to the stage of leaf litter decomposition recording a minimum concentration of EPA (1.08 and 1.65 mg g^{-1}) and DHA (0.71

and 1.18 mg g^{-1}) in mangrove leaf litter which had decomposed for a period of 5 and 6 weeks. The overall decline of EFA in shrimp tissue may imply a deficiency of fatty acids in the food substrates for the shrimp postlarvae either in the decomposed leaf litter or in the biota associated with the periphytic biofilm.

In the present study, the FA profiles of shrimp foraging in the open creek were characterized by markers for diatoms, 20:5ω3, (Parrish et al., 2000) and zooplankton, 20:1 + 22:1, (Falk-Petersen et al., 2002), pointing to their role as food. Shrimp feeding in decomposing mangrove litter also portrayed fatty acid ratios characteristic for phytobenthos, Σ16/Σ18 > 1 and an overall non-planktonic feeding, 20:5ω3/22:6ω3 > 1 (Alfaro et al., 2006), confirming reliance on benthic food sources, including both primary producers and consumers. Diatoms are typically rich in PUFA and HUFA fatty acids (Parrish et al., 2000). Periphytic biofilm may contain a mix of microalgae including nitrogen fixing cyanobacteria. Some penaeid shrimp species larvae such as *Penaeus merguiensis* have been observed to feed on the non-toxic blue-green alga *Trichodesmium* sp., without deriving observable benefits in growth or survival (Preston et al., 1998). Rothlisberg (1998) observed that the diet of juvenile and adult shrimp consisted of a wide variety of zoobenthos and macro-invertebrates (gastropods, bivalves, crustaceans and polychaetes) and plant material (Dall et al., 1990) suggesting that penaeid shrimp postlarvae can ingest what is available but tend to be more selective as they progress through growth stages (Hill and Wassenberg, 1987). For instance, juveniles of *P. indicus* and *P. merguiensis* (Angsupanich et al., 1999) and *P. esculentus* (O'Brien, 1994) showed an ontogenetic shift in diet. Small juveniles eat micro-invertebrates and some plant material (mangrove detritus, epiphytes on seagrasses and seagrass seeds), while larger juveniles and adults eat mainly larger invertebrates and less plant material. The diet may also vary seasonally, depending on the prey availability.

4.5. Ecological implication of potential natural food sources to shrimp postlarvae

Shrimp foraging in the open creek recorded significantly higher EPA and DHA concentrations than shrimp foraging on decomposed mangrove litter. Foraging zones of penaeid shrimp postlarvae may be widely spread and the entry into mangrove habitats may be limited by the tidal currents. Penaeid shrimp larvae are reported to enter coastal areas through diurnal vertical migration coupled to inshore currents, while postlarval migration is closely linked to lunar phases and tidal amplitudes (Rothlisberg, 1998). The entry into the mangrove habitats may not necessarily be influenced by presence of good feeding grounds. Ronnback et al. (2002) investigated the distribution of *P. indicus* in an *Avicennia marina* forest and observed that although juveniles and adults had a preference for vegetated mangrove habitat especially along the fringes, the postlarvae also dominated the adjacent sand flats. The lower values of EFA in shrimp foraging on decomposed mangrove litter underlines the ecological importance of the open creek, including sand flats and seagrass beds, as preferred feeding ground for shrimp postlarvae. The presence of decomposed leaf litter in mangrove habitats may not necessarily attract shrimp postlarvae. Ronnback et al. (2002) observed that the distribution of the postlarvae of *P. indicus* did not vary between the sediments of low and high organic content.

Fatty acid analysis demonstrated that shrimp postlarvae seem to derive higher quality of food, in terms of EFA, while foraging in the open creek than when feeding on decomposing mangrove litter. Studying food web structure of a mangrove forest and adjacent seagrass beds in Gazi bay, Kenya, (Marguillier et al., 1997), observed a carbon isotopic ratio gradient from mangroves (−26.75‰) to seagrass beds (−16.23‰). Using mixing model, our study was able to trophically link the shrimp postlarvae sampled from the three feeding zones; mangrove litter, bare mangrove zones and open creek, to seagrass and biofilm food sources whereas mangrove litter did make a major contribution. Such scenario corroborates the tendency of selective feeding of shrimp postlarvae in mangrove estuaries where ^{13}C rich food sources are preferentially ingested. For instance, Schwamborn et al. (2002), observed postlarvae of *Litopenaeus schmitti* from far inside the estuary were not more ^{13}C depleted compared to those caught in the inlets suggesting that the postlarvae avoided direct feeding on mangrove litter and were selecting ^{13}C rich sources such as diatoms (*Coscinodiscus centralis*), copepods, rotifers, brachuryan zoeae, cirripedian nauplii etc. Our study observed that postlarvae stocked in mangrove litter were isotopically closer to biofilm developing on the decomposing mangrove litter. This could suggest that the shrimp postlarvae fed on the biofilm targeting its constituent bacteria, phyto- and zoobenthos. However they also ingested reasonable amount of ^{13}C depleted mangrove litter in the form of both particulate and dissolved organic matter mixed in the biofilm which consequently depleted their tissue ^{13}C. However the high proportion of $\delta^{13}C$ from the seagrass food sources in the shrimp foraging in the bare mangrove zones indicates that organic matter imported from the seagrass beds plays a major role as food for shrimp in the mangrove systems. In assessing the nutritive importance of mangrove litter and associated periphytic biofilm to shrimp postlarvae, it is also important to consider that the feeding response may be influenced by litter and biofilm palatability which may be on their turn be influenced by the level of tannins in decomposing mangrove leaves (Rajendran and Kathiresan, 2000). In fact, several studies have made direct observations where penaeid shrimp postlarvae (Primavera, 1996), and other benthic invertebrates (Bouillon et al., 2002) have shown a reduced preference for mangrove derived carbon to local and imported algal sources.

5. Conclusion

The present study underlines the ecological importance of decomposing mangrove leaf litter to support an epiphytic biofilm. This biofilm may have a potential nutritive value for penaeid shrimp postlarvae during the early stages of decomposition, as changes in the shrimp bacterial FA profile were observed. Shrimp postlarvae assimilate organic matter, algal and bacterial components of the mangrove litter and biofilm which may not only impact growth but also contribute to other biochemical properties important to their physiological performance. The higher content of essential fatty acids and seagrass derived carbon sources in shrimp postlarvae foraging in the open creek and bare mangrove zones further highlights that shrimp foraging in mangrove forest cannot rely on decomposing mangrove litter and biofilm to satisfy their requirement for essential fatty acids but that they have to shift to the nearby ecosystems.

Further growth experiments with shrimp feeding on the biofilm are needed, in order to support potential aquaculture applications. However, it is clear that aquaculture management should control the residence time of decomposing mangrove litter in shrimp ponds and select sites which would allow linkage with other ecosystems.

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