FEEDING ECOLOGY OF PENAEID SHRIMP IN KENYAN MANGROVE ECOSYSTEMS

Implications for biological shrimp aquaculture

Warima Charles Gatune

UNIVERSITEIT GENT

FACULTY OF SCIENCES

Marine Biology
Ghent University

FEEDING ECOLOGY OF PENAEID SHRIMP IN KENYAN MANGROVE ECOSYSTEMS

Implications for biological shrimp aquaculture

Warima Charles Gatune

Promoter: Prof. Dr. Ann Vanreusel (Gent University, Belgium)

Promoter: Dr. Renison K. Ruwa (KMFRI, Kenya)

Co-Promoter: Prof. Dr. Peter Bossier (Gent University, Belgium)

Co-Promoter: Dr. Marleen De Troch (Gent University, Belgium)

Academic year 2012 - 2013

Thesis submitted in partial fulfillment of the requirements for the degree of Doctor in Science: Biology

Members of the reading committee

Prof. Magda Vincx
Gent University, Gent, Belgium

Prof. Tom Moens
Gent University, Gent, Belgium

Prof. Patrick Sorgeloos
Gent University, Gent, Belgium

Members of the examination committee

Prof. Dominique Adriaens
Gent University, Gent, Belgium

Prof. Ann Vanreusel
Gent University, Gent, Belgium

Prof. Magda Vincx
Gent University, Gent, Belgium

Prof. Tom Moens
Gent University, Gent, Belgium

Prof. Patrick Sorgeloos
Gent University, Gent, Belgium

Dr. Reninson K. Ruwa
Kenya Marine and Fisheries Research Institute, Kenya

Dr. Marleen De Troch
Gent University, Gent, Belgium

Prof. Peter Bossier
Gent University, Gent, Belgium

Prof. Nico Koedam
Vrije Universiteit Brussel

FEEDING ECOLOGY OF PENAEID SHRIMP IN KENYAN MANGROVE ECOSYSTEMS

Implications for biological shrimp aquaculture

Ghent University
Faculty of Science
Marine Biology Research Group
Campus Sterre – S8
Krijgslaan 281
B-9000 Gent
Belgium
Email: kgatune@yahoo.com

Publically defended on November 19th, 2012
Chairman of the defense: Prof. Dr. Dominique Adriaens

For citation, please refer as below:

Gatune, W.C., 2012. Feeding ecology of penaeid shrimp in Kenyan mangrove ecosystems: Implications for biological shrimp aquaculture. Ghent University. Belgium. 153 pp.

Table of Contents

Acknowledgements

No work is difficult, nothing is impossible unless I would rather do some other work.

God always answers my prayers and gives me strength, I will always believe in you.

My late father, Paul Simon Warima, sincerely believed in my academic capability, I will forever respect you.

My mother, Enestina Njeri, loves me and never losses hope in me, I will forever adore you.

My wife, Wairimu, Son; Warima, Daughters; Njeri and Wangui, have undergone long periods of pain in my absence but they have never lost faith in me, I will forever love you.

I, the author of this PhD thesis express my sincere gratitude to the Kenya Ministry of Fisheries Development for awarding me study leave and infrustracture to pursue my PhD studies. I feel indebted to Flemish InterUniversity Council (VLIRUOS) of Belgium for the financial support to follow an international PhD program in Biology at Ghent University, Belgium. I am much pleased by the staff of VLIRUOS, Micheline, Helke, Sam Geert, Karolien for your tireless assistance.

I sincerely feel obliged to the much tireless guidance, patience and encouragement i received from my promoters Prof. Ann Vanreusel and Dr. Marleen De Troch of Marine Biology at Gent University, Belgium; Dr. Renison Ruwa of Kenya Marine and Fisheries research Institute (KMFRI), Prof. Peter Bossier and Prof. Patrick Sorgeloos of the Laboratory of Aquaculture and Artemia Reference Center at Gent University, Belgium.

I feel humbled by youth of the Majaoni Silvofishery project based in Mtwapa creek in Mombasa, Kenya for providing me space for the field research and especially Daniel, Patrick and Kalton for their unconditional assistance in the field. Deep felt thanks goes to the KMFRI laboratory technicians Eric Magara, Omondo, Mwendwa Mbaluka, Boaz, Onduso, Kilonzi, Andugosi and Emily.

Acknowledgements

I cannot forget to thank Mr. Glen based at Mbengani Fisheries Development Centre in Bagamoyo Tanzania, Dr. Mahika, Director of Aquaculture Tanzania; Mr.Vageri and Ari of Alphakrust, Tanzania, for facilitating export and donating the test shrimp post larvae.

I salute Dirk Van Gansbeke and Bart of the laboratory of Marine Biology at Gent University for the much practical training and assistance in the analysis of the fatty acids and the C/N nutrients. Special thanks go to Clio Cnudde for assisting in the microbiological analysis and acknowledging the difficulty times that the PhD studies can sometimes unleash.

I am grateful to the staff of the Marine Biology at Gent University and especially Annick, Guy, Isolde for making my study life comfortable at Gent University.

You have occupied a special place in my heart,

Charles Gatune Warima

Ghent, November 19th 2012

Summary

Ecological shrimp aquaculture derives its feed input from the naturally occurring food sources. This practice has an environmental advantage of reducing the use of fish meal as the main food input. Use of fish meal in aquaculture has a negative effect on the coastal fisheries in the sense of depleting fish stocks. It also has a tendency to discharge wastes that pollutes the receiving coastal ecosystems. Ecological shrimp aquaculture therefore impacts low burden to the coastal ecosystems since it is integrated into the estuarine food chain. The potential of shrimp aquaculture in Kenya is high. However the initial development attempts heavily relied on the mangrove ecosystem and expensive technology. The environmental efforts to conserve the mangrove forest and the inability of the poor community to adopt the expensive technology led to the collapse of the shrimp aquaculture in Kenya. The potential approach to revive this practice therefore depends on defining ways which are not destructive to the mangrove ecosystem and which falls within the economic capability of the local community. Ecological shrimp aquaculture uses naturally generated and locally available inputs making it less costly to develop and manage. For instance, the food inputs can be derived from the estuarine ecosystem or enhanced by promoting microbial proteins. A microbial intercept would also prevent discharge of nitrogenous pollutants. However this would depend on the general setting of the shrimp ponds so that most appropriate inputs to enhance the supply of the natural food is optimised.

The following study therefore attempt to explore to what extent the penaeid shrimp post larvae would rely on the natural food derived from the decomposing leaf litter of the mangrove, *Rhizophora mucronata*. It is hoped that the observations of this interaction can be employed in the design and management of an ecological shrimp culture practice in the Kenyan mangroves. The role of micro-biota such as the microbial community, micro-algae and meiofauna in supporting a potential food chain with the penaeid shrimp post larvae as an apex consumer is hereby explored. Specific attention is focused on the nutritional requirement of *Penaeus monodon* and *Penaeus indicus*. These penaeid shrimp species are of commercial food importance both globally and along the Kenyan coast. The study derives insight from recent studies on bacteria and micro-algae in the processing of nutrients resulting in the production of a potential diet for small invertebrates and penaeid shrimp. Moreover, ***an ecological approach to shrimp aquaculture cannot be accomplished without considering the policy issues governing the utilization of the coastal resources in Kenya.*** The motivation

for this study is to recommend an ecological approach to shrimp aquaculture as a fishing activity in Kenya. It is visualised that this approach has a potential to increase income and food security to the local communities deriving livelihood from the Kenyan mangrove ecosystems.

The overall aim of this PhD study was to understand the feeding ecology of penaeid shrimp in a mangrove system and to apply this knowledge to design an ecological shrimp aquaculture system which can derive biological food resources from the mangrove ecosystem.

Five specific research themes were assessed: 1) the extent to which shrimp post larvae can ingest and assimilate organic matter from decomposing mangrove leaf litter given the option of a bacterial biofilm as a food source; 2) the microbial contribution to the nutritive value of the decomposing mangrove leaf litter and the associated biofilm to shrimp post larvae in comparison to an estuarine food source; 3) the temporal variation in the biota associated with the biofilm developing on the decomposing mangrove litter and their food-value-potential to support the growth of shrimp post larvae; 4) the effect of exposure to the different environmental conditions of sunlight and sediment to the temporal variation in the diversity and abundance of biota associated with the biofilm developing on the decomposing leaf litter; 5) the nutritive effect of the biofilm associated with the decomposing mangrove leaf litter on the physiological performance of shrimp post larvae.

The detailed concept and the interacting areas of interest which are investigated in the various studies are introduced in **Chapter 1,** whereas, the synthesis of all observations, recommendations and areas that require further investigation is discussed in **Chapter 7.**

Chapter 2 used a ^{13}C label method to investigate whether the mangrove detritus and the associated bacteria can be ingested and assimilated by shrimp post larvae. The biofilm developing on the decomposing mangrove leaf litter of *Rhizophora mucronata* was labeled with ^{13}C stable isotope sodium acetate and fed to the shrimp post larvae of the *Penaeus indicus*. Bacterial fatty acid biomarkers were also analysed. Shrimp post larvae feeding on the biofilm recorded higher uptake of the ^{13}C into their tail tissue. The uptake of the ^{13}C was confirmed by the abundance of the bacterial fatty acid biomarkers in the same treatments. Shrimp feeding on the decomposing mangrove litter had higher uptake of the ^{13}C compared to

the unfed shrimp controls. The shrimp post larvae assimilated both the bacteria and the mangrove detritus in the absence of any other food.

Chapter 3 investigated the hypothesis that the nutritive importance of the mangrove organic matter and the associated bacterial biofilm, to shrimp post larvae, may depend on the extent of mangrove leaf litter decomposition and the presence of an alternative natural food source. The study therefore 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 ($3^{o}57'S$; $39^{o}42'E$). 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 remineralisation, 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 week 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 towards 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, more specificallyweek 3 - 4. The results of this study suggests that 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.

Chapter 4 explored the argument that the ability of biofilm, associated with the decomposing mangrove litter, to support the growth of shrimp post larvae may depend on the specific type of the associated biota which may have a temporal variation. In this chapter, growth and survival of post larvae (PL) of *Penaeus monodon* were tested on (1) mangrove leaf litter of

Rhizophora mucronata with biofilm at 1, 3, 4, 6 and 8 weeks of decomposition; (2) commercial compound feed (CP) and (3) no food available as control. PL were analyzed for specific growth rate (SGR %) and percentage survival (SR %). Biofilm was analyzed for biomass and composition of micro-algae and epifauna. Micro-algae biomass increased with the progress of litter decomposition. Diatoms dominated the first 6 weeks of litter decomposition with their percentage cover ranging from 88 to 99% during the 3rd and 4th week. Diatoms *Navicula* spp. and *Nitzschia* spp. dominated and replaced each other during the progress of litter decomposition. Cyanobacteria dominated over the diatoms by 61% in the 8 weeks old biofilm. Copepoda dominated the epifauna during the first 3 weeks of litter decomposition. Polychaeta dominated during the 4th and 5th week whereas Nematoda dominated during the 8th week of litter decomposition. PL growing on CP had the overall best growth and survival with SGR of 6.1 ± 0.3% and SR of 97.2 ± 2.7%. PL foraging on 1, 3, 6 and 8 weeks old leaf litter had reduced growth and survival. PL foraging on 4 weeks old litter had a better SGR of 1.6 ± 0.5% and SR of 39.8 ± 4.8% and coincided with the peak period of the micro-algae and epifauna abundance. The study illustrated that 1) shrimp post larvae living on the decomposing mangrove leaf litter perform better when foraging on the 4 weeks old biofilm; 2) the quality biofilm improves during the 4th week of mangrove leaf litter decomposition and is dominated by diatoms, polychaetes, harpacticoid copepods and oligochaetes; and 3) quality biofilm food for the shrimp post larvae is limited by the collapse of the epifauna and subsequent colonization by nutritionally low quality Cyanobacteria, if decomposing mangrove leaf litter is retained in the pond water for longer than 5 weeks.

Chapter 5 argued that since application of ecological shrimp aquaculture in a mangrove system implies abundant supply of leaf litter from the mangrove trees, then the food value of biofilm associated with the decomposing mangrove leaf litter would depend on the extent of shading by the mangrove tree and the contact with the sediment. In this experiment the degradation of the leaf litter of *Rhizophora mucronata* and the assembly of micro-algae and epifauna were assessed under various conditions of direct sunlight, shade and the presence/absence of sediment. This study contributes to the potential use of mangrove leaf litter and biofilm in providing favorable culture conditions and natural diet to shrimp post larvae of *Penaeus monodon*. Mangrove leaf litter incubated with sediment and exposed to sunlight was rapidly degraded compared to the litter incubated without sediment and in the shade. Decomposing litter exposed to the sunlight in the presence of sediment supported the highest biomass and diversity of micro-algae and epifauna, and the highest abundance of

diatoms, polychaetes and nematodes during the 4th week. Cyanobacteria of the genus *Microcystis* dominated the mangrove litter incubated without sediment in the presence of sunlight after a decomposition period of 5 weeks. Diatoms *Navicula* spp. and cyanobacteria (*Anabaena* spp. and *Oscillatoria* spp.) continued to grow in the shade. The water in the microcosms supporting mangrove litter decomposing in the shade was rich in total ammonium nitrogen (TAN) but had low levels of dissolved oxygen (DO), temperature and pH. The study illustrated that there is a synergistic effect between sediment and direct sunlight in promoting the proliferation of a wide range of micro-algae and polychaetes, inhibiting the growth of cyanobacteria and maintaining water quality which is favorable to the culture of shrimp post larvae. This implies that ecological shrimp culture management practices should consider locating ecological shrimp ponds in less forested areas to promote primary production and aerating bottom layers of shaded ponds receiving litter fall. Moreover, our findings suggest that shrimp culture microcosms using mangrove leaf litter substrates should include sediment to prevent cyanobacteria blooms.

Chapter 6 explored a rather concluding question of whether it is possible to practically culture shrimp post larvae solely on the decomposing mangrove leaf litter and the associated biofilm. In this study shrimp post larvae were fed with mangrove leaf litter and the associated biofilm, under laboratory conditions, at three nutritionally important stages of decomposition (1, 6 and 10 weeks). The growth performance of shrimp was assessed by means of fatty acids as trophic markers. Biofilm on decomposing mangrove litter was found to be a source of bacterial fatty acids during the 6th week. Overall, shrimp post larvae feeding on the mangrove litter derived food were characterised by higher levels of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), arachidonic acid-ARA (20:4ω6) and eicosapentanoic acid-EPA (20:5ω3) than shrimps feeding on the compound feed. However, mangrove litter derived food suppressed the growth and survival of shrimp post larvae due to the unavailability of DHA and linoleic acid (18:2ω6).

After conducting the various experiments and measurements, it was concluded that decomposing mangrove leaf litter of *Rhizophora mucronata* can biologically support penaeid shrimp post larvae if they are connected with another ecosystem such as seagrass beds and if the duration of decomposition is routinely controlled. This study therefore recommended that, ecological shrimp aquaculture located in a mangrove zone should consider routine removal of the mangrove litter fall at 6 week intervals. Ecological shrimp aquaculture located in a

mangrove zone should include supplementary input that would enhance the supply of natural food which is rich in essential fatty acid DHA and linoleic acid. Moreover, ecological shrimp aquaculture would perform better if located in an area which has not only input of mangrove organic matter but is also linked to the seagrass ecosystem.

Samenvatting

In ecologische aquacultuur van garnalen bestaat de voedselinput uit natuurlijk voorkomende voedselbronnen. Deze manier van kweken heeft een belangrijk ecologisch voordeel omdat er minder vismeel als belangrijkste voedselbron gebruikt wordt. Het gebruik van vismeel in aquacultuur heeft immers een negatief effect op de kustvisserij vooral wanneer het leidt tot de uitputting van visbestanden. Daarenboven impliceert het gebruik van vismeel vaak een toevoer van afvalstoffen die vervolgens de nabije kustecosystemen vervuilen. Ecologische garnalenaquacultuur heeft daarom een beperkte impact op kustecosystemen omdat het geïntegreerd wordt in de estuariene voedselketen. Het potentieel van garnalenaquacultuur in Kenia is hoog. Echter, de eerste pogingen om deze vorm van aquacultuur te ontwikkelen was sterk afhankelijk van het mangrove-ecosysteem en dure technologie. De milieu-inspanningen om het mangrovebos te beschermen en het onvermogen van de arme gemeenschap om de dure technologie toe te passen leidde tot de ineenstorting van de garnalenaquacultuur in Kenia. De mogelijke aanpak om deze ecologische aquacultuur succesvol te maken is afhankelijk van het definiëren van manieren die niet destructief zijn voor het mangrove-ecosysteem en die binnen het economische vermogen van de lokale gemeenschap liggen. Ecologische garnalenaquacultuur maakt gebruik van natuurlijke gegenereerde en lokaal beschikbare voedsel waardoor het minder duur is om te ontwikkelen en te beheren. Zo kan de voedselinput afgeleid worden van het estuarien ecosysteem of versterkt worden door de bevordering van microbiële eiwitten. Een microbiële interactie zou ook lozingen van stikstofhoudende verontreinigende polluenten kunnen voorkomen. Dit zou echter afhangen van de algemene toestand van de garnalenkwekerijen zodat de meest geschikte toevoer van natuurlijk voedsel geoptimaliseerd wordt.

Deze studie heeft daarom als doel om te onderzoeken in welke mate de penaeid garnaallarven afhankelijk zijn van het natuurlijke voedsel afgeleid van de ontbindend bladafval van de mangrove, *Rhizophora mucronata.* Daarbij hopen we dat de waarnemingen van deze interactie gebruikt kan worden in het ontwerp en het beheer van een ecologische garnalenkweek in Keniaanse mangrovebossen. De rol van micro-biota, zoals de microbiële gemeenschap, micro-algen en meiofauna voor de ondersteuning van een mogelijke voedselketen met de penaeid garnaalpostlarven als topconsument wordt daarbij onderzocht. Specifieke aandacht is gericht op de nutritionele behoefte van *Penaeus monodon* en *Penaeus indicus.* Deze penaeid garnaalsoorten zijn van commercieel belang als voedsel, zowel

wereldwijd als langs de Keniaanse kust. De studie leidt deels inzicht af van de recente studies op bacteriën en micro-algen in de verwerking van voedingsstoffen resulterend in de productie van een mogelijk dieet voor kleine ongewervelden en penaeid garnalen. Bovendien ***kan een ecologische benadering van garnalenaquacultuur niet worden bereikt zonder de beleidskwesties inzake het gebruik van de kustgebieden in Kenia in beschouwing te nemen.*** De motivatie voor deze studie is om een ecologische benadering van de garnalenaquacultuur als een visserijactiviteit in Kenia te adviseren. Het is duidelijk dat deze aanpak potentieel de inkomsten en voedselzekerheid van de lokale gemeenschappen, die voor hun levensonderhoud afhankelijk zijn van de Keniaanse mangrove-ecosystemen, kan verhogen.

Het algemene doel van dit doctoraatsonderzoek was om de voedselecologie van penaeid garnalen te begrijpen in een mangrovesysteem en om deze kennis toe te passen op een ecologische garnalenaquacultuur die biologische voedselbronnen gebruikt van het mangrove-ecosysteem.

De volgende vijf specifieke vraagstellingen werden bestudeerd: 1) de mate waarin garnaalpostlarven organisch materiaal kunnen opnemen en assimileren uit ontbindend mangrovebladafval waarbij een bacteriële biofilm een potentiële voedselbron vormt; 2) de microbiële bijdrage aan de voedingswaarde van het ontbindend mangrovebladafval en de bijhorende biofilm voor garnaalpostlarven in vergelijking met een estuariene voedselbron; 3) de temporele variatie in de biota geassocieerd met de ontwikkeling van de biofilm op het ontbindend mangrovebladafval en hun voedselwaarde die potentieel de groei van garnaallarven kan ondersteunen; 4) het effect van de blootstelling aan verschillende omgevingsomstandigheden in termen van zonlicht en sediment op de temporele variatie in de diversiteit en abundantie van biota geassocieerd met de biofilm die zich ontwikkeld op het ontbindend bladafval; en 5) beoordeling van de voedingseffect van de biofilm geassocieerd met het ontbindend mangrovebladafval op de fysiologische prestaties van garnaalpostlarven.

Het gedetailleerde concept en de inter-agerende interessegebieden die worden onderzocht in verschillende studies worden ingeleid in **hoofdstuk 1,** terwijl de synthese van alle waarnemingen, aanbevelingen en gebieden die nader bestudeerd moeten worden, besproken worden in **hoofdstuk 7.**

Summary

Hoofdstuk 2 gebruikt een ^{13}C-aanrijkingsmethode om te onderzoeken of het mangrovedetritus en de bijhorende bacteriën kunnen worden ingenomen en geassimileerd door garnaalpostlarven. De biofilm die zich ontwikkelt op het ontbindend mangrovestrooisel van *Rhizophora mucronata* werd gelabeld met ^{13}C natriumacetaat en toegevoegd aan de garnaalpostlarven van *Penaeus indicus*. Bacteriële vetzuurbiomerkers werden ook geanalyseerd. Garnaalpostlarven die zich voeden met biofilm vertoonden een hogere opname van ^{13}C in hun staartweefsel. De opname van ^{13}C werd bevestigd door de abundantie van de bacteriële vetzuurbiomerkers in dezelfde behandelingen. Garnalen die zich voeden met ontbindende mangrovebladeren hadden een hogere opname van ^{13}C ten opzichte van de controlegarnalen die niet gevoed werden. De garnaalpostlarven assimileerden zowel de bacteriën als het mangrovedetritus in de afwezigheid van enig ander voedsel.

Hoofdstuk 3 test de hypothese dat het belang van mangrove organische stof en de geassocieerde bacteriële biofilm als voedsel voor garnaalpostlarven afhangt van de mate van afbraak en de aanwezigheid van een alternatieve natuurlijke voedingsbron. De studie onderzocht daarom de mogelijkheden van mangrovedetritus van *Rhizophora mucronata* en de geassocieerde microbiële biofilm als voedsel voor garnaalpostlarven van *Penaeus indicus* en *Penaeus monodon* in een gemeenschapsgebaseerde ecologische garnalenkwekerij in Mtwapa Creek, Kenia (3° 57' Z, 39° 42' O). Verouderde mangrovebladeren werden gedurende 6 weken geïncubeerd, samen met garnaalpostlarven, PL 15-25, in ondiepe mangrovepoelen. Bladdegradatie, koolstof en stikstof nutriëntenremineralisatie, bacteriële gemeenschapsstructuur en biomassa van de algen in de perifytische biofilm werden wekelijks onderzocht gedurende 6 weken. Voedselopname en -assimilatie werden beoordeeld door vetzuurprofielen en δ ^{13}C isotoopwaarden in garnaalweefsel, detritus en biofilm te vergelijken. Postlarven van de open kreek werden gebruikt als controle. Ontbindende mangrovebladeren ondersteunen de groei van microalgen en bacteriën in de vorm van perifytische biofilm met een maximale groei in de 3e en 4e week wanneer de bladeren voor 43% afgebroken waren. De bacteriële gemeenschap varieerde in structuur naarmate de afbraak van de bladeren vorderde door een vermindering in abundantie na de 3e week met een minimum in de 6e week. De diversiteit van de bacteriële kolonies veranderde eveneens van een hoge dominantie in de vroege stadia van de afbraak van de bladeren naar meer gelijkmatig diverse kolonies na meer dan 5 weken van afbraak. Garnalen die gestockeerd werden in een mangrovebos hadden 1) de hoogste concentratie aan linolzuur, linoleenzuur en meervoudig onverzadigde vetzuren (HUFA) in behandelingen met 3 tot 4 weken oude

bladeren, 2) lager vetzuurniveaus in vergelijking met de garnalen uit de kreek en waren 3) isotopisch meer gelijkend op zeegras en biofilm. In termen van voedingswaarde, ondersteunen mangrovebladeren penaeid garnaalpostlarven met een perifytische biofilm tijdens de vroege stadia van ontbinding, meer bepaald in week 3 - 4. De resultaten van deze studie suggereren dat de toevoer van voedingsstoffen naar de ecologische garnalenaquacultuur in mangrove systemen kunnen worden geoptimaliseerd door het controleren van de verblijfstijd van mangrovebladeren in garnalenkweekvijvers en door de plaatsen zodanig te selecteren dat ze gekoppeld zijn aan andere ecosystemen zoals kreken en open zee.

Hoofdstuk 4 onderzoekt het argument dat het vermogen van de biofilm geassocieerd met het ontbindend mangrovestrooisel, om de groei van garnaalpostlarven te ondersteunen, kan afhangen van het specifieke type van de geassocieerde biota die temporeel kan variëren. In dit hoofdstuk, werden groei en overleving van postlarven (PL) van *Penaeus monodon* getest in behandelingen met als voedsel (1) mangrovebladafval van *Rhizophora mucronata* met biofilm na 1, 3, 4, 6 en 8 weken van decompositie; (2) commercieel mengvoeder (CP) en (3) geen eten als controle. PL werden geanalyseerd op specifieke groeisnelheid (SGR%) en percentage overleving (SR%). Biofilm werd geanalyseerd in termen van biomassa en samenstelling van micro-algen en epifauna. Micro-algen biomassa nam toe met de verdere afbraak van de mangrovebladeren. Diatomeeën domineerden gedurende de eerste 6 weken van de afbraak van de bladeren met een aandeel van 88 tot 99% tijdens de 3^{e} en 4^{e} week. De diatomeeën *Navicula* spp. en *Nitzschia* spp. domineerden en vervingen elkaar tijdens de afbraak van het bladafval. Cyanobacteriën domineerden boven de diatomeeën met 61% in 8 weken oude biofilm. Copepoden domineerden de epifauna tijdens de eerste 3 weken van afbraak van de bladeren. Polychaeta domineerden tijdens de 4^{e} en de 5^{e} week terwijl Nematoda domineerden tijdens de 8ste week van de afbraak van het bladafval. PL die op CP groeiden hadden algemeen de beste groei en overleving met een SGR van 6.1 ± 0.3% en SR van 97.2 ± 2.7%. PL die zich voedden met 1, 3, 6 en 8 weken oud bladafval hadden een verminderde groei en overleving. PL die zich voedden met 4 weken oude bladeren hadden een beter SGR van 1.6 ± 0.5% en SR van 39.8 ± 4.8%. Dit overlapte met de piekperiode van de micro-algen en epifauna densiteiten. De studie toonde aan dat 1) garnaalpostlarven die op ontbindend mangrovebladafval leven, beter presteren wanneer ze zich voeden met 4 weken oude biofilm, 2) de kwaliteit van de biofilm verbetert tijdens de 4^{e} week van de mangrovebladafbraak en wordt gedomineerd door diatomeeën, borstelwormen, harpacticoide roeipootkreeftjes en

oligochaeten, en 3) de kwaliteit van de biofilm als voedsel voor de garnaalpostlarven beperkt wordt door de ineenstorting van de epifauna en de daaropvolgende kolonisatie door nutritioneel armere cyanobacteriën, indien het ontbindend mangrovebladafval langer dan 5 weken in het water blijft.

Aangezien de toepassing van ecologische garnalenaquacultuur in een mangrove-ecosysteem een overvloedige toevoer van bladafval van mangrovebomen impliceert, was de vooropgestelde hypothese voor **hoofdstuk 5** dat de voedingswaarde van de biofilm op het ontbindend mangrovebladafval zou afhangen van de mate van verduistering door de mangroveboom en het contact met het sediment. In dit experiment werden de afbraak van de strooisellaag van *Rhizophora mucronata* en de gemeenschap van micro-algen en epifauna beoordeeld onder verschillende omstandigheden van direct zonlicht, schaduw en de aan- en afwezigheid van sediment. Deze studie draagt bij tot het mogelijk gebruik van mangrovebladafval en biofilm als gunstige kweekomstandigheden van garnaalpostlarven van *Penaeus monodon* op basis van hun natuurlijke dieet. Mangrovebladafval dat geïncubeerd werd met sediment en blootgesteld werd aan zonlicht werd snel afgebroken in vergelijking met de bladeren geïncubeerd zonder sediment en in de schaduw. Ontbindend bladafval dat blootgesteld werd aan zonlicht in de aanwezigheid van sediment bevatte de hoogste biomassa en diversiteit aan micro-algen en epifauna, en de hoogste abundantie van diatomeeën, borstelwormen en nematoden in de 4^{e} week. Cyanobacteriën van het genus *Microcystis* domineerden mangrovedetritus wanneer geïncubeerd zonder sediment maar in zonlicht gedurende een incubatieperiode van 5 weken. Diatomeeën *Navicula* spp. en cyanobacteriën *(Anabaena* spp. en *Oscillatoria* spp.) bleven groeien in de schaduw. Het water in de microkosmos ondersteunde de afbraak van mangrovebladeren in de schaduw en was rijk aan totale ammonium stikstof (TAN), maar had lage niveaus van opgeloste zuurstof (DO), temperatuur en pH. De studie toonde aan dat er een synergetisch effect was tussen sediment en direct zonlicht om de proliferatie van diverse micro-algen en borstelwormen te bevorderen, waardoor de groei van cyanobacteriën en het behoud van de waterkwaliteit gunstig is voor de cultuur van garnaalpostlarven. Deze bevindingen impliceren dat het management van ecologische garnalenaquacultuur zou moeten overwegen om garnalenkweekvijvers in minder beboste gebieden te lokaliseren om de primaire productie te promoten en de beluchting van de onderste lagen van schaduwrijke plaatsen met veel bladval te bevorderen. Bovendien suggereren onze bevindingen dat microkosmosopstellingen voor garnaalkweek met mangrovebladafval sediment zouden moeten bevatten om cyanobacteriënbloei te voorkomen.

Hoofdstuk 6 onderzocht de algemene vraag of het praktisch mogelijk is om garnaalpostlarven uitsluitend op ontbindend mangrovebladafval en de bijhorende biofilm te kweken. In deze studie werden garnaalpostlarven onder laboratoriumomstandigheden gevoed met mangrovebladafval en de geassocieerde biofilm tijdens drie nutritioneel belangrijke stadia in de afbraak (1, 6 en 10 weken). De groei van garnalen werd beoordeeld aan de hand van vetzuren als trofische merkers. Biofilm op ontbindend mangrovebladafval bleek een bron van bacteriële vetzuren te zijn tijdens de 6^{e} week. Algemeen vertoonden de garnaalpostlarven die zich voedden met mangrovebladafval hogere concentraties van verzadigde vetzuren (SAFA), enkelvoudig onverzadigde vetzuren (MUFA), arachidonzuur ARA (20:4ω6) en eicosapentaeenzuur EPA (20:5ω3) dan garnalen die zich voedden met samengesteld voedsel. Echter, mangrovebladafval als voedselbron onderdrukte de groei en de overleving van garnaalpostlarven als gevolg van het ontbreken van DHA en linolzuur (18:2ω6).

Na het uitvoeren van de verschillende experimenten en metingen, werd geconcludeerd dat ontbindend mangrovebladafval van *Rhizophora mucronata* penaeid garnaalpostlarven kan ondersteunen op voorwaarde dat ze verbonden zijn met een ander ecosysteem, zoals zeegrasvelden en wanneer de duur van de afbraak van de bladeren routinematig wordt gecontroleerd. Deze studie beveelt daarom aan dat ecologische garnalenaquacultuur in een mangrovezone rekening dient te houden met routinematig verwijderen van mangrovedetritus *a rato* van 6-weken intervallen. Daarenboven zou ecologische garnalenaquacultuur in een mangrovezone aanvullende input moeten promoten die de toevoer van natuurlijk voedsel, rijk aan essentiële vetzuren DHA en linolzuur, ten goede komt. Daarenboven zou ecologische garnalenaquacultuur beter presteren indien gelokaliseerd in een gebied dat niet is alleen input van mangrove organisch materiaal krijgt maar ook gelinkt is met het zeegrasecosysteem.

1. Chapter 1

1.1 General Introduction

Collapsed shrimp farm at Ngomeni and the currently established small scale community shrimp farms at Vanga and Majaoni show case the potential of shrimp aquaculture in Kenya coastal zone (Photo courtesy Gatune C. 2009)

1.1.1 Potential of penaeid shrimp aquaculture in Kenya

Many reports have criticized intensive and extensive shrimp aquaculture due to overreliance on fish meal despite the decreasing wild fish stocks (Naylor et al., 2000; Ronnback, 2001; Tacon, 1996b). It's a common occurrence where 2 times more protein, in the form of fish meal, is used to feed the shrimp than is ultimately harvested. This type of shrimp culture undermines its profitability (Tacon, 1998). The use of high protein fish meal results to a discharge of high nitrogen wastes which pollute the receiving ecosystems (Lin, 1989; Primavera, 2006; Primavera et al., 1993). Shrimp aquaculture also causes mangrove deforestation and ecosystem modification (Primavera, 2006; Spalding et al., 1997). According to Spalding et al. (2010), in areas where vast tracts of mangrove have been cleared for shrimp

aquaculture, fast profits often left a legacy of long-term debts and poverty, which are hard to reverse. The negative effect of full fed shrimp aquaculture has necessitated support for an ecological friendly practice that would maintain a balance between the utilization and conservation of the immediate ecosystems (Folken et al., 1998; Hamilton et al., 1989; Kautsky et al., 1997; Larsson et al., 1994; Primavera, 1998).

In the year 2007, a once lucrative semi-industrial shrimp trawling activity of the commercial species *Penaeus monodon* and *P. indicus* along the Kenyan coast, of the Indian Ocean, was officially closed due to assumed depletion of shrimp stocks (GOK, 2006a). 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). The shrimp fishery of *P.monodon* and *P. indicus* in Kenya is a major commercial activity in both local and foreign markets (GOK, 2006a). As a result, artisanal shrimp fishing along the Kenyan coast remains a continuous practice in the estuaries, creeks and shallow intertidal zones. These zones are preferred nursery grounds for fish and shrimp juveniles (Ronnback, 2001; Wakwabi and Jaccarini, 1993). Such fishing therefore contravenes the Kenyan Fisheries Act 378 which prohibits fishing in fish breeding and nursery grounds. To mitigate such conflicts, an alternative fishing method, such as farming penaeid shrimp performed in way that would impact minimal damage to the estuarine and mangrove ecosystems, is therefore a major necessity. If approached from an ecological point of view, shrimp aquaculture can promote a sustainable production and trade in penaeid shrimp resources along the Kenyan coast.

The Ngomeni shrimp culture project generated a promise of prevailing potential of shrimp aquaculture in Kenya (Le Bitoux, 1985). An average production of 426 kg/ha of *Penaeus monodon* was achieved in 1984 without application of fertilizers (FID, 1989; Le Bitoux, 1985). This production was very promising (Le Bitoux, 1985) and fell within the range of comparable productivity of extensive shrimp culture in the more experienced countries (FAO/NACA, 2012; Primavera, 1998) (Table1)

Country	Year	Productivity (kg/ha)
Ecuador	1996	923
Indonesia	1996	257
China	1996	667
India	1996	350
India	2007	660
Bangladesh	1996	250
Vietnam	1996	150
Philippines	1996	417
Others	1996	807
Kenya	1984	426

Table 1: The extensive production of cultured penaeid shrimp in Kenya in relation to the more experienced countries

Despite the achieved potential, Ngomeni shrimp project was blamed for the destruction of 60 -100 ha of mangrove forest (Kairo and Abuodha, 2001). In an effort to remedy the negative perception of shrimp aquaculture in Kenya, Rasowo (1992) recommended a shift from tide-fed to pump-fed pond systems in order to divert the shrimp culture from the mangroves to higher grounds. This recommendation was based on a general review of the existing shrimp culture practices and the ecological conflicts at hand. Scientifically, this recommendation has an added advantage of increased shrimp production due to the reduced effect of sulphides and acid sulphate soils typical of mangrove soils (Chien, 1992). In the Ngomeni shrimp project, Le Bitoux (1985) actually observed low shrimp production in the shrimp ponds excavated in the mangrove areas compared to the ponds on a higher ground which was less impacted by the mangrove organic matter. Although the pumping system may be beyond the investment capability of the local community, tide-fed small-scale mangrove-friendly shrimpculture can be developed in the open mangrove areas within the estuaries. Planting of mangrove seedlings in the inner flooded side of the shrimp pond dykes would be an effective system to re-afforest the open mangrove areas (Fig 1). It is important to note that ecological aquaculture in the Kenyan mangrove areas is supported by the Kenya Forest Act. 2005. However it is subject to fulfilment of the provided conditions.

The small-scale shrimp culture in the open mangrove areas has the potential to contribute to the economic well being of the local communities. The reduction of acid sulphate soils and the proliferation of the natural food to feed shrimp post in the ponds can be achieved ecologically by periodic treatment of the ponds bottom soil with ecologically harmless techniques such as the use of organic matter from the livestock yards, agricultural lime and management of pond water levels to allow adequate sunlight and primary production (Burford, 1997; Le Bitoux, 1985; Preston et al., 1992). Primary production is the basis of the ecological friendly biofloc, periphyton and biofilm technology which can be applied to naturally feed the shrimp (Azim and Wahab, 2005a; Burford et al., 2004; Crab et al., 2010). High carbon content (starch) agriculture by products are locally affordable and can be used to supplement feed inputs as a direct diet or substrate for harnessing the microbial protein (Asaduzzaman et al., 2008; Hari et al., 2006). Shrimp ponds integrated into mangrove forests are subject to intercept considerable amount of leaf litter fall. The leaf litter is a potential source of nutrients and may play an important role in enhancing the supply of the natural food. However this hypothesis needs to be tested.

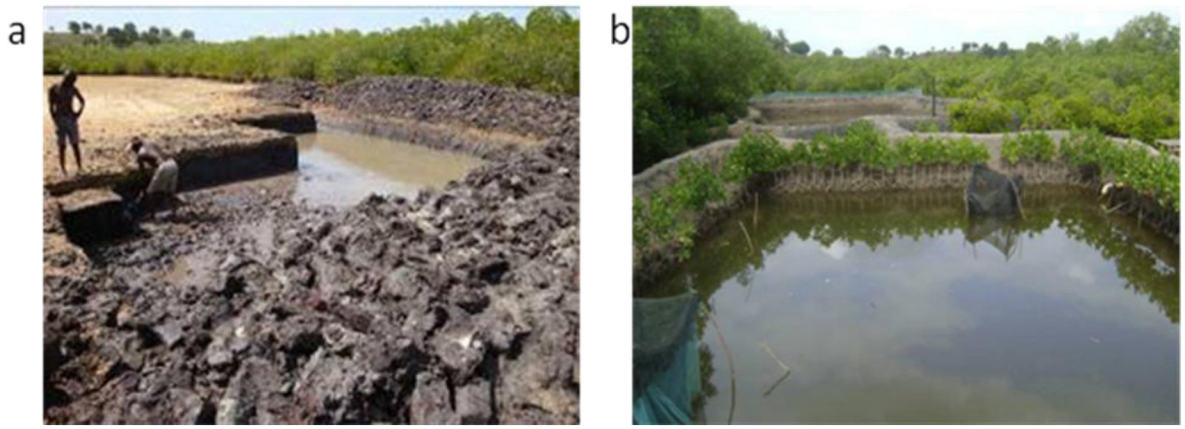


Fig 1: (a) Shrimp culture can be developed in the open mangrove areas within the estuaries. (b)Mangrove planted at the inner flooded side of the shrimp pond dyke is an effective system to re-afforest the open mangrove areas.

1.1.2 Shrimp biology of relevance to ecological aquaculture

1.1.2.1 Life cycle

In order to develop an appropriate system to manage the supply of natural food in the mangrove shrimp ponds, one must understand the various ecological and biological elements involved. For instance, ecological shrimp aquaculture cannot be developed without understanding the biology and the ecology of the penaeid shrimp of interest (Primavera, 2006; Rothlisberg, 1998). Penaeid shrimp inhabit shallow and inshore tropical and subtropical waters and comprise most of the total world catch of shrimp (Dall et al., 1990). Tropical penaeids spawn twice a year (Rothlisberg, 1998).

The two penaid shrimp species studied, *Penaeus monodon* (tiger shrimp) and *Penaeus indicus* have a similar life cycle. Adult penaeid shrimp migrate into the open water to spawn. Fertilized eggs (diameter less than 0.4mm) are shed free into the water where they settle at the bottom and hatch into various stages ranging from nauplius, protozoea and mysis. There are five nauplius stages. The first stage is about the size of the egg and the succeeding stages are slightly larger. Nauplius have limited swimming ability and usually become part of the oceanic plankton. There are three protozoea stages. Protozoea have undergone development of their mouth parts and the abdomen has begun to develop. Protozoea and nauplii are found in the oceanic waters and primarily feed on the micro-algae (phytoplankton). There are three mysid stages. Mysis have an early development of legs and antennae. During the mysis stage the feeding changes from primary herbivorous to carnivorous. The mysis larvae develop into post larvae. The post larvae have walking and swimming legs which are fully developed and appear as miniature shrimps. The post larvae develop into juvenile stage where growth is rapid and they soon resemble the adults. The shrimp-like post larvae and juvinile reach inshore waters about two weeks after hatching where they become demersal and settle on various habitats including seagrass beds and the muddy banks of mangrove-lined estuaries. The post larvae and juviniles are primarily carnivorous. However they feed on phytoplankton, zooplankton, detritus and small macro-invertebrates (Dall et al., 1990; Lavens and Sorgeloos, 1996). The postlarvae settle in the upper parts of the tidal creeks (mangrove communities) whereas the juvenile remain in the marsh creeks and move to the deeper rivers where they become sub-adults. The adult shrimp are totally mature to produce sperm and eggs and are usually found in the ocean (Fig 2).

Decomposing mangrove litter leaches large amounts of dissolved organic material which supports a microbial food web that ultimately may feed shrimp postlarvae (Benner and Hodson, 1985a; Keshavanath and Gangadhar, 2005b). However, shrimp undergo an ontogenetic shift in diet in the different larvae stages even within the progressing postlarvae stage (Rothlisberg, 1998). 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 omega-3 poly unsaturated fatty acids (PUFA)(Sorgeloos and Lavens, 2000; Watanabe et al., 1983). This essential element is ultimately derived from the natural food sources such as the phytoplankton, zooplankton and macro-invertebrates (Parrish, 2009). A highlight of the potential natural food sources for shrimp post larvae is important to understand whether a mangrove estuary is an important destination in the shrimp life cycle.

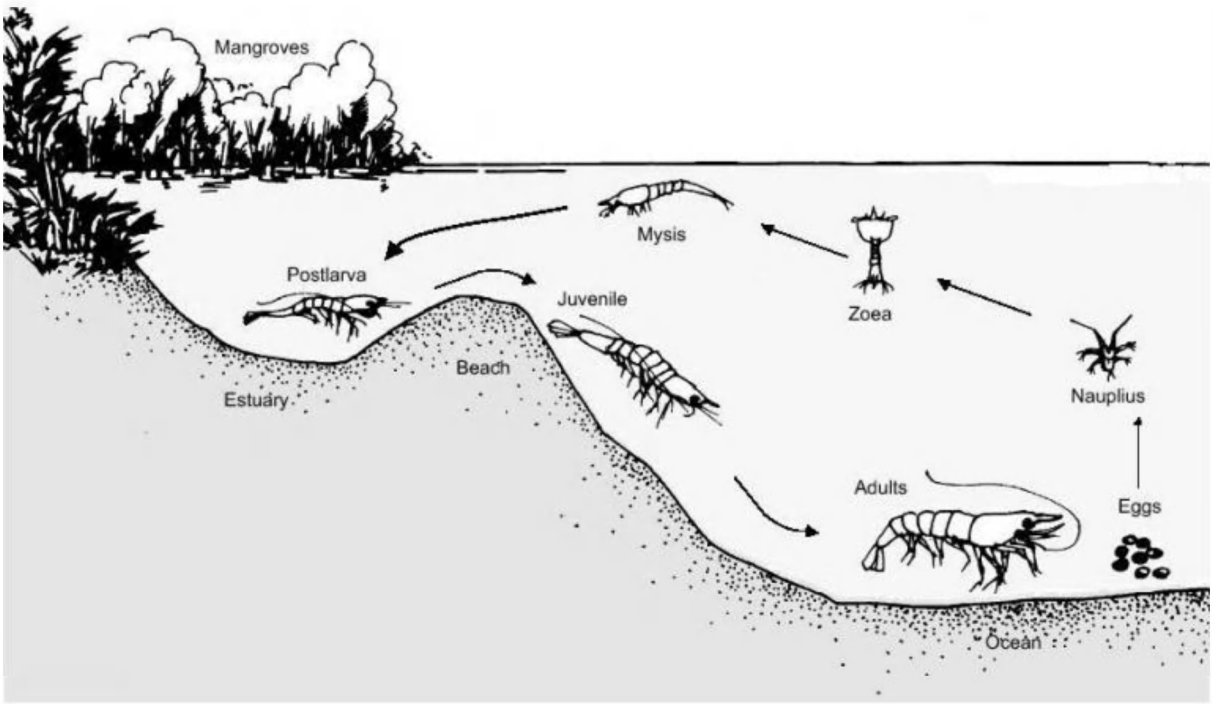


Fig. 2: The life cycle of penaeid shrimp in relation to their habitat (Bailey-Brock and Moss, 1992)

1.1.3 Nutritional requirements for penaeid shrimp

1.1.3.1 Proteins

In shrimp farming, quality and quantity of available food affects survival of cultured shrimp and inadequate nutrition is one of the main limitations (Vogt et al., 1985). This observation

should be emphasized in promoting supply of natural food in a mangrove system. For instance the important natural feed for *P. monodon* post larvae should resemble the shrimp tissue in proximate composition of 65-71% crude protein and 4.9 - 6.5% lipids (Focken et al., 1998) and should therefore match the shrimp dietary requirement for protein of 39% (Focken et al., 1998) or 32-48% (Davis and Kureshy, 2002; Watanabe, 2002). The natural diet should also be higher in digestibility compared to plant tissue and detrital material which has higher fiber content (Focken et al., 1998). The high body protein composition of shrimp may dictate dietary requirements for high protein content. The high protein requirements could emanate from the fact that to a large extent shrimp just like fish use proteins for energy production (Hepher, 1988). The protein digestive enzyme trypsin response was observed to increase with size and stage of development in *P. indicus* post larvae (Ribeiro and Jones, 2000). A sharp increase in tryptic enzyme was observed to coincide with the completion of hepatopancreas development in the later post larval development (from substage PL 20) in *P. setiferus* (Lovett and Felder, 1990b). Al Azad et al. (2002) found that the phototrophic bacteria *Rhodovulum sulfidophilum* contained 62.3% crude protein and was able to contribute substantially to the growth of *P. monodon* post larvae. Mohamed (1996) and Rengpipat et al.(1998) reported similar observations.

1.1.3.2 Feed digestibility

The feeding habits and the physical nature of the gut may play a big role in dictating digestibility requirements of the natural feed. The gut length of shrimp can be compared to that of fish which is short and the ratio of gut length to body length is small i.e. 2.0–2.5 times longer than the body in carp (Hertrampf and Piedad-Pascual, 2000), hence the natural feed must be highly digestible. Using a mixture of four strains of *Rhodopseudomonas* diet to produce *Penaeus chinensis*, Cui et al.(1997) ranked grazing ability among the requirements for improved physical and physiological performance of the shrimp post larvae. Penaeid shrimp are known to consume diatoms. Diatoms are readily digestible by shrimp because of their low fiber content and they contain high levels of essential lipids (Moss, 2004).

1.1.3.3 Lipids

Shrimp have an essential nutritional requirement for 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 elongate FA chains and desaturate PUFA to HUFA. Therefore an exogenous source of HUFA is necessary as part of the essential fatty acids. Past studies have specified two classes of PUFA, linoleic acid (18:2ω-6) and linolenic acid (18:3ω-3), and HUFA, eicosapentanoic acid (EPA; 20:5ω-3) and docosahexanoic acid (DHA; 22:6ω-3) as essential for the growth of the shrimp *P. japonicus,* (Guary et al., 1976; Kanazawa et al., 1977; Kanazawa et al., 1979; Kanazawa et al., 1978) 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 post larvae of *P. monodon* resulted in improved post larvae quality by increasing their ability to survive exposure to salinity shocks.

1.1.4 Potential natural diet for penaeid shrimp in a mangrove ecosystem

The concept of farming down* the food chain emanates from the various reports on the natural diets of penaeid shrimp of commercial importance such as the *P .indicus* and *P. monodon* in the mangrove habitats. Using stable carbon isotopes, a remarked contribution from phytoplankton, epiphytic algae, benthic algae and micro-algae compared to carbon from mangrove detritus has been reported (Bouillon et al., 2002; Mohan et al., 1997; Primavera, 1996; Stonner and Zimmerman, 1988). *P. monodon* juveniles have been observed to feed heavily on zooplankton in both culture ponds and laboratory containers (Chen and Chen, 1992).

Some studies have shown post larval of *P. monodon* to have grown well when fed 'lablab', a micro-benthic biofilm complex found in mangrove mudflats (Fig 3). This biofilm consists of blue-green algae, diatoms and other small plants and animals (Apud, 1988). Gut content and $\delta^{13}C$ stable isotope analyses of *P. monodon* reared without supplementary feeding in extensively managed ponds showed detritus to be the most common food, followed by animal remains, diatoms, cyanobacteria and green algae (Bombeo-Tuburan et al., 1993). *P. indicus* shifts in feeding behaviour from mainly herbivorous at early larval stage to omnivorous/carnivorous during post-larval development (PL20 and PL22, 28 and35 days old) (Lovett and Felder, 1990a) or opportunistic feeders depending on what dominates the

environment (Preston et al., 1992). However, they may not necessarily assimilate into the tissue what they ingest, for instance, in the case of some species of Cyanobacteria such as the *Trichodesmium* spp. (Preston et al., 1998). The post larvae of *P. monodon* consume phytoplankton, zooplankton, detritus, periphyton and small macro-invertebrates (Lavens and Sorgeloos, 1996).

Reports on the natural diets of *P. monodon* and *P .indicus* clearly points out the ranking of these shrimp as secondary consumers in a complex food web within the mangrove system. However, their feeding is not necessarily concentrated on macro-invertebrates but also tend to move down the trophic levels with significant uptake of micro-algae and the detrital fraction. Mangrove detritus has low nutritional importance to penaeid shrimp (Bouillon et al., 2002; Mohan et al., 1997; Primavera, 1996). The nutritional role of detritus could probably be understood by appreciating its importance as a substrate supporting bacterial proliferation in the sediment. Mangrove detritus and sediment fraction has been found to support a consortium of bacteria and meiofauna and has attracted much attention in nutrition studies of penaeid shrimp (Abraham et al., 2004; Ansari et al., 1993; Hari et al., 2006; Somerfield et al., 1998). This could be due to the complex nature of bacteria in supporting the base of the food web by enhancing primary production through re-mineralization (Avnimelech and Gad, 2003; Moriarty, 1997). Bacteria enhance cycling of organic detritus to a digestible state available to depositfeeders such as meio- (38µm-1mm) and macrofauna (>1mm) which are then consumed by shrimp postlarvae (Dittel et al., 1997). The role of bacteria in mangrove food web should be emphasized because of its high potential to enable the culture of penaeid shrimp down the food chain.

*Using primary trophic levels as the energy sources for the secondary consumers.

1.1.5 Microbial diet for penaeid shrimp

Ecological studies are scarce on the importance of bacteria as a direct food source to *P. monodon* and *P. indicus* post larvae in mangrove systems. Many studies have concentrated on bacteria as a premix supplement in artificial feeds (Aujero et al., 1985; Cui et al., 1997; Mohamed, 1996; Ochoa- Solano and Olmos-Soto, 2006; Rengpipat et al., 1998), flocculated mass or biofloc in high intensive shrimp culture ponds and tanks (Burford et al., 2004; Crab et al., 2010), biofilm in recirculation systems (Abreu et al., 2008; Azim et al., 2001) and periphyton on structures in semi-intensive and extensive ponds (Azim and Wahab, 2005a; Bratvold and Browdy, 2001a; Keshavanath and Gangadhar, 2005b). Although these studies

have demonstrated bacteria as an important nutrition source for penaeid shrimp in promoting growth and survival (Aujero et al., 1985) and grazing ability (Cui et al., 1997), the motivating factor has been to use bacteria as a direct source of cheap protein in artificial feeds or to optimize nitrogen retention from the culture water. Bacteria are known to have a balanced nutritional value and can increase the survival of *P. monodon* larvae if used to supplement plankton feed (Aujero et al., 1985). However recent studies by Al Azad et al. (2002), demonstrates insufficient levels of tryptophan and poly unsaturated fatty acids (PUFA) in the bacteria *Rhodovulum sulfidophilum* to support better growth and survival of *P. monodon* post larvae. The study recommended that the deficient nutrients in the bacterial biomass could be supplemented by marine diatoms which are rich in PUFA (Parrish, 2009).

1.1.6 Microbial biofilm in mangrove systems

In mangrove systems the natural food for penaeid shrimp post larvae may occur in the form of a biofilm consisting of a mixture of bacteria, micro-algae and meiofauna. The biofilm would be found on the bottom mud flats as lab lab, attached to vertical structures such as the decomposing litter as periphyton (Fig 4), or flocculated organic matter suspended in the water column as lumut or biofloc.

1.1.6.1 'Lablab'

In a natural setting, bacteria may not occur as separate entity but in combination with micro-algae, fungi, organic matter and meiofauna (Azim and Wahab, 2005a; Burford et al., 2003). In mangroves systems this complex mass has been observed to occur as 'lablab', which is a benthic biofilm of mangrove detritus, cyanobacteria, diatoms and associated fauna such as copepods, rotatoria, nematodes, and crustacean larvae (Focken et al., 1998) (Fig 3). Studies have shown that post larval *P. monodon* grew well when fed 'lablab' (Apud, 1988). 'Lablab' is found growing on the mangrove mudflats in shallow pools or pond bottom and later floats at the water surface (Focken et al., 1998). 'Lablab' could be a major food source for species which are then consumed by shrimp post larvae (Dittel et al., 1997; Moorthy and Altaff, 2002).

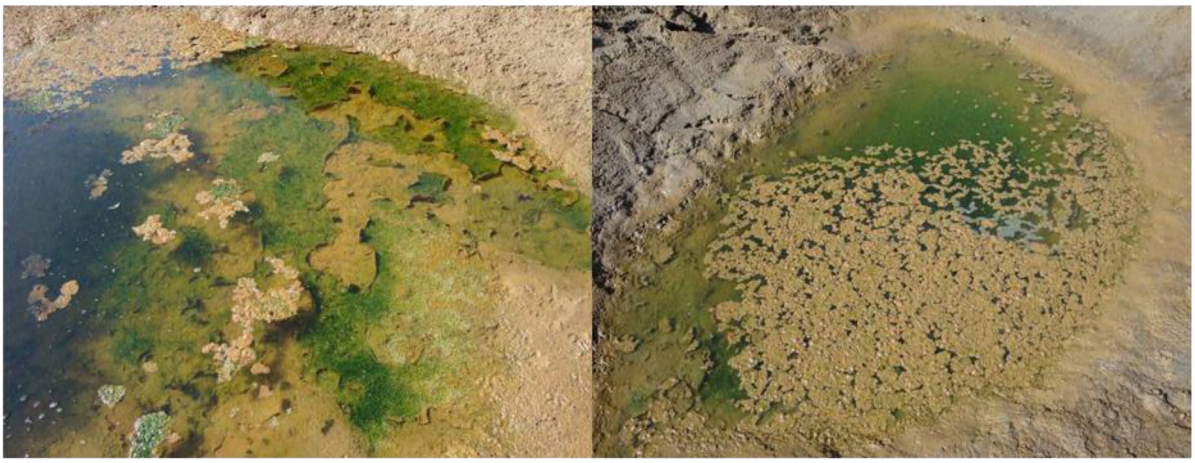


Fig 3: Biofilm ('lablab') on a mangrove mudflat

1.1.6.2 Periphyton

Periphyton consists of microbiota attached on submerged substrates and is composed of algae, bacteria, fungi, protozoa, zooplankton and other invertebrates (Azim and Wahab, 2005a) (Fig 4). Protein content of periphyton is around 25% of the dry matter (Azim et al., 2002). Shrimp post larvae of *P. monodon* grew better when fed mangrove leaf litter with dead or living periphyton (Nga et al., 2004). The presence of structures with epiphytic growth increased production in *Litopenaeus vannamei* culture (Bratvold and Browdy, 2001a). Based on stable carbon isotope ratios, Primavera (1996) observed that *P. monodon* were closer to phytoplankton and epiphytic algae, that are both part of periphyton layer, than to mangrove leaves and detritus.

Fig 4: Periphyton or biofilm on the decomposing mangrove leaf litter of *Rhizophora mucronata*

1.1.6.3 'Lumut'and biofloc

'Lumut' is a community of filamentous green algae and attached organisms that form a tangled mass in the water column (Focken et al., 1998). Samples of 'lablab' and 'lumut' have

been analyzed for proximate composition and were found to contain 3.4 and 2.6% crude protein, respectively (Focken et al., 1998). 'Lablab' and 'lumut' have very low crude protein because it is low in animal and bacterial fraction (Focken et al., 1998). The bulk of 'lablab' is composed of 'flakes' and seems to originate from the water column. Flake is probably digested at a higher rate than the cells of plant tissue (Focken et al., 1998). On the other hand, biofloc, a flocculated material in intensive shrimp culture ponds, contain large numbers of bacteria and senescent phytoplankton (Burford et al., 2003). Bioflocs contain high protein content (43%) and the levels of some essential amino acids are similar to those found in the whole shrimp homogenates, i.e. arginine (2%), methionine (0.5%) and lysine (2.1%) (Jory et al., 2001). Bioflocs as a natural food source can contribute substantially to the nutrition of *Litopenaeus vannamei* (Burford et al., 2004) and *P. monodon* (Epp et al., 2002).

Is it possible to promote nutritional value of 'lablab' and 'lumut' from the studies on bioflocs? The fact that reports are scarce on the nutritional value of 'lablab' and 'lumut' points to the need for an extended study, especially in evaluating their proximate value of proteins and lipids which are the most important qualities in determining their feasibility as feed in ecological shrimp culture. Such study should also focus on intervention methods to optimize their nutritional value and digestibility. As observed for biofloc, nutritional value of lablab and lumut may be achieved by increasing the bacterial and micro-algae load. Microbial protein can be promoted by maintaining a good microbial C/N ratio by adding carbohydrates to enhance nitrogen uptake and vice versa (Avnimelech, 1999; Hari et al., 2006). The nitrogen uptake by bacteria and algae decreases the ammonium concentration more rapidly than nitrification with an additional benefit of enhancing shrimp production by improved water quality (Hargreaves, 2006). Most fish farmers use complete diets comprising proteins (18–50%), lipids (10–25%), carbohydrates (15–20%), ash (<8.5%), phosphorus (<1.5%), and trace amounts of vitamins and minerals (Craig and Helfrich, 2002). As advised for bioflocs (Schryver et al., 2008), the nutritional composition of 'lablab' and 'lumut' should be improved to these values.

1.1.7 Biological shrimp culture and the mangrove ecosystem health

The reviewed studies demonstrate the importance of bacteria, micro-algae and meiofauna as food for penaeid shrimp which can be applied in ecological shrimp aquaculture. However the challenging issue is how to promote such application while maintaining the ecological health of a coastal ecosystem. While using phototrophic bacterium *Rhodovulum sulfidophilum*

supplemented with *Artemia* in feeding post larvae of the giant tiger shrimp *P. monodon,* Al Azad et al.(2002) observed that diets comprising 3-5% bacteria biomass had lowest growth and survival and gave the highest levels of ammonia-nitrogen in the culture water. Microbial bio-flocs and periphyton improves water quality by removing total ammonia nitrogen (TAN) naturally (Avnimelech, 1999; Ebeling et al., 2006; Hargreaves, 2006; Schneider et al., 2005). By facilitating development of heterotrophic bacteria, sustainability of extensive shrimp aquaculture can be improved through reduced concentrations of potentially toxic TAN and nitrite nitrogen (NO_2-N) and the reduced water based nitrogen pollutants (Hari et al., 2004; Hari et al., 2006). In semi-intensive *Litopenaeus vannamei* and *Penaeus setiferus* ponds, 52% of nitrogen was retained by the pond biota (Parker et al., 1989; 1991).

It seems an area of interest to use the low capital investment approach of balancing the carbohydrate and nitrogen to the microbial C/N ratio to enhance proliferation and nutritional value of biofilm as well as improve the water quality. Such an approach would largely contribute to the success of ecological shrimp aquaculture in the mangrove systems. To enhance natural proliferation of biofilm in a mangrove system, decomposing leaf litter would definitely play a major role. The studies performed in this PhD project attempts to evaluate the extent to which a mangrove derived substrate can enhance the proliferation of a quality biofilm to feed the shrimp post larvae.

The proceeding chapters of this thesis will use the term biofilm to represent the terms periphyton, lablab, lumut and biofloc as a general acronym defining the complex mixture of bacteria, microfauna and low molecular weight organic matter associated with both organic and inorganic substrates in a mangrove habitat.

1.2 Aims, objectives and thesis outline

1.2.1 General aim

This PhD study has derived its primary motivation from the fact that use of fish meal in shrimp aquaculture has a negative effect on the coastal fisheries in the sense of depleting fish stocks, inability to make economic sense and its high potential in discharging wastes that pollutes the receiving coastal ecosystems. So far i have briefly described the potential and benefit of using natural food sources to culture shrimps. This approach is referred to as an ecological shrimp aquaculture since it derives its feed input from the naturally occurring food sources. This practice has an economic and an environmental advantage of reducing the use of

fish meal as the main food input. **The overall aim of this PhD study is therefore to understand the feeding ecology of penaeid shrimp in a mangrove system and apply this knowledge to design an ecological shrimp aquaculture system which can derive biological food resources from the mangrove ecosystem.**

1.2.2 Specific aims and thesis outline

The extent to which the natural food can be applied in culturing penaeid shrimp in a mangrove ecosystem depends on the biochemical influences of the decomposing mangrove leaf litter. **Specific objective 1** is explored in ***Chapter 2*** where this PhD study specifically aims at assessing to what extent shrimp post larvae can ingest and assimilate organic matter from decomposing mangrove leaf litter given the option of a bacterial biofilm as a food source. This study uses ^{13}C stable isotope and fatty acid profiling to selectively label bacteria and monitor the uptake into the shrimp tissue. The nutritive importance of the mangrove organic matter and the associated bacterial biofilm, to shrimp post larvae, may depend on the extent of decomposition and the presence of an alternative natural food source. **Specific objective 2** is therefore explored in ***Chapter 3*** where hypothesis generated in chapter 2 develops the aim of assessing the microbial contribution to the nutritive value of the decomposing mangrove leaf litter and the associated biofilm to shrimp post larvae in comparison to an estuarine food source. Nevertheless, the nutritive value of the biofilm to shrimp post larvae may depend on the specific type of the associated biota which may show temporal variation. **Specific objective 3** is therefore investigated in ***Chapter 4*** which is aimed at assessing the temporal variation in the biota associated with the biofilm developing on the decomposing mangrove litter and their food-value-potential to support the growth of shrimp post larvae. This objective is investigated by characterizing the microalgae and epifauna associated with the decomposing mangrove leaf litter at the different stages of biofilm development. The time based food value of the biofilm is assessed by observing the growth and survival performance of shrimp post larvae foraging on such a biofilm. It is important to consider that, food value of biofilm may also depend on the location of the decomposing mangrove leaf litter which may vary depending on the exposure to the ambient environmental conditions. Among the important environmental conditions is the exposure to sunlight, sediment and water quality. **Specific objective 4** of this PhD study was therefore investigated in ***Chapter 5.*** The aim of this study was to assess the effect of exposure to the different environmental conditions of sunlight and sediment to the temporal variation in the diversity and abundance of biota associated with the biofilm developing on the decomposing leaf litter.

The diversity and abundance of the biota and the quality of the associated water body is inferred to predict the potential impact it would pose onto the growth and survival of shrimp post larvae.

In chapters 2, 3, 4 and 5; the nutritive potential of decomposing mangrove leaf litter and the associated biofilm to shrimp post larvae at the different conditions of space and time is explored. However, is it possible to practically culture shrimp post larvae solely on the decomposing mangrove litter and the associated biofilm? **Specific objective 5** of this PhD study is investigated in ***Chapter 6*** which aimed at assessed the nutritive effect of the biofilm associated with the decomposing mangrove leaf litter on the physiological performance of shrimp post larvae. ***Chapter 7*** links the observations made, from the various chapters, to the opportunities available in developing ecological shrimp aquaculture in relation to the management of mangrove ecosystems along the Kenyan coast. Suggestions for further research to ellaborate on the current findings and recommendations are provided.

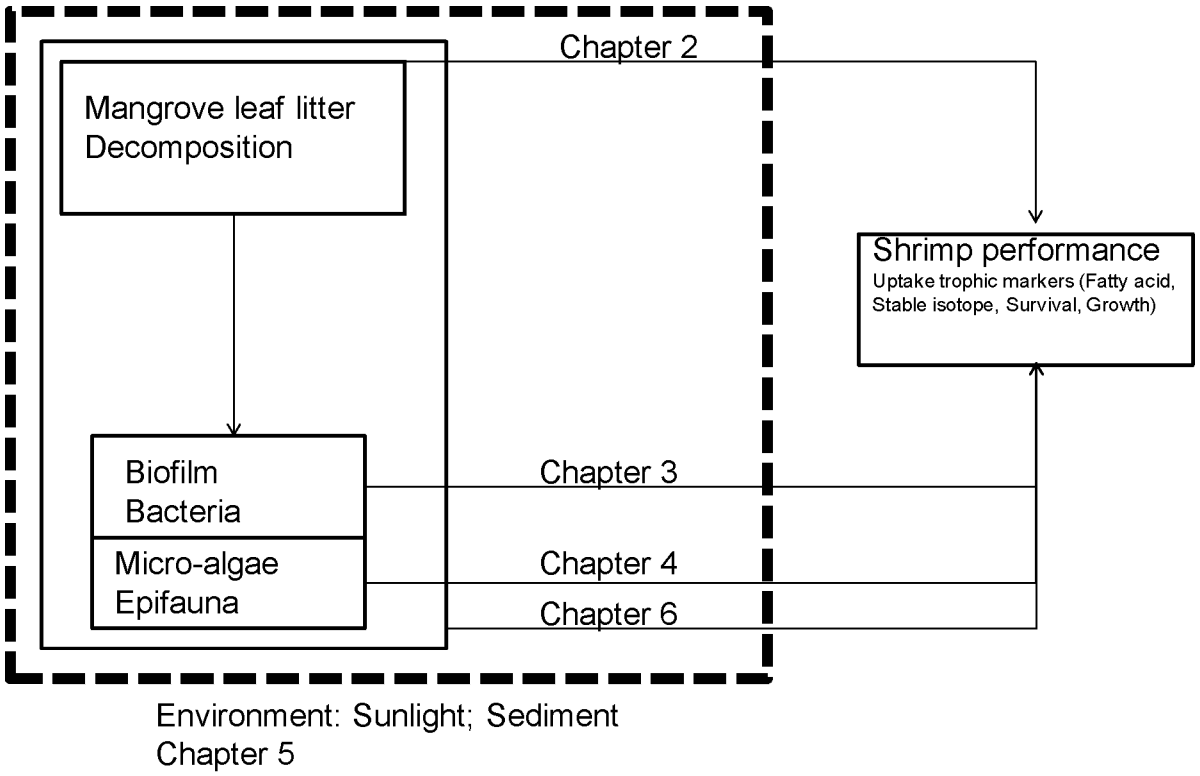


Fig 5: Conceptual scheme of the interaction between ecological parameters hypothesised to provide the ecosystem function of the mangrove leaf litter in providing dietary support to shrimp post larvae.

Chapter 1

2 Chapter 2

2.1 ^{13}C stable isotope labeled bacteria highlight that shrimp post larvae can alternatively assimilate mangrove detritus

Charles Gatune [a,c]*, Ann Vanreusel[a], Renison Ruwa[b], Peter Bossier[d] and Marleen De Troch[a]

[a] Ghent University, Biology Department, Marine Biology, Campus Sterre, Krijgslaan 281-S8, B-9000, Gent, Belgium.
[b] Kenya Marine and Fisheries Research Institute, P.O Box 80100, 81651, Mombasa, Kenya.
[c] Ministry of Fisheries Development, P.O. Box 90423 – 80100, Mombasa, Kenya.
[d] Ghent University, Faculty of Bioscience Engineering, Laboratory of Aquaculture and Artemia Reference Centre, Rozier 44, B-9000, Gent, Belgium.

*Corresponding Author: Email address: kgatune@yahoo.com

The set up of the controlled shrimp feeding experiment in the laboratory tanks at the Kenya Marine and Fisheries research Institute (Photo courtesy Gatune C. 2010)

2.1.1 Abstract

Mangrove detritus (decomposing leaf litter) was previously found to be of minor importance as food to penaeid shrimp post larvae. However, it remains undocumented whether mangrove detritus could contribute to the diet of the shrimp post larvae when other food sources are scarce. In the present study, the biofilm developing on the decomposing mangrove leaf litter of *Rhizophora mucronata* was labeled with ^{13}C stable isotope sodium acetate and fed to the shrimp post larvae of the *Penaeus indicus*. Together with the analysis of specific bacterial fatty acid biomarkers, this experiment allowed to analyze the contribution of bacteria from mangrove detritus as food source to shrimp post larvae. Shrimp post larvae feeding on the biofilm recorded higher uptake of the ^{13}C into their tail tissue. The uptake of the ^{13}C was supported by the abundance of the bacterial fatty acid biomarkers in the same treatments. The shrimp post larvae assimilated both the bacteria and the mangrove detritus in the absence of any other food.

Keyword: pulse-chase experiment; stable isotopes; fatty acids; shrimp; mangrove; bacteria

2.1.2 Introduction

Isotopic studies generally rank mangrove detritus as being of minor importance as food source to secondary consumer production in estuarine systems but do not rule out low-level use of mangroves in the estuarine food webs (Fry and Ewel, 2003). However, these studies have not tested whether mangrove detritus could contribute to the diet of consumers when other food sources are scarce. Therefore, more detailed experimental studies by means of pulse-chase tracking of uptake (stable isotopes) (Boecklen et al., 2011) and by the measurement of trophic biomarkers e.g. fatty acids (Kelly and Scheibling, 2012) are useful to get more precise information on the use of particular food sources.

In mangrove forests and estuarine systems, most carbon is typically lost to the microbial loop (Benner and Hodson, 1985b) whereas carbon imported from other estuarine sources offers a reasonable competition (Bouillon and Boschker, 2006). In large-scale growth installations of shrimp (e.g. aquaculture ponds), bacteria are known to play an important role especially through nutrient budgets (Moriarty, 1997). In integrated mangrove-shrimp aquaculture, bacteria would predominate re-mineralization of nitrogen and phosphorous from decomposing mangrove leaf litter (YiMing and Sternberg, 2007). The nutrients would play an important role in further nourishing the edible benthic bacteria and algae (Twilley and Pelegri, 1998). Consequently, these microbiota are recognized to support the shrimp pond food web. The precise nature and main source of bacteria for uptake by shrimp is however not documented as such.

Bacteria associated with the decomposing mangrove litter typically form a biofilm consisting of the mangrove organic matter in combination with microalgae and bacteria, forming a potential food source for shrimp post larvae (Abreu et al., 2008; Abreu et al., 2007; Azim and Wahab, 2005a; Thompson et al., 2002). The mangrove organic matter associated with the microbiota is ingested by the shrimp post larvae (Bombeo-Tuburan et al., 1993). The extent of assimilation of mangrove organic matter determines the net nutritive importance of mangrove litter in an ecological shrimp pond. Furthermore, knowledge on the specific contribution of bacteria in this energy transfer would be pivotal information for an optimal turnover in the pond. The present study labeled the bacterial biofilm on the decomposing mangrove leaf litter using ^{13}C stable isotope labeled acetate (Boschker et al., 2001). According to the 'you are what you eat' principle (Fry, 2008), the assessment of the uptake of the ^{13}C into the shrimp tissue provides insight in the preferential ingestion and assimilation of bacteria in the presence

of mangrove detritus. Such observation could assist in assessing the importance of bacteria and mangrove detritus as a food source for shrimp post larvae. Bacterial fatty acid biomarkers (Rajendran et al., 1995; Taylor and Parkes, 1983) can provide additional in-depth evidence of the presence and the assimilation of the bacterial constituent of the biofilm.

2.1.3 Material and methods

2.1.3.1 Biofilm labeling with ^{13}C

Senescent mangrove leaves were dried in the shade to a constant weight and incubated for 2 weeks to allow for bacterial attachment (seeding) in a shrimp pond situated in in a mangrove forest at Majaoni Silvofishery and the mangrove conservation Farm located in Mtwapa creek, Northern coastal region of Kenya ($3^{o}57'S$; $39^{o}42'E$). After 2 weeks a clear biofilm was observed that could be tested as potential food source for shrimp (see experimental design for different ways of offering as food).

The seeded leaf litter was then transferred and incubated in wide 350 l laboratory tanks at a leaf concentration of 1 g l^{-1} with aeration for 8 days under natural light regime. During this period the natural water in the tank was enriched with ^{13}C sodium acetate solution ($^{13}C_2$ acetate, both carbon atoms labeled; 99% ^{13}C at labeled position, 0.1µM l^{-1} final concentration) (Hall and Meyer, 1998). This concentration of acetate was intended to selectively label bacteria from fungi in the biofilm developing on the incubated mangrove leaf litter. Fungi has a 1000 times higher half saturation constant for labile dissolved carbon molecules compared to bacteria, i.e 0.1 to 1 µM l^{-1} for fungi and 0.001 µM l^{-1} for bacteria (Hall and Meyer, 1998). Hence because acetate concentrations are 0.1 µM l^{-1} it is unlikely that fungi can take up the label. Algae also exhibit similar acetate diffusion kinetics to fungi, thus cannot take up acetate at low concentrations (Hall and Meyer, 1998). This labeling technique by means of impregnation yielded an average increase of ^{13}C signal from –24.88 ± 0.79 ‰ (control) to 12.25 ± 0.40 ‰ (labeled biofilm).

2.1.3.2 Experimental design

A shrimp feeding experiment was set up in triplicate with the following 4 treatments as food source for shrimp: 1) leaf litter without biofilm (SL); 2) ^{13}C labelled biofilm (SB); 3) litter with ^{13}C labelled biofilm (SLB); 4) no feeding as control (CS). Shrimp post larvae of *Penaeus indicus*, PL 15-25 hatched from the same brooder were obtained from Bagamoyo fisheries shrimp hatchery, Tanzania. The post larvae from the same brooder met the requirement of the standardization of the test organism.

The feed was prepared as follows; 1) SB: Labeled biofilm was gently scraped from the leaf litter surface and rinsed with distilled water to remove excess of ^{13}C sodium acetate; 2) SL: mangrove leaf litter was stripped off the biofilm and rinsed 3 times with distilled water to remove biofilm and any excess label; 3) SLB: mangrove leaf litter and the attached biofilm were gentle rinsed with distilled water to remove the excess label. Feed pellets were then made by finely grinding and mixing the leaf litter and biofilm with 2.5% cellulose binder (AQUACOP and Cuzon, 1989). The resulting paste was oven dried at 70 °C for 24 h and ground into pellets not exceeding 1 mm. Shrimp post larvae of *Penaeus indicus*, PL 15-25, were starved overnight and stocked in each treatment at a stocking density not exceeding 2PL l^{-1}. The stocked post larvae were fed on the different feeds at a daily rate of 10% wet body weight, twice a day, respectively. The experiment was carried out for a period of 3 weeks under 12h/12h dark: light regime at a temperature of 27°C. 3 week duration was used to prevent abnormal statistical variation from the unexpected mortality within the starved control. The period was also adequate to exclude short term variability in $\delta^{13}C$ of the tail tissue.

2.1.3.3 Stable isotope analysis

Stable isotope measurements ($\delta^{13}C$) were performed on the biofilm, mangrove litter and shrimp tail muscle based on oven-dried samples (60°C for 24 h). For shrimp tissue, at least 3 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, biofilm and litter were ground to fine powder, weighed in triplicates and wrapped in tin capsules (8x5 mm, Elemental Microanalysis Limited) as follows: 1mg shrimp tail tissue; 10.5-66mg biofilm as sediment organic; 4.9-5.9 mg ground leaf litter as decaying plant material. 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). Incorporated ^{13}C is reflected as excess (above ground) ^{13}C, expressed as total uptake (I) in micrograms of ^{13}C per unit carbon of the sample organic carbon biomass. Excess ^{13}C is the difference between the fraction of ^{13}C of the control i.e the shrimp which were not fed ($F_{control}$, i.e signature of shrimp that were not fed) and the sample (F_{sample}), where $F=^{13}C/ (^{13}C+^{12}C) = R/(R+1)$. The carbon isotope ratio (R) was derived from the measured $\delta^{13}C$ values as $R = (\delta^{13}C /1000+1)$ x

R_{VPDB} with R_{VPDB} =0.0112372 as $\delta^{13}C$ is expressed relative to Vienna Pee Dee Belemnite (VPDB).

Since the offered food sources (leaf litter and biofim) had different initial $\delta^{13}C$ signatures, the uptake per shrimp tissue biomass was further standardized taking into account the proportion of ^{13}C in each food source. The amount of carbon that was taken up by shrimp and expressed per unit carbon biomass of shrimp tissue was multiplied with a factor 92.06, 91.91 and 88.91 for the treatments with labelled litter, litter with the associated biofilm and biofilm, respectively. These correction factors were derived from the difference in the atomic percentage of ^{13}C in the three food sources (i.e on average 1.09% ^{13}C for the litter, 1.09% ^{13}C for the litter and the associated biofilm and 1.12% ^{13}C for the biofilm) (De Troch et al., 2008).

2.1.3.4 Fatty acid extraction and analysis

Samples of shrimp tail tissue, mangrove litter and biofilm were stored at -80 °C and later on freeze-dried (lyophylised) and weighed prior to analysis. Fatty acids were extracted and methylated to fatty acid methyl esters (FAMEs) by a modified one-step derivatisation method after Abdulkadir and Tsuchiya (2008). The boron trifluoride-methanol reagent was replaced by a 2.5% H_2SO_4-methanol solution since BF_3-methanol can cause artefacts or loss of PUFA (Eder 1995). The fatty acid methylnonadecanoate C19:0 was added as an internal standard for later quantification (Fluka 74208). 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 analysed using a Hewlet Packard 6890N GC linked to a mass spectrometer (HP 5973). The mangrove litter and biofilm samples were run in splitless mode injecting 1 µl extract per run, whereas the shrimp tissue samples were run in split x10 mode by injecting o.1 µl extract per run. The samples were run at an injector temperature of 250 °C using a HP88 column (60m x 0.25mm internal diameter x 0.20µm 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 analysed 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). Odd carbon fatty acids (C15:0 and

C17:0) were used as biomarkers for bacteria (Rajendran et al., 1995) present in the biofilm and assimilated by the shrimp post larvae.

2.1.3.5 Water quality parameters

Water quality was monitored by weekly measurements of temperature, dissolved oxygen, pH, salinity and total ammonium nitrogen. Temperature, dissolved oxygen, pH were measured using meters, salinity was measured using a refractometer whereas total ammonium nitrogen was analysed in the laboratory according to Eaton et al.(2005)

2.1.3.6 Data analysis

Statistical analyses (One way ANOVA) was conducted with Statistica 7.0 software to compare the specific uptake ($\Delta\delta^{13}C$) of ^{13}C into the tail tissue of shrimp which were fed different food types that were ^{13}C prelabeled. One way ANOVA was also used to compare the abundance of the bacterial fatty acids both between the mangrove derived feed and between these feed and the tail tissue of shrimp foraging on it. All data were checked for normality and homogeneity requirements for parametric analysis. Data that did not meet normality requirements after being transformed were analysed non-parametrically following Kruskal-Wallis ANOVA & Median Test.

2.1.4 Results and discussion

2.1.4.1 Uptake of ^{13}C label

Biofilm (B) recorded a high ^{13}C delta value of 12.3±0.4‰ whereas mangrove leaf litter (L) recorded the lowest value (-22.7±0.2‰) after removal of the biofilm. Mangrove leaf litter with the associated biofilm (LB) was more enriched in ^{13}C with delta value of -21.1 ± 0.5‰ than the mangrove leaf litter alone which recorded a lower delta value of -22.7 ± 0.2‰. The shrimp which were not fed (CS) were more depleted of the ^{13}C ($\delta^{13}C$ of -21.4 ± 0.1‰) than the shrimp which were fed the labeled food regardless of the source i.e shrimp fed labeled litter (SL*) had $\delta^{13}C$ of -19.1 ± 1.3‰; shrimp fed labeled biofilm (SB*) had $\delta^{13}C$ of -18.0 ± 0.9‰ and shrimp fed labelled litter with attached biofilm (SLB*) had $\delta^{13}C$ of -19.3 ± 1.4‰.. The biofilm recorded significantly higher specific uptake ($\Delta\delta^{13}C$) of the ^{13}C from the labeled acetate than the labelled litter with and without biofilm which were not statistically different (One-way ANOVA: $F_{(2, 6)} = 856.4$; $p<0.05$; Tukey post hoc; $p=0.0002$) (Fig 1). This implies that the ^{13}C labeled sodium acetate was preferentially incorporated in the biofilm associated with the decomposing mangrove leaf litter. Decomposing mangrove leaf litter provides

organic matter which is a favorable site for microbial development (Avnimelech and Gad, 2003). The mineralization of organic matter under anoxic conditions is a stepwise process, in which low-molecular intermediates produced by fermentative bacteria play an important role (Boschker et al., 2001). Acetate is among the main intermediate during fermentation or mineralization of the organic matter and is consumed by bacteria (Cottrell and Kirchmann, 2000). The bacterial constituent of the biofilm would therefore have played a major role in the incorporation of the ^{13}C labeled sodium acetate. This observation therefore provides prove of the presence of bacteria in the biofilm.

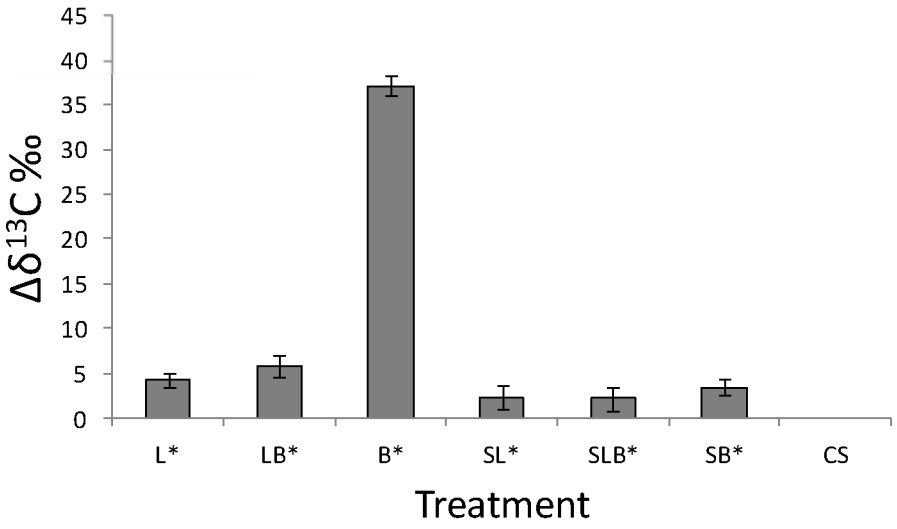


Fig 1: Specific uptake $\Delta\delta^{13}C$: proportion of ^{13}C in the shrimp tail tissue and in the different food types. S: shrimp feeding on; decomposing mangrove leaf litter (L); decomposing mangrove leaf litter with the associated biofilm (LB) biofilm scrapped from the leaf litter (B) and the control shrimp (CS). The * sign represents feeding treatments labeled with ^{13}C acetate.

Based on the tail tissue, shrimp post larvae which were fed on the labeled biofilm (SB*) had the highest average uptake of ^{13}C per unit carbon (0.0008 ± 0.0003 µg) compared to shrimp fed mangrove leaf litter (SL*and SLB*) (0.0005 ± 0.0003 µg) (Fig. 2). Although the shrimps feeding on the labeled biofilm (SB*) had a higher mean uptake of ^{13}C, the uptake was not statistically different to that of the shrimps feeding on the mangrove leaf litter with and without biofilm ($p>0.05$) (Fig. 2). The higher uptake of ^{13}C by shrimp post larvae feeding on the biofilm, can be used to infer the importance of bacteria in the natural diet of the shrimp. The lack of a clear statistical difference between the shrimp feeding on the biofilm and those

feeding on the mangrove litter could be attributed to the label absorbed into the decomposing mangrove litter. To label the biofilm, decomposing mangrove leaf litter were incubated in water containing ^{13}C labelled sodium acetate. Some of the label could have been absorbed into the leaf cell remains, which is hard to remove even after rinsing. The higher uptake of ^{13}C by shrimp feeding on labeled mangrove leaf litter (SL*) compared to the control shrimp (CS) underlines the role of the mangrove detritus to the diet of shrimp post larvae. Although formerly reported as an unimportant diet to shrimp post larvae (Schwamborn et al., 2002), mangrove derived food seem to have contributed to the diet of the shrimp post larvae in the current experimental set-up. This observation supports the reasoning by Fry and Ewel (2003) that mangrove detritus could supply some of the diet of the consumers when other food is scarce.

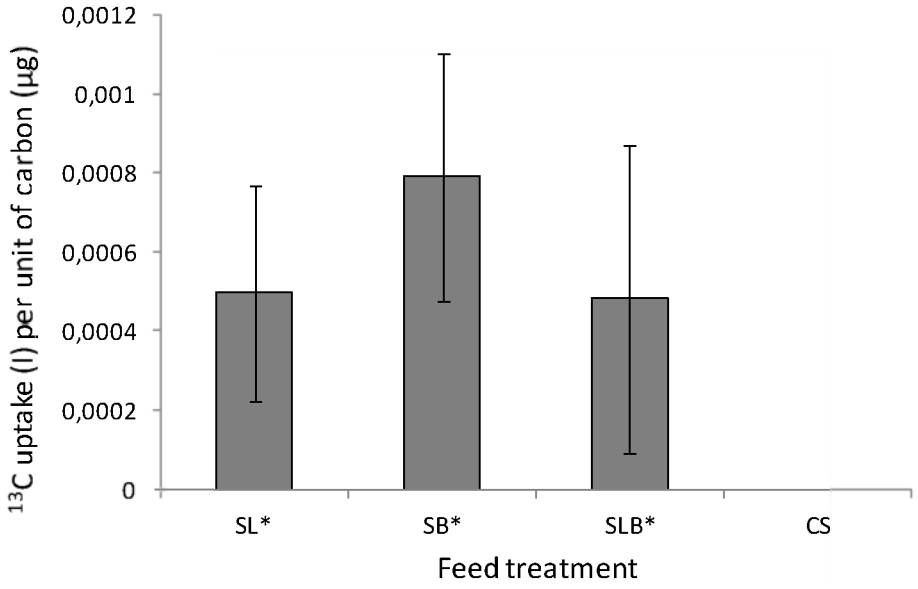


Fig 2: Uptake of ^{13}C per unit carbon (I) into the shrimp tail tissue after feeding on ^{13}C labeled decomposing mangrove leaf litter derived food. SL*: shrimp feeding on labeled leaf litter (by impregnation); SB*: shrimp feeding on biofilm; SLB*: shrimp feeding on litter with the associated biofilm; CS: control shrimp

2.1.4.2 Bacterial fatty acids

Biofilm (B) recorded higher proportion of the odd carbon numbered branched fatty acids C15:0 and C17:0 than the mangrove leaf litter with the associated biofilm (LB) (Fig. 3). The fatty acid pool of biofilm was characterised by 84.6% of bacterial biomarkers whereas the mangrove litter with the associated biofilm (LB) had only 15.3% of odd carbon numbered branched fatty acids (Fig 3).

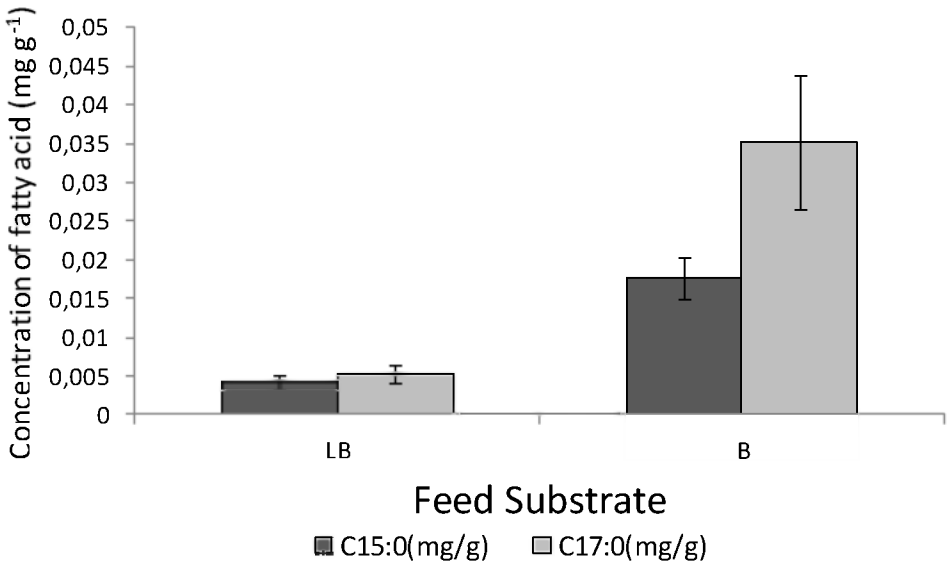


Fig 3: Bacterial biomarker fatty acids in the mangrove derived feed. LB: decomposing mangrove leaf litter with the associated biofilm; B: biofilm

Consequently, shrimp foraging on the biofilm (SB*) recorded a higher abundance of the summed bacterial fatty acids (C15:0, C17:0 (0.53±0.09 mg g^{-1}) whereas shrimp foraging on the mangrove litter only (SL*) recorded a lower abundance (0.50±0.06 mg g^{-1},) (Fig. 4). The starved shrimp (CS) had the lowest abundance of the bacterial fatty acids (0.47±0.07 mg g^{-1}). The difference in the abundance of the bacterial fatty acids in control shrimp, shrimp foraging on the mangrove litter and biofilm (CS, SLB* and SB*) could not be statistically separated (C15:0, One way ANOVA: $F_{(3, 8)} = 3.12$; p=0.087; C17:0, Kruskal-Wallis ANOVA: $H_{(3, 12)} =0.54$; p=0.91). Bacterial fatty acid biomarker in the shrimps feeding on biofilm and the mangrove litter derived food points at the same patterns as the ones concluded from the ^{13}C pulse-chase experiment. This similarity highlights the complimentary importance of both methods. 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). Previous studies have used fatty acids as biomarkers for bacteria (Rajendran et al., 1995; Taylor and Parkes, 1983). It should be noted with caution despite their conservation, fatty acid also undergo bioconversion in the tissues (Kelly and Scheibling, 2012). The use of compound-specific stable isotope analyses therefore acts to clarify the true assimilation of the ingested food. Moreover, one should be aware that shrimp had already fatty acids in their tissues at the start of this experiment. The comparison

of the fatty acids in the control shrimp helps to account for this. However, in the case of bacterial uptake, the control shrimp had lower biomarker content. This concludes uptake of bacteria by shrimps foraging on the biofilm.

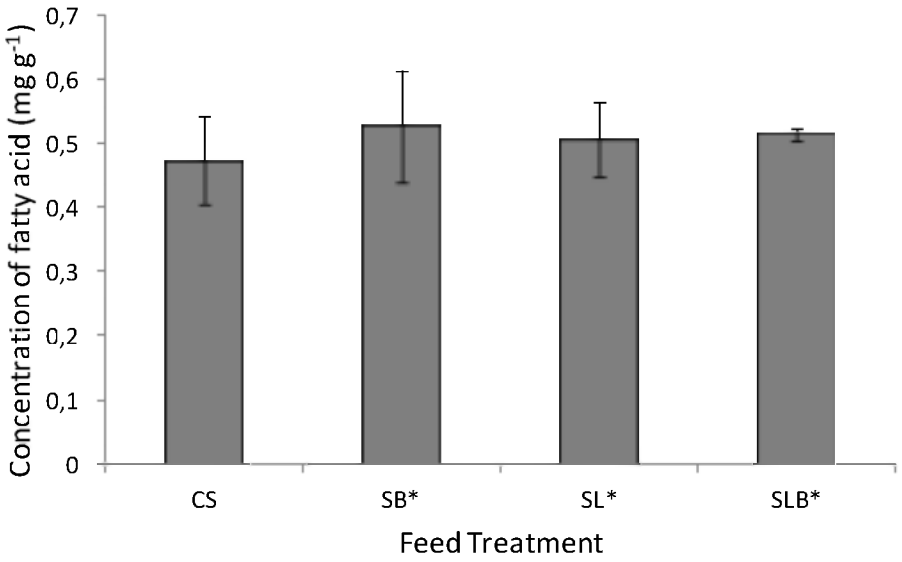


Fig 4: Bacterial biomarker fatty acids in the tail tissue of shrimp feeding on the decomposing mangrove leaf litter derived food. CS: unfed control shrimp; SL*: shrimp feeding on labeled leaf litter (by impregnation); SB*: shrimp feeding on labeled biofilm; SLB*: shrimp feeding on litter with the associated biofilm

2.1.5 Acknowledgements

The first author thanks the Flemish InterUniversity Council (VLIR) for the financial support to follow an international PhD program in Biology at Ghent University, Belgium. The present study is part of a PhD project in the *FEEDING ECOLOGY OF SHRIMP IN TROPICAL MANGROVE SYSTEMS OF KENYA: The nutritive value of detritus and its use in biological shrimp aquaculture.* The last author is a postdoctoral fellow financed by the Special Research Fund of the Ghent University (GOA project 01GA1911W). Thanks to Dirk Van Gansbeke (UGent, Marine Biology) for assisting in the analyses of the fatty acids.

Chapter 2

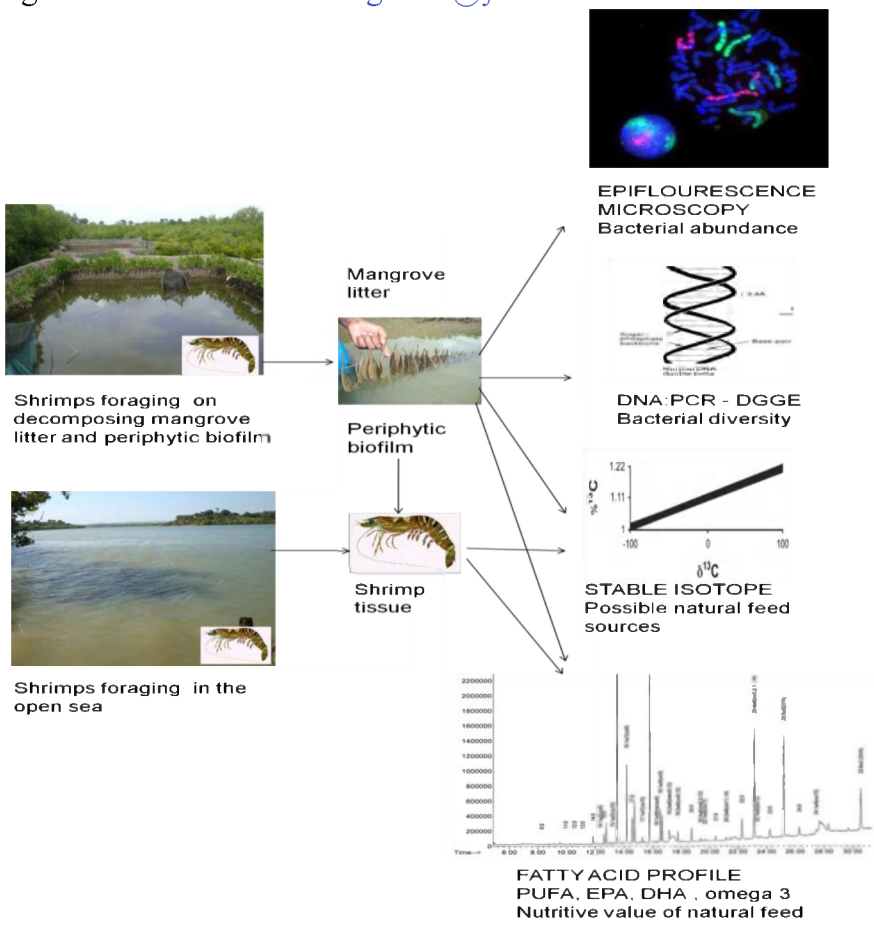
3 Chapter 3

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

Charles Gatune [a,c]*, Ann Vanreusel[a], Clio Cnudde[a], Renison Ruwa[b], Peter Bossier[d] and Marleen De Troch[a]

[a] Ghent University, Biology Department, Marine Biology, Campus Sterre, Krijgslaan 281-S8, B-9000, Gent, Belgium.
[b] Kenya Marine and Fisheries Research Institute, P.O Box 80100, 81651, Mombasa, Kenya.
[c] Ministry of Fisheries Development, P.O. Box 90423 – 80100, Mombasa, Kenya.
[d] Ghent University, Faculty of Bioscience Engineering, Laboratory of Aquaculture and Artemia Reference Centre, Rozier 44, B-9000, Gent, Belgium.

*Corresponding Author: Email address: kgatune@yahoo.com



Modified after :

Gatune, C., Vanreusel, A., Cnudde, C., Ruwa, R., Bossier, P., De troch, M., 2012. Decomposing mangrove litter supports a microbial biofilm with potential nutritive value to penaeid shrimp post larvae. Journal of Experimental Marine Biology and Ecology 426-427, 28-38.

3.1.1 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 ($3^{o}57'$S; $39^{o}42'$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 remineralisation, 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 week 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 towards 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 (week 3 - 4). The results of this study suggests 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.

Key words: shrimp; bacteria; biofilm; mangrove; nutrition; isotope

3.1.2 Introduction

In the year 2007, a once lucrative semi-industrial shrimp trawling activity of the commercial species *Penaeus monodon* and *P. indicus* along the Kenyan coast (Indian Ocean) was officially closed due to assumed depletion of shrimp stocks (GOK, 2006b). 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 practises (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, *Penaeus monodon,* which may exceed 200g and a total length of 336mm 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 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, 2005a; Burford et al., 2004; Keshavanath and Gangadhar, 2005a). 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, 1996b), 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; Primavera, 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, 1985b). 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.

3.1.3 Materials and methods

3.1.3.1 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 ($3^{o}57'$S; $39^{o}42'$E). The study site is reforested with mangrove trees of the species *Rhizophora mucronata*. The penaeid shrimp species *Penaeus indicus* and *P. monodon* are commonly fished within this creek.

3.1.3.2 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 $10gl^{-1}$. The shallow mangrove pools (0.6m deep during low tide) were enclosed with a netting cage (hapa) measuring $4m^2$ base area, 2m high and covered at the top to prevent content overflow during high tide. The hapa had a mesh size of 2 mm. The 2 mm mesh size was small enough to prevent exit of the shrimp post larvae and entry of large invertebrates. The mesh was also large enough to allow adequate water exchange, microbial colonisation and entry of small benthic invertebrates. Shrimp postlarvae (PL) of *Penaeus indicus* (PL 25-35), fished from the open creek, were stocked in one set of hapa containing mangrove litter and another set without litter in triplicate at a density not exceeding 1 PLl^{-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.

3.1.3.3 Litter degradation and nutrient analysis

Senescent mangrove leaves were dried in the shade to a constant weight placed in litter bags (30x30cm, 2mm 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 hrs 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 analysed for carbon and nitrogen content using a Flash 2000 Organic Elemental Analyser (Thermo Scientific, Italy).

3.1.3.4 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 lyophylised GF/F filters at 4°C in the dark and the supernatant was analysed for chlorophyll a according to a modified protocol of Granger and Lizumi (2001).

3.1.3.5 Fatty acid extraction and analysis

Samples of shrimp tissue, mangrove litter and biofilm were freeze- dried (lyophylised), weighed and fatty acids extracted and methylated to fatty acid methyl esters (FAMEs) by a modified one-step derivatisation 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 artefacts 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, 240mbar, then dissolved into 1ml hexane and analysed using a Hewlet 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 (60m x 0.25mm internal diameter x 0.20µm 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 analysed 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; Kanazawa et al., 1979; Kanazawa et al., 1978; 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)Σ16/Σ18>2 and 16:1/16:0>1 as proxy for microalgae; (2) 16:1/16:0 >1.6, Σ16/Σ18>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).

3.1.3.6 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 hrs. 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).

3.1.3.7 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 hrs 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, 0.5g 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, $Na_2B_4O_7$.$10H_2O$). Samples were sonicated three times (Branson Sonifier 2200; 60W for 1 min) with intervals of 30 sec 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 x 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%.

3.1.3.8 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). The primers 357F and 518R targets the ribosomal RNA gene sequence of the bacterial cell (Van Hoorde et al., 2008) with a GC-clamp (5′CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGG3′) (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 analysed 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.

3.1.3.9 Water quality parameters

Water quality was monitored by weekly measurements of temperature, dissolved oxygen, pH, salinity and total ammonium nitrogen. Temperature, dissolved oxygen, pH were measured using meters, salinity was measured using a refractometer whereas total ammonium nitrogen was analysed in the laboratory according to Eaton et al.(2005).

3.1.3.10 Data analysis

One way ANOVA was used to compare the abundance of bacterial cells and micro-algae in the biofilm developing on mangrove litter after different periods of decomposition. Statistica 7.0 software was used. One way ANOVA was also used to compare the concentration of both essential fatty acid biomarkers and the bacterial fatty acids in the decomposing mangrove litter, biofilm and tail tissue of shrimp feeding on the mangrove litter and the associated biofilm at the different periods of development. All data were checked for normality and variance homogeneity requirements for parametric analysis. Data that did not meet normality requirements after transformation were analysed non-parametrically with Kruskal-Wallis ANOVA & Median Test. The Bionumerics software was used to normalize and analyse the digitized DGGE bands that represented the bacterial community colonizing the biofilm developing on the mangrove litter at the different periods of decomposition. The calculation of the Pearson correlation coefficient and application of the unweighted pair group method with arithmetic mean (UPGMA) resulted in a dendrogram visualizing similarity between the bands pattern of the biofilm scraped from mangrove litter at the different weeks of decomposition. Multidimensional scaling (MDS) and analysis of similarity (ANOSIM) were used to compare similarity in the distribution of fatty acids in the mangrove litter, biofilm and the tail tissue of shrimp feeding on them at the different periods of the decomposition of the mangrove litter and the growth of the associated biofilm. Primer 6.0 software was used for the MDS and ANOSIM analysis. 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). A mean trophic enrichment factor (fractionation/ discrimination factor) of 1 ±1.2‰ for $\delta^{13}C$ was considered for the food sources as suggested by Vander Zanden and Rasmussen (2001) and adopted by Ouisse et al.(2012) .

3.1.4 Results

3.1.4.1 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 5^{th} and 6^{th} week when they were already decomposed for 53±3% and 61±1%, N=3; respectively. In a period of 6 weeks, nitrogen and carbon content increased by 300%

and 16.6% respectively in 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.

3.1.4.2 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% per day) (Table1). The maximum number of bacteria recorded at this stage reached an abundance of 3.99 x 10^9±8.03 x 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±0.63µg 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 x 10^9±1.21 x 10^8cells 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±0.009µg 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).

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	2.1	1.5	1.5	1.4
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)

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

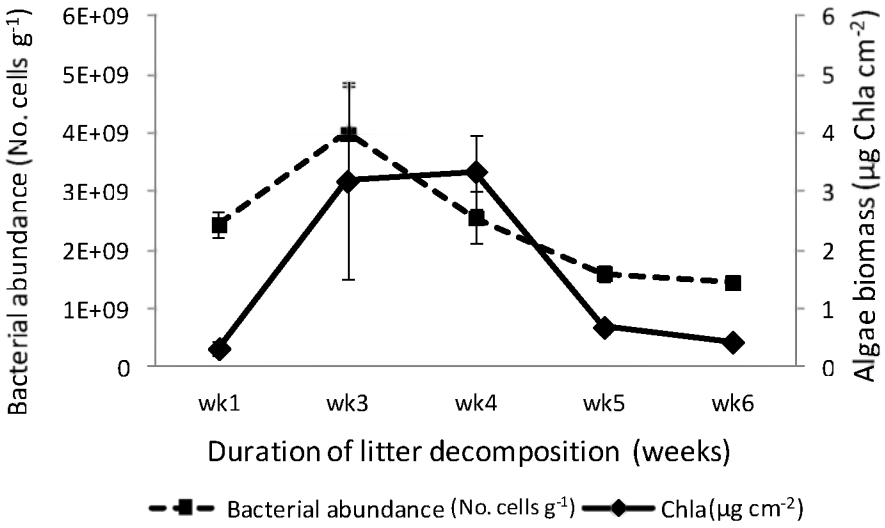


Fig 1: Abundance of bacteria cells and microalgae in the biofilm developing on mangrove litter at different periods of decomposition

3.1.4.3 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.

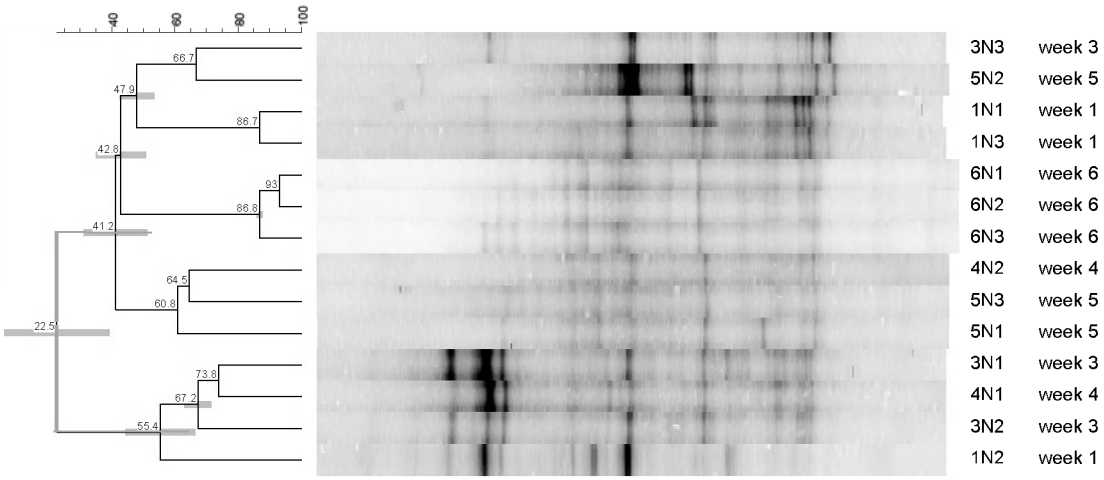


Fig 2: Dendrogram of DNA bands derived from Pearson's correlation coefficient. Similarity levels are indicated per cluster. Bands represent bacterial community in periphytic biofilm developing on decomposing mangrove litter of *Rhizophora mucronata* at different periods of decomposition. Significant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

3.1.4.4 Fatty acid biomarkers

3.1.4.4.1 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.

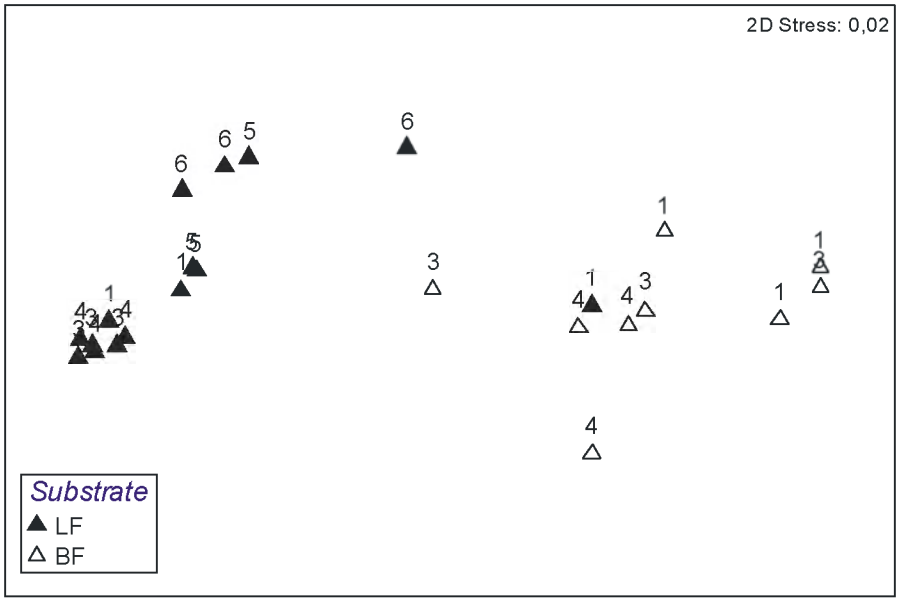


Fig 3: MDS plot of similarity (Bray-Curtis) in fatty acids in LF (mangrove litter) and BF (periphytic biofilm developing on the decomposing mangrove litter). Numbers refer to the duration of the litter decomposition in weeks. BF plots for the week 5 and 6 are not reflected due to the outlier effect.

3.1.4.4.2 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 weeks 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.

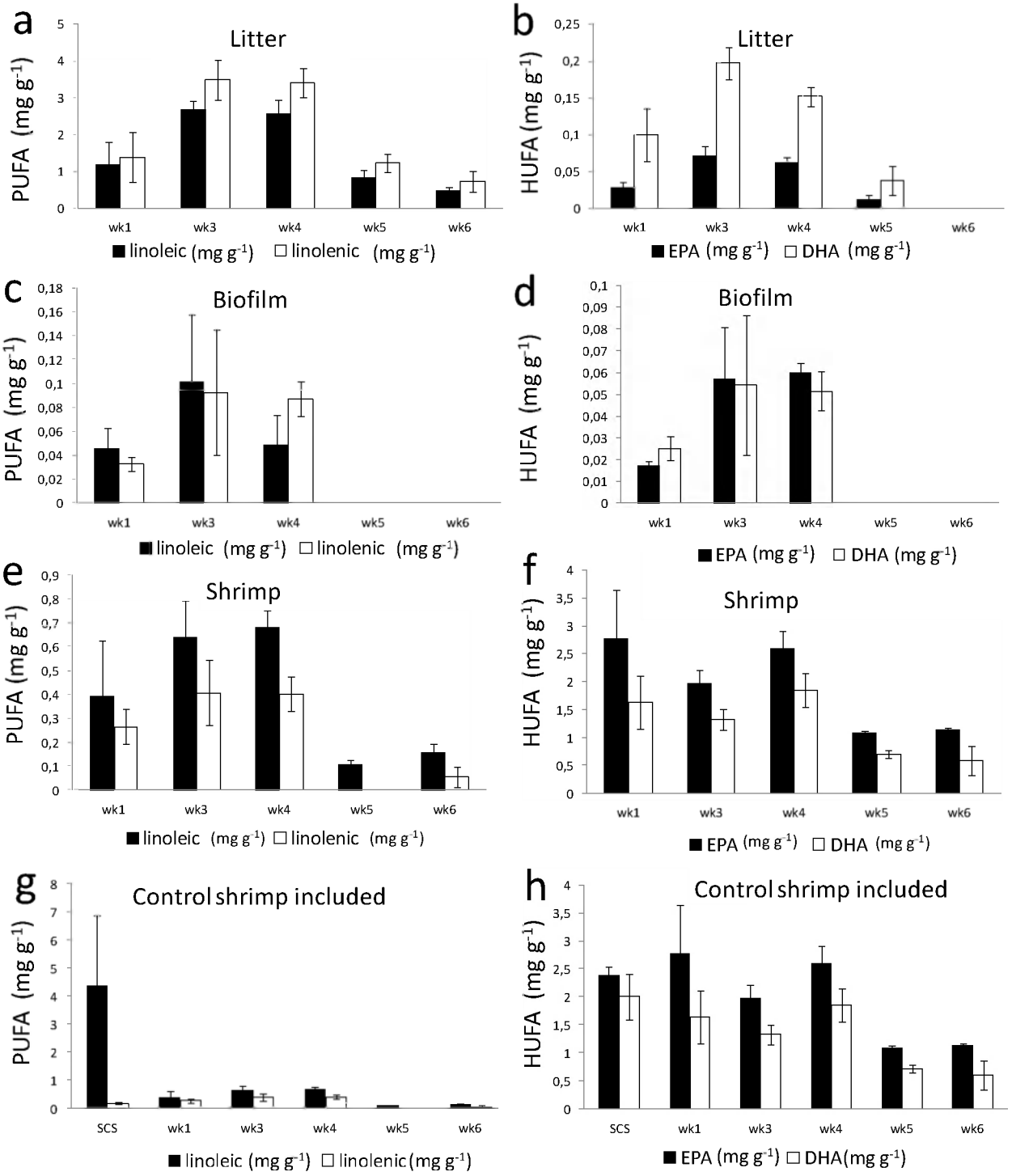


Fig 4: Concentration (mean and standard error) of Essential Fatty Acid biomarkers in potential food substrates; (mangrove leaf litter (a-b) periphytic biofilm (c-d)) and shrimp tissue (e-h) at different stages of decomposition of the mangrove leaf litter. SCS (control shrimp from the open creek)

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 (1.08-1.65 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).

	Open creek (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 & 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 & SBF

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

3.1.4.4.3 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).

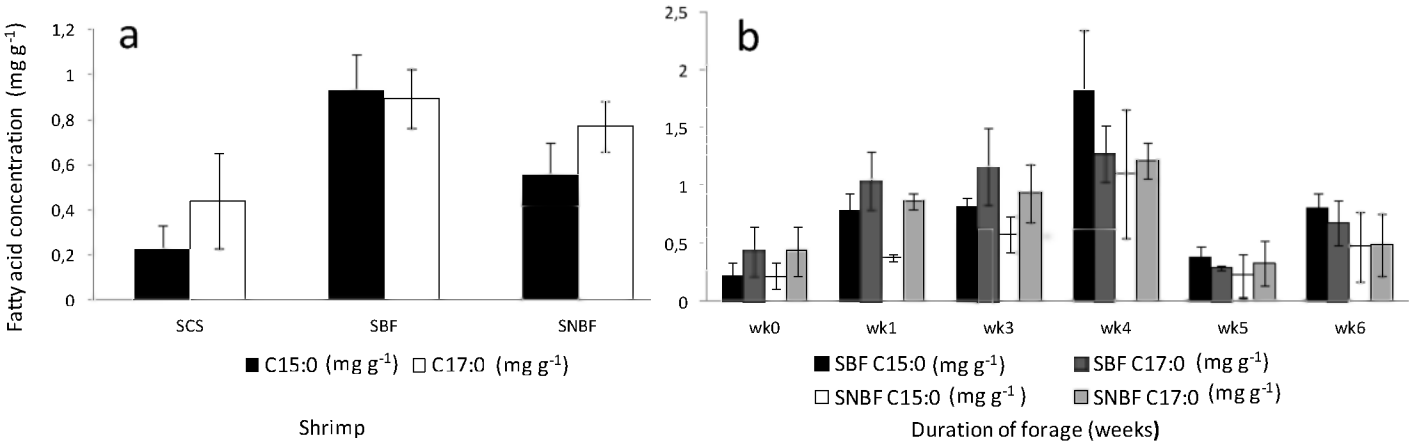


Fig 5: Concentration of bacterial biomarkers in shrimp tissue at different zones and periods of forage; SCS, control shrimp from the open creek; SBF, shrimp stocked with decomposing mangrove litter; SNBF, shrimp stocked without mangrove litter.

3.1.4.5 Isotope tracing

Mangrove litter was significantly more depleted in ^{13}C ($\delta^{13}C$= -28.4±0.6‰, N=3) than biofilm ($\delta^{13}C$ =-25.2±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±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).

Shrimp tissue		**δ13C ‰**
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
Sea Grass	SG	-16.23 ±0.16
Biofilm	BF	-25.16 ± 0.24
Decomposed mangrove Litter	LF	-28.43 ± 0.59

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

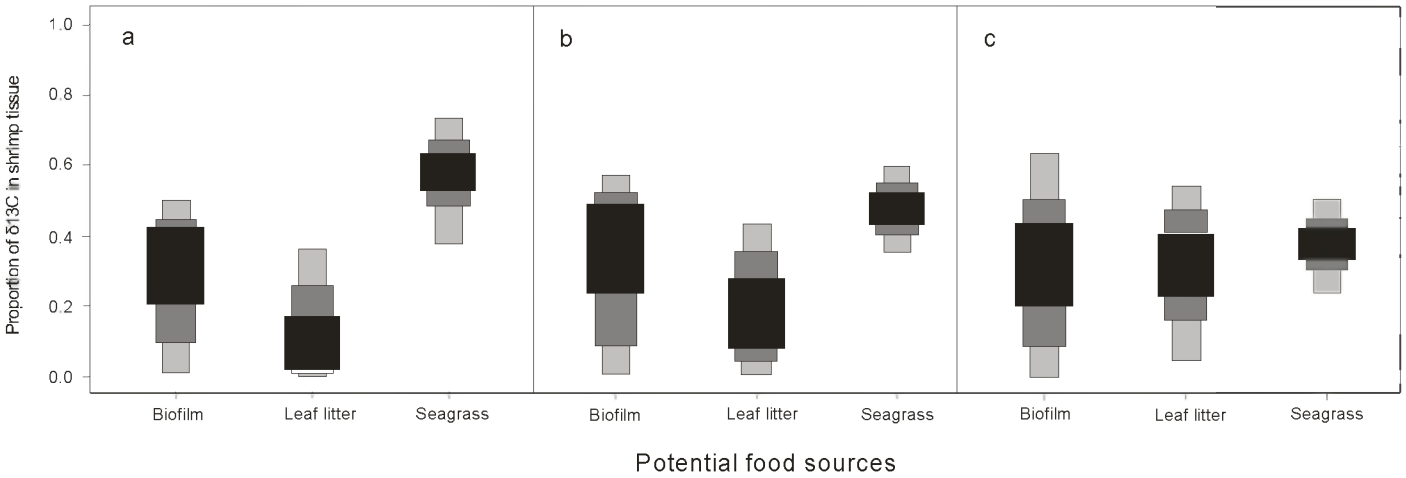


Fig 6: Box Plot showing proportion of $\delta^{13}C$ in the shrimp post larvae foraging in various estuarine habitats; a) shrimp foraging in the open creek; b) shrimp foraging in the bare mangrove zones; c) shrimp foraging in the decomposing mangrove litter.

3.1.5 Discussion

3.1.5.1 Mangrove leaf litter degradation

The extent of leaf litter decomposition of *Rhizophora mucronata* have been found to range between 48% and 98% during the dry and rainy season 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.

In the present study, mangrove leaf litter decomposed rapidly within the first 3 weeks with a percentage weightloss of 2.5% and 1.8% day^{-1} which declined to 1.0% day^{-1} between the 5^{th} and the 6^{th} week. A similar observation was made by Bosire et al. (2005) in Gazi bay (Kenya) where weekly leaf litter weight loss was highest in the 2^{nd} 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, 2000b). 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 immobilisation 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.

3.1.5.2 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 mineralisation 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 is 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, week 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 mineralisation. According to Benner and Hodson (1985b), 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.

3.1.5.3 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, 2005a; Bratvold and Browdy, 2001b; Keshavanath and Gangadhar, 2005a; Nga et al., 2004). Juvenile penaeid 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 epifluoresence 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, 2005a; Burford et al., 2003). According to the present study, mangrove leaf litter decomposed for 3 and 4 weeks are 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 are 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.

3.1.5.4 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 already fatty acid in their tissues at the start of 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 chort 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; Kanazawa et al., 1979; Kanazawa et al., 1978) 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 *P. 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.

3.1.5.5 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 *Penaeus 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 *Penaeus 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 tendancy 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 play 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, 2000b). 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.

3.1.6 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 may not necessarily fully rely on decomposing mangrove litter

and biofilm to satisfy their requirement for essential fatty acids but that they have to alternate between the mangrove forest and 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.

3.1.7 Acknowledgements

The first author thanks the Flemish InterUniversity Council (VLIR) for the financial support to follow an international PhD course program in Biology at Ghent University, Belgium. The present study is part of a PhD project entitled '*FEEDING ECOLOGY OF SHRIMP IN TROPICAL MANGROVE SYSTEMS OF KENYA: The nutritive value of detritus and its use in biological shrimp aquaculture*'. The last author is a postdoctoral fellow financed by the Special Research Fund of the Ghent University (GOA project 01GA1911W). Dirk Van Gansbeke (UGent, Marine Biology) assisted in the analysis of the fatty acids. Deep felt thanks goes to KMFRI laboratory technicians Kilonzi, Eric, Mary, Onduso, Akisha, Mwendwa, Boaz, Emily, the youth and community members of the Majaoni silvofishery project, Kenya, for their field assistance. The authors are grateful to the two anonymous reviewers for their constructive remarks that helped us to improve an earlier version of the manuscript.

Chapter 3

4 Chapter 4

4.1 Effect of micro-algae and epifauna biofilm on decomposing mangrove litter on the growth and survival of shrimp post larvae of *Penaeus monodon*

Charles Gatune [a,c]*, Ann Vanreusel[a], Renison Ruwa[b], Peter Bossier[d] and Marleen De Troch[a]

[a] Ghent University, Biology Department, Marine Biology, Campus Sterre, Krijgslaan 281-S8, B-9000, Gent, Belgium.
[b] Kenya Marine and Fisheries Research Institute, P.O Box 80100, 81651, Mombasa, Kenya.
[c] Ministry of Fisheries Development, P.O. Box 90423 – 80100, Mombasa, Kenya.
[d] Ghent University, Faculty of Bioscience Engineering, Laboratory of Aquaculture and Artemia Reference Centre, Rozier 44, B-9000, Gent, Belgium.

*Corresponding Author: Email address: kgatune@yahoo.com

The incubation of the decomposing mangrove leaf litter at Majaoni shrimp culture ponds (Photo courtesy Gatune C. 2011)

Submitted as:
Gatune, C., Vanreusel, A., C., Ruwa, R., Bossier, P., De troch, M. Effect of micro-algae and epifauna biofilm on decomposing mangrove litter on the growth and survival of shrimp post larvae of *Penaeus monodon*. *Aquaculture*.

4.1.1 Abstract

The importance of mangroves on shrimp post larvae have been focused on their role in providing shelter and food from the sediment organic matter and biota. Biofilm associated with the decomposing mangrove leaf litter is also a potential natural food source. Growth and survival of post larvae (PL) of *Penaeus monodon*, were tested on (1) mangrove leaf litter of *Rhizophora mucronata* with biofilm at 1, 3, 4, 6 and 8 weeks of decomposition; (2) commercial compound feed (CP) and (3) no food available as control. PL were analyzed for specific growth rate (SGR %) and percentage survival (SR %). Biofilm was analyzed for biomass and composition of micro-algae and epifauna. Micro-algae biomass increased with the progress of litter decomposition. Diatoms dominated the first 6 weeks of litter decomposition with their percentage cover ranging from 88 to 99% during the 3rd and 4th week. Diatoms *Navicula* spp. and *Nitzschia* spp. dominated and replaced each other during the progress of litter decomposition. Cyanobacteria dominated the diatom by 61% in the 8 weeks old biofilm. Copepoda dominated the epifauna during the first 3 weeks of litter decomposition. Polychaeta dominated during the 4th and 5th week whereas Nematoda dominated during the 8th week of litter decomposition. PL growing on CP had the overall best growth and survival with SGR of 6.1 ± 0.3% and SR of 97.2 ± 2.7%. PL foraging on 1, 3, 6 and 8 weeks old leaf litter had reduced growth and survival. PL foraging on 4 weeks old litter had a better SGR of 1.6 ± 0.5% and SR of 39.8 ± 4.8% and coincided with the peak period of the micro-algae and epifauna abundance. The study illustrated that; 1) shrimp post larvae living on the decomposing mangrove leaf litter perform better when foraging on the 4 weeks old biofilm; 2) the quality biofilm is developed during the 4th week of mangrove leaf litter decomposition and is dominated by diatoms, polychaetes, harpacticoid copepods and oligochaetes; and 3) quality biofilm food for the shrimp post larvae is limited by the collapse of the epifauna and subsequent colonization by nutritionally low quality Cyanobacteria, if decomposing mangrove leaf litter is retained in the pond water beyond a period of 5 weeks.

Key words: mangrove; decomposition; biofilm; micro-algae; epifauna; shrimp

4.1.2 Introduction

The natural diet of penaeid shrimp post larvae has been widely studied and characterized at different stages of the life cycle from nauplius, protozoea, mysis to post larvae (Dall et al., 1990; Rothlisberg, 1998). Protozoea are generally herbivorous, while the mysis and post larvae become increasingly carnivorous. However, the post larvae are opportunistic feeders. For example, if diatoms dominate the environment, they will dominate the diet of the shrimp too (Preston et al., 1992). The composition and the diversity of micro-algae may both directly and indirectly determine the nutritional quality of the natural food for shrimp post larvae. For instance, diatom species have an effect on growth and survival (Brown and Farmer, 1994; Brown and Jeffrey, 1995; Burford, 1997; Sorgeloos and Lavens, 2000; Wouters et al., 2001; Ying et al., 2000). Differences in the micro-algae taxonomic composition rather than size or shape appear to be the critical factor. For instance, in case of diatoms, both *Achnanthes* sp. (50 μm^3) and *Thalassionema frauenfeldii* (1260 μm^3) provided good survival and growth, while *Fragilaria pinnata* (60 μm^3) and *Thalassionema nitzschioides* (780 μm^3) would not support their development (Rothlisberg, 1998). Not all algae species ingested may support growth whereas the potential to support growth may depend on the level of nutrients in the growth medium used. For instance the non-toxic blue-green alga *Trichodesmium* sp. was found to be ingested by shrimp larvae of *Peneaus merguiensis* although it did not support growth or survival (Preston et al., 1998). Chlorophyta of the species *Tetraselmis suecica* grown on high nitrogen medium were found to be nutritionally rich in proteins and essential fatty acids and resulted in better growth when fed to larvae of *Penaeus semisulcatus* compared to same algae grown on low nitrogen medium which had high carbohydrate levels (D'Souza and Kelly, 2000). Previous research by Burford (1997) and Primavera (1998) suggested to use a variety of micro-algae indicators, such as their abundance and species diversity as proxy for the actual food conditions and water quality (Case et al., 2008) in the shrimp culture ponds. Penaeid shrimp post larvae also eat a wide variety of small invertebrates and plant material (Chen and Chen, 1992; Nunes and Parsons, 2000; Rothlisberg, 1998). Prey animals that comprised the natural diet of juvenile *Penaeus esculentus* in seagrass beds include gastropod species, bivalves, crustaceans and polychaetes. They were found to be high in protein content, relatively low in mean carbohydrate concentrations while a high proportion of polyunsaturated fatty acids (PUFA) was observed in the lipid fraction (Dall et al., 1991). Polychaetes of the families Spionidae, Capitellidae, Eunicidae, Nereidae, Pilargidae and Seballidae have successfully been used in penaeid shrimp predation experiments especially with *Penaeus subtilis* (Nunes and Parsons, 2000).

In view of this wide variety of food sources for shrimp post larvae, the importance of mangrove leaf litter to the food for shrimp larvae may be linked to the associated periphytic biofilm. Stable isotope studies have clearly disclosed that *Peneaus monodon* was feeding on phytoplankton and epiphytic micro-algae in a riverine mangrove in Guimaras (Central Philipines)(Primavera, 1996). In the Itamaraca estuary, Brazil, Schwamborn et al. (2002) found penaeid shrimp post larvae to have the highest $\delta^{13}C$ among the decapod larvae indicating a negligible contribution of mangrove carbon to their nutrition. Mangrove epiphytes and highly decomposed mangrove leaf material were found to be too depleted in $\delta^{13}C$ and $\delta^{15}N$ to be contributing to the mangrove sesarmid crab diet whereas a three-source mixing model indicated mainly benthic organic material as their primary food source (Mazumder and Saintilan, 2010).

In asserting the nutritive importance of mangrove leaf litter and the associated periphytic biofilm to shrimp post larvae, it is also important to consider that the feeding response may be influenced by the litter and biofilm palatability, which may be on their turn influenced negatively by increasing level of tannins in decomposing mangrove leaves (Rajendran and Kathiresan, 2000b). In fact, several studies have made direct observations where penaeid shrimp post larvae (Primavera, 1996), and other benthic invertebrates (Bouillon et al., 2002) have shown a reduced preference for mangrove derived carbon compared to local and imported algal sources. In fact the presence of decomposed leaf litter in mangrove habitats may not necessarily attract shrimp post larvae. Ronnback et al. (2002) observed that the distribution of the penaeid shrimp juveniles of *Penaeus indicus* did not vary between the sediments of low and high organic content. However juvenile penaeid shrimp have been observed to aggregate around the mangrove litter colonized with bacteria (Kathiresan and Rajendran, 2007; Rajendran and Kathiresan, 2004). On average 60-75% of nitrogen and 20-40% of 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). In natural systems, bacteria may not occur as a separate functional or ecological entity but they are present in combination with other micro-biota forming benthic and epiphytic biofilms or a flocculated mass in suspension (Azim and Wahab, 2005a; Burford et al., 2003).

The above observations suggest that the importance of decomposing mangrove litter in providing natural food to penaeid shrimp post larvae lies in the associated periphytic biofilm.

Biofilm is composed of a network of primary and secondary trophic levels mediated by a microbial loop (Azim and Wahab, 2005a). However the various biological components of the biofilm on decomposing mangrove leaf litter and their potential nutritive value to shrimp post larvae in ecological shrimp culture ponds have not been studied so far. Such knowledge is important in identifying an intervention time line in managing mangrove litter fall into the ecological shrimp culture ponds. Because it is desirable that shrimp culture ponds and other waters support phytoplankton and zooplankton for maintaining water quality as well as providing a quality food source for the other consumers (Burford, 1997; Case et al., 2008), it is important to elucidate the temporal species dominance and the probable factors controlling the community structure. The present study therefore hypothesized that the food value of mangrove leaf litter and the associated biofilm to shrimp post larvae depends on the assemblage and biomass of micro-algae and epifauna as the decomposition of the mangrove litter progresses. We therefore investigated the major taxa of microalgae and invertebrate fauna colonizing the decomposing mangrove litter of *Rhizophora mucronata* and their potential application in providing natural diet to shrimp post larvae of *Penaeus monodon.*

4.1.3 Materials and methods

4.1.3.1 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 ($3^{o}57'$S; $39^{o}42'$E). The study site is characterized by a reforested mangrove forest dominated by *Rhizophora mucronata.* The post larvae of penaeid shrimp species *Penaeus indicus* and *P. monodon* are commonly fished within this creek.

4.1.3.2 Standard test organism

Shrimp post larvae (PL) of *Penaeus monodon*, PL 15-25 hatched from the same brooder (cohort) were obtained from Alphakrust shrimp hatchery situated at Mafia Island, Tanzania (see www.alphaafrica.com). They were transported in plastic bags with a pure oxygen headspace for a duration not exceeding 6 hours and acclimatized to local conditions for a period of 1 week before the start of the experiment. During the acclimatization period the shrimp post larvae were fed on a shrimp larvae compound feed (CP) imported from India (Higashimaru zoea to PL 20 feed; crude protein over 52%, see www.aquafeed.com/ documents/1254938830_1.pdf).

4.1.3.3 Litter incubation and biofilm biomass

Senescent mangrove leaves (hereafter referred to as mangrove leaf litter) which had just turned yellow-brown and dropped from the trees were dried in the shade to a constant weight and incubated hanging in a shrimp pond for a period of 8 weeks. The associated biofilm was sampled weekly by carefully scrapping 3 leaves per replicate in triplicates, dried in an oven at 70°C for 48 hours and quantified as weight per unit leaf surface area to estimate biomass.

4.1.3.4 Micro-algae abundance and taxa

Mangrove litter was sampled, weekly, in triplicates by pooling 3 decomposing leaves per replicate. The biofilm was gently washed from the surface of the decomposing mangrove leaf litter with a known volume of filtered sea water and preserved in 2% Lugol's iodine solution (1:2 iodine:iodide: glacial acetic acid solution). Micro-algae were classified and counted in the laboratory by first diluting each replicate sample 5 to 10 times. Five sub-replicates of 0.02 ml were then sub-sampled and examined under an inverted microscope.

4.1.3.5 Epifauna abundance and taxa composition

The present study emphasized on the epifauna which included both the meiofauna (metazoans that can pass unharmed through a 1 mm sieve and are retained on a 38 µm sieve) and macrofauna (organisms retained on 1mm sieve) living on the decomposing mangrove litter. Mangrove leaf litter was sampled weekly in triplicates by pooling 9 decomposing leaves per replicate in a plastic bag. Each group of the decomposing leaves was immediately mixed with 8% magnesium chloride to shock the attached epifauna, thoroughly agitated and subsequently sieved through 1 mm and 38 µm mesh size sieves. The sieved fauna was gentle washed from the 38 µm sieve with a soft filtered fresh water spray, preserved in 4% formaline and stained with a few drops of 1% solution Bengal rose. Epifauna were identified and counted at the major taxa level using a binocular microscope and recorded as number per cm^2 of leaf surface.

4.1.3.6 Species diversity and evenness of the main micro-algae and epifauna groups

The Shannon-Wiener index (H') and equitability index (EH) were used to estimate the micro-algae and epifauna community diversity and evenness based on natural log (ln) (Shannon, 1948). The following formula was used:

$$H' = -\sum_{i=1}^{n} p_i \ln p_i$$

$$EH = \frac{H'}{\ln S}$$

Where S is the total number of species in the community; p_i is the proportion of S made up by the i^{th} species. Species equitability or evenness (EH) was interpreted within the range of 0 to 1 with values close to 0 signifying dominance by single species and close to 1 signifying many species present in equal numbers.

4.1.3.7 Micro-algae biomass

Mangrove leaf litter was sampled, weekly, in triplicates by pooling 3 leaves per sample. The periphytic biofilm was gently scraped from the surface of the mangrove leaf litter with a known volume of filtered sea water and filtered over a glass fiber filter GF/F (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 Chla µg l^{-1} to mg cm^{-2} of total leaf surface. Phytopigments were extracted from the collected biofilm after adding 10 ml 90% acetone to the lyophylised GF/F filters at 4°C in the dark and the supernatant was analysed for chlorophyll a according to the modified protocol of Granger and Lizumi (2001).

4.1.3.8 Shrimp feeding experiment

Shrimp postlarvae (PL) of *Penaeus monodon*, PL 15-25 were starved for 24 hours then stocked into 70 liter laboratory tanks in triplicate at a density not exceeding 2 PL l^{-1}.The following food treatments were tested: (1) mangrove leaf litter with biofilm at 1, 3, 4, 6, 8 weeks of decomposition; (2) commercial compound feed (CP); and (3) no food, as control treatment. The post larvae were supplied every 4 days with new mangrove leaf litter (after removing the old ones) at different stages of decomposition (and biofilm development) maintaining a litter density not exceeding 1 g l^{-1} (Hai and Yakupitiyage, 2005). Shrimp post larvae were sampled weekly for specific growth rate (SGR %) and at the end of the experiment for percentage survival (SR %).

The growth and survival indices were calculated using the following formula (Busacker et al., 1990),

$$SR\% = \frac{N_t}{N_0} * 100$$

$$SGR\% = \frac{\ln(BW_t) - \ln(BW_0)}{T} * 100$$

Where SR is the survival (in %); N_t is the number of shrimp collected at sampling time t; N_0 is the number of shrimp initially stocked; SGR is the specific growth rate (% BW day^{-1}); BW_t

being the final body weight (g); BW_0 is the initial body weight (g); and T is duration of the experiment (days).

4.1.3.9 Water quality parameters

Water quality was monitored by weekly measurements of temperature, dissolved oxygen, pH, salinity and total ammonium nitrogen. Temperature, dissolved oxygen, pH were measured using meters, salinity was measured using a refractometer whereas total ammonium nitrogen was analysed in the laboratory according to Eaton et al.(2005).

4.1.3.10 Data analysis

The comparison of biomass and abundance in micro-algae and epifauna colonizing the decomposing mangrove litter at different time period was analysed using ANOVA in Statistica 7.0 software. ANOVA was also used to compare the specific growth rates and survival of shrimp post larvae foraging on the biofilm developing on mangrove litter at different time intervals. All data were checked for normality and variance homogeneity requirements for parametric analysis. Data which did not meet normality requirements after being transformed were analysed non-parametrically using Kruskal-Wallis ANOVA & Median Test. Multidimensional scaling (MDS) and analysis of similarity (ANOSIM) was used to compare similarity in the taxonomic distribution of the micro-algae and epifauna species in the biofilm developing on the mangrove litter at different period of decomposition. Primer 6.0 software was used for the MDS and ANOSIM analysis (Clarke and Gorley, 2006). Shannon-Wiener index was used to estimate the diversity and evenness of the community of the micro-algae and epifauna colonizing the decomposing mangrove litter.

4.1.4 Results

4.1.4.1 Water quality

In all shrimp feeding treatments, dissolved oxygen ranged from 4.33 ± 0.3 mg l^{-1} in the 8^{th} week treatment to 5.87 ± 0.12 mg l^{-1} in the 1^{st} week treatment. Temperature ranged from 26.7 ± 0.03°C in the 3^{rd} week treatment, to 26.9 ± 0.03°C in the 6^{th} week treatment. Total ammonium nitrogen (TAN) ranged from 1.57µg l^{-1} in the 3^{rd} week treatment to 1.70µg l^{-1} in the CP treatment. All water quality parameters did not statistically differ between the treatments receiving mangrove leaf litter, CP feed and the control ($P>0.05$).

4.1.4.2 Micro-algae biomass

Micro-algae biomass *(chl a)* increased throughout the period of leaf litter decomposition with the highest biomass (1383.6 ± 137.6µg l^{-1} Chla, N=3) being recorded in the leaf litter

decomposed for 8 weeks which was significantly different from the biomass recorded on the leaf litter which were decomposed for a period of less than 6 weeks 241.1 ± 8.9 µg l^{-1}, N=3, (ANOVA: $F_{(6, 14)}$ =145.43; p=0.000) and Tukey Post Hoc: P=0.000175). Further comparisons showed that algae biomass on the leaf litter decomposed for 4, 5 and 6 weeks was not significantly different from each other (Tukey Post Hoc: P=0.0656; 0.83; 0.107) but it was significantly higher from the micro-algae biomass on the leaf litter decomposed for a shorter period of 1 and 2 weeks (Tukey Post Hoc: p=0.000174) (Fig. 2a)

4.1.4.3 Micro-algae taxonomic composition

Five micro-algae classes were identified as dominating the periphytic biofilm developing on the decomposing mangrove leaf litter. The five micro-algae classes were diatoms, Cyanobacteria, dinoflagellates, coccolithales and flagellates. The micro-algae taxa colonizing the biofilm differed significantly in the different weeks of mangrove leaf litter decomposition (ANOSIM: R=0.602; p=0.001) (Fig.1a). Diatoms dominated the biofilm during the first 6 weeks of leaf litter decomposition (Fig. 1b). They reached a relative abundance of 88 and 99% during the 3rd and 4th week of the biofilm development. However, cyanobacteria dominated in the 8 weeks old biofilm with an abundance of 61% whereas diatoms declined to 38%. Diatoms varied significantly in abundance during the 8 weeks of leaf litter decomposition (Kruskal-wallis test, $H_{(6, 21)}$ =13.26, p=0.039) with a maximum abundance in the 3rd week ($1.9x10^4 \pm 1.6x10^3$ cells ml^{-1}) and the 6th week ($2.1x10^4 \pm 2.2x10^3$ cells ml^{-1}). Cyanobacteria recorded lower abundance during this period with a sharp increase during the 8th week reaching an abundance of $2.4x10^4 \pm 1.1x10^4$ cells ml^{-1} which exceeded the abundance of diatoms ($1.5x10^4 \pm 6.2x10^3$ cells ml^{-1}). The other 3 classes of phytoplankton occupied lower percentage cover of less than 10% during the entire period of litter decomposition (Fig. 2b). *Navicula* spp. and *Nitzchia* spp. were the main diatom species representing the majority of the diatoms with the highest abundance reaching $2.0x10^4$ cells ml^{-1} during the 6th week in the period between the 3rd and the 8th week (Fig. 2c). *Microcystis* spp. dominated the cyanobacteria constituting $2.0x10^4$ cells ml^{-1} of the total micro-algae abundance of $2.4x10^4 \pm 1.1x10^4$ cells ml^{-1} during the 8th week (Fig. 2d). *Peridinium* spp. dominated the dinoflagelletes constituting $4.0x10^2$ out of $6.3x10^2 \pm 2.4x10^2$ cells ml^{-1} during the 6th week (Fig. 2e)

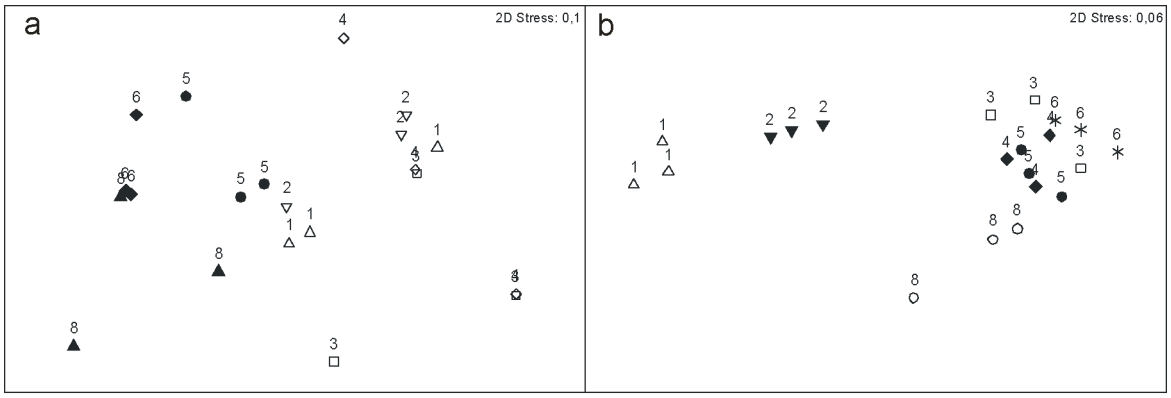


Fig 1: MDS plot of similarity in (a) microalgae and (b) fauna colonizing biofilm at the different stages of mangrove leaf litter decomposition. Numbers refer to duration of litter decomposition in weeks.

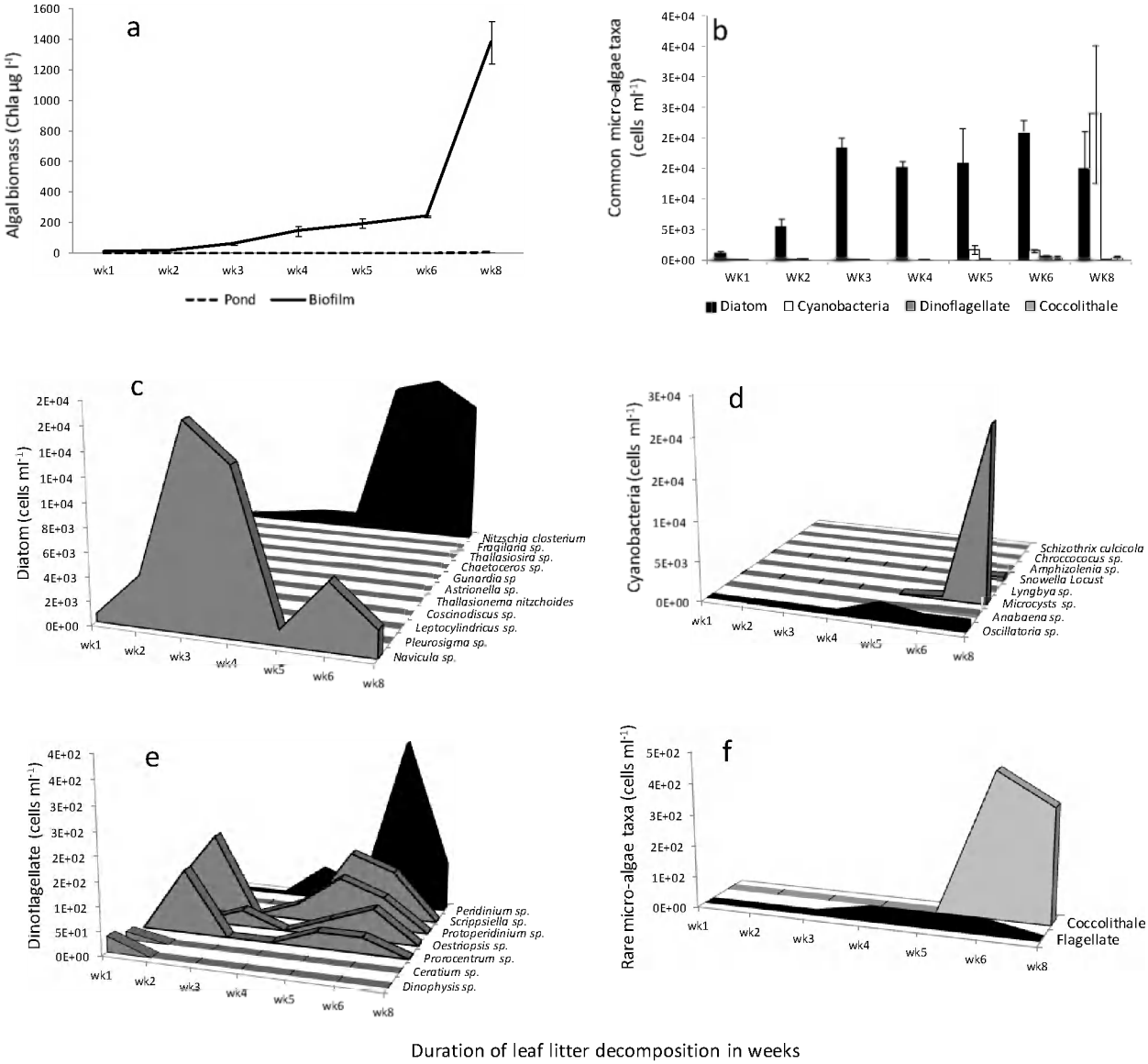


Fig 2: Biomass and assemblage of microalgae in biofim developing on mangrove leaf litter of *Rhizophora mucronata* at different stages of decomposition.

4.1.4.4 Micro-algae species diversity

Among the 5 phototrophic algae classes, 32 species were identified including 15 diatom species, 8 cyanobacteria species, 7 dinoflagellate species, 1 cocolithale and 1 flagellate species. Among the diatom species, *Navicula spp.* and *Nitzschia* spp. dominated and replaced each other as the decomposition of the leaf litter progressed. *Navicula spp.* dominated the first 4 weeks of decomposition reaching a maximum proportional cover of 90.8% during the 3rd week whereas *Nitzschia* spp. dominated the later period of decomposition from week 5 to 8 with a maximum proportional cover between 64.2% and 78.7% during the 5th and 6th week of the leaf litter decomposition. However among the Cyanobacteria, *Anabaena* spp. dominated the 8th week old litter with a proportional cover of 55.3%. The lowest micro-algae diversity was recorded during the weeks 3, 4 and 5 (H'=0.391, 0.445, 0.861) which also recorded the lowest species evenness (EH'=0.036, 0.041, 0.079) (Table 1)

Week	`	1	2	3	4	5	6	8
Diatom	*Nitzschia closterium*	0,133	0,102	0,061	0,078	0,787	0,642	0,321
	Nitzchia spp.	0,096	0,011	0,002	0,892			
	Navicula spp.	0,518	0,733	0,908		0,072	0,237	0,057
	Pseudonitzschia spp.	0,024	0,006			0,002		0,001
	Rhyosolenia spp.	0,036						
	Pleurosigma spp.	0,024	0,034	0,023	0,017	0,006		0,002
	Leptocylindricus spp.			0,002				
	Coscinodiscus spp.	0,012			0,004	0,006	0,001	
	Thallasionema nitzchoides					0,002		
	Astrionella spp.	0,024				0,002	0,001	
	Gunardia spp.					0,007		
	Chaetoceros spp.						0,003	
	Thallasiosira spp.	0,012					0,001	
	Fraglaria spp.		0,006					0,001
	Halsea spp.		0,051					
Cyanobacteria	*Oscillatoria* spp.			0,002		0,093	0,035	0,024
	Anabaena spp.	0,048	0,006				0,013	0,010
	Microcystis spp.						0,017	0,553
	Lyngbya spp.							0,005
	Snowella Locust							0,013
	Amphizolenia spp.					0,004	0,001	
	Chroccococus spp.							0,001
	Schizothrix culcicola							0,001
Dinoflagellate	*Dinophysis* spp.	0,024						
	Ceratium spp.	0,012						
	Peridinium spp.				0,004	0,002	0,016	0,003
	Protoperidinium spp.	0,036	0,028		0,002	0,006	0,003	
	Prorocentrum spp.	0,023				0,002	0,001	
	Scrippsiella spp.					0,007	0,004	
	Oestriopsis spp.			0,002		0,002	0,003	
Coccolithale	*Coccolithophoroid* spp.						0,020	0,009
Flagellate	*Choanoflagelate* spp.				0,002	0,002	0,001	
Diversity Index		1,739	1,054	0,391	0,445	0,861	1,140	1,166
Species evenness		0,209	0,108	0,036	0,041	0,079	0,102	0,100

Table 1: Proportional assemblage of microalgae in biofilm developing on mangrove leaf litter of *Rhizophora mucronata* at different stages of decomposition

4.1.4.5 Epifauna abundance and diversity

Nineteen major taxa were identified: Polychaeta, Oligochaeta, Copepoda, Nematoda, Amphipoda, Turbellaria, Gastropoda, Appendicularia, Kinorhyncha, Cnidaria, Ostracoda, Bivalvia, Tunicata, Foraminifera, Chaetognatha, Siphonophores, Nemertina, Holothuroidea and Insecta. The epifauna species colonizing the biofilm differed significantly in the different weeks of mangrove leaf litter decomposition (ANOSIM: R=0.887; p=0.001) (Fig.1b). Copepoda dominated the epifauna colonizing the biofilm during the first 3 weeks of leaf litter decomposition with a proportional percentage cover ranging from 59 to 68% while Polychaeta dominated during the 4th and 5th week with a proportional percentage cover of 90 to 93% (Table 2). Nematoda were the dominant fauna in the biofilm developing on litter decomposed for 8 weeks with a percentage cover of 40% (Fig. 3c). The biofilm developing on the litter decomposed for 4 and 5 weeks also had the lowest diversity of H'=0.3339 and 0.4102 and species evenness of 0.0275 and 0.0349, respectively (Table 2). The maximum abundance of epifauna was recorded in the biofilm on the leaf litter decomposed for a period of 4 and 5 weeks recording an abundance of 68 ± 10 and 63 ± 17 ind. cm^{-2} which significantly declined in the biofilm on the leaf litter decomposed for 6 and 8 weeks (ANOVA, $F_{(6, 14)}$ =93.26, $p<0.05$; Tukey Post Hoc: p=0.000174) (Fig. 3a). Epifauna on the 4 and 5 weeks old biofilm was dominated by meiofauna of the lower size class ranging between 38 and 250 µm with an abundance of 43±9 and 49±18 ind. cm^{-2} compared to the meiofauna of the size class between 250 µm and 1mm which had a lower abundance of 25 ± 9 and 15 ± 2 ind. cm^{-2} respectively (Fig. 3b).

week	1	2	3	4	5	6	8
Polychaeta	0,0418	0,0699	0,0827	0,9259	0,9021	0,3975	0,3584
Oligochaeta	0,0096	0,0670	0,1015	0,0002	0,0004	0,0020	
Copepoda	0,6833	0,5917	0,6777	0,0372	0,0503	0,4996	0,1204
Nematoda	0,2315	0,2232	0,0367	0,0327	0,0430	0,0831	0,3986
Amphipoda					0,00003		
Turbellaria	0,0338	0,0445	0,0889	0,0024	0,0014	0,0076	0,0648
Gastropoda				0,0001	0,0001	0,0002	0,0068
Appendicularia					0,00002		
Kinorhyncha			0,0002	0,0001			
Cnidaria		0,0038	0,0101	0,0003	0,0010	0,0030	0,0199
Ostracoda			0,0005	0,0008	0,0016	0,0039	0,0310
Bivalve			0,0006	0,0003	0,00001	0,0015	0,0001
Oikopleura					0,00002		
Foraminifera			0,0003			0,0017	
Chaetognatha			0,0001		0,00001		
Siphonophores					0,00001		
Nemertina			0,0001				
Holothuroidea			0,0003				
Insecta			0,0001				
Diversity Index	0,8908	1,1718	1,1027	0,3339	0,4102	1,0308	1,3871
Species evenness	0,1385	0,1529	0,1186	0,0275	0,0349	0,0899	0,1561

Table 2: Proportional assemblage of epifauna on biofilm developing on mangrove leaf litter of *Rhizophora mucronata* at different stages of decomposition

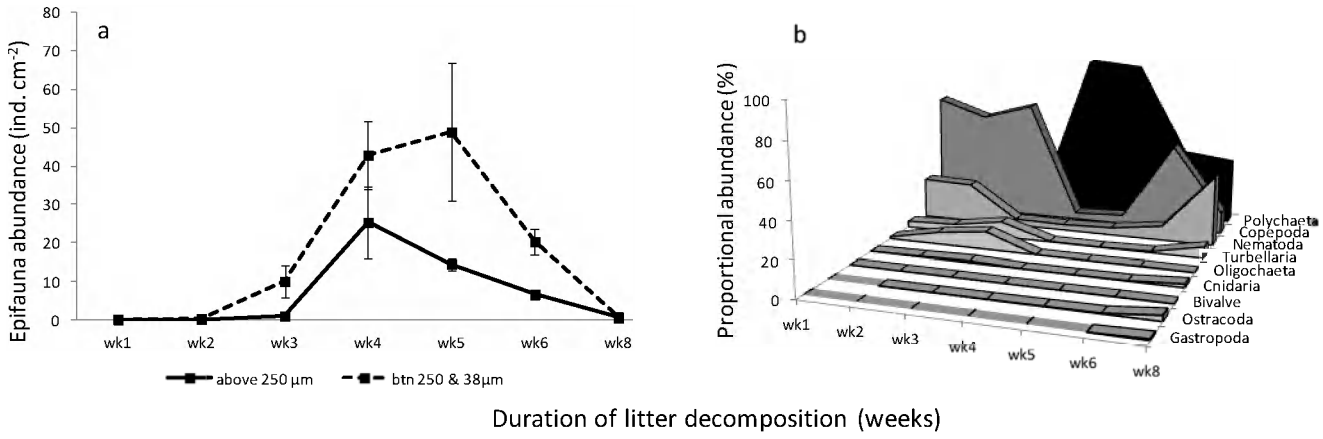


Fig 3: Abundance and assemblage of the major epifauna taxa in biofilm developing on mangrove leaf litter of *Rhizophora mucronata* at different stages of decomposition.

4.1.4.6 Shrimp performance

4.1.4.6.1 Growth rate

Shrimp foraging on the biofilm developing on the mangrove leaf litter at the different stages of decomposition recorded significantly lower specific growth rate (SGR) ranging from -0.009 ± 0.5% to 1.6 ± 0,5% compared to shrimp that were fed with compound feed (CP) (SGR: 6.1 ± 0.3%)(Anova $F_{(6,14)}$ =30.423; $p<0.05$; Tukey Post Hoc: P=0.000174). However among the treatments receiving the mangrove leaf litter, shrimp foraging on the biofilm on the 4 weeks decomposed leaf litter had an overall better performance with an SGR of 1.6 ± 0.5% (Tukey Post Hoc: P=0.029). Shrimp foraging on the biofilm on the leaf litter decomposed for a period of 1, 3 and 6 weeks deteriorated in growth recording negative SGR. Although shrimp foraging on the leaf litter decomposed for 8 weeks had a positive growth, their SGR was not significantly different from the treatments recording the negative growth rates and the shrimp under starvation treatments (NF) (Wk 8: 0.95 ± 0.74% vs No feeding: -0,88 ± 0,46%) (Tukey Post Hoc: P= 0.107)(Fig. 4)

4.1.4.6.2 Survival

Shrimp fed compound feed (CP) recorded the highest significant survival (SR) of 97.2 ± 2.7% (ANOVA, $F_{(6, 14)}$ =73.85; $p<0.05$; Tukey Post Hoc: P=0.000174). Among the shrimp foraging on the biofilm on the decomposing mangrove leaf litter, shrimp foraging on the 4 weeks old leaf litter recorded the highest mean SR of 39.8 ± 4.8% although they did not differ statistically from shrimp foraging on the leaf litter decomposed for 1, 3 and 6 weeks where the SR ranged from 23.3 ± 3.2 to 38.9 ± 4.6% (Tukey Post Hoc: p=0.06; 0.99; 0.57). However

pair wise comparisons revealed significantly lower SR in shrimp foraging on the leaf litter decomposed for 8 weeks which recorded an SR of 23.3 ± 3.2% (Tukey Post Hoc: P=0.04). Shrimp in the starvation treatments (NF) recorded the lowest SR of 8.4 ± 0.9% (Tukey Post Hoc: P=0.003) but they did not differ significantly from the shrimp foraging on the leaf litter decomposed for 8 weeks (Tukey Post Hoc: p=0.0745) (Fig. 4).

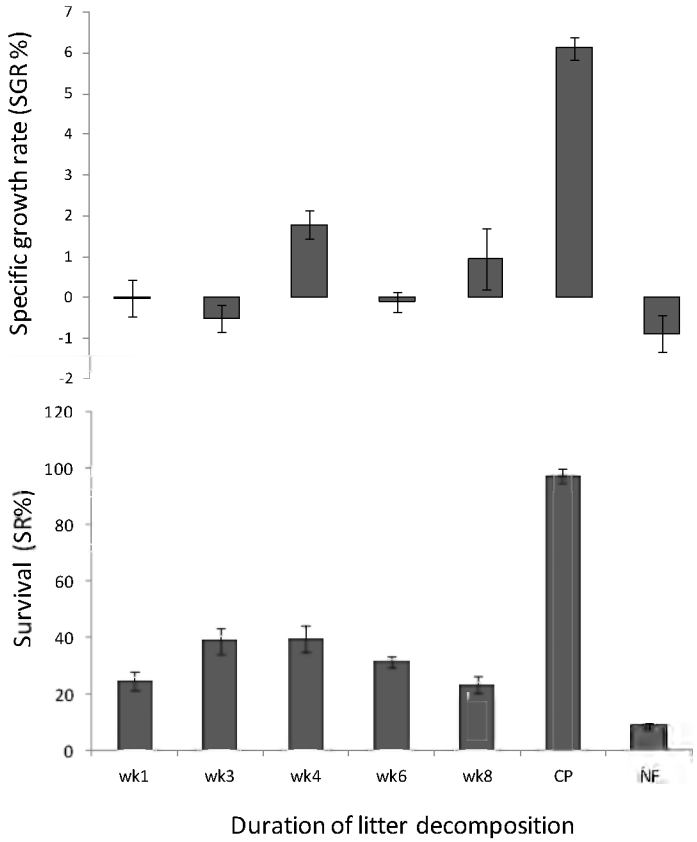


Fig 4: Performance of shrimp post larvae of *Penaeus monodon* in different feeding treatments. Where; wk1 to wk8: shrimp foraging on biofilm on mangrove leaf litter at different stages of decomposition; CP: shrimp fed a commercial compound feed; NF: shrimp starved as control.

4.1.5 Discussion

4.1.5.1 Micro-algae

In the first 6 weeks of the experiment there was a clear dominance of diatoms but also a shift in the community composition with *Navicula* spp. dominating the leaf litter until the 4th week of decomposition. After the 4th week, *Navicula* spp. was replaced by *Nitschia* spp. at weeks 5, 6 and 8. The reason for this shift in the species of diatom is not understood although its implication to the diet of shrimp post larvae could have a significant application. *Nitzschia*

spp. has been reported to be richer in proteins (Brown and Jeffrey, 1995) and therefore could imply a better source of proteins to the immediate grazers.

Micro-algae succession from diatoms to cyanobacteria, was prominent at the later stages of the leaf litter decomposition as observed in this experiment. This succession may have been a micro-scale bloom triggered by a sudden increase in nutrients leached from the decomposed litter such as the ammonified particulate and dissolved organic nitrogen (Alongi et al., 2000) and phosphorus (Lin and Sternberg, 2007). In addition, the first weeks of the biofilm development are further characterized by a strong growth of copepods which could have been feeding on the diatoms. Diatoms have a C/N ratio of about 5 and could influence carbon and nitrogen contents of copepod fecal pellets (Dam and Siuda, 2010; Morales, 1987b). Such a case would imply that the copepods grazing on diatoms could have also released a lot of nutrients especially ammonium. This may have stimulated the cyanobacterial blooms which commonly result from eutrophic conditions and changes in specific environmental conditions such as high water temperature and pH values, low turbulence and high nutrient input (Amado and Monserrat, 2010).

Apart from the increase in cyanobacteria, there was a general decline in the other micro-algae species belonging to the diatoms, dinoflagellates and flagellates at the advanced stages of litter decomposition. It seems that the microalgae reached a stationary phase between the 4^{th} and the 6^{th} week of decomposition and progressed to the death phase during the 8^{th} week. The decline of the micro-algae could have been a result of grazing by epifauna. However the similar decline in epifauna after the 5^{th} week (Fig 3a) suggests that grazing may not have been the only reason. Decomposing mangrove litter leaches a phenolic compound tannin (Hernes et al., 2001; Lin et al., 2007; Rajendran and Kathiresan, 2000b). Tannin concentrations above 18 mg l^{-1} was found to inhibit the growth of micro-algae *Skeletonema* spp. and *Dunnaliella* spp. in Celestun lagoon in the Gulf of Mexico (Herrera-Siveira and Ramirez-Ramirez, 1996). The leaching of tannins from the decomposing mangrove litter could have partially contributed to the decline in certain species of micro-algae and in this way prevailing the space and reduced competition for the proliferation of cyanobacteria.

4.1.5.2 Epifauna

Epifauna identified on the biofilm included several juvenile stages of macroinvertebrates such as gastropods, bivalves, crustaceans, polychaetes, insects, annelid worms and mollusks which are potential food for shrimp post larvae (Rothlisberg, 1998; Tacon, 1996a). In the present study adult copepods were the major component of the biofilm during the first 3 weeks of leaf litter decomposition and mainly consisted of representatives of the order Harpacticoida which occur in sediments but they are also very diverse in epiphytic communites such as on seagrass leaves (De Troch et al., 2003). Juvenile stages of copepods (nauplius larvae), egg cocoons of polychaetes and oligochaetes, bivalve trochophore and veliger larvae, although enumerated together with the adult species, were observed to occur at this stage and are therefore the possible contributors to the increase of epifauna in the size range of 38 µm and 250 µm which dominated the 4th and 5th week old biofilm. The increase in juvenile stages points to the recruitment at this phase of the decomposition process. The increase in polychaetes after the 3rd week points that, at this stage of litter decomposition, polychaetes have an increased potential in contributing to the food web (Fig 3b). According to a previous study (Gatune et al., 2012), mangrove leaf litter decomposed for 3 and 4 weeks supported a climax microbial abundance and essential fatty acids which may nutritionally influence a healthy assemblage of epifauna. At this stage of mangrove leaf litter decomposition, there is also a large pool of nitrogen, amino sugars and amino acids (Rajendran and Kathiresan, 2000b) which would definitely nourish the primary trophic levels occupied by bacteria and micro-algae. These micro-biota are potential food sources for the epifauna (Woitchik et al., 1997).

The decline in the abundance of epifauna after the recruitment stage (week 4 and 5) could be due to the reduced survival of juveniles going in to adult stages. Inadequate supply of quality food could be a possible reason especially supported by the decline and aging of diatoms, concomitant with an increase of non-palatable cyanobacteria. Mangrove leaf litter decomposed for more than 4 weeks could be characterized by low levels of reduced amino sugars and amino acids (Rajendran and Kathiresan, 2000b; Tremblay and Benner, 2006) and may not be nutritionally sufficient to support a climax community. For instance, it has been noted that the meiofauna (between 38 and 250 µm) communities are not related to the mechanical process of litter decay but may be responding to chemical changes and/or development of microorganisms associated with the decay process (Gee and Somerfield, 1997). Other direct chemical interactions could support such trend by impairing the energy flows in the lower trophic links. For instance condensed tannin form complexes with proteins

and enzymes leading to antimicrobial and antiviral properties (Lin et al., 2007) and impairing immediate leaching of nutrients through nitrogen and phosphorus immobilization (Lin and Sternberg, 2007). A negative effect of the tannin leaching on the mangrove associated benthic fauna has been suggested before (Lee, 1999) especially for meiofauna (Alongi, 1987) and macrofauna (Dittmann, 2001) where lower species number and diversity have been recorded in mangrove sites compared to the adjacent mudflat.

4.1.5.3 Performance of shrimp post larvae foraging on biolfilm

Shrimp post larvae foraging on biofilm of mangrove leaf litter decomposed for a period of less than 3 weeks seem to experience retarded and emaciated growth. This scenario is also repeated in the shrimp foraging on 6 weeks old leaf litter. A notable improvement in growth and survival is observed in shrimp foraging on 4 weeks old litter. Growth performance of shrimp post larvae can be predicted from quality and/or palatability of food which may be influenced by the ambient environmental conditions. The climax quality micro-algae and epifauna community during the 4^{th} and the 5^{th} week may have provided shrimp post larvae with a rich source of nutrition enabling them to withstand adverse water quality conditions such as chemical oxygen demand (COD), tannins and hydrogen sulphide (H_2S) which are common in decomposing mangrove litter and are known for their negative effect on shrimp growth (Hai and Yakupitiyage, 2005). Later stages of the shrimp growth, just like the initial stages, could have been affected by meager and dwindling of the quality food sources with a consequential deterioration in the general health of the shrimp post larvae.

4.1.5.4 Potential Impact of micro-algae on shrimp growth and survival

The diatoms *Chaetoceros* spp. and *Thalassiosira* spp. occurred in small proportions in the biofilm developed on the leaf litter decomposed for 1 and 5 weeks (table1). These diatom species are among the microalgae species which are commonly used in aquaculture (Borowitzka, 1997; Brown and Miller, 1992; Brown and Farmer, 1994). In the microcosm study by Brown and Jeffrey (1995), *Nitzschia closterium,* was found to be richest in proteins (38% content) compared to 6 other diatoms belonging to *Navicula* spp., *Skeletonema* spp., *Lauderia* spp. and *Cylindrotheca spp.* Such observation emphasizes the importance of *Nitzchia* spp. as a food source to shrimp post larvae foraging on the mangrove litter decomposed for a period of less than 6 weeks. The ecological role of the dominating diatoms on the less decomposed litter cannot be ignored given the importance of diatoms in the energy transfer to the consumer trophic levels. For instance, the characteristic fatty acid composition of diatoms is readily distinguishable from those of other micro-algal groups (Ying et al.,

2000). Diatoms are typically rich in polyunsaturated fatty acids (PUFA) such as the Eicosapentanoic acid (EPA: 20:5ω3) (Parrish et al., 2000). The nutritional quality of diatoms may deteriorate at the advanced age as could be the case of diatoms in the old biofilm on the litter decomposed beyond 6 weeks. While comparing three growth phases in four marine diatoms, among them, *Nitzschia closterium* and *Chaetoceros gracilis*, Liang and Mai (2005) observed that saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) increased while the polyunsaturated fatty acids (PUFA) decreased with the culture age from the exponential growth phase, early stationary phase to late stationary phase. Shrimp have an essential nutritional requirement of lipids. Certain fatty acids such as the 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); consequently these fatty acids are referred to as essential fatty acids (EFA) (Parrish, 2009). The significant contribution of diatoms to the growth of the biofilm at the early stages of litter decomposition is an important scenario to infer the good performance of shrimp post larvae foraging on the 4 week old biofilm. However, the retarded and emaciated growth in the shrimp post larvae foraging on the leaf litter decomposed for 1 and 3 weeks could raise suspicion of a prevailing intoxicating condition. For instance the popular negative biological and chemical aspects of tannin leachate typical of the decomposing leaf litter of mangroves of the *Rhizophora* spp. cannot be ignored. During the first stages of mangrove leaf litter decomposition there is a rapid leaching of tannins, among other compounds, which reduces at the later stages of decomposition (Rajendran and Kathiresan, 2000b). Tannins reduce the digestibility of feed (Mandal and Ghosh, 2010) and negatively affect growth responses of shrimp in mangrove leaf litter treatments (Hai and Yakupitiyage, 2005). It has even been postulated that high tannin concentrations may be potentially toxic to shrimp cultured in the integrated mangrove–shrimp farming system (Fitzgerald Jr., 2000).

Shrimp post larvae foraging on the 6 and 8 weeks old biofilm may also have been adversely affected by the replacement of diatoms as 'excellent' food source by cyanobacteria. Some penaeid shrimp species post 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). In the present study, the cyanobacteria *Microcystis* spp. was dominant. *Microcystis* spp. is known to produce a potent hepatotoxin, a microcystin that is also produced by a number of planktonic cyanobacteria genera such as the *Anabeana* spp., *Anabaenopsis* spp., *Nostoc* spp. and *Planktothrix* spp. *(Oscillatoria)* (Amado and Monserrat, 2010) and other bioactive metabolites with the potential to degrade the nutritional status of aquaculture species (inhibitors of proteases and grazer deterrents) (Smith et al., 2008). Both *Anabeana* spp. and *Oscillatoria* spp. were also found in the biofilm analysed from the decomposing mangrove leaf litter. *Oscillatoria* spp. produces the musty, off-flavor compound 2-methylisoborneol, which can taint the flesh of channel catfish and render them unmarketable (Schrader et al., 1998a). Consequently, green algae are preferred over cyanobacteria in aquaculture ponds since they do not produce 2-methylisoborneol and because they are better in maintaining the primary productivity in pond ecosystems (Schrader et al., 1998a; Schrader et al., 1998b).

4.1.5.5 Potential Impact of epifauna on shrimp growth and survival

Shrimp foraging on the litter decomposed for 4 weeks may have benefited from the ample food supply from the increased recruitment of epifauna. Zooplankton and epibenthos are known to contribute to the nutrition of shrimp post larvae in aquaculture installations (Chen and Chen, 1992). The establishment of an abundant assemblage of epifauna is therefore an important prerequisite to stocking (Coman et al., 2003; Tacon, 1996a) and can be part of the supplementary feeds that can directly be consumed by cultured shrimp, ranging from live to fresh, natural food items such as insects, annelid worms, crustaceans and mollusks (Tacon, 1996a). Meiofauna and small macrofauna can constitute a major food source for juvenile fish and shrimp (Dittmann, 2001). 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 post larvae eat what is available but are also selective (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 seagrass 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. As discussed above the reduced epifauna abundance could have led to starvation with a consequent

deterioration in growth and survival of shrimp foraging in the litter decomposed for more than 4 weeks.

There is an overall low species diversity and evenness in the biofilm biota during the 4th and the 5th week of growth. This observation could point at this period as being the most important stage in deriving biofilm with a high quality natural food source from the decomposing mangrove leaf litter. The quality of such food source and how it would impact onto an immediate trophic level would definitely depend on the prevailing nutritional requirements.

4.1.5.6 Water quality

Tannin has previously been observed to increase shrimp survival by reducing predation as a result of colouring the water which provided favourable conditions for hiding from predators (Hai and Yakupitiyage, 2005; Nga et al., 2004). In the present study we used whole mangrove leaf litter which also provided good hiding places for the shrimp post larvae. The reduced survival and growth performance of shrimp foraging in mmangrove leaf litter in relation to the shrimp feeding on the compound feed was observed suggesting that a different condition rather than physical was limiting the overall shrimp performance. Furthermore there was no difference in the growth limiting water quality factors, such as the salinity, temperature, dissolved oxygen and total ammonia nitrogen, between the best performing and poor performing treatments which were not below or above the lethal concentration levels (Chien, 1992). The water temperature during the leaf litter incubation and shrimp foraging test periods fell within the range for optimal primary production of EFA as observed by Renaud (1995) where cultures of *N. closterium* and *Isochrysis* spp. gave maximum production of PUFA and EPA (20:5ω3) at 25°C and 20 °C, respectively.

4.1.6 Conclusion

1. Shrimp post larvae foraging on the decomposing mangrove leaf litter perform better when foraging on the 4 weeks old biofilm. .
2. The quality of the biofilm is fully developed during the 4th week of mangrove leaf litter decomposition and is dominated by diatoms, polychaetes, harpacticoid copepods and oligochaetes.
3. The ecological function of decomposing mangrove leaf litter of *Rhizophora mucronata* in providing quality natural food to shrimp post larvae is limited by the

collapse of the epifauna and subsequent colonization by cyanobacteria (considered to be of low nutritional quality) if retained in the pond water beyond a period of 5 weeks.

4.1.7 Acknowledgements

The first author thanks the Flemish InterUniversity Council (VLIR) for the financial support to follow an international PhD program in Biology at Ghent University, Belgium. The present study is part of a PhD project in the *FEEDING ECOLOGY OF SHRIMP IN TROPICAL MANGROVE SYSTEMS OF KENYA: The nutritive value of detritus and its use in biological shrimp aquaculture*. Deep felt thanks go to Dr. Mahika, Director of Aquaculture Tanzania; Mr.Vageri and Ari of Alphakrust, Tanzania, for facilitating export and donating the test shrimp post larvae. KMFRI laboratory technicians James Kilonzi, Eric Magara, Mary Mkonu, George Onduso, Mary Adungosi, Mwendwa Mbaluka and the youth and community members of the Majaoni silvofishery project, Kenya, for their field assistance. The last author acknowledges the Special Research Fund of the Ghent University (BOF-GOA 01GA1911W).

5 Chapter 5

5.1 The importance of light intensity and the presence of sediment for ecological shrimp aquaculture

Charles Gatune [a,c]*, Ann Vanreusel[a], Renison Ruwa[b], Peter Bossier[d] and Marleen De Troch[a]

[a] Ghent University, Biology Department, Marine Biology, Campus Sterre, Krijgslaan 281-S8, B-9000, Gent, Belgium.
[b] Kenya Marine and Fisheries Research Institute, P.O Box 80100, 81651, Mombasa, Kenya.
[c] Ministry of Fisheries Development, P.O. Box 90423 – 80100, Mombasa, Kenya.
[d] Ghent University, Faculty of Bioscience Engineering, Laboratory of Aquaculture and Artemia Reference Centre, Rozier 44, B-9000, Gent, Belgium.

*Corresponding Author: Email address: kgatune@yahoo.com

The set up of the experimental microcosms to study the effect of light and sediment on the biofilm and the decomposing mangrove leaf litter at Majaoni shrimp culture ponds (Photo courtesy Gatune C. 2011)

Submitted as:
Gatune, C., Vanreusel, A., C., Ruwa, R., Bossier, P., De troch, M. Sunlight and sediment influences the biophysicochemical value of ecological shrimp aquaculture in a mangrove ecosystem. *Journal of Experimental Marine Biology and Ecology.*

5.1.1 Abstract

Ecological shrimp aquaculture in mangrove forest implies the integration of shrimp culture ponds between mangrove trees in a (semi)-natural forest. Consequently, mangrove leaf litter fall may have an important impact on the ecological processes in the pond. Various studies have focused on the physical and chemical influences of mangrove litter fall to the receiving ecosystems. However, little attention is paid to the environmental settings of the interactions, e.g. the exposition to sunlight and the role of sediment at the bottom of the pond. The degradation of the leaf litter of *Rhizophora mucronata* and the assembly of micro-algae and epifauna were assessed under various conditions of direct sunlight, shade and the presence/absence of sediment. This experiment contributes to the potential use of mangrove leaf litter and biofilm in providing favorable culture conditions and natural diet to shrimp post larvae of *Penaeus monodon*. Mangrove leaf litter incubated with sediment and exposed to sunlight was rapidly degraded compared to the litter incubated without sediment and in the shade. Decomposing litter exposed to the sunlight in the presence of sediment supported the highest biomass and diversity of micro-algae and epifauna, and the highest abundance of diatoms, polychaetes and nematodes during the 4th week. Cyanobacteria of the genus *Microcystis* dominated the mangrove litter incubated without sediment in the presence of sunlight after a decomposition period of 5 weeks. Diatoms of the *Navicula* spp. and cyanobacteria of the *Anabaena* spp. and *Oscillatoria* spp. continued to grow in the shade. The water in the microcosms supporting mangrove litter decomposing in the shade was rich in total ammonium nitrogen (TAN) but had low levels of dissolved oxygen (DO), temperature and pH. The study illustrated that there is a synergistic effect between sediment and direct sunlight in promoting the proliferation of a wide range of micro-algae and polychaetes, inhibiting the growth of cyanobacteria and maintaining water quality which is favorable to the culture of shrimp post larvae. This implies that ecological shrimp culture management and conservation practices should consider locating ecological shrimp ponds in less forested areas to promote primary production and aerate bottom layers of shaded ponds receiving litter fall. Moreover, our findings suggest that shrimp culture microcosms using mangrove leaf litter substrates should include sediment to prevent cyanobacteria blooms.

Key words: sunlight; sediment; mangrove; leaf litter; shrimp; micro-algae; epifauna

5.1.2 Introduction

The importance of decomposing mangrove leaf litter in providing natural food to penaeid shrimp post larvae lies mainly in the associated periphytic biofilm (Gatune et al., 2012). The biofilm constitutes of phyto- and zoo-biota which is obviously mediated by a microbial loop (Azim and Wahab, 2005b; Burford et al., 2003; Pascal et al., 2008). The various biological components of the biofilm and their potential nutritive value to shrimp post larvae at different stages of litter decomposition were assessed in an earlier experiment (Chapter 4). This baseline study allowed us to identify an intervention time line in managing mangrove litter fall into the ecological shrimp culture ponds. Ecological shrimp aquaculture in mangrove systems has gained environmental support because of its tendency to conserve the mangrove forest (Fitzgerald Jr., 2000; Primavera, 1998). Silvo-culture is a major ecological aquaculture practice in the mangrove forests and involves rearing fish or shrimp in the presence of mangrove trees (Primavera, 1998). This integrated approach implies that the shrimp ponds constantly intercept mangrove litter fall which would proceed to decompose under the influence of the present micro-biota biofilm (Benner and Hodson, 1985a; Rajendran and Kathiresan, 2006). The mangrove trees would also shade the shrimp pond from the direct sunlight either partially or completely. Since the mangrove leaf litter falling into the pond would definitely sink to the bottom water layers. , the micro-algae accumulating at the water surface or other suspended particles in the water column would further shield them from the light. Sunlight has been demonstrated to accelerate the rate of decomposition of leaf litter either solely, by photochemical oxidation action (Gallo et al., 2009) or in combination with the accelerated extracellular enzymatic action of the associated microbial communities (Francoeur et al., 2006). Light intensity also increases the algae biomass, density and composition creating a rich energy source for shredders which enhances the rate of plant litter decomposition by their grazing activity (Franken et al., 2005)

In an attempt to comply with the ecological requests to reduce aquaculture activities in mangrove systems, shrimp culture practices were pushed further inland. Shrimp aquaculture further inland would definitely lack the influence of the natural services from the mangrove based estuarine ecosystems (Kautisky et al., 2001). This has caused an increase in shrimp culture practices which have adopted artificial systems to mimic nature in promoting proliferation of natural food sources. Such microcosm facilities are using artificial substrates

in the absence of sediment (Asaduzzaman et al., 2008; Bratvold and Browdy, 2001a; Otoshi et al., 2006). The use of bio-floc technology to enhance bacterial mediated natural food supply has successfully applied high carbon source substrates to reduce nutrient loading in the absence of sediment (Crab et al., 2007; Crab et al., 2010). Hypothetically, if the artificial substrate may come inform of decomposing mangrove leaf litter, the impact of the lack of sediment on the growth and composition of biofilm on the decomposing leaf litter substrate may depend on the exposure to sunlight.

It is essential that ecological shrimp ponds, whether in mangrove systems or as inland shrimp culture microcosms, support phyto- and zoobiota for maintaining water quality as well as providing a quality food source for the shrimp post larvae (Bratvold and Browdy, 2001a; Burford, 1997; Thompson et al., 2002). For this reason, it would be quite important to assess the potential of the decomposing mangrove leaf litter in supporting a biofilm contributing to the natural diet for shrimp post larvae when the environmental conditions are modified. The present study therefore hypothesized that the status of the water quality and the natural food value provided by the biofilm associated with decomposing mangrove leaf litter depends on the presence of direct sunlight and sediment. We therefore investigated the assembly of the major taxa of microalgae and invertebrate fauna colonizing the decomposing mangrove leaf litter of *Rhizophora mucronata*, under limited sunlight and sediment conditions. The potential application of the various microalgae and fauna in providing natural diet to shrimp post larvae of *P. monodon* was then extrapolated to the natural food types commonly used in shrimp aquaculture.

5.1.3 Materials and methods

5.1.3.1 Study site

The study was carried out in an integrated mangrove and shrimp culture pond at Mtwapa creek, Northern coastal region of Kenya ($3^{o}57'$S; $39^{o}42'$E). The study site is characterized by a reforested mangrove forest dominated by *Rhizophora mucronata.*

5.1.3.2 Experimental design

Senescent mangrove leaf litter was carefully selected to include leaf litter of similar surface area. The leaf litter was then incubated in pond water innoculated in two sets of microcosms consisting of 70 l tanks at a litter loading density not exceeding 1g l^{-1} (Hai and Yakupitiyage, 2005). One set was carefully shaded with fronds not to allow direct sunlight. The second was left exposed to the direct sunlight. Each set consisted of two treatments, with 5 cm layer of

sediment and without sediment, in triplicates. The sediment, which was predominantly clay-fine silt, was obtained from the bottom of the pond which had previously been used to culture shrimps. The sediment was not treated in order to exclude the natural microbiota. The set up provided the following treatments; sunlight with sediment (LS); sunlight without sediment (LX); shaded with sediment (DS) and shaded without sediment (DX). The tank water was additionally seeded with microbiota retained on 38 µm sieve after passing 50 l of pond water through a 250 µm sieve to exclude larger particles. The litter incubation, 70l microcosms, were set floating in an open sunlight exposed shrimp culture pond to prevent build up of excess temperature.

5.1.3.3 Litter degradation

Senescent mangrove leaf litter was dried in the shade to a constant weight and incubated hanging 3 cm above the sediment in each microcosm for a period of 8 weeks. 3 decomposing leaves were weekly sampled at random in triplicates. Biofilm on mangrove litter was scraped off and gently washed with distilled water and oven dried at 70^{o}C for 48 hrs and weighed. Litter degradation was recorded as percentage weight loss of the initial, intact leaf, after the removal of biofilm.

5.1.3.4 Micro-algae abundance and taxa composition

Mangrove leaf litter was sampled from each microcosm, weekly, in triplicates by pooling 3 decomposing leaves per replicate. The biofilm was gently washed from the surface of the mangrove leaves with a known volume of filtered seawater and preserved in 2% Lugol's iodine solution (1 : 2 iodine/iodide: glacial acetic acid solution). Micro-algae were classified and counted in the laboratory by first diluting each replicate 5 to 10 times. 5 sub-replicates of 0.02 ml were then sub-sampled and examined under an inverted microscope.

5.1.3.5 Epifauna abundance and taxa composition

The present study emphasized on the epifauna which included both the meiofauna (metazoans that can pass through a 1 mm sieve and are retained on a 38 µm sieve) and macrofauna (organisms retained on1 mm sieve) occurring on the decomposing mangrove leaf litter.

Mangrove leaf litter was sampled weekly in triplicates by pooling 3 decomposing leaves per replicate in a plastic bag. Each group of decomposing leaves was immediately mixed with 8% magnesium chloride to shock the attached epifauna, thoroughly agitated then sieved through 1mm and 38µm mesh size sieves. The sieved fauna was gentle washed from the 38µm sieve

with a soft filtered fresh water spray, preserved in 4% formaline and stained with a few drops of 1% solution Bengal rose. Epifauna were identified and counted at major taxa level using a binocular microscope and recorded as number per cm^2 of leaf surface.

5.1.3.6 Species diversity and evenness of the main micro-algae and epifauna groups

The Shannon Wiener index (H') and equitability index (EH) was used for the estimation of micro-algae and epifauna community diversity and evenness based on natural log (ln) (Shannon, 1948). Shannon Wiener diversity index (H') is an accurate measure of species diversity in an area since it not only considers the variety of species but also the number of the specific species. In simple terms it considers the relation between the number of species and the number of individuals (in a given area or in a given sample)(Spellerberg and Fedor, 2003). The following formula was used:

$$H' = -\sum_{i=1}^{s} Pi * \ln Pi$$

$$EH = \frac{H'}{\ln S}$$

Where S is the total number of species in the community; P_i is the proportion of S made up by the i^{th} species. Species equitability or evenness (EH) was interpreted within the range of 0 to 1 with values close to 0 signifying dominance by a single species and close to 1 indicating many species present with equal numbers (high eveness).

5.1.3.7 Micro-algae biomass

Mangrove leaves were sampled, weekly, in triplicates consisting of 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 filter GF/F (0.45 µm mesh, 47 mm diameter). Phytopigments were extracted from the collected biofilm after adding 10 ml 90% acetone to the lyophylised GF/F filters at 4°C in the dark and the supernatant was analysed for chlorophyll a according to the protocol of Granger and Lizumi (2001).

5.1.3.8 Water quality parameters

Water quality was monitored by weekly measurements of temperature, dissolved oxygen, pH, salinity and total ammonium nitrogen. Temperature, dissolved oxygen, pH were measured using meters, salinity was measured using a refractometer whereas total ammonium nitrogen was analysed in the laboratory according to Eaton et al.(2005). Unionised ammonia (NH_3) toxicity was assessed by using TAN (NH_4-N) to ammonia-nitrogen (NH_3-N) (pH, temperature and salinity-thermodynamic dependent) ratio as described by Spotte and Adams (1983).

5.1.3.9 Data analysis

Three-way ANOVA was used to compare the effect of time period (week), light and sediment on the extent of degradation, biomass and abundance of micro-algae and epifauna qualities of the mangrove litter incubated in seawater microcosms at different ambient conditions of shade, sunlight and sediment. Three-way ANOVA was also used to compare the effect of time(week), light and sediment on the dissolved oxygen, temperature, pH and total ammonium nitrogen (TAN) of the seawater used to incubate the decomposing mangrove litter. All data were checked for normality and variance homogeneity requirements for parametric analysis. Data which did not meet normality requirements after being transformed were analysed non-parametrically with Kruskal-Wallis ANOVA & Median Test. Multidimensional scaling (MDS) and analysis of similarity (Two way ANOSIM) was used to compare the similarity in the taxonomic distribution of the epifauna colonizing the decomposing mangrove litter due to the effect of time and light. Primer 6.0 software was used for the MDS and ANOSIM analysis (Clarke and Gorley, 2006). Shannon-Wiener index was used to estimate the diversity and evenness of the community of the micro-algae and epifauna colonizing the decomposing mangrove litter under different conditions of sunlight and sediment.

5.1.4 Results

5.1.4.1 Litter degradation

Mangrove leaf litter incubated with sediment and direct sunlight were significantly more degraded compared to the litter incubated without sediment and in the shade especially at the 5^{th} and the 8^{th}weeks of decomposition (Three way ANOVA; Week, light and sediment; $F_{(3, 32)}$ =6.048; p=0.0022). For complete F ratio see Appendix 1 (Fig. 1). Leaf litter incubated with sediment and exposed to sunlight (series LS) recorded the highest percentage of weight loss of 70.3±2.3% after 8 weeks of decomposition. Although the litter incubated in the sunlight without sediment recorded the lowest mean weight loss of 42.5±4.2% it did not significantly differ in weight loss compared to the litter incubated in the shade with or without sediment (Tukey post hoc: $p>0.05$) (Fig 1)

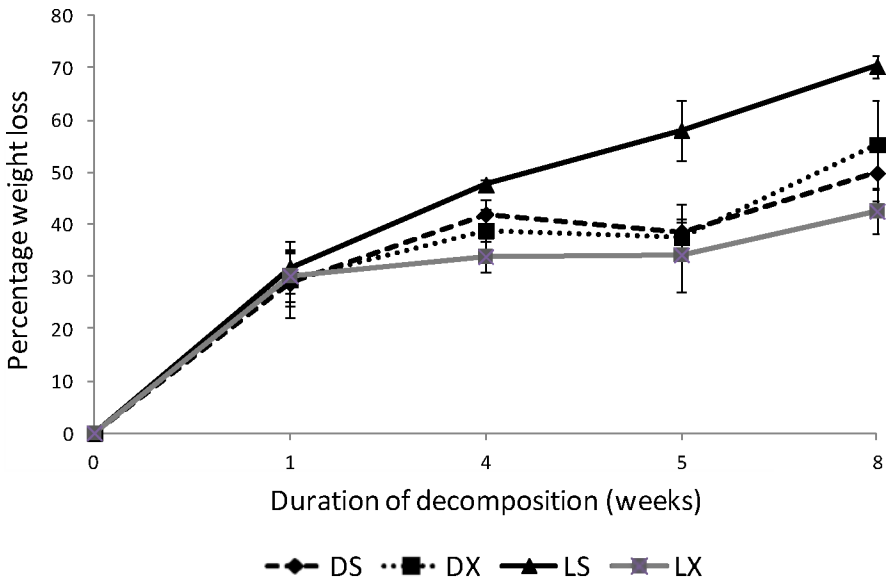


Fig 1: Degradation of the mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX).

5.1.4.2 Micro-algae biomass

Microalgae biomass was significantly higher in the decomposing mangrove leaf litter exposed to the sunlight than those decomposing in the shade (Kruskal-Wallis test: $H_{(3, 36)} = 27.64$; p=0.000) (Fig. 2). Mangrove leaf litter decomposing in the sunlight without sediment recorded the highest biomass (92.82± 61.53µg l^{-1} Chla, N=3) which was recorded during the 4th week. The highest macro-algae biomass in the mangrove litter decomposing in the shade was 7.3±5.0 µg l^{-1} Chla, N=3). Further comparisons showed that micro-algae biomass did not differ significantly between the treatments exposed to the similar light and shade conditions with or without sediments present (p>0.05) (Fig. 2)

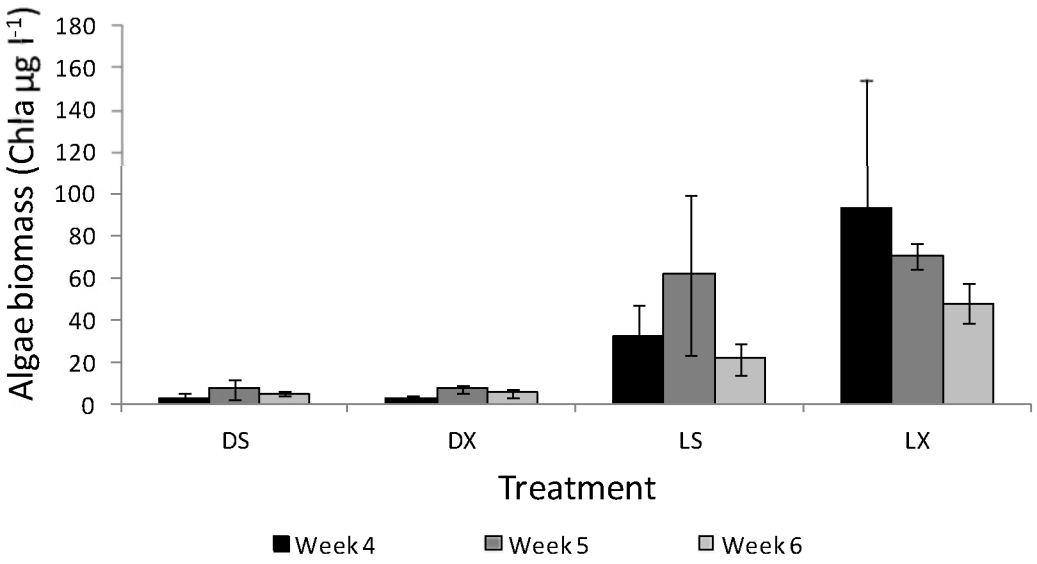


Fig 2: Biomass of micro-algae in biofim developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at three time intervals and at different ambient conditions of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX).

5.1.4.3 Microalgae taxonomic composition

Nine phototrophic algae classes were identified as dominating the periphytic biofilm present on the decomposing mangrove leaf litter. In order of decreasing abundance, the nine micro-algae classes were diatoms, Cyanobacteria, dinoflagellates, Zygnemopheceae, Chlorophyceae, Chrysophyceae, flagellates, Euglenozoa and coccolithales. At all time intervals, except for week 6, and in all treatments diatoms dominated the micro-algae community (Fig. 3). Litter decomposing in the direct sunlight supported the highest abundance of micro-algae with the litter incubated with sediment supporting the highest abundance of diatoms during the 4th week (1.5 10^4±7.3 10^3 cells ml^{-1}) while litter without sediment supported the highest abundance of cyanobacteria especially during the 6th week when it reached the highest abundance of 3.3 10^8±2.8 10^8 cells ml^{-1}. The abundance of diatoms increased in both the sunlight and shade treatments to a maximum during the 4th week (Fig. 3). The abundance of the microalgae on the leaf litter decomposing in the sunlight was significantly higher than on the litter decomposing in the shade especially due to the week effect during weeks 3, 4 and 6 (Three way ANOVA: $F_{(3, 32)} = 17.961$; $p = 0.000001$). For complete F ratio see appendix 2.

Diatoms dominated the litter decomposed for less than 6 weeks in both the shade and the direct sunlight treatments with and without sediment especially due to the week effect (Three way ANOVA: $F_{(3, 32)} = 4.064$; $p=0.014$). For complete F ratio see Appendix 3) whereas Cyanobacteria dominated the decomposing litter exposed to the sunlight in the absence of sediment with the highest abundance reached during the 6th week (Three Way ANOVA: Tukey post hoc: $p< 0.0074$)). The abundance of the Cyanobacteria on the mangrove litter decomposing in the shade without sediment was similar to that of the litter decomposing in the sunlight in the presence of sediment across all week groups (Two way crossed ANOSIM: $R=-0.127$; $p=0.77$).

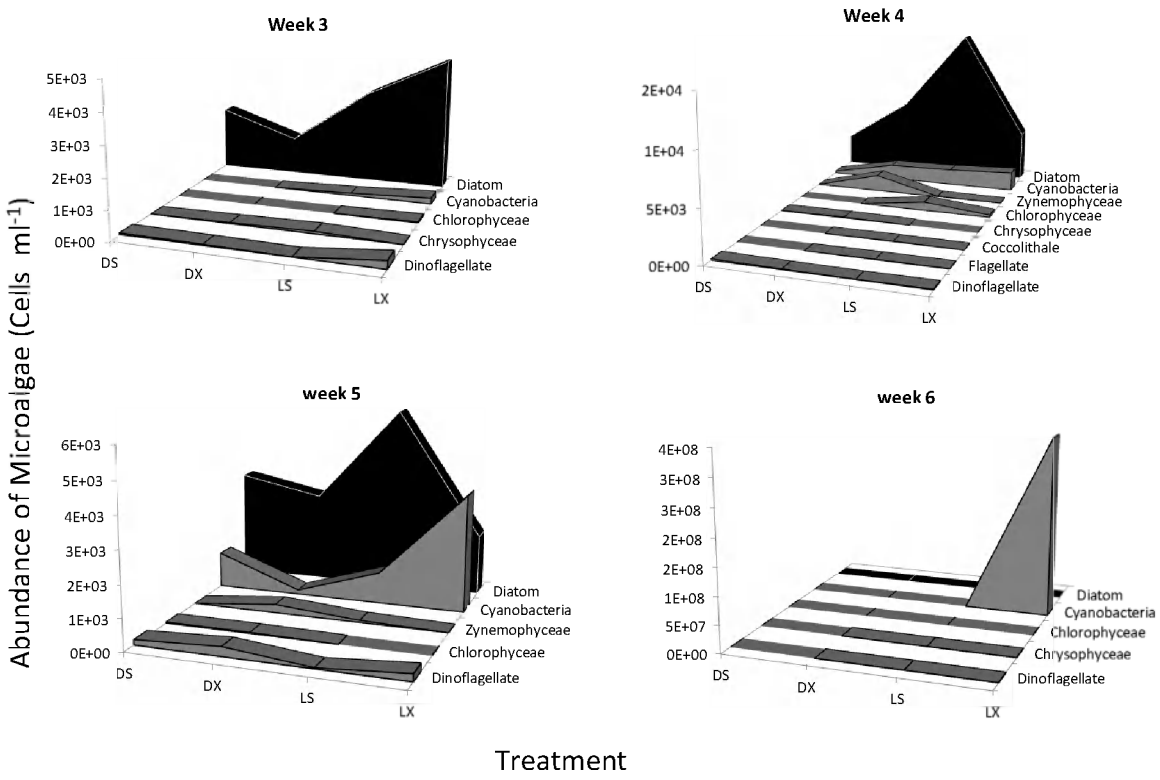


Fig 3: Abundance of different microalgae taxa colonizing biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX).

5.1.4.4 Micro-algae species diversity

Among the nine phototrophic algae classes, 23 species were identified including 6 diatom species, 5 Cyanobacteria species, 5 dinoflagellate species, 1 Zygnemopheceae species, 3 Chlorophyceae species, 1 Chrysophyceae species, 1 flagellate species and 1 coccolithale species. Among the diatom species, *Navicula* spp. and *Pleurosigma* spp. were the most predominant species. *Navicula* spp. dominated the litter decomposing in the shade with a proportion of 85% and 79% in the presence and absence of sediment, respectively. Among the Cyanobacteria, *Microcystis* spp. dominated the decomposing litter exposed to sunlight in the absence of sediment reaching a proportional cover of 99.9% during the 6th week. Cyanobacteria of *Anabaena* spp. dominated the litter incubated in the shade without sediment while *Oscillatoria* spp. was dominant on the litter incubated in the shade in the presence of the sediment. The highest micro-algae diversity was recorded in the decomposing mangrove litter exposed to the sunlight in the presence of sediment (H'=1.08), concomitantly with the highest species evenness (EH'=0.092) (Table 1).

Micro-algae taxa		DS	DX	LS	LX
Diatoms	*Nitzchia* spp.		0.04		
	Navicula spp.	84.95	78.57	65.87	
	Pleurosigma spp.	5.81	1.46	20.66	
	Coscinodiscus spp.	0.89	0.46	0.08	
	Fragilaria spp.	0.30			
	Striatella unipuctata	0.15			
Cyanobacteria	*Anabaena* spp.	0.15	5.93	3.15	
	Pseudo-anabaena spp.			0.24	
	Oscillatoria spp.	4.17	0.29	0.65	
	Microcystis spp.	0.00	2.46	6.21	99.99
	Spirulina spp.	1.49	0.72	0.40	
Dinoflagellates	*Gyrodinium* spp.			0.08	
	Protoperidinium spp.	1.34	1.32	0.08	
	Prorocentinum spp.	0.15	0.14		
	Oestropsis spp.		0.14		
	Peridinium spp.	0.15			
Flagellates	*Choanoflagelate* spp.			0.08	
Cocolithales	*Cocolitus* spp.			0.08	
Zygnemophyceae	*Cosmarium* spp.		8.04	0.24	
Chlorophyceae	*Scenedesmus* spp.		0.14		
	Dunaliela spp.	0.15		2.02	
	Colomonas spp.				
Chrysophyceae	*Dinobryon* spp.	0.30	0.29	0.16	
Diversity index (H')		0.6824	0.8953	1.0841	0.0007
Species evenness (EH')		0.0614	0.0803	0.0924	0.0000

Table 1: Proportional assemblage of microalgae in biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX).

5.1.4.5 Epifauna abundance and diversity

Eighteen major taxa were identified in the meiofauna fraction: Rotifera, Copepoda, Foraminifera, Polychaeta, Nematoda, Insecta, Cnidaria, Gastropoda, Oligochaeta, Turbellaria, Amphipoda, Mysida, Ostracoda, Bivalvia, Oikopleura, Tanaidacea, Kinorhyncha and Cumacea.

The epifauna colonizing the decomposing mangrove leaf litter differed significantly between the different treatments across the week groups especially due to the effect of the light (Two way crossed ANOSIM: R=0.426-0.704; p=0.001) (Fig. 4). Decomposing mangrove litter exposed to sunlight supported significantly higher abundance of epifauna compared to the litter incubated in the shade (Kruskal-Wallis test: $H_{(3, 48)} = 19.68$; p=0.0002). The abundance of epifauna in the shade increased after the 4th week. During the 6th week, decomposing litter exposed to the sunlight in the presence of sediment supported the highest mean abundance of the epifauna (2.2 ± 1.1 ind. cm^{-2}) compared to the litter incubated in the shade without sediment which supported a lower abundance of 1.45±0.86 ind. cm^{-2}. Litter incubated in the shade with sediment supported the lowest abundance of the epifauna (0.4 ± 0.3 ind. cm^{-2}). Further comparisons showed that the abundance of the epifauna did not differ significantly between the treatments within the similar light conditions (Kruskal-Wallis; Multiple Comparison, p > 0.05) (Fig. 5).

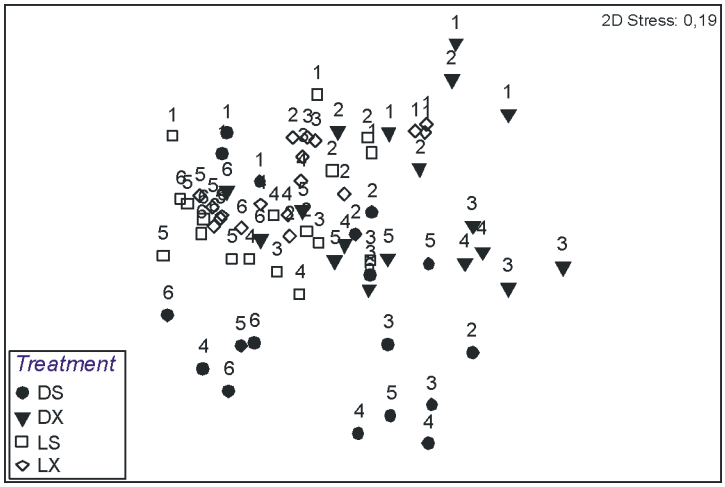


Fig 4: MDS plot of similarity (Bray-Curtis) of epifauna composition on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX). Numbers refer to the duration of litter decomposition in weeks.

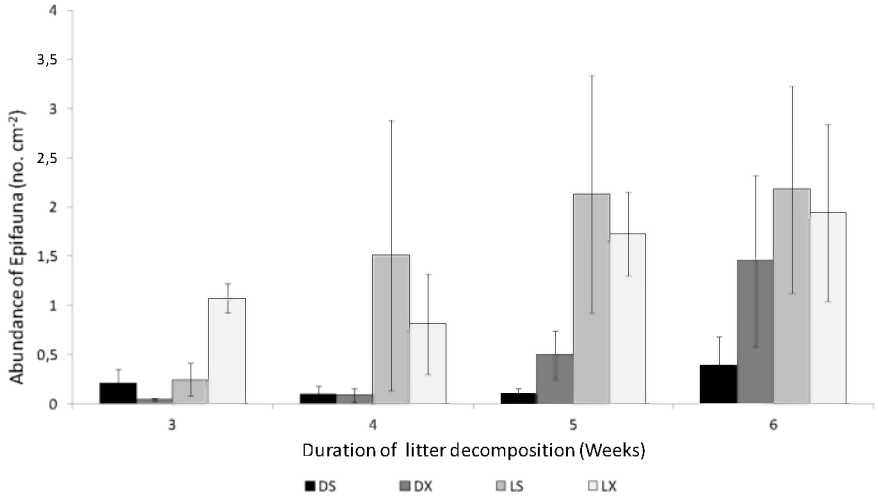


Fig 5: Abundance of epifauna in biofilm developing on the mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX).

Rotifera and Copepoda dominated the shaded mangrove litter decomposing in the absence of sediment with a proportion of 34.2% and 55% respectively whereas Polychaeta preferred conditions receiving direct sunlight. Nematoda preferred conditions with sediment. The litter decomposing in the presence of the sediment supported the highest diversity and an even assembly of the different classes of the epifauna regardless of the light conditions (Table 2).

Epifauna taxa	DS	DX	LS	LX
Rotifera	10.98	34.21	21.03	40.30
Copepoda	40.74	55.08	33.06	11.09
Foraminifera	14.79	0.30	2.43	0.23
Polychaeta	5.81	4.59	14.50	15.57
Nematoda	23.99	3.53	24.54	30.81
Insecta	0.12	0.04	0.01	0.00
Cnidaria	0.18	0.00	0.06	0.31
Gastropoda	0.18	0.30	0.47	0.41
Oligochaeta	0.12	0.04	0.00	0.00
Turbellaria	1.90	1.43	3.56	1.19
Amphipoda	0.00	0.04	0.01	0.00
Mysiidae	0.06	0.00	0.00	0.00
Ostracoda	0.25	0.19	0.28	0.09
Bivalve	0.00	0.00	0.01	0.00
Oikopleura	0.00	0.00	0.01	0.00
Tanaidacea	0.12	0.19	0.02	0.00
Kinorhyncha	0.12	0.00	0.00	0.00
Cumacea	0.72	0.08	0.00	0.00
Diversity index (H)	1.59	1.09	1.58	1.38
Species evenness (HE)	0.21	0.13	0.17	0.15

Table 2: Proportional assemblage of epifauna on biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions

of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX).

5.1.4.6 Water quality

The water in the microcosms supporting decomposing mangrove litter exposed to the sunlight had high levels of dissolved oxygen, high temperature and pH but low levels of the total ammonium nitrogen (TAN). The concentration of dissolved oxygen in the sunlight treatments ranged between 6.5±0.8 and 3.0 ± 0.5 mg l^{-1} whereas it was much lower in the shade treatments where it ranged between 2.9±0.4 and 0.2±0.1 mg l^{-1}. The higher level of dissolved oxygen in the sunlight treatment differed significantly from the levels in the shade treatments (Kruskal-Wallis test: $H_{(3, 60)}$ =39.87; $p < 0.05$) (Fig. 7a). Although there was no statistical difference in the levels of temperature (Kruskal-Wallis test: $H_{(3, 60)}$ =0.587; p =0.899) and pH (Kruskal-Wallis test: $H_{(3, 60)}$ = 6.26; p=0.0993) between the sunlight and the shade treatments, the temperature and pH was generally low in the shade treatments (Fig. 6b,c). The lowest pH level (5.4±0.1) was recorded in the shade microcosm without sediment during the 4th week of litter decomposition. In the same treatment there was an increase in TAN which reached the highest concentration of 0.0078±0.0055 mg l^{-1} during the 5th week of decomposition. However, the highest levels of TAN, 0.038±0.006 mg l^{-1}, although declined later, were recorded in the shade treatments with the sediment during the first week of litter decomposition (Fig. 6d).

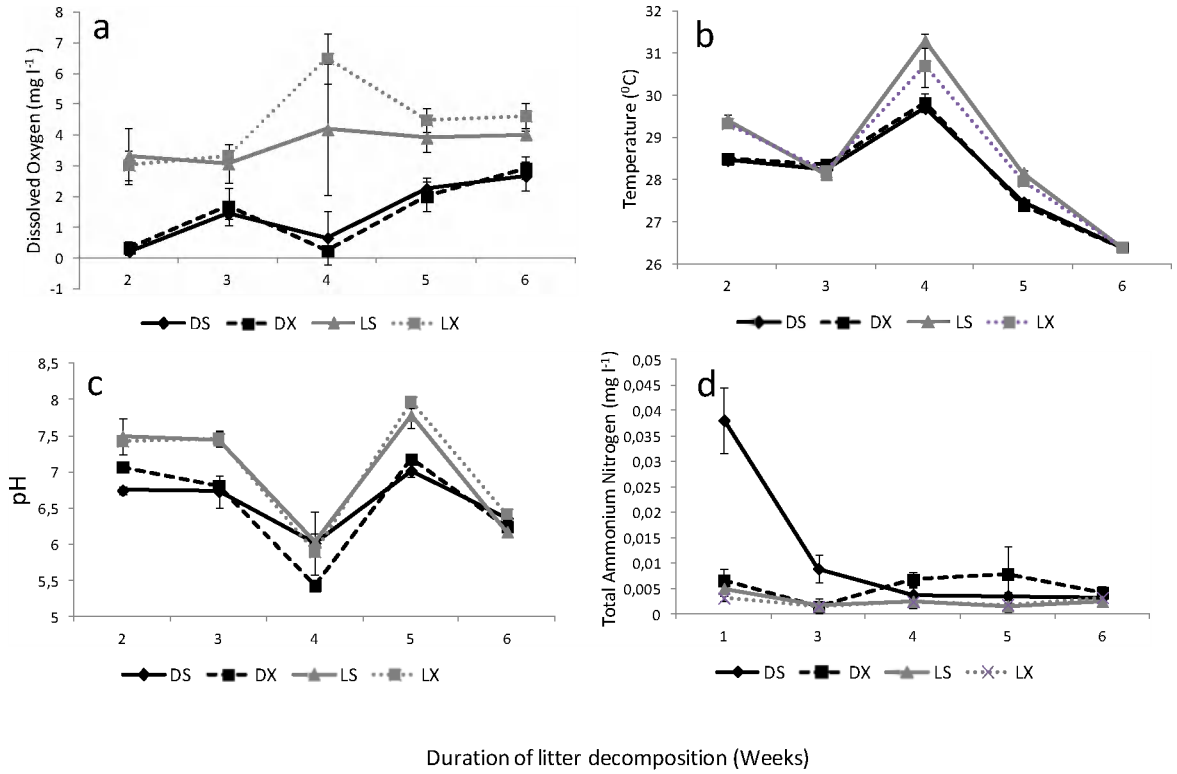


Fig 6: Water quality parameters of the seawater in the microcosms with decomposing mangrove leaf litter of *Rhizophora mucronata* at the different ambient conditions of shade with sediment (DS), shade without sediment (DX), sunlight with sediment (LS) and sunlight without sediment (LX).

5.1.5 Discussion

5.1.5.1 Litter degradation

The ecological approach of shrimp aquaculture in mangrove forest implies that mangrove leaf litter falls into the shrimp pond, and plays a potentially important function in the ecological processes within the pond. Various studies on the ecological function of the decomposing mangrove litter in both mangrove integrated shrimp aquaculture (Fitzgerald Jr., 2000; Schneider et al., 2005) and the natural estuarine ecosystems (Bosire et al., 2005; Loneragan et al., 1997; Schwamborn et al., 2002) have focused on the chemical aspects. It is important to recognise that mangrove litter falling into the integrated shrimp ponds have different destination zones of light intensity depending on their direct exposure to sunlight. This exposure to sunlight depends on the location, for instance depth, of the litter in the pond water column and will vary as the litter decomposes and becomes denser.

The exposure of the decomposing mangrove leaf litter to the sunlight will also depend on the extent of the shading of the shrimp pond by the mangrove trees. In our present study we

observed that mangrove leaf litter exposed to the sunlight were more degraded than the litter decomposing in the shade. Various studies have observed that sunlight enhances the rate of degradation of the plant leaf litter either directly through photo-degradation (Gallo et al., 2009) or indirectly mediated by associated macrobiota (Franken et al., 2005) and microbiota (Francoeur et al., 2006). Gallo et al. (2009) observed higher degradation of plant litter exposed to the ambient sunlight regardless of the water treatments and the microbial extracellular enzyme activities of the fungal communities. The study by Gallo et al.(2009) observed that the principal driver of the degradation was the photo-degradation of cellulose, poly-phenols and polysaccharides andtherefore concluded that photochemical oxidation can supplement enzymatic oxidation and increase the decomposition rates of plant litter.

In our study, the litter exposed to the sunlight was further characterised by a higher abundance of microalgae and epifauna compared to the litter incubated in the shade. Our study did not focus on the microbial abundance but instead concentrated on the micro-algae assembly and abundance. Francoeur et al.(2006) observed that the extracellular enzyme activity in microbial communities colonizing natural organic substrata is stimulated by light and photosynthesis, which is common in the microbial communities associated with the natural decaying plant litter in wetlands. By stimulating the microbial activities, exposure to sunlight is therefore an important physical parameter influencing the microbial mediated decomposition of mangrove leaf litter in the ecological shrimp ponds. The effect of sunlight to the abundance and activities of the epifauna in the litter decomposition is rather an indirect process as explained by Franken et al. (2005). The light intensity first influences the algal biomass, density and composition which in turn positively influence the growth of the epifauna which are efficient shredders of the decomposing leaf litter.

The higher degradation of the mangrove leaf litter in the presence of sediment, compared to the litter without sediment, may suggest the additional importance of sediment and the associated organisms in enhancing the decomposition of the submerged mangrove leaf litter. According to Avnimelech and Gad (2003), the concentrations of nutrients including the organic carbon compounds in the shrimp pond bottom soil are higher than those found in the water column. Hence the bottom becomes the favourable site for microbial development due to the availability of organic matter. In this study the sediment was not sterilized. The rapid degradation of the mangrove leaf litter incubated with sediment may also having been due to the higher microbial load contributed by the sediment. Although the sediment grain size was

not measured, the sediment used in the study was obtained from the shrimp pond bottom which is characterized by small grain size (Burford et al., 1998). The smaller sediment grain size may have also influenced a high microbial load (Burford et al., 1998). Mangrove leaf litter exposed to the sunlight in the absence of sediment seems to have degraded slowly, defying the potential photodegradation influences of the sunlight. The possible influence of the predominant cyanobacteria on the slow degradation cannot be connected to a possible disruption of the shredding effect of the epifauna. This is because the abundance of the epifauna community was similar to that on the fast decomposing mangrove leaf litter in the presence of sediment. Further analysis is required to assess the bio-chemical interaction of the cyanobacteria with the various decomposition processes of mangrove leaf litter in the absence of sediment.

5.1.5.2 Effect of direct sunlight on the natural food supply

The exposure of pond water to sunlight is crucial in promoting the primary production which is an important pathway in the transfer of energy to the upper trophic levels in the pond food web. The relevance of this pathway would depend on the nutritional value of the primary producer to the immediate (primary) or secondary consumers. In our present study, decomposing mangrove litter exposed to the sunlight supported the largest biomass of the micro-algae compared to the litter decomposing in the shade. This observation is an expected scenario since sunlight is important in primary productivity. The importance of sunlight in promoting the supply of natural food of relevance to the shrimp post larvae in ecological shrimp ponds seems to make ecological sense, however only in the presence of sediment. Mangrove leaf litter decomposing in the presence of sediment supported a higher diversity of both micro-algae and epifauna. This would imply a possibility of a stable micro-environment. In the absence of sediment, the growth of the micro-algae is bent on a single species which is normally an indicator of an unstable environmental condition. Sediment therefore seems to have a buffering effect on the pond food web and seems important in maintaining the ecological stability of the shrimp pond habitat.

Litter decomposing in the sunlight further supported the presence of polychaetes which are potential natural food for penaeid shrimp post larvae (Nunes and Parsons, 2000). Leaf litter decomposing in the presence of sediment is observed to support diatoms of *Navicula* and *Pleurosigma* species which are of nutritional importance to the ecological shrimp aquaculture (Abu Hena and Hishamuddin, 2012; Bombeo-Tuburan et al., 1993). Litter decomposing in the

absence of sediment supported only cyanobacteria which was dominated by *Microcystis* spp. *Microcystis* is known to produce a potent hepatotoxin, a microcystin that is also produced by a number of planktonic cyanobacteria genera such as *Anabeana*, *Anabaenopsis*, *Nostoc* and *Planktothrix (Oscillatoria)*(Amado and Monserrat, 2010) and other bioactive metabolites with a potential to degrade the nutritional status of aquaculture species (inhibitors of proteases and grazer deterrents)(Schrader et al., 1998a; Smith et al., 2008).

Diatoms, especially of *Navicula* spp., continued to grow in the shade, although in lower abundance compared to the sunlight conditions, and could be the reason behind the continued growth of copepods on the litter decomposing in the shade. Diatoms of *Navicula* and *Nitzschia* spp. are capable of growing in the shade since they can adopt a heterotrophic mode of feeding (Admiraal and Peletier, 1979; Lewin, 1953). The cyanobacteria species of *Anabaena* and *Oscillatoria* were also found to thrive on the litter decomposing in the shade. The filamentous nitrogen-fixing cyanobacterium *Anabaena variabilis* is capable of heterotrophic growth in complete darkness (Mannan and Pakrasi, 1993). Nevertheless, our present study revealed reduced abundance of cyanobacteria on the litter decomposing in the presence of sediment. This observation elucidates the importance of coupling sediment and sunlight in enabling the decomposing mangrove litter to support the growth of quality food micro-algae in the ecological shrimp pond. The application of substrates to promote the growth of biofilm in the shrimp culture microcosms without sediment should therefore be approached with caution since the low food quality cyanobacteria may dominate. The sediment play an important role in providing a whole range of nutrients required to support growth of different types of micro-algae (Avnimelech and Gad, 2003). The concentrations of nutrients in sediment is higher than those recorded in pond water (Avnimelech and Gad, 2003). Sediment that accumulated in shrimp ponds is typically highly enriched in nitrogen and phosphorous (Boyd et al., 1994) which are important nutrients in supporting the proliferation of micro-algae. Although light limits the growth of micro-algae, micro-algae communities have also been found to vary with changing nitrogen to phosphorous ratios (Burford, 1997). Sediment acts as a nutrient sink whereby it buffers the fluctuation of nutrients in the water column by accumulating carbon, nitrogen and phosphorous (Avnimelech and Gad, 2003). In the absence of sediment the remineralized nutrients from the decomposing mangrove leaf litter may increase in the water column. Case et al. (2008) observed that enhanced nutrient input affected plankton density and composition in shrimp pond whereby diatom dominance was replaced by cyanobacteria as nutrient concentrations

increased. In the present study, the dominating cyanobacteria in the sunlight litter decomposed for 6 weeks, in the absence of sediment, may have partially been triggered by increased nutrients leached from the decomposed litter such as the ammonified particulate and dissolved organic nitrogen (Alongi et al., 2000) and phosphorus (Lin and Sternberg, 2007) .

5.1.5.3 Effect of sunlight on water quality

Sunlight is capable to influence the dynamics of various water quality parameters and therefore plays an important role in determining the ecological health of a shrimp pond in the mangrove forest. For instance, in the present study, microcosms supporting decomposing litter in the shade were low in dissolved oxygen compared to those exposed to the sunlight. Sunlight promotes photosynthesis whereby carbon dioxide is absorbed and oxygen is released as a result (Dawes, 1998). This process can oxygenate the sediment at the pond bottom (Avnimelech and Gad, 2003). According to Avnimelech and Gad (2003), algae produce oxygen during the day leading to oxygen enrichment of the water column especially the top layer. The author adds that although the diffusion of oxygen to the sediment is slow, wind and mechanically driven water currents mix the water column and bring some of the oxygen to the bottom layers. The importance of micro-algae in promoting oxygen in water depends on the type of algae. Cyanobacteria are not a preferred food by epifauna and shrimp (Preston et al., 1998). They are therefore likely to bloom in the biofilm and then collapse. During decomposition by bacteria, they would consume a lot of dissolved oxygen from the water. On the other hand, diatoms are preferred food for epifauna and shrimp (Borowitzka, 1997; Brown and Jeffrey, 1995; De Troch et al., 2010). Diatoms would therefore not tend to bloom in the biofilm if the grazing by the epifauna is adequate. Epifauna and shrimp feeding on the diatoms would consume less oxygen than bacteria resulting into a positive oxygen balance. When cyanobacteria die they float and form a layer on the water surface and thus cut off sunlight to the pond water reducing the amount of photosynthesis in the water. Dead diatoms would remain attached to the litter or sink (Smetacek, 1985) to the bottom of the pond. The water above would remain clear and exposed to sunlight, allowing photosynthesis to continue. In the presence of sediment, diatoms would therefore contribute as the net producers of oxygen and as potential regulators of water quality whereas cyanobacteria are not.

In the absence of adequate sunlight, the bottom sediment and the decomposing mangrove litter provides large amounts of organic matter which are favorable conditions for microbial development (Avnimelech and Gad, 2003). Bacteria consume large amounts of oxygen

(Avnimelech and Gad, 2003) and since there is no oxygen production due to the absence of photosynthetic activity, the water and the sediment become anoxic. In our study, the litter decomposing in the shade was characterized by relatively higher total ammonium nitrogen (TAN) and low dissolved oxygen levels. According to Reddy et al. (1986) when oxygen is depleted denitrification occurs with the nitrate as an electron acceptor. Due to microbial metabolism of nitrogenous compounds, an increase of ammonium in the water can be expected (Chien, 1992). Another pathway of ammonium accumulation is when sulphate is used as an electron acceptor and sulfide is released (Chien, 1992). The released sulfide inhibits nitrification (Joye and Hallibaugh, 1995). These reactions could have largely contributed to the increased levels of TAN in the microcosms with mangrove leaf litter decomposing in the shade.

5.1.5.4 Potential toxicity to shrimp post larvae

The water quality in the shaded microcosms has the potential to build a toxic environment for macro-invertebrates including shrimp post larvae. When oxygen is depleted, other terminal electron acceptors such as nitrate, iron, manganese, sulphate and CO_2 can be used to mediate microbial decomposition of organic matter (Reddy et al., 1986). This could lead to the production of reduced and potentially toxic compounds such as nitrite, unionized ammonia, reduced divalent manganese, hydrogen sulfide, organic acids and methane (Avnimelech and Gad, 2003; Boyd, 1998; Chien, 1992; Nix and Ingols, 1981). Another important reaction under anaerobic conditions, is the fermentation of organic substrates by fermenting bacteria releasing reduced acids, alcohols, carbon dioxide and hydrogen which produce offensive odor and some are toxic to shrimp (Moriarty, 1997). The potential to produce reduced acids, carbon dioxide, hydrogen sulphides and hydrogen could have contributed to the reduced levels of pH in the microcosms with mangrove litter decomposing in the shade.

Litter decomposing in the shade was characterized by pH values as low as 5.4. Low pH below 6.0 can stress shrimp, cause limited calcification and poor survival (Kater et al., 2006). It increases nitrite toxicity to fish and have similar effects on shrimp (Kater et al., 2006). The combined toxicity of both TAN (NH_4-N) and Ammonia-N (NH_3-N) occurs at pH<8.3 (Kater et al., 2006). Unionised ammonia (NH_3) is the more toxic form of ammonia to penaeid shrimp post larvae and occurs at elevated temperature and pH>8.3 (Chien, 1992). The safe level of unionized ammonia for *Penaeus monodon* post larvae PL 6 to 25 is 0.1 mg l^{-1} (Chien, 1992) or 1.11 mg l^{-1} TAN at a pH of 8.2, temperature level of 29.5 and salinity level of 34

(Spotte and Adams, 1983). In our experiment, the levels of TAN were lower than the allowable upper limit. However the higher TAN levels observed in the shaded treatments suggest an increased potential of TAN toxicity if the incidence of sunlight is limited. Low pH levels as observed in the shaded microcosms can increase the fraction of unionized hydrogen sulfide (H_2S) which is toxic to shrimp post larvae (Chien, 1992).

The above observations demonstrate the importance of exposing the decomposing mangrove litter to the sunlight. Caution should therefore be observed while using mangrove leaf litter in the ecological shrimp ponds which are shaded by mangrove trees. The use of multiple layers of leaf litter, shading each other, should also be avoided. Some observations have been made indicating that a moderate load, not exceeding 1g l^{-1}, of mangrove litter could play an important role in promoting shrimp growth and survival in aerobic conditions (Hai and Yakupitiyage, 2005). In this respect, our study has specifically demonstrated the additional advantage of the sunlight and sediment in enhancing proliferation of quality natural food from the mangrove leaf litter.

5.1.6 Conclusion

1. There is a synergistic effect between sediment and sunlight in maintaining a high diversity of microalgae and water quality which is favorable for the culture of shrimp post larvae.
2. In the presence of sunlight, lack of sediment enhances the growth of cyanobacteria of the *Microcystis* spp. on mangrove litter decomposed for a period longer than 5 weeks.
3. Cyanobacteria of the *Anabaena* spp. and *Oscillatoria* spp. colonize mangrove litter decomposing in the shade.
4. Shading of decomposing mangrove litter inhibits the growth of polychaetes which is a potential food for shrimp post larvae.

The study therefore recommends adopting the following management practices in the integrated mangrove and shrimp aquaculture;

1. Locate ecological shrimp ponds in less forested areas to promote primary production.
2. Aerate bottom layers of the shaded shrimp ponds receiving litter fall.
3. Control mangrove litter fall probably by reducing the load of litter input.
4. Include sediment in the shrimp culture microcosm using mangrove leaf litter substrates to prevent cyanobacteria blooms.

5.1.7 Acknowledgements

The first author thanks the Flemish InterUniversity Council (VLIR) for the financial support to follow an international PhD program in Biology at Ghent University, Belgium. The present study is part of a PhD project in the *FEEDING ECOLOGY OF SHRIMP IN TROPICAL MANGROVE SYSTEMS OF KENYA: The nutritive value of detritus and its use in biological shrimp aquaculture*. The last author is a postdoctoral fellow financed by the Special Research Fund of the Ghent University (GOA project 01GA1911W). Deep felt thanks go to Dr. Mahika, Director of Aquaculture Tanzania; Mr.Vageri and Ari of Alphakrust, Tanzania, for facilitating export and donating the test shrimp post larvae. KMFRI laboratory technicians James Kilonzi, Eric Magara, Mary Mkonu, George Onduso, Mary Adungosi, Douglas Okemwa, Ben Ogolaand the youth and community members of the Majaoni silvofishery project, Kenya, for their field assistance. The last author acknowledges the Special Research Fund of the Ghent University (BOF-GOA 01GA1911W).

6 Chapter 6

6.1 Overall performance of *Penaeus monodon* shrimp post larvae feeding on decomposing mangrove litter as evidenced by fatty acid biomarkers

Charles Gatune [a,c]*, Ann Vanreusel[a], Renison Ruwa[b], Peter Bossier[d] and Marleen De Troch[a]

[a] Ghent University, Biology Department, Marine Biology, Campus Sterre, Krijgslaan 281-S8, B-9000, Gent, Belgium.
[b] Kenya Marine and Fisheries Research Institute, P.O Box 80100, 81651, Mombasa, Kenya.
[c] Ministry of Fisheries Development, P.O. Box 90423 – 80100, Mombasa, Kenya.
[d] Ghent University, Faculty of Bioscience Engineering, Laboratory of Aquaculture and Artemia Reference Centre, Rozier 44, B-9000, Gent, Belgium.

*Corresponding Author: Email address: kgatune@yahoo.com

The set up of the controlled shrimp feeding experiment in the laboratory tanks at the Kenya Marine and Fisheries research Institute (Photo courtesy Gatune C. 2010)

Submitted as:
Gatune, C., Vanreusel, A., C., Ruwa, R., Bossier, P., De troch, M. Overall performance of *Penaeus monodon* shrimp post larvae feeding on decomposing mangrove litter as evidenced by fatty acid biomarkers. Aquaculture.

6.1.1 Abstract

Ecological shrimp ponds integrated in mangrove forests are an important point of interception of mangrove leaf litter fall. The importance of the leaf litter input to the shrimp pond ecology would largely depend on the rate of the accumulated nitrogen from micro-biota and the availability of essential fatty acids. In this study shrimp post larvae were fed with mangrove leaf litter and the associated biofilm, under laboratory conditions, at three nutritionally important stages of decomposition (1, 6 and 10 weeks). The growth performance of shrimp was assessed by means of fatty acids as trophic markers. Biofilm on decomposing mangrove litter was found to be a source of bacterial fatty acids during the 6th week. Overall, shrimp post larvae feeding on the mangrove litter derived food were characterised by higher levels of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), arachidonic acid-ARA (20:4ω6) and eicosapentanoic acid-EPA (20:5ω3) than shrimps feeding on the compound feed. However, mangrove litter derived food suppressed the growth and survival of shrimp post larvae due to the unavailability of DHA and linoleic acid (18:2ω6).

Key words: mangrove; leaf litter; biofilm; shrimp; fatty acids; growth

6.1.2 Introduction

Ecological shrimp aquaculture involves integrating shrimp pond in between mangrove trees (Fitzgerald Jr., 2000). Shrimp ponds would therefore become major intercepting areas for the mangrove litter fall. Consequently, mangrove leaf litter has a potential major role in driving the food web in the shrimp ponds. Following the litter fall the mangrove litter is utilized by the macrofauna, which also includes shrimp post larvae, after it has undergone considerable modification by bacteria and fungi (Wilson, 2002). During this process a combination of bacteria, micro-algae, epifauna and attracted organic matter would form a so-called biofilm (Thompson et al., 2002). This leaf-associated biofilm would largely influence the supply of natural food to shrimp post larvae (Abreu et al., 2007; Azim and Wahab, 2005b).

The availability of nutrients to the shrimp pond food web from the mangrove litter is variable. For instance the palatability of the litter may depend on the accumulated microbial nitrogen (Tremblay and Benner, 2006), the concentration of tannin which is a feeding deterrent (Lee and Meziane, 2006) and the availability of fatty acids (FA) (Mfilinge et al., 2003; Tsuchiya et al., 2005). The highly unsaturated fatty acids (PUFA) are known to be essential for the growth of shrimp post larvae (Kanazawa et al., 1977; Sorgeloos and Lavens, 2000; Wouters et al., 2001). During decomposition of mangrove litter, total lipids and FA concentrations are changed either in terms of decreasing abundance (Tsuchiya et al., 2005) or nutritional quality such as the increase in monounsaturated fatty acids (MUFA) and bacterial FA (Mfilinge et al., 2003).

Although shrimp have been often observed to rework detrital material and use flocculant material, such as bioflocs(Crab et al., 2010), the use of biofilms per se are understudied so far. The periphytic biofilm developing on decomposing mangrove leaf litter may enhance the nutrition quality of decomposing mangrove litter to the penaeid shrimp post larvae. Detailed knowledge on the contribution of biofilm to the transfer of energy from the mangrove detritus to shrimp is pivotal for integrating shrimp culture into the mangrove ecosystems.. Few studies attempted to investigate this approach and found that the post larvae of *Penaeus monodon* performed poorly in growth when they were fed the pure periphyton (biofilm) compared to when they were fed in the combined state with mangrove litter (Nga et al., 2004). However, temporal quality changes in the nutrition composition of the periphyton, such as the proteins and FA, were not taken into account.

Biomarkers such as stable isotopes and FA are up-to-date tools that can elucidate the nutritional importance of decomposing mangrove litter and biofilm. These methods can explain the preferential assimilation of natural food sources into the shrimp tissue. Based on carbon isotope ratios Primavera (1996) observed that the penaeid shrimp *P. monodon* preferred phytoplankton and epiphytic algae over mangrove detritus. The latter case-study dealt only with microalgae and did not consider the nutritional potential of the associated epifauna.

In the present study we attempt to use carbon/nitrogen ratio, protein levels and FA profiling to assess the contribution of the mangrove leaf litter and the associated biofilm at different stages of decomposition to the growth and physiological performance of shrimp postlarvae. The following timelines were considered because of their biotic and nutrients characteristics. 1 week decomposed litter when there is low microbiota abundance and low levels of unsaturated FA (Gatune et al., 2012; Mfilinge et al., 2003). Between 4 and 6 weeks when the decomposed litter has high microbiota abundance and high levels of unsaturated FA (Alikunhi et al., 2010; Gatune et al., 2012) high bacteria immobilized nitrogen, high leaching of nitrogen, amino sugars and amino acids (Tremblay and Benner, 2006; Woitchik et al., 1997). Above 70 days when there is 94.7% loss of tannins (Rajendran and Kathiresan, 2000a). Shrimp provided with mangrove litter and biofilm derived food sources were compared to shrimp post larvae fed a shrimp compound feed with known optimal nutritional quality. The endpoints to evaluate shrimp performance were growth and survival. The parameters were linked to the biochemical composition of shrimp in terms of FA as trophic markers.

6.1.3 Materials and methods

6.1.3.1 Biofilm nutrient analysis

Senescent mangrove leaves (hereafter referred to as mangrove leaf litter) which had just turned yellow-brown and dropped from the trees were dried in the shade to a constant weight and incubated hanging in a shrimp pond for a period of 10 weeks. Biofilm was sampled at 3 litter decomposition periods of 1, 6 and 10 weeks. Biofilm on mangrove litter was scraped off and gently washed with distilled water, oven dried at 70^{o}C for 48 hrs and weighed to get its total biomass. The dried biofilm was treated for nutrient analysis by weighing sub samples which were then placed in silver capsules and analysed for carbon and nitrogen content using a Flash 2000 Organic Elemental Analyser (Thermo Scientific, Italy).

6.1.3.2 Shrimp feeding experiment Standard test organism

Shrimp postlarvae (PL) of *Penaeus monodon*, PL 15 hatched from the same brooder (cohort) were obtained from a shrimp hatchery situated at Mbengani Fisheries Development Centre in Bagamoyo, Tanzania. They were transported packed in oxygen for duration not exceeding 6 hours and acclimatized to local conditions for a period of 7 days before the experiment. During the acclimatization period the shrimp postlarvae were fed on a pelleted shrimp larvae compound feed (CP) imported from India (Higashimaru zoea to PL 20 feed; crude protein over 52% (see http://www.aquafeed.com/documents/1254938830_1.pdf).

6.1.3.3 Feed preparation

Leaf litter from mangrove *Rhizophora mucronata* were dried under shade for 7 days and then after, continuously submerged in pond sea water.. The incubated leaf litter and the associated biofilm was removed at different periods of decomposition (1, 6, 10 weeks). Loose biofilm was obtained from a parallel batch of decomposed leaf litter by scraping the periphyton layer. Different combinations of the leaf litter and loose biofilm was then dried at 70°C for 48 hours then ground into a fine powder. The powder was then mixed with 1% cellulose binder and 3% attractant premix (Chen, 1993). The mixture was moistened and extruded into 0.01-1mm pellets. This size is comparable to the size of the mouth of the shrimp PL 15-25 (Bailey-Brock and Moss, 1992) and detritus with a similar size have been found in gut content of post larvae shrimp (Bombeo et al., 1993). The processed pelleted feed was then stored at -18°C until the time of feeding.

6.1.3.4 Experimental design

Shrimp postlarvae (PL) of *Penaeus monodon*, PL 15-25 were starved for 24 hours then stocked into laboratory tanks (70 l) in triplicate at a density not exceeding 2 PL l^{-1} and treated with the following food types: 1) 1 week pelleted decomposed mangrove leaf litter with biofilm (BL1); 2) 6 week pelleted decomposed mangrove leaf litter with biofilm (BL6); 3) 10 weeks pelleted decomposed mangrove leaf litter with biofilm (BL10); 4) 6 weeks pelleted biofilm (B6); 5) 10 weeks pelleted biofilm (B10); 6) compound feed (CP); 7) no food, as control (NF). The feeding with leaf litter derived food was adjusted to ensure maintaining a leaf litter density not exceeding 1 g l^{-1} (Hai and Yakupitiyage, 2005) by observing a feeding rate of 20% of dry body weight per day and removal of remnant food and feacal pellets every day in the morning before feeding. However with CP feed a feeding rate was adjusted to 10% dry body weight. Food items were offered twice a day (morning and afternoon) and doubled every 3.5 days. The experiment was performed for a period of 15 days.

6.1.3.5 Shrimp growth and survival

Shrimp post larvae were sampled weekly for specific growth rate (SGR %) using dry weight recordings after drying in the oven at 70°C for 48 hours. At the end of experiment the percentage survival (SR %) was estimated.

The growth and survival indices were calculated using the following formula (Busacker et al., 1990),

$$SR\% = \frac{N_t}{N_0} * 100$$

$$SGR\% = \frac{\ln(BW_t) - \ln(BW_0)}{T} * 100$$

Where: SR is the survival (in %); N_t is the number of shrimp collected at sampling time t; N_o is the number of shrimp initially stocked; SGR is the specific growth rate (% $BW_{day\text{-}1}$); BW_t being the final body weight (g); BW_0 is the initial body weight (g); and T is duration of the experiment (days).

6.1.3.6 FA extraction and analysis

Samples from the shrimp tail tissue, pelleted mangrove litter and biofilm were stored at -80^{o}C. To extract the FA the frozen samples werefreeze-dried (lyophylised) andweighed. TheFA was then extracted and methylated to FA methyl esters (FAMEs) by a modified one-step derivatisation method after Abdulkadir and Tsuchiya (2008). The FA 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 artefacts 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^{o}C, 240mbar, then dissolved into 1 ml hexane and analysed using a Hewlet 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 (60m x 0.25mm internal diameter x 0.20µm 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), and analysed 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).

FA biomarkers that yield information on the physiological performance of shrimp post larvae were identified (Kanazawa et al., 1977; Kanazawa et al., 1979; Kanazawa et al., 1978; Sorgeloos and Lavens, 2000): polyunsaturated fatty acid-PUFA (linoleic acid: 18:2ω6, linolenic acid: 18:3ω3, arachidonic acid: 20:4ω6) and highly unsaturated fatty acids-HUFA (eicosapentanoic acid-EPA: 20:5ω3, docosahexanoic acid-DHA: 22:6ω3).

6.1.3.7 Crude protein analysis

Kjeldahl technique is the method which was used to analyse the percentage protein content of the various test feeds.

6.1.3.8 Water quality parameters

Water quality in the experimental units was monitored by weekly measurements of temperature, dissolved oxygen, pH, salinity and total ammonium nitrogen. Temperature, dissolved oxygen, pH were measured using meters, salinity was measured using a refractometer whereas total ammonium nitrogen was analysed in the laboratory according to Eaton et al.(2005).

6.1.3.9 Data analysis

One way ANOVA was used to compare the abundance of the fatty acid biomarkers in the tail tissue of shrimp fed different feed types derived from decomposing mangrove litter and biofilm at different periods of development, a commercial feed (CP) and shrimp which had not received any feed and thus acted as control. One way ANOVA was also used to compare specific growth rate and survival of shrimp fed different feed types. The statistical analyses were conducted with Statistica 7.0 software. All data were checked for normality and variance homogeneity requirements for parametric analyses. Data which did not meet normality requirements after being transformed were analysed non-parametrically following Kruskal-Wallis and Median Test. Multidimensional scaling (MDS) and analysis of similarity (ANOSIM) was used to compare similarity (Bray-Curtis) between the absolute fatty acid (FA) compositions of shrimp tissue from the different diet treatments. Primer 6.0 software was used for the MDS and ANOSIM (Plymouth Marine Laboratory; Clarke and Gorley 2006).

6.1.4 Results

6.1.4.1 Feed nutrient composition

C/N ratio in biofilm declined from 21.8 ± 0.76 to 6.5 ± 0.65 in the leaf litter decomposed for a period of 1 and 10 weeks, respectively (Table 1). Microalgae biomass increased over the entire period of biofilm development. The proportion of crude protein increased in the decomposing leaf litter in the range of 6.06 ± 0.28% to 7.69 ± 0.62% over the entire 10 weeks and was highest in the biofilm on the leaf litter decomposed for 6 weeks (crude protein 15.10 ± 0.71%) (Table 1). Contrary to crude protein, relative total lipids concentration decreased in the decomposing leaf litter from 7.2 to 3.76 % over the 10 weeks of decomposition. Parallel, in the biofilm the total lipid level declined from 0.85 to 0.63% between weeks 6 and 10. Commercial shrimp feed (CP) recorded highest levels of crude protein and total lipids of 62.74 ± 1.46% and 10.89%, respectively (Table 1).

	N %	TOC %	C/N	Chla (mg l^{-1})	Crude protein %	Lipid %	Ash %
BL1					6.06 ± 0.28	7.2	24.84 ± 0.04
BL6					6.79 ± 0.18	5.68	37.98 ± 0.1
BL10					7.69 ± 0.62	3.76	42.65 ± 0.06
B1	0.2 ± 0.06	3.7 ± 1.36	21.8 ± 0.76	0.03 ± 0.01			
B6	0.5 ± 0.0	6.9 ± 0.62	13.8 ± 1.25	1.4 ± 0.1	15.10 ± 0.71	0.85	70.16 ± 0.12
B10	1 ± 0.1	6.4 ± 0.21	6.5 ± 0.65	1.6 ± 0.4	7.24 ± 0.30	0.63	74.83 ± 0.02
CP					62.74 ± 1.46	10.89	18.12 ± 0.02

Table 1: Composition of the decomposing mangrove leaf litter and biofilm derived feed at different periods of litter decomposition (1, 6 and 10 weeks). Values are calculated on dry weight base. BL: litter with the associated biofilm, B: biofilm and CP: compound feed.

6.1.4.2 Fatty acids (FA)

6.1.4.2.1 Feed

The overall proportion of the total lipids decreased in the more decomposed mangrove litter and the associated biofilm (Table 1). In mangrove litter and biofilm, 6 FA contributed >5% to the total FA composition. These included C16:0, C16:1ω7 (most abundant in 10 weeks old biofilm), C18:0 (Abundant only in 6 weeks decomposed litter and the associated biofilm), C18:1ω9, C18:2ω6 and C18:3ω3 (abundant only in the least decomposed litter). In the compound feed, the FA C16:0, C18:1ω9 and C18:2ω6 were comparatively present in proportions >5%. Highly unsaturated fatty acid-HUFA (EPA: 20:5ω3 and DHA: 22:6ω3) were the most abundant. The compound feed was therefore low in specific FA which was

abundant in the mangrove litter and the associated biofilm, at certain stages of decomposition. These were C18:0, C16:1ω7 and C18:3ω3 (Table 2).

Feed	BL1	BL6	BL10	B6	B10	CP	Shrimp	BL1	BL6	BL10	B6	B10	NF	CP
15:0	0.5	0.7	0.7	0.7	1.0	0.5	15:0	0.0	0.6	0.4	1.5	0.3	0.4	0.2
16:0	42.6	41.3	33.3	42.8	35.4	18.8	16:0	15.0	13.2	13.3	12.3	12.3	13.0	13.2
16:1ω7	0.9	1.9	6.3	2.7	14.5	3.2	16:1ω9	1.5	1.6	2.1	1.7	2.1	1.7	1.0
17:0	1.7	1.7	1.3	1.6	0.5	0.6	17:0	0.2	0.2	0.1	0.2	0.1	0.1	0.1
18:0	4.9	5.7	4.9	5.5	2.6	4.8	18:0	13.4	12.7	11.6	10.9	10.5	11.3	9.1
18:1ω9	9.8	9.3	11.6	10.1	12.5	12.2	18:1ω9	16.1	15.1	16.0	14.7	15.0	15.9	12.3
18:2ω6 (Linoleic)	6.7	4.2	5.8	6.9	3.7	15.5	18:2ω6	7.3	5.9	5.5	5.9	5.4	8.4	10.2
18:3ω3 (Linolenic)	5.3	2.3	2.4	4.4	1.2	2.4	18:3ω3	-	-	-	-	-	-	-
20:4ω6 (ARA)	0.0	0.1	0.5	0.3	0.8	0.9	20:4ω6	9.2	9.6	9.5	9.3	8.7	8.5	5.6
20:5ω3 (EPA)	1.0	1.0	1.1	0.3	1.5	5.6	20:5ω3	20.6	21.5	21.9	21.4	21.7	21.5	16.6
22:6ω3 (DHA)	0.0	0.1	0.6	0.3	0.5	11.3	22:6ω3	13.0	15.7	16.5	18.8	20.8	15.1	29.5
ΣSAFA	*58.2*	*57.7*	*49.7*	*58.7*	*47.6*	*29.5*	*ΣSAFA*	*32.4*	*30.6*	*28.5*	*28.2*	*26.3*	*28.8*	*24.9*
ΣMUFA	*13.6*	*16.4*	*23.3*	*18.1*	*32.2*	*19.6*	*ΣMUFA*	*17.6*	*16.7*	*18.1*	*16.4*	*17.0*	*17.7*	*13.3*
ΣPUFA	*12.3*	*6.9*	*8.9*	*11.7*	*5.8*	*18.7*	*ΣPUFA*	*16.5*	*15.5*	*15.0*	*15.2*	*14.1*	*16.9*	*15.8*
ΣHUFA	*1.0*	*1.1*	*1.7*	*0.6*	*1.9*	*16.9*	*ΣHUFA*	*33.5*	*37.2*	*38.4*	*40.2*	*42.6*	*36.6*	*46.0*

Table 2: Proportion of fatty acids (%) in the feed derived from decomposing mangrove leaf litter and biofilm and in the shrimp tissue fed the different feed types; BL: leaf litter with the associated biofilm; B: biofilm and CP: compound feed. Numbers 1, 6 and 10 refer to the period of litter decomposition in weeks whereas NF refers to the starved shrimp used as a control.

6.1.4.2.2 Shrimp

The concentration of FA in shrimp foraging on the different feed types was significantly different (One-way ANOSIM; Global R=0.523; p=0.001) (Fig. 1). Shrimp feeding on compound feed were more similar in FA composition to the starved shrimp and shrimp feeding on biofilm than to shrimp feeding on decomposed mangrove leaf litter (Pairwise ANOSIM; R=0.852: p=0.1; R=0.87: p=0.012; R=0.911: p=0.005, respectively). Shrimp feeding on decomposing mangrove leaf litter were generally low in total FA and polyunsaturated fatty acid-PUFA (EPA and DHA included) compared to the starved shrimp and those feeding on biofilm and compound feed. However, they had a relatively higher content of saturated FA (SAFA) and monounsaturated FA (MUFA) which were low in the shrimp feeding on the compound feed (Fig. 2). Total FA and PUFA contributed most to the dissimilarity in FA between the shrimp and the mangrove litter derived food (SIMPER: 50% and 32.66% respectively) whereas SAFA contributed the least (SIMPER: 8.76%) due to palmitic acid C16:0 (SIMPER: 8.62%)

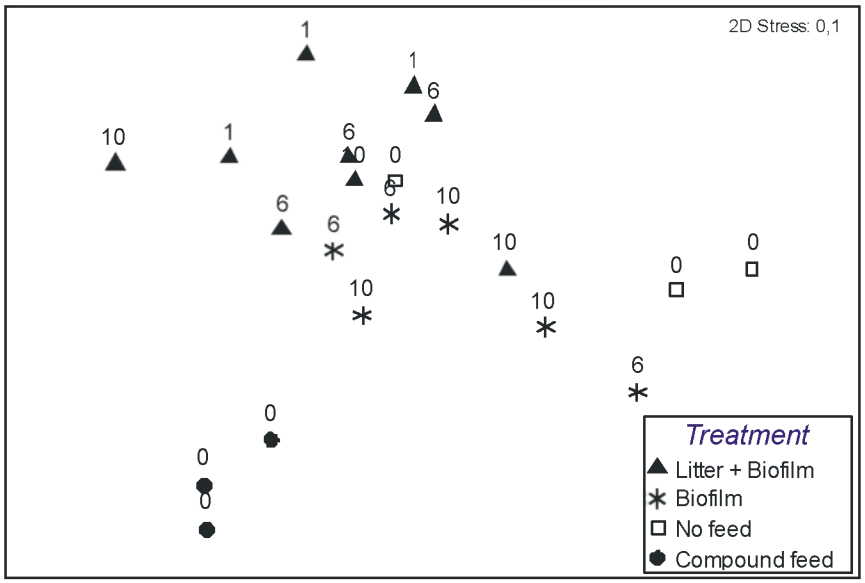


Fig 1: MDS plot based on similarity (Bray-Curtis) between absolute FA composition of shrimp tissue from different diet treatments: (1) decomposing mangrove leaf litter and the associated biofilm at the different periods of litter decomposition, (2) biofilm, (3) no feed and (4) compound feed. Numbers refer to the duration of litter decomposition in weeks.

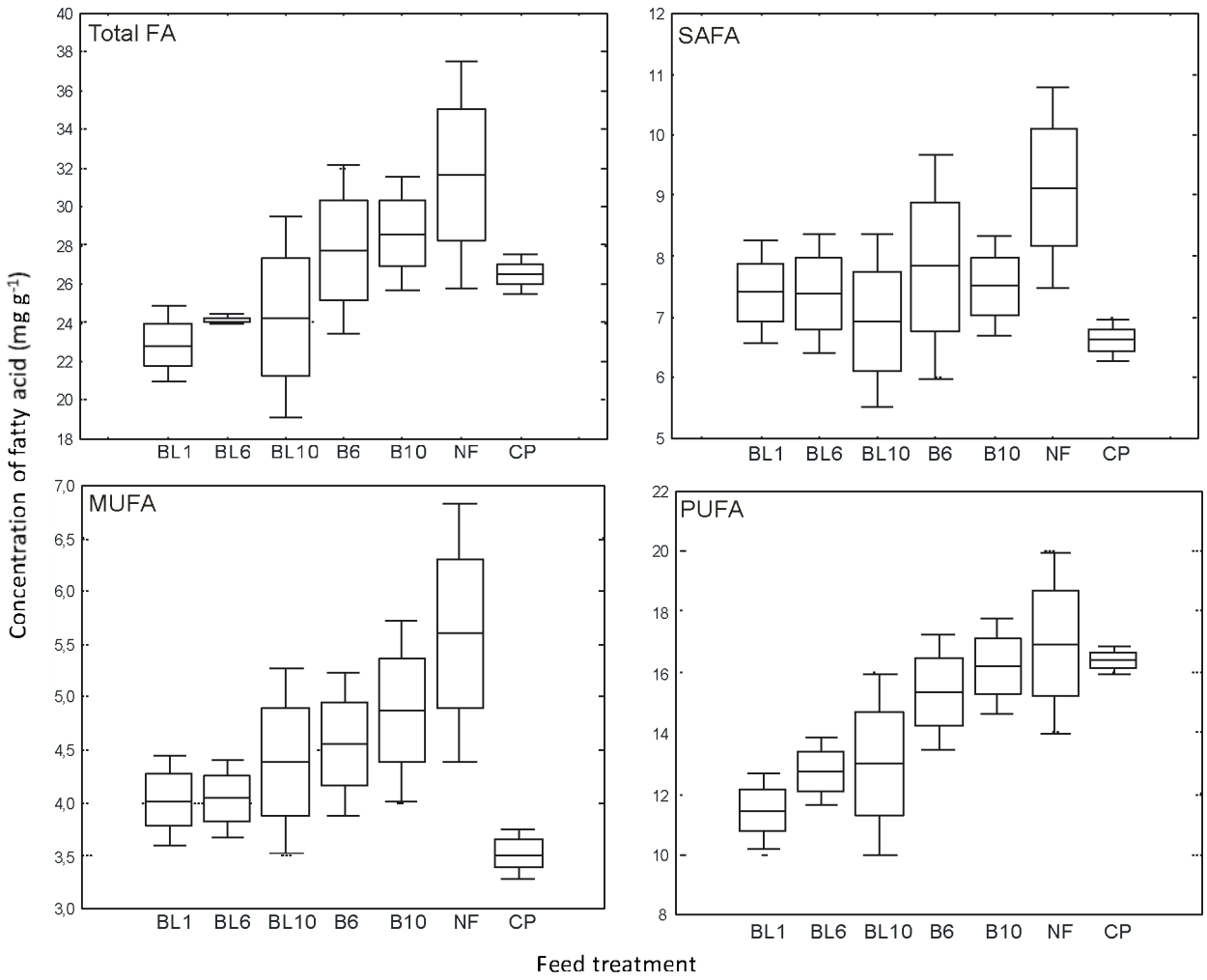


Fig 2: FA groups in shrimp tissue fed different feed types derived from compound feed, the decomposing mangrove leaf litter and the associated biofilm at the different periods of litter decomposition (1, 6 and 10 weeks). BL: shrimp feeding on litter with the associated biofilm, B: shrimp feeding on biofilm, CP: shrimp feeding on compound feed and NF: starved shrimp used as a control.

6.1.4.3 Essential FA

6.1.4.3.1 Highly unsaturated fatty acid (HUFA)

Proportion of the total HUFAin shrimp feeding on decomposed leaf litter and the associated biofilm increased, between week 1 and 6, as it also slightly increased in the food source (Table 2). Shrimp feeding on compound feed had significantly higher levels of DHA (7.8±0.1 mg g^{-1}) than shrimp feeding on litter mixed with biofilm (ANOVA, $F_{(6, 14)} = 8.72$; $p<0.003$). During the 6th week shrimp feeding on the biofilm recorded higher mean levels of DHA (5.2±0.7 mg g-1) than shrimp feeding on mangrove litter mixed with biofilm (3.8±0.8 mg g^{-1}). The abundance of DHA in shrimp feeding on 6 and 10 weeks old biofilm was not

significantly different from the shrimp feeding on the compound feed (Tukey, Post Hoc; p>0.05). DHA was most abundant in the shrimp feeding on the biofilm compared to starved shrimp and those feeding on the feed derived from mangrove leaf litter at all the stages of litter decomposition (Fig. 3).

EPA was abundant in the starved shrimp and shrimp feeding on the mangrove litter and biofilm while it was relatively low in the shrimp feeding on the compound feed (Tukey Post Hoc; p=0.02).EPA and DHA contributed most to the similarity in shrimp feeding on different feed sources (SIMPER: 21.42% and 16.97% respectively) and most to the dissimilarity between shrimp and the litter derived food sources (SIMPER: litter EPA 25.63%, DHA 18.28%; biofilm EPA 24.91%, DHA 22.8%).

6.1.4.3.2 Omega- 6 FA

Arachidonic acid-ARA (20:4ω6) was abundant in both the starved shrimp and shrimp feeding on mangrove litter and pure biofilm. However it was significantlylow in the shrimp feeding on the compound feed (ANOVA: $F_{(6, 14)}$ =5.58; p=0.003). On the contrary, linoleic acid (18:2ω6, Fig. 3d) was high in the shrimp feeding on the compound feed and the starved shrimp but low in the shrimp feeding on the litter derived food (Fig. 3). Compared to ARA, Linoleic acid (18:2ω6) contributed most to the similarity between shrimp and the litter/biofilm food sources (SIMPER: Linoleic; litter 94.23%, biofilm 94.82%: ARA; litter 83.38 %; biofilm 82.82%) and most to the dissimilarity between shrimp feeding on the litter, the biofilm in comparison to the starved shrimps (SIMPER: Linoleic; litter 14.51, biofilm 14.03%: ARA; litter 5.71, biofilm 6.41%).

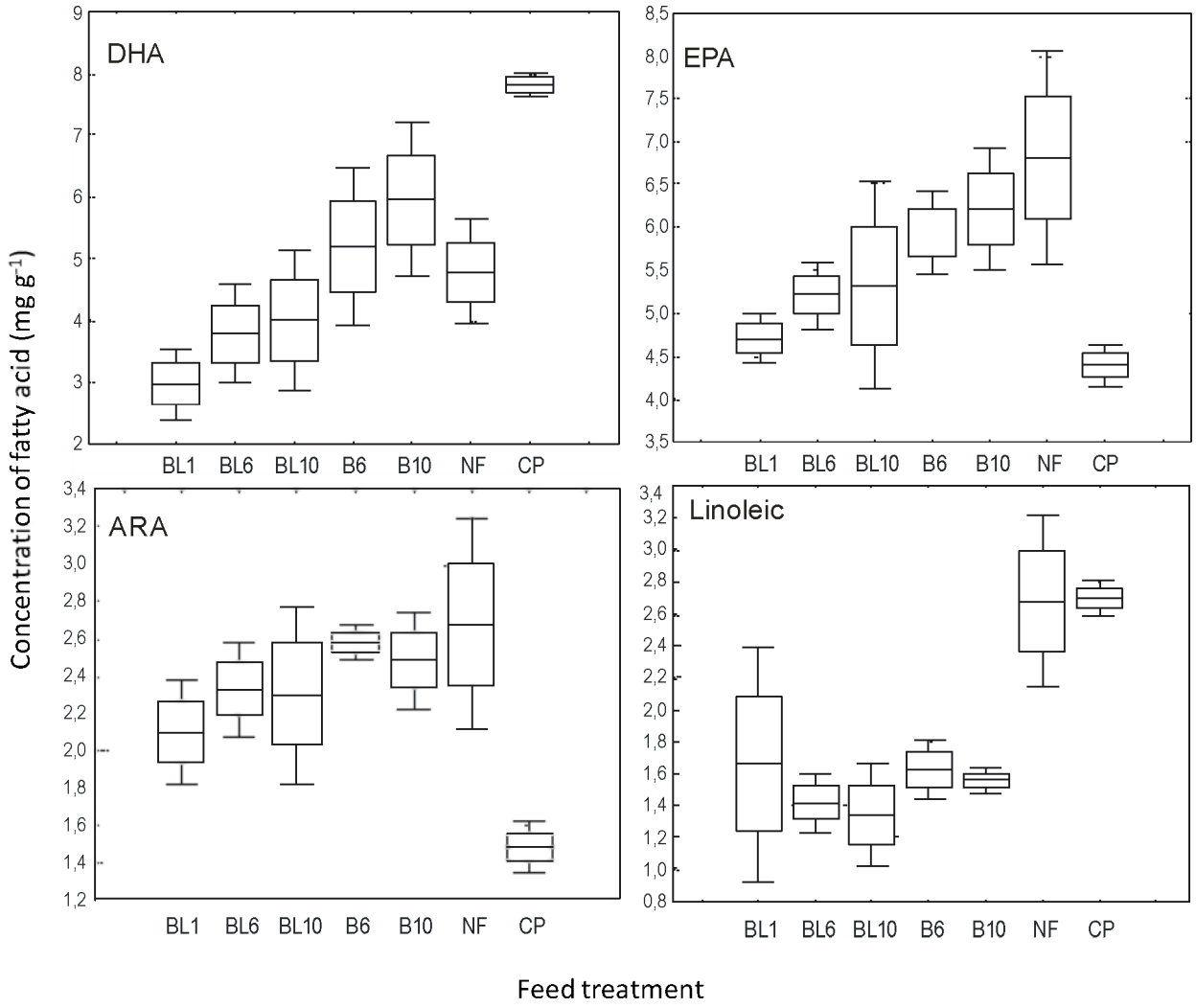


Fig 3: Essential FAs in shrimp tissue fed different feed types derived from compound feed, the decomposing mangrove leaf litter and the associated biofilm at the different periods of litter decomposition (1, 6 and 10 weeks). BL: shrimp feeding on litter with the associated biofilm, B: shrimp feeding on biofilm, CP: shrimp feeding on compound feed and NF: starved shrimp used as a control.

6.1.4.3.3 Odd carbon chain FA (C15:0 and C17:0)

C15:0 and C17:0 FA (Fig. 4) was abundant in the shrimp receiving mangrove leaf litter decomposed beyond 1 week and their respective biofilms. C15:0 FA content was higher in shrimp feeding on the leaf litter decomposed for 6 weeks and recorded the highest mean levels in the shrimp fed 6 week old biofilm but was not detected in the shrimp from treatments receiving 1 week decomposed mangrove leaf litter. As these FA concentrations were characterised by very high within-group variances, no statistical difference between the different feed treatments was found (Kruskal-Wallis test, $H_{(8, 26)} = 13.43$; $p=0.0977$) (Fig. 4).

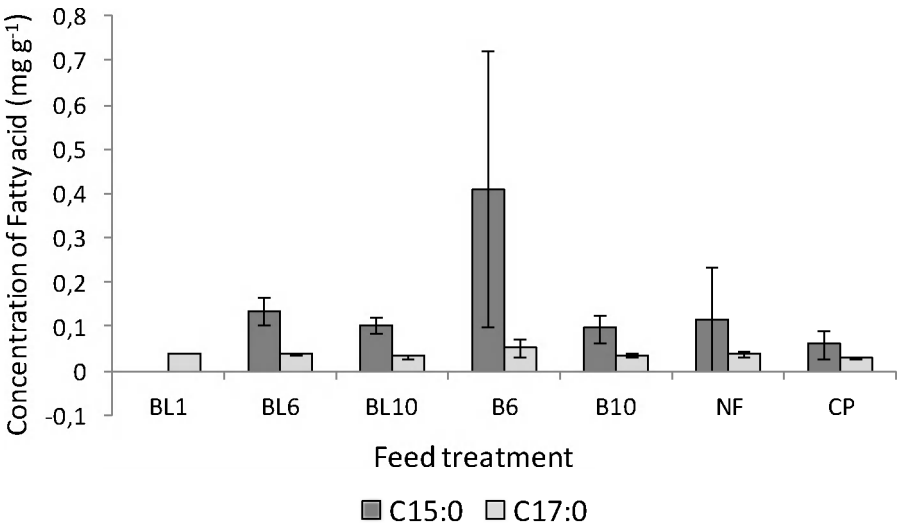


Fig 4: Odd carbon chain FAs in shrimp tissue fed different feed types derived from compound feed, the decomposing mangrove leaf litter and the associated biofilm at the different periods of litter decomposition (1, 6 and 10 weeks). BL: shrimp feeding on litter with the associated biofilm, B: shrimp feeding on biofilm, CP: shrimp feeding on compound feed and NF: starved shrimp used as a control.

6.1.4.4 Shrimp performance

6.1.4.4.1 Survival (SR)

Shrimp feeding on the compound feed recorded the highest survival of 100±0.0% which was significantly different from the shrimp feeding on the mangrove litter and biofilm (Kruskal-Wallis test, $H_{(6,\ 21)}$ =15.21; p=0.0186) (Fig. 5b). Survival of shrimp feeding on 1 week decomposed mangrove litter was higher than that of shrimp feeding on the 6 and 10 weeks litter (SR: 31.1±4.4%, 25.9±3.4%, 20.7±2.6% respectively). In shrimp feeding on the 10 weeks biofilm was higher than that of shrimp feeding on 6 weeks old biofilm (37.7±4.4% and 28.8±9.6%). Although survival of shrimp feeding on 10 weeks old biofilm (B10) was significantly higher than that of the starved shrimp (NF)(Tukey post hoc; p=0.032), all shrimp feeding on mangrove litter at all the stages of decomposition and 6 weeks old biofilm did not survive significantly better than the starved shrimp (p>0.05)(Fig (5a)

6.1.4.4.2 Specific growth rate (SGR)

Shrimp feeding on the commercial feed recorded a significantly higher (5.3±0.4%) than the shrimp feeding on mangrove litter and biofilm with a SGR ranged between 1.3±0.1% and 2.2±0.5% (ANOVA, $F_{(6,\ 14)}$ =11.23; p<0.001) (Fig. 5a). Shrimp feeding on the 6 weeks decomposed mangrove litter and 6 weeks old biofilm recorded a higher SGR of 2.2±0.5% and

2.1±0.5% than shrimp feeding on the 1 and 10 weeks litter and biofilm (SGR: 1.3±0.1%; 2.1±0.2%; 1.4±0.1%, respectively). Starved shrimp recorded the lowest SGR of 0.9±0.8%, but did not differ significantly from the ones feeding on the mangrove litter and biofilm ($p>0.05$) (Fig. 5b).

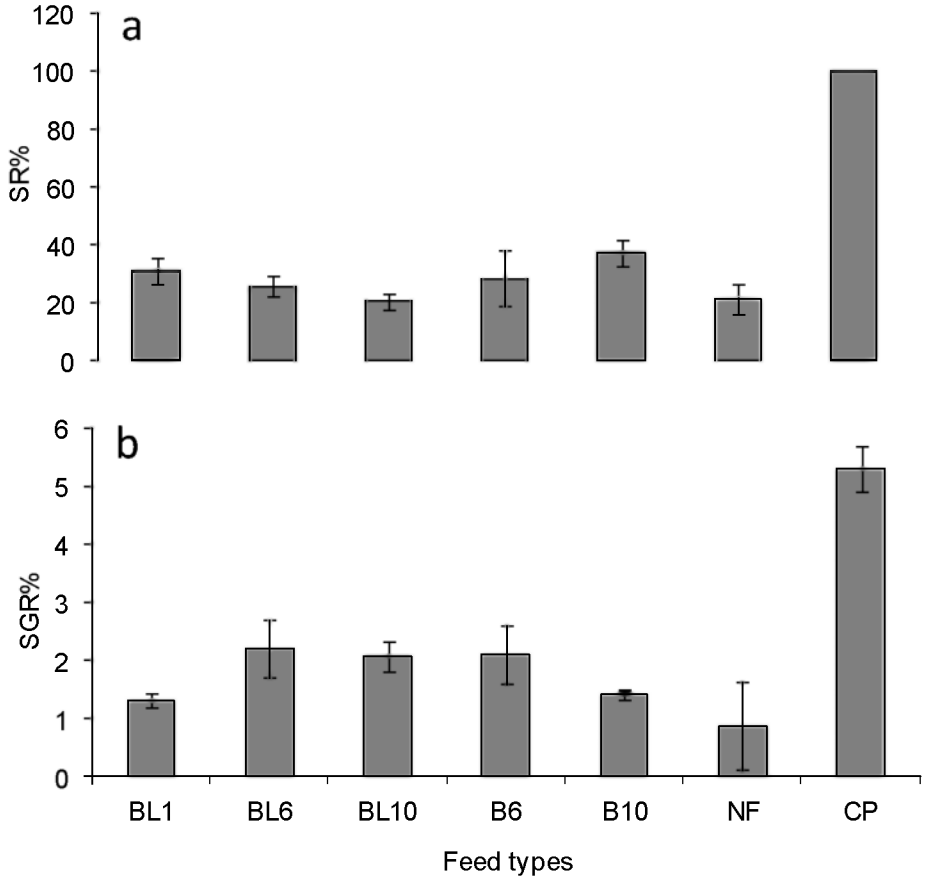


Fig 5: Performance of shrimp post larvae of *Penaeus monodon* fed different feed types derived from compound feed, the decomposing mangrove leaf litter and the associated biofilm at the different periods of litter decomposition (1, 6 and 10 weeks). BL: shrimp feeding on litter with the associated biofilm, B: shrimp feeding on biofilm, CP: shrimp feeding on compound feed and NF: starved shrimp used as a control.

6.1.5 Discussion

6.1.5.1 Feed nutrients

The biofilm developing on the decomposing mangrove litter showed a progressive increase in nitrogen and consequent decline in C/N ratio. The shift in C/N ratio to 6.5 in the more developed biofilm is indicative of increased colonisation by microbiota such as bacteria, epiphyton and epifauna. During litter decomposition, N-immobilization is particularly promoted by microbial activity (Tremblay and Benner, 2006) with a resultant reduction in C/N ratio. On the other hand, epibiotic biomass from diatoms and copepods would largely contribute since their C/N ratio could be as low as 5.5 (Morales, 1987a), 6.6 for marine phytoplankton and between 4 and 6 for diatoms (Dam and Siuda, 2010). The growth of biofilm on the decomposing mangrove litter also meant an increase in the foraging activity of the epifauna grazing on the micro-algae (diatoms) with a consequential increase of nitrogen from the feacal pellets. In general, using the carbon to nitrogen ratio, it is possible to elucidate that both the biomass and the foraging activity of the microbiota was predominant in the 10 week old biofilm. This fact is supported by the observed increase in the biomass of the micro-algae and crude protein levels in the more decomposed litter (Table 1). The higher crude protein content in the 6 weeks old biofilm points to increased mobilization of protein rich sources at this point. For instance protein rich diatoms such as *Navicula* spp. and *Nitschia* spp. (Brown and Jeffrey, 1995) were observed to dominate 4 to 6 weeks old biofilm but were later replaced by cyanobacteria (Gatune et al., PhD Thesis Chapter 4) which is low in nutrients (Smith et al., 2008). On the other hand, comparing to our previous study (Gatune et al., PhD Thesis Chapter 4), the observed high abundance of macroinvertebrate polychaetes at this stage of litter decomposition may also have largely influenced the increased crude protein.

6.1.5.2 FA profiling of food sources

It is generally observed that total lipids tend to be less abundant in the more decomposed mangrove litter and the more developed biofilm (Table 1). The decrease in lipids would imply that mangroves are important sources of FA in the estuarine ecosystems. However, the impact of this decrease to the estuarine food web would depend on the nature and type of limiting fatty acid in terms of palatability and nutritional quality. For instance, saturated FA (SAFA) is the abundant FA in the food source, litter and biofilm, and is likely to nutritionally influence the contribution and the importance of litter and biofilm in the lower trophic levels. SAFA were observed to decrease whereas monounsaturated forms (MUFA) increased with the decomposition of mangrove litter. Similar observation was made in a previous study

(Mfilinge et al., 2003) where during decomposition, FA composition in mangrove leaf litter changed from predominantly SAFA to MUFA. SAFA is not essential since they can be bio-synthesised in the tissue. According to D' Abramo (1989), shrimp like other animals possess a delta 9-desaturase enzyme system which can convert SAFA to MUFA. However caution should be observed at this juncture, since although penaeid shrimp don't have a definite lipids requirement, dietary levels of lipids ranging from 6% to 10% have been suggested (Shi-Yen, 1998). Moreover one unique aspect of lipid nutrition in crustaceans is that cholesterol is essential for penaeid and a level of 0.5% cholesterol is recommended in the diet for *P. monodon* juveniles (Shi-Yen, 1998). Hence decomposition is essential in mobilizing both saturated and unsaturated FA and therefore improving the diet value of the litter to penaeid shrimp post larvae. The further processing of the decomposing mangrove litter by bacteria also enhances the levels of essential FA in litter (Mfilinge et al., 2003). While interacting with decomposing mangrove litter bacteria do not degrade polyunsaturated fatty acid (PUFA) but instead they tend to conserve them hence enriching the detritus with nutrients (Mfilinge et al., 2003).

Essential FA such as the highly unsaturated FA (HUFA) tends to increase, although slightly, in the more decomposed litter and biofilm. HUFA are important in a wide range of physiological performance of an organism, for instancein the growth of macrofauna such as the penaeid shrimp (D' Abramo, 1989; Kanazawa et al., 1979; Sorgeloos and Lavens, 2000). HUFA are therefore the best suited fatty acid category to highlight the ecological function of the decomposing mangrove litter. Such function would start at the micro level in the biofilm developing on the decomposing litter and would later spread to a wider ecosystem such as the estuarine food web or ecological shrimp culture system. The contribution of FA from decomposing mangrove litter to the ecosystem may be largely limited by the palatability of the mangrove litter caused by the presence of tannins which deter grazers (Scalbert, 1991). However the growth of the periphytic biofilm would reduce the effect of the deterrents and enhance the ecological function through the provision of essential FA. Essential FA suh as HUFA is found in the micro-algae and epifauna (Parrish, 2009) which are among the major constituents of the biofilm. In our previous study (Gatune et al., PhD Thesis Chapter 4) , diatoms, copepods and polychaetes were observed to dominate the biofilm in the early stages of mangrove litter decomposition. Diatoms and copepods are typically rich in eisosapentanoic acid-EPA (20:5ω3) and docosahexanoic acid-DHA (22:6ω3)(Parrish et al., 2012; 2000). Polychaetes which are important food for penaed shrimp (Nunes and Parsons, 2000) were

found to dominate the biofilm. Hence the ecological importance of mangrove litter to penaied shrimp post larvae is defined by the nutritional value of the biota constituent of the biofilm.

6.1.5.3 FA profiling of shrimp post larvae

The low level of total FA in the shrimp foraging on mangrove litter in comparison to the best performing shrimp feeding on the compound feed, may question the efficiency of the decomposing mangrove litter in meeting the lipid requirement of the penaied shrimp. Lipids play a major role in the physiological performance of shrimp in providing energy and growth (Sandifer and Joseph, 1976; Sorgeloos and Lavens, 2000) and its scarcity could impair the overall production of the ecologically cultured shrimp. However, this observation should be approached with caution since shrimp have a specific requirement in the level of lipids in the diet. For instance high dietary levels of oil are associated with retarded growth (ref) and since food uptake is thought to be an energy supplement, excessive dietary lipid may therefore inhibit appetite (D' Abramo, 1989).

Ecological shrimp culture imply a strong reliance on natural food supply and its success would be determined by the abundance of preferred nutrients among which would be the essential FA (Kanazawa et al., 1979; Rothlisberg, 1998). The fact that EPA and arachidonic acid-ARA (20:4ω6) were abundant in starved shrimp and shrimp feeding on mangrove litter and biofilm would likely suggest that these FA are less utilized under stressful conditions than when the shrimp post larvae interacts with the decomposing mangrove litter. The fact that linoleic acid was abundant in starved shrimp and low in shrimp foraging on mangrove litter and biofilm derived food could imply that certain conditions, specific to the mangrove derived food, influenced excessive utilization of linoleic acid in the shrimp tissue. Mangrove litter derived food was low in both EPA and DHA compared to the compound feed. Kanazawa et al. (1979), found that *Penaeus japonicus* had some ability to convert linolenic acid to EPA and DHA in situation where they are lacking in the diet. The low linoleic acid (18:2ω6) in shrimp foraging on mangrove litter derived food could have been a result a bioconversion metabolism in desaturating and elongating linoleic acid to compensate for the scarcity of EPA and DHA in the mangrove food sources. This kind of metabolism would definitely expend energy causing a high utilization of omega-6 FA since they are important in providing energy to the shrimp (Sandifer and Joseph, 1976). Moreover, these omega-6 FA are actually the main starting points for elongation and desaturation reaction during bioconversion towards HUFA.

The starved shrimps had higher EPA, DHA and linoleic acid implying that they stored these FAs and they didn't go for the bioconversion of the linoleic acid.

Shrimp feeding on the 6 weeks decomposed mangrove litter and its associated biofilm recorded higher levels of odd carbon chain FA (C15:0 and C17:0) which are important fatty biomarkers for bacteria (Alikunhi et al., 2010; Ederington et al., 1995). This implies that most bacteria were mobilised at this stage of leaf litter decomposition and were contributing to the nutrition of the shrimp by providing the typical bacterial FA. It also presents the period around the 6th week as an important turning point in time when the decomposing mangrove litter is a major source of bacterial biomass to the ecological shrimp pond food web.

6.1.5.4 Shrimp performance

Shrimp grew better when foraging on the mangrove litter decomposed for 6 weeks but did not survive better compared to when foraging on the less decomposed mangrove litter and on the advanced biofilm. The better growth but low survival could be attributed to the presence of nutrient rich less abundant food source. Our earlier study on the assembly of epifauna (Gatune et al., PhD Thesis Chapter 4) observed a high abundance of polychaete and larvae stages in the 4 weeks old litter which declined towards the 5th and the 6th week of decomposition. The better growth but low survival could therefore be attributed to the reduction of the nutrient rich epifauna. A higher diversity index and evenness of epifauna were also observed on mangrove litter decomposed for less than 4 weeks. Such diversity could imply a wide range of food sources but of moderate nutritional quality just enough to maintain the general survival of the emanciated shrimp post larvae. A high biomass of low quality micro-organisms dominated by cyanobacteria has also been previously observed in biofilm associated with mangrove litter decomposed beyond 6 weeks (Gatune et al., PhD Thesis Chapter 4). This could imply that the shrimp post larvae feeding on the 10 weeks old biofilm were supplied with large amounts of low nutritional quality cyanobacteria maintaining survival without any reasonable increase in growth.

Shrimp foraging on mangrove litter and associated biofilm did not perform outstandingly better compared to the shrimp which were not feeding at all. This observation gives an insight into the limitation of the single role of decomposing mangrove litter of *Rhizophora mucronata* in supporting shrimp post larvae nutritively. Mangrove leaves of *Avicennia marina* have been observed to be a less important source of dietary carbon and nitrogen for crabs

(Mazumder and Saintilan, 2010). In our study, mangrove litter derived food is limited in the supply of essential FA especially DHA which is important for the growth performance of penaeid shrimp post larvae (Kanazawa et al., 1979). Consequently, shrimp have to seek alternative sources of DHA, for instance by possibly bio-converting the linoleic acid. This could definitely lead to emaciation of the shrimp post larvae from the energy they could be expending in driving this process. This study therefore finds shrimp post larvae to be physiologically antagonised if mangrove litter is used as a sole food source.

Biofilm which was 6 weeks old had comparatively higher protein content (15.10 ± 0.71%) yet the shrimp feeding on it did not perform better compared to the starved shrimp. The cause of the poor performance in shrimp relying on mangrove derived food could therefore also be a combined effect of nutrition and other ambient influences. For instance, tannin which is leached from the decomposing mangrove litter (Rajendran and Kathiresan, 2000a). Future studies should investigate the nature of interaction between the chemical attributes of decomposing mangrove litter and the physiological performance of the penaied shrimp post larvae. For instance; 1) the antimicrobial properties of tannin (Gonzalez-Farias and Mee, 1988) which may slow down the microbial breakdown and further processing of mangrove litter by bacteria and fungi (Wilson, 2002); 2) the direct inhibitory effect of tannin on the assembly of meiofauna (Coull, 1999) and its effect on the supply of quality food to shrimp post larvae; 3) the tendency of tannin to inhibit availability of nutrients to shrimp post larvae. Tannin binds proteins, starch, cellulose and minerals interefering with the nutritional quality of feeds (NRC, 2011) Tannin also inhibits digestive enzymes such as trypsin and amylase and also inhibits arginase enzyme responsible for L-arginine metabolism and biosynthesis through chlorogenic acid pathways (Kandil et al., 2004; NRC, 2011)(Ronald et al 2011; Kandil et al., 2004). By antinutritional tendencies tannin can thereforeadverselyeffect the performance of shrimp post larvae by suppressing growth and survival for instance in shrimp ponds receiving mangrove leaf litter fall.

Since mangrove litter is most abundant in the mangrove aquaculture systems, their contribution as substrates for the growth of biofilm would greatly increase the efficiency of an ecological practise by reducing operational costs. The importance of biofilm and bacteria mediated systems in aquaculture has gained much interest in both scientific and culture practices (Crab et al., 2010; Thompson et al., 2002) due to the reduction of the production costs (Asaduzzaman et al., 2008). To promote biofilm, hard substrates have been used and

higher survival and growth of cultured organisms have been reported (Asaduzzaman et al., 2008). However the negative interaction observed in this study, between shrimp postlarvae and mangrove litter derived food, call for caution in selecting mangrove leaf litter, which may be species specific, as substrates in developing ecological shrimp aquaculture in mangrove systems. Similar studies with leaf litter from other species of mangroves would assist in profiling species specific interactions and the strategies for application.

6.1.6 Conclusion

1. Biofilm developing on the decomposing mangrove litter provides bacterial FA during the 6th week of litter decomposition and influences the mobilization of the essential fatty acid DHA in the shrimp tissue.
2. Shrimp post larvae feeding on the mangrove litter derived food and shrimp under starvation are characterised by higher levels of SAFA, MUFA, ARA and EPA than shrimps feeding on compound feed.
3. Mangrove litter derived food suppresses the growth and survival of shrimp post larvae due to the unavailability of DHA and possible energy consuming bioconversion of linoleic acid.

6.1.7 Acknowledgements

The first author thanks Flemish InterUniversity Council (VLIR) for the financial support to follow an international PhD program in Biology at Ghent University, Belgium. The present study is part of a PhD project in the *FEEDING ECOLOGY OF SHRIMP IN TROPICAL MANGROVE SYSTEMS OF KENYA: The nutritive value of detritus and its use in biological shrimp aquaculture*. The last author is a postdoctoral fellow financed by the Special Research Fund of the Ghent University (GOA project 01GA1911W). Deep felt thanks to Mr. Glen based at Mbengani Fisheries Development Centre in Bagamoyo Tanzania, for facilitating export and donating the test shrimp post larvae. Dirk Van Gansbeke (UGent, Marine Biology) assisted in the analysis of the fatty acids. KMFRI laboratory technicians Eric Magara, Omondo, Mwendwa Mbaluka, Boaz and the youth and community members of the Majaoni silvofishery project, Kenya, for their field assistance.

Chapter 6

7 Chapter 7

7.1 Synthesis

Prospects for implementing shrimp aquaculture in Kenya may heavily depend on locating shrimp ponds out side the mangrove areas (Photo courtesy Gatune C. 2010)

7.1.1 Prospects for implementation

The prospect to develop biological shrimp aquaculture in mangrove systems of Kenya emanates from the prevailing potential at the local level. The fishery produce being targeted has high food and commercial value both for the local community and the international market. The produce, which is the penaeid shrimp of *Penaeus monodon* and *P. indicus* species, is locally available along the Kenyan coast. The fishery of these two penaeid species is an established industry and an important export product comparable to the famous Nile perch fishery from the Lake Victoria (a fishery so vibrant at the local and international market, that it motivated the 2006 Oscar nominated documentary "Darwin's Nightmare"). The shrimp fishery is a field full of conflicts especially within Ungwana bay of the Kenyan Indian Ocean. The industrial shrimp trawling is an exclusive export based activity controlled by a cartel of foreign companies. The economic proceeds from the shrimp trawling industry do not benefit the local community. Yet the daring shrimp fishing vessels are constantly illegally trespassing into the shallow fishing grounds which are the only possible operation zones for the local small-scale or rather artisanal shrimp fishery. The destruction of the shallow coastal zones by the shrimp trawling vessels and the constant conflict with the local community is a major ecological foot print which has generated a lot of concern. The social, ecological destructive nature of this foot print led to the search for alternatives that would ensure that the economic gains from the shrimp fishery trickles down to the local community. Among the alternatives was the development of a shrimp aquaculture practice by FAO in the year 1978. This trial project failed 10 years later. Some sources which have attempted to evaluate the reason behind the failure of this project predominantly blame the high level of technology that could not be readily adopted by the local community. This is a credible argument given the project was based on a large scale technology requiring a high capital to invest and manage. Given the low financial capability of the local community and the lengthy capital recovery period, against the much needed daily bread, the idea was just another foreign monster just like the shrimp trawling industry. This oversight befell a community which had also suffered from the eviction by the salt making companies which have acquired almost the entire land, along the Ungwana bay. This land has high potential to develop the shrimp aquaculture. Probably the salt companies were also not comfortable with what would result if the community realized the importance of shrimp aquaculture and started demanding back their land. Despite having contributed most to the destruction of the mangrove forest, salt harvesting seem always to receive a clean bill of health whereas the smaller shrimp

aquaculture project in Ungwana bay is constantly blamed for the destruction of the Kenyan mangrove forest.

Development of shrimp aquaculture along the Kenyan coast is a feasible fishery practice in empowering the local community economically. What needs to be done is to address the social ecological issues that can be employed to strategize on its development. The search for probable answers to develop shrimp aquaculture may not necessarily be straight forward but would create an entry point. A search and try scenario, a scientifically based argument that would generate a step towards the final achievement. 'You may never find your way out if the ray from the rising sun falls on your back'.

The blame on the destructive nature of shrimp aquaculture on the Kenyan mangrove forest can be addressed by promoting ecological shrimp aquaculture. The capital requirement of an ecological aquaculture practice can be adjusted to suit the local capability by designing a management tool that would allow a convenient cost of implementation and production. For instance site selection, food and shrimp post larvae quality and availability at the local level are the main pillars worth considering in developing ecological shrimp aquaculture. With this in mind, various studies were performed in an attempt to design a strategic approach in developing a shrimp aquaculture practice that would co-exist with a mangrove forest and that would be accepted by the local community. Food supply is a major factor determining the success of a shrimp aquaculture. Fish meal has a high nutrition value to shrimp post larvae and is an important food that results to a high production of cultured shrimp. However, fish meal is a costly input in a shrimp culture farm and increases the potential of the water discharged from the shrimp farm to pollute the receiving environment. The use of fish meal can be minimized by searching for an alternative feed that would be locally affordable and which would not pollute the environment.

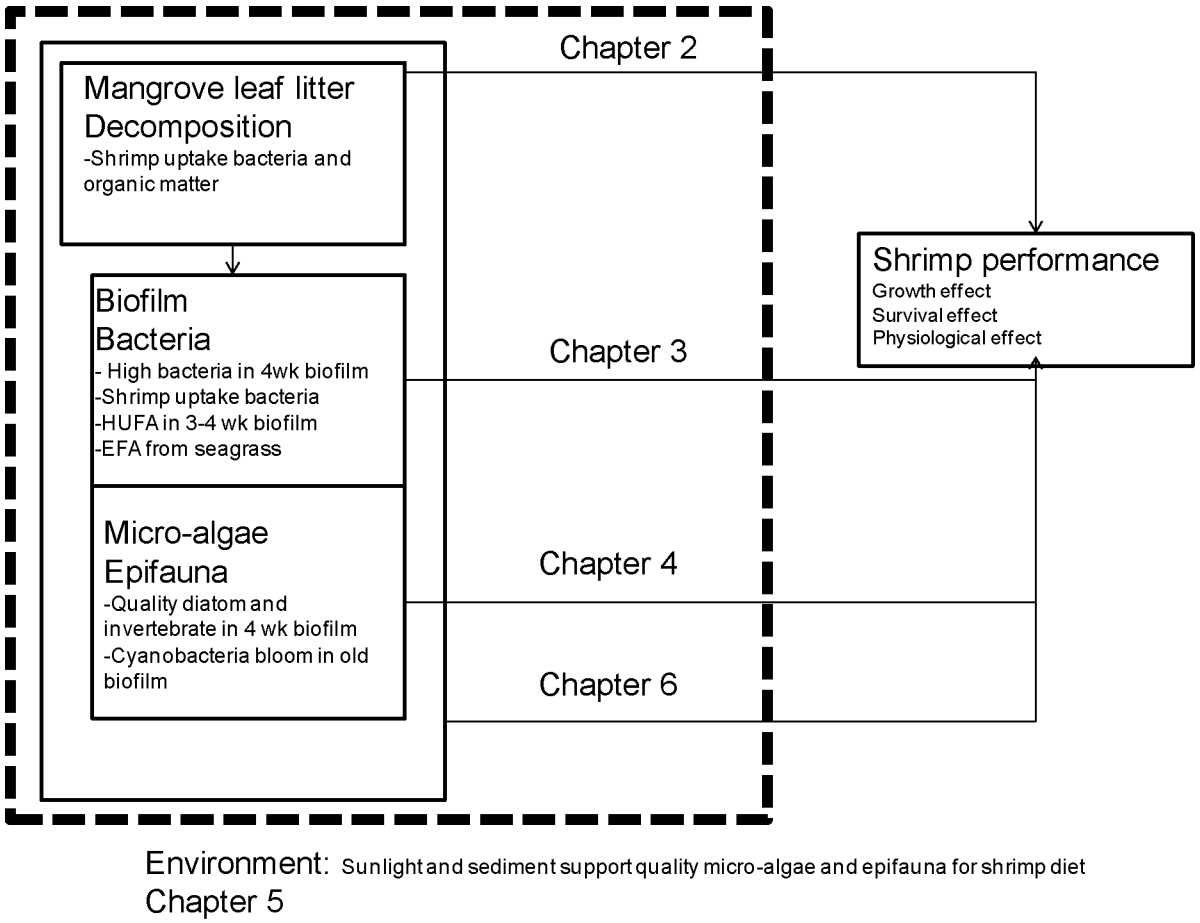


Fig 1: Schematic representation of the specific parameters observed to support the ecological function of the mangrove leaf litter in providing dietary support to shrimp post larvae.

Developing ecological shrimp aquaculture in a mangrove zone implies that the mangrove system would be relied upon in providing food that would replace the fish meal. The food would definitely be of biological nature and directly or indirectly connected to the mangrove system. Food is among the ecological services that would be derived from the mangrove forest and is the motivating factor to the **specific objective 1** which was investigated in **Chapter 2** and aimed at assessing to what extent shrimp post larvae can ingest and assimilate organic matter from decomposing mangrove leaf litter given the option of a bacterial biofilm as a food source. This objective was based on an effort to apply the natural feeding ecology of shrimp in a mangrove ecosystem and the possible application in a biologically based, ecological shrimp aquaculture. This chapter is based on the reasoning that mangrove forest has a considerable litter fall and this litter would be nutritive to bacteria and in turn to the shrimp postlarvae. When the mangrove leaf litter decomposes in a shrimp pond,

it would develop a microbial biofilm. If no other food is available, would the shrimp post larvae feed and assimilate this leaf litter and the associated biofilm? This study made an important observation that shrimp post larvae actually assimilate both the bacteria and the mangrove detritus in the absence of any other food. The observation made from this study supported the food importance of the mangrove detritus and its associated bacteria to shrimp post larvae. However, this observation is relative to the absence of any other food and it should not be used to solely emphasize the mangrove detritus as an important food. However the pulse-chasing method used in this study by the use of ^{13}C stable isotope labeled food, has not been commonly applied in the mangrove and shrimp related studies. Another important point emphasized in this chapter is that, the decomposing mangrove litter is associated with bacteria which are not only ingested but are nutritive to the shrimp post larvae. ***It is worth noting that, although studies on penaeid shrimp nutrition are numerous, very few have investigated the biological quality dynamics of the biofilm associated with the decomposing mangrove litter. In addition to this view, the studies which have invested in assessing the nutritive importance of this biofilm to shrimp post larvae are very scarce and it is most likely that our current study, as a whole, could be the only one of the kind.*** The confirmation of the presence of a bacterial biofilm developing on the decomposing leaf litter gave a hint of the importance of the leaf litter fall from the mangrove forest into shrimp ponds. This study therefore motivated the **specific objective 2** of this PhD study where a further investigation into the time based microbial contribution to the nutritive importance of the decomposing mangrove leaf litter and the associated biofilm to the diet of shrimp post larvae was performed given the option of a natural food source from seagrass. In the process of making this investigation an interesting observation is made in **Chapter 3.** In this chapter, the decomposing mangrove leaf litter seems to have a timeframe in supporting the biofilm. The biochemical qualities of the biofilm seem to be really defined along certain time lines which are of great relevance to the nutritive driven performance of the shrimp post larvae. For instance, when the mangrove leaf litter is almost half way decomposed they have the best capability to support the highest bacteria and micro-algae biomass. It is also at this juncture that the decomposing mangrove leaf litter provides the shrimp post larvae with the most abundant fatty acids which are essential for their growth. The timeline for this scenario is clearly set as occurring at the 3rd and the 4th week of decomposition. ***As mentioned earlier, the establishment of a time line is very important in developing a management tool. For instance a time line can be used to design a check list to control the duration of residence of***

the leaf litter fall into the shrimp pond. Such a check list can guide an ecological shrimp farmer on how frequent to remove the mangrove leaf litter from the shrimp pond. The study described in chapter 3 also went further to compare the nutritive quality of the biofilm, developing on the decomposing mangrove leaf litter, with the biological food imported from an ecosystem outside the mangrove forest. The ultimate importance of a mangrove forest would be to fully provide the shrimp post larvae with their nutritional requirements. However, when compared with a neighboring ecosystem such as seagrass beds, mangrove forest is found to be deficient in the supply of the essential nutrients such as the essential fatty acids to the shrimp post larvae. In this study, special focus is directed to a specific type of the mangrove leaf litter, the *Rhizophora mucronata.* So it is possible to argue that such deficiency is not a whole some scenario of the mangrove forest. However, the *Rhizophora mucronata* is the most common tree in the mangrove system of Kenya and is actually the tree species that the Ungwana bay shrimp aquaculture project is blamed as having destroyed. It is also the tree type which is commonly found interacting with the shrimp ponds where this study was conducted. The observation of the importance of food supply from the seagrass is the second important management tool that can be designed from this study. It was earlier mentioned that site selection is an important factor in determining the success of an ecological shrimp aquaculture. This could be on the basis of accessibility and proximity to raw material which includes food supply. If the proximity to the mangrove forest is of less nutritional importance compared to seagrasses, then it is better to select a site which would allow the supply of natural food from the seagrass ecosystem. Furthermore, such consideration would select a site which is far from the mangrove forest which would reduce the ecological conflict related with the conservation of the mangrove forest. ***This observation scientifically proves that locating shrimp ponds away from the mangrove forest but adopting a design that would link it to the seagrass would increase shrimp production. This is a very important management tool for ecological shrimp aquaculture which can be included in the GIS platform for selecting sites which are benign to the destruction of the mangrove forest.*** This tool can be included to the other geographic variables as e.g. no mangroves, no wetlands, within 1 km of water front, more than 3 km from urban development, elevation < 15m or slope < 5%, temperature and salinity tolerance or optima, restricted areas such as the national parks and infrastructural needs such as the proximity to the international airport. This view seems to be favorable to a high capital investment shrimp aquaculture. However in this same study, open areas within the mangrove forest and the biofilm were better in the supply of essential nutrients to the

shrimp post larvae. This opens a window for developing small-scale ecological shrimp ponds in the open areas within the mangrove forest. It also implies that there is a chance that biofilm found within the mangrove forest could support the nutritional needs of the shrimp post larvae. This observation prompted further growth experiments with shrimp feeding on the biofilm in order to support potential aquaculture applications. **Chapter 4** describes a study which was performed in line with the **specific objective 3** which was aimed at assessing the time line based biological constituents of the biofilm and their potential to support the growth of the shrimp post larvae. This study investigates whether it is possible to biologically apply the biofilm developing on the decomposing mangrove litter in the small-scale ecological shrimp aquaculture ponds. Just like the previous study, this study similarly observed the best performance of the shrimp post larvae when they foraged on the biofilm associated with mangrove leaf litter decomposed for 4 weeks. The 4^{th} week time line seems to be repeated here and tends to reproduce the observation made in chapter 3. Apart from the influence by the bacteria, this study established the actual biota involved. At this juncture, this study observed this important timeline in the development of the biofilm as being characterized by a consortium of organisms of nutritional importance to the shrimp post larvae. For instance the diatoms, copepods and polychaetes which have high nutritional value to the shrimp post larvae are abundant at this timeline in the decomposing mangrove litter. This biota is the reason behind the previously observed high prevalence of the essential fatty acids in the shrimp foraging on the leaf litter at the 3^{rd} and the 4^{th} week of decomposition. However the previous experiment (Chapter 3) could only predict the fate of the shrimp foraging on the leaf litter decomposed beyond a period of 5 weeks from the declining supply of essential nutrients. However it was not possible to pin point the actual biological drivers. The study in chapter 4 makes it possible to pin point the actual biological culprits behind the declining supply of the essential nutrients. The cyanobacteria, which is a poor natural food source for the shrimp post larvae is clearly observed as occurring in the decomposing leaf litter decomposed beyond a period of 5 weeks. This chapter not only characterizes the biofilm but also highlights the timeline that the quality natural food for shrimp post larvae can be derived from the biofilm developing on the decomposing mangrove litter. This chapter stresses the point that the benefit of deriving natural food from the decomposing mangrove leaf litter is short lived and cannot be sustained beyond 5 weeks. An aquaculture management requirement is to remove the litter from the shrimp pond after this period. The question that would arise at this junction is whether it is possible to extend this period and whether it is necessary to do so in the first

place. To extend this period would require an intervention that would promote sustained growth of the diatoms and epifauna and suppress the growth of the cyanobacteria. This is a gap that would require further investigation. The prolongation of this period would be justified if it does not make economic sense to control the residence time of the decomposing leaf litter in the shrimp pond. Since the decomposing mangrove leaf litter was observed to have very high C/N ratio (chapter 3), the addition of nitrogen may probably sustain the growth of the quality microalgae, that is the diatoms, and suppress the growth of the atmospheric nitrogen fixing cyanobacteria. Nitrogen addition would probably extend the supply of quality natural food from the most decomposed mangrove leaf litter through the crop period of the cultured shrimp until the appropriate time for pond draining and preparation for the next crop.

As the thought of the nitrogen intervention runs through our mind, probably a quick answer to sustain the quality micro-algae can be sought from the ambient conditions of the sunlight and sediment. Exposing the decomposing litter to a balanced condition of sunlight and sediment may probably sustain the growth of quality natural food without the addition of nitrogen which has a polluting potential. **Chapter 5** describes a study which was performed to explore the **specific objective 4** of this PhD study and managed to establish the extent to which the varying conditions of sunlight and sediment may impact on the biological quality of biofilm in a week 4 timeline of decomposing mangrove leaf litter. This study simulated the biological changes that biofilm may undergo depending on the extent of exposure of the decomposing leaf litter to the sunlight and the sediment. The study was motivated by the fact that ecological shrimp culture is in direct contact with the mangrove trees which would supply litter fall and attenuate sunlight. In addition, the decomposing leaf litter may be subjected to conditions without sediment. This study observed a synergistic effect between the sunlight and sediment in increasing the rate of litter decomposition, improving the water quality and promoting the proliferation of a wide range of food quality micro-algae and epifauna. ***This study also highlighted the 4th week as important period in the decomposition of the mangrove leaf litter when the micro-algae and epifauna which are of nutritional importance to the shrimp post larvae is most abundant.*** This observation reproduces the finding of the previous studies and strengthens the 4th week as an important time line in deriving the biological benefit of the natural food supply from the decomposing mangrove leaf litter of *Rhizophora mucronata*. ***The specific conditions for this time line are defined in***

this chapter, as being the presence of sunlight and sediment. In the absence of either of these two conditions, it is impossible to observe this time line especially due to rapid proliferation of cyanobacteria. The specific condition favoring the rapid proliferation of cyanobacteria is established in this study where abundant sunlight in the absence of sediment may be an important cue to trigger proliferation of cyanobacteria on the decomposing mangrove leaf litter. The observation of the rapid proliferation of cyanobacteria in the absence of sediment is an important caution to an ecological shrimp farmer in controlling the leaf litter decomposing in the absence of sediment such as the litter floating on the water surface. This caution is also important to culturist who would intend to use decomposing mangrove leaf litter as substrate in the development of the periphyton technology in the microcosms without sediment. It was observed in this experiment that presence of cyanobacteria suppressed the rate of decomposition of the mangrove leaf litter. It was impossible to logically explain the reason behind this effect. This seems to have opened a gap that requires further investigation. An assessment of the bio-chemical interaction of the cyanobacteria with the various decomposition processes of the mangrove leaf litter in the absence of sediment would provide a basis for understanding the relevant ecological function emanating from this interaction.

So far, we are able to establish the ecological function of the decomposing mangrove leaf litter in supporting the nutritional requirements of the shrimp post larvae. This ecological function has been derived from the biochemical attributes of the biofilm associated with the decomposing mangrove leaf litter. The nutritive importance of the decomposing mangrove leaf litter to shrimp post larvae has been contested by the seagrass ecosystem. So is it worthwhile to use decomposing mangrove leaf litter in the ecological shrimp aquaculture ponds? This question prompted a study described in **Chapter 6** which explored the **specific objective 5** which assessed the nutritive effect of the biofilm associated with the decomposing mangrove leaf litter on the physiological performance of shrimp post larvae. The use of fatty acid biomarkers in evaluating the nutritional performance, assisted in identifying the actual nutrition pathway that the mangrove litter and the associated biofilm, would impact on the growth performance of the shrimp post larvae. This study made an important revelation that would assist in understanding the extent to which mangrove decomposing leaf litter would support an ecological shrimp culture system. The mangrove litter derived food suppressed the growth and survival of shrimp post larvae due to the unavailability of DHA and linoleic acid (18:2ω6). This study revealed the specific nutrient which is deficient in the decomposing

mangrove leaf litter and the associated biofilm. This is an important revelation since it pin points the actual nutrient that require intervention in promoting ecological shrimp culture in a mangrove ecosystem. ***This observation tends to corroborate the importance of involving other ecosystems, such as seagrass beds (witnessed in chapter 3) in supplementing the nutrients which are deficient in a mangrove system.*** This observation is also important in the search for appropriate inputs to elevate the DHA and linoleic fatty acid nutrients which are in short supply. This management tool is important in a situation where the ecological shrimp culture must be performed in a zone which is heavily impacted by mangrove organic matter. Further studies on the dynamics of essential fatty acids in ecological shrimp ponds receiving different preparation methods and inputs which are hypothesised to promote dietary sources of essential fatty acid would be an important follow up to the findings from this study.

7.1.2 General conclusion and recommendation

The various studies performed in this PhD project seem to direct to the following specific recommendations.

1. Ecological shrimp aquaculture located in a mangrove zone dominated by *Rhizophora mucronata* should avoid overloading the shrimp pond with mangrove leaf litter by considering some routine removal of the leaf litter in at least 6 week intervals.

2. Ecological shrimp aquaculture located in a mangrove zone especially dominated by *Rhizophora mucronata* should consider including feed input that would supplement or enhance the supply of natural food which is rich in essential fatty acids.

3. Ecological shrimp aquaculture would perform better if located in an area which has less input of organic matter from the mangrove species of *Rhizophora mucronata* but most importantly the area should be linked to other rich food sources such as the seagrass ecosystem.

   The knowledge of how mangrove organic matter impacts on the growth and survival performance of penaeid shrimp is important in formulating effective management and conservation strategies of the mangrove related shrimp aquaculture.

   The third conclusion is based on observations of antagonizing effect of the decomposing mangrove litter on both physical and physiological performance of shrimp post larvae. This third observation is the most important lesson learnt from this study especially in the application of management and conservation of shrimp

aquaculture in a mangrove forest. However ***this observation should be approached with caution since only one species of mangrove trees was considered in this study and would not necessary apply with other mangrove species.*** In Kenya the intergrated coastal zone management (ICZM) advocates for sustainable utilization of the coastal resources which includes development of aquaculture within or adjacent to a mangrove forest. The identification of areas which can be utilized for shrimp aquaculture with minimal negative impact on the mangrove forest is paramount in realizing the food security objective of the Kenya's Vision 2030 from the coastal fishery sector.

The phase II of the VLIR-IUC-UoN Project in aquatic science, undertaken by University of Nairobi, Kenya, was used to answer a specific research question related to the search for alternatives to reduce human pressure on mangrove ecosystem as a vulnerable natural resource. The study gave a scientific prove that the mangrove ecosystem and the related biodiversity is degraded by the local community accessing the fishing sites leading to low productivity of the various sources that provide food and livelihood for the local community leading to a cycle of poverty. In view of this the Kenya Government through the Ministry of Fisheries Development initiated an Economic Stimulus Program (ESP) and Kenya Coastal Development Project (KCDP) in the coastal region focusing on marine and fresh water aquaculture and ICZM. It was anticipated that aquaculture will provide the much needed fish to improve both the community health through improved diet and their livelihood through provision of business in aquaculture farming particularly for those with small pieces of land. Development of coastal aquaculture is an ecosystem service that is envisaged to reduce fishing pressure in the environment such as the marine ecosystems. It is thus foreseen that the high degradation of the ecosystem will be reduced through provision of alternative sources of fish. Hence coastal aquaculture in Kenya must be encouraged at all means.

The aquaculture policy on the utilization of the coastal resources especially the mangrove ecosystems is at the present time not well defined and the present regulations are not based on research findings. Therefore there is no clear guideline to promote the shrimp aquaculture in the vicinity of the mangrove areas. The finding of this study highlights a scientific based justification of the redundancy in the use of

mangrove areas dominated by *Rhizophora mucronata* in developing shrimp aquaculture whereas there are alternative areas with eco-geographical potential where shrimp aquaculture can be developed without destroying the mangrove forest. Hence, using the findings of this study, it would make scientific sense to consider areas which are not necessarily populated by mangroves but which are topographically located to allow some linkage with the estuarine ecosystems. This kind of consideration would make a logical input into the aquaculture and ICZM policy in the development of shrimp aquaculture in the coastal region of Kenya.

Further investigations are required to:

1. Sustain the proliferation of diatoms and epifauna and suppress the growth of cyanobacteria in the biofilm associated with the decomposing mangrove leaf litter.

2. Assess the bio-chemical interaction of the cyanobacteria with the various processes involved in the decomposition of the mangrove leaf litter in the presence and absence of sediment.

3. Assess the dynamics of essential fatty acids in shrimp ponds associated with mangrove ecosystems especially in relation to the site selectionand choice of inputs which are hypothesised to promote supply of natural sources of essential fatty acid for penaeid shrimp post larvae.

4. Assess ecological function of mangrove ecosystem in relation to the distribution and residence of penaeid shrimp post larvae of relevance to the development of aquaculture.

BIBLIOGRAPHY

8 References

Abdulkadir, S., Tsuchiya, M., 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. J. Exp. Mar. Biol. Ecol. 354, 1-8.

Abraham, T.J., Shubhadeep Ghosh, Nagesh, T.S., Sasmal, D., 2004. Distribution of bacteria involved in nitrogen and sulphur cycles in shrimp culture systems of West Bengal, India. Aquaculture 239, 275-288.

Abreu, P.C., Fernandes da Silva, C., Ballester, E., Monserrat, J., Geracitano, L., Wasielesky JR, W., 2008. Contribution of microorganisms to the biofilm nutritional quality: protein and lipid contents. Aquacult. Nutr. 14, 507-514.

Abreu, P.C., Ballester, E.L.C., Odebrecht, C., Wasielesky, W., Cavalli, R.O., Graneli, W., Anesio, A.M., 2007. Importance of biofilm as food source for shrimp (*Farfantepenaeus paulensis*) evaluated by stable isotopes (δ13C and δ15N). J. Exp. Mar. Biol. Ecol. 347, 88-96.

Abu Hena, M.K., Hishamuddin, O., 2012. Food selection preference of different ages and sizes of black tiger shrimp, *Penaeus monodon* Fabricus, in tropical aquaculture ponds in Malaysia. Afr. J. Biotechnol. 11, 6153-6159.

Admiraal, W., Peletier, H., 1979. influence of organic compounds and light limitation on the growth rate of estuarine benthic diatoms. Br. phycol. J. 14, 197-206.

Al Azad, S., Chong, V.C., Vikineswary, S., 2002. Phototrophic Bacteria as Feed Supplement for Rearing *Penaeus monodon* Larvae. J. World Aquac. Soc. 33.

Alfaro, A.C., Thomas, F.O., Sergent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar. Coast. Shelf Sci. 70, 271-286.

Alikunhi, N.M., Narayanasamy, R., Kandasamy, K., 2010. Fatty acids in an estuarine mangrove ecosystem. Rev. Biol. Trop. 58, 577-587.

Alongi, D.M., 1987. The influence of mangrove-derived tannins on intertidal meiobenthos in tropical estuaries. Oecologia 71, 537-540.

Alongi, D.M., Johnston, D.J., Xuan, T.T., 2000. Carbon and nitrogen budgets in shrimp ponds of extensive mixed shrimp-mangrove forestry farms in the Mekong delta, Vietnam. Aquac. Res. 31, 387-399.

Amado, L.L., Monserrat, J.M., 2010. Oxidative stress generation by microcystins in aquatic animals: Why and how. Environ. Int. 36, 226-235.

Bibliography

Angsupanich, S., Chiayvareesajja, S., Chandumpai, A., 1999. Stomach contents of Banana prawns (*Penaeus indicus and P.merguiensis*) in Tammalang Bay, Southern Thailand. Asian Fish. Sci. 12, 257-265.

Ansari, Z.A., Sreepada, R.A., Motandkar, S.G.P., Perulekar, A.H., 1993. Meiofaunal stratification in relation to microbial food in tropical mangrove mud flat. Trop. Ecol. 34, 204-216.

Apud, F.D., 1988. Prawn grow-out practices in the Philippines. In: SEAFDEC (Ed.), Biology and culture of *Penaeus monodon*. Southeast Asian Fisheries Development Center (Makati), Tigbauan.

AQUACOP, Cuzon, G., 1989. Selected ingredients for shrimp feed. Advances in Tropical Aquaculture 9, 405-412.

Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Huque, S., Salam, M.A., Azim, M.E., 2008. C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. Aquaculture 280, 117-123.

Aujero, E.J., Tech, E., S, J., 1985. Nutritional values of marine yeast fed to larvae of *Penaeus monodon* in combination with algae. In: Taki, Y., Primavera, J.H., Liobrera, J.A. (Eds.), Proceeding of the first international conference on the culture of penaeid shrimp. Aquaculture Department, Southeast Asian Fisheries Development Center, Iloiol, Philippines, pp. 197-197.

Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176, 227-235.

Avnimelech, Y., Gad, R., 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220, 549-567.

Azim, M.E., Wahab, M.A., 2005a. Periphyton based pond polyculture In: Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton: Ecology, exploitation and management. CABI Publishing, Wallingford, pp. 207-222

Azim, M.E., Wahab, M.A., 2005b. Periphyton based pond polyculture. In: Azim, M.E., Verdegem, Boenigk, J., Arndt, H. (Eds.), Bacterivory by heterotrophic flagellates: community structure and feeding strategies International journal of general and molecular microbiology, pp. 465-480.

Azim, M.E., Wahad, M.A., Van Dan, A.A., Beveridge, M.C.M., Milstein, A., Verdegem, M.C.J., 2001. Optimization of fertilization rate for maximizing periphyton production

on artificial substrates and the implications for periphyton-base aquaculture. Aquac. Res. 32, 749 -760.

Azim, M.E., Wahad, M.A., Verdegem, M.C.J., Van Dan, A.A., Van Rooij, J.M., Beveridge, M.C.M., 2002. The effects of artificial substrates on freshwater pond productivity and water quality and the implications for periphyton-base aquaculture. Aquat. Living Resour. 15, 231-241.

Bailey-Brock, J.H., Moss, S.M., 1992. Penaeid taxonomy, biology and zoogeography. In: Fast, A.W., Lester, L.J. (Eds.), Marine shrimp culture: principles and practices. Developments in aquaculture and fisheries science. Elsevier Science Publisher B.V, The Netherlands, pp. 9-27.

Bell, J.D., Pollard, D.A., Burchmore, J.J., Pease, B.C., Middleton, M.J., 1986. Structure of a fish community in a temperate tidal mangrove creek in Botanu bay, New South Wales. Aust. J. Mar. Freshw. Res. 35, 33-46.

Benner, R., Hodson, R.E., 1985a. Microbial degradation of the leachable and lignocellulosic components of leaves and wood from Rhizophora mangle in a tropical mangrove swamp. Mar. Ecol. Prog. Ser. 23, 221-230.

Benner, R., Hodson, R.E., 1985b. Microbial degradation of the leachable and lignocellulosic components of leaves and wood from Rhizophora mangle in a tropical mangrove swamp. Mar. Ecol. Prog. Ser. 23, 221-230.

Boecklen, W.J., Yarnes, C.T., Cook, B.A., James, A.C., 2011. On the use of stable isotopes in Trophic Ecology. Annu. Rev. Ecol. Syst. 42, 411-440.

Bolter, M., Bloem, J., Meiners, K., Moller, R., 2002. Enumeration and biovolume determination of microbial cells: A methological review and recommendations for applications in ecological research. . Biol. Fertil. Soils. 36, 249-259.

Bombeo-Tuburan, I., Guanzon Jr, N.G., Schroeder, G.L., 1993. Production of *Penaeus monodon* (Fabricius) using four natural food types in an extensive system. Aquaculture 112, 57-65.

Bombeo, I., Guanzon, N.G., Schroeder, G.L., 1993. Production of *Penaeus monodon* (Fabricius) using four natural food types in an extensive system. Aquaculture 112, 57-65.

Borowitzka, M., 1997. Microalgae for aquaculture: Opportunities and constraints. J. Appl. Phycol. 9, 393-401.

Bibliography

Boschker, H.T.S., de Graaf, W., Koster, M., Meyer-Reil, L.A., Cappenberg, T.E., 2001. Bacterial populations and processes involved in acetate and propionate consumption in anoxic brackish sediment. Microb. Ecol. 35, 97-103.

Bosire, J.O., Dahdouh-Guebas, F., Kairo, J.G., Kazungu, J., Dehairs, F., Koedam, N., 2005. Litter degradation and CN dynamics in reforested mangrove plantations at Gazi Bay, Kenya. Biol. Conserv. 126, 287-295.

Bouillon, S., Boschker, H.T.S., 2006. Bacterial carbon sources in coastal sediments: a cross system analysis based on stable isotope data of biomarkers. Biogeosciences 3, 175-185.

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

Boyd, C.E., 1998. Pond water aeration systems. Aquacult. Eng. 18, 9-40.

Boyd, C.E., Tanner, M.E., Madkour, M., Masuda, K., 1994. Chemical characteristics of bottom soils from fresh water and brackishwater aquaculture ponds. J. World Aquac. Soc. 25, 517-534.

Bratvold, D., Browdy, C.L., 2001a. Effects of sand sediment and vertical surfaces (Aquamats) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. Aquaculture 195, 81-94.

Bratvold, D., Browdy, C.L., 2001b. Effects of sand sediment and vertical surfaces (Aquamats) on production, water quality, and microbial ecology in an intensive Litopenaeus vannamei culture system. . Aquaculture 195, 81-94.

Brito, E.F., Moulton, T.P., De Souza, M.L., Bunn, S.E., 2006. Stable isotope analysis indicates microalgae as the predominant food source of fauna in a coastal forest stream, South-east Brazil. Austral Ecol. 31, 623-633.

Brown, M., Miller, K., 1992. The ascorbic acid content of eleven species of microalgae used in mariculture. J. Appl. Phycol. 4, 205-215.

Brown, M., Farmer, C., 1994. Riboflavin content of six species of microalgae used in mariculture. J. Appl. Phycol. 6, 61-65.

Brown, M.R., Jeffrey, S.W., 1995. The amino acid and gross composition of marine diatoms potentially useful for mariculture. J. Appl. Phycol. 7, 521-527.

Burford, M.A., 1997. Phytoplankton dynamics in shrimp ponds. Aquac. Res. 28, 351-360.

Bibliography

Burford, M.A., Peterson, E.L., Baiano, J.C.F., Preston, N.P., 1998. Bacteria in shrimp pond sediments: their role in mineralizing nutrients and some suggested sampling strategies. Aquac. Res. 29, 843-849.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219, 393-411.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (Litopenaeus vannamei) nutrition in a high-intensity, zero-exchange system. Aquaculture 232, 525-537.

Busacker, G.P., Adelman, T.R., Goolish, E.M., 1990. Growth. In: Schreck, C.B., Moyle, P.B. (Eds.), Methods for Fish Biology. American Fishery Society, Bethesda, USA, pp. 363–387.

Case, M., Leca, E.E., Leitao, S.N., Sant Anna, E.E., Schwamborn, R., Travassos de Moraes Junior, A., 2008. Plankton community as an indicator of water quality in tropical shrimp culture ponds. Mar. Pollut. Bull. 56, 1343-1352.

Chen, H.Y., 1993. Recent advances in nutrition of *Penaeus monodon*. J. World Aquac. Soc. 24, 231-240.

Chen, Y.L.L., Chen, H.Y., 1992. Juvenile *Penaeus monodon* as effective zooplankton predators. Aquaculture 103, 35-44.

Chien, Y.H., 1992. Water quality requirements and management for marine shrimp culture. In: Wyban, J. (Ed.), Proceedings of the special session on shrimp farming. World Aquaculture Society, Baton Rouge, LA USA, pp. 144-156.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6. In: PRIMER-E (Ed.), User Manual/Tutorial, Plymouth.

Coman, F.E., Connolly, R.M., Preston, N.P., 2003. Zooplankton and epibenthic fauna in shrimp ponds: factors influencing assemblage dynamics. Aquac. Res. 34, 359-371.

Cottrell, M.T., Kirchmann, D.L., 2000. Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low and high molecular weight dissolved organic matter. Appl. Environ. Microbiol. 66, 1692-1697.

Coull, B.C., 1999. Role of meiofauna in estuarine soft-bottom habitats. Aust. J. Ecol. 24, 327-343.

Coutteau, P., Kontara, E.K.M., Sorgeloos, P., 1999. Comparison of phosphatidylcholine purified from soybean and marine fish roe in the diet of postlarval *Penaeus vannamei* Boone. Aquaculture 181, 331-345.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270, 1-14.

Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W., 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquac. Res. 41, 559-567.

Craig, S., Helfrich, L.A., 2002. Understanding fish nutrition, feeds and feeding. Virginia Cooperative Extension, Yorktown, Virginia, pp. 9.

Cui, J.J., Ding, M.L., Sun, W.L., Liu, H., Wand, D.B., Yu, H.R., F, S.L., 1997. The application of photosynthetic bacteria to the seed production of *Penaeus chinensis*. J. Ocean Univ. Quingdao 27, 191-194.

D'Souza, F.M.L., Kelly, G.J., 2000. Effects of a diet of a nitrogen-limited alga (*Tetraselmis suecica*) on growth, survival and biochemical composition of tiger prawn (*Penaeus semisulcatus*) larvae. Aquaculture 181, 311-329.

D' Abramo, 1989. Lipid requirements in Shrimp. Advances in tropical aquaculture, Tahiti, Feb. 20 Mar. 4 1989; AQUACOP, IFREMER. Actes de Colloque 9, 271-285.

D'Croz, L., Del Rosaria, J., Ricchardo, H., 1989. Degradation of red mangrove (*Rhizophora mangle L*) leaves in the bay of Panama. Rev. Biol. Trop. 37, 101-104.

Dall, W., Smith, D.M., Moore, L.E., 1991. Biochemical composition of some prey species of Penaeus esculentus Haswell (Penaeidae: Decapoda). Aquaculture 96, 151-166.

Dall, W., Hill, B.J., Rothlisberg, P.C., Staples, D.J., 1990. The Biology of the Penaeidae. Adv. Mar. Biol. 27, 489.

Dam, H.G., Siuda, A.N.S., 2010. Effects of omnivory and predator-prey elemental stoichiometry on planktonic trophic interactions. Limnology and Oceanography 55, 2107-2116.

Danovaro, R., Armeni, M., Dell'Anno, A., Fabiano, M., Manini, E., Marrale, D., Pusceddu, A., Vanucci, S., 2001. Small-scale distribution of bacteria, enzymatic activities, and organic matter in coastal sediments. Microb. Ecol. 42, 177-185.

Davis, D.A., Kureshy, N., 2002. Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei* Aquaculture 204, 125-143.

Dawes, C.J., 1998. Marine Botany. 2nd Edition. John Wiley and Sons Inc, New York, NY.

De Mesel, I., Derycke, S., Moens, T., Van De Gucht, K., Vincx, M., Swings, J., 2004. Top-down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. Environ. Microbiol. 6, 733-744.

De Troch, M., Fiers, F., Vincx, M., 2003. Niche segregation and habitat specialisation of harpacticoid copepods in a tropical seagrass bed. Mar. Biol. 142, 345-355.

De Troch, M., Chepurnov, V.A., Vincx, M., Olafsson, E., 2008. The effects of *Fucus vesiculosus* on the grazing of harpacticoid copepods on diatom biofilms. J. Sea Res. 60, 139-143.

De Troch, M., Cnudde, C., Willems, A., Moens, T., Vanreusel, A., 2010. Bacterial colonization on feacal pellets of Harpacticoid copepods and on their diatom food. Microb. Ecol. 60, 581-591.

Dittel, A.I., Epifanio, C.E., Cifuentes, L.A., Kirchmen, D.L., 1997. Carbon and Nitrogen sources for shrimp post larvae fed natural diets from a tropical mangrove system. Estuar. Coast. Shelf Sci. 45, 629-637.

Dittmann, S., 2001. Abundance and distribution of small infauna in mangroves of Missionary Bay, North Queensland, Australia Rev. Biol. Trop. 49, 535-544.

Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., Franson, A.H., 2005. Standard Methods for the Examination of Water and Waste Water. American Public Health Association - ALPHA-AWWA-WEF, Washington, D.C, 1368 pp.

Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257, 346-358.

Ederington, M.C., MCManus, G.B., Harvey, H.R., 1995. Trophic transfer of fatty acids, sterols and triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limno. Oceangr. 40, 860-867.

Epp, M.A., Ziemann, D.A., Schell, D.M., 2002. Carbon and nitrogen dynamics in zero-water exchange shrimp culture as indicated by stable isotope tracers. Aquac. Res. 33, 839-846.

Falk-Petersen, S., Dahl, T.M., Scott, C.L., Sargent, J.R., Gullisken, B., Kwasniewsky, S., Hop, H., Millar, R., 2002. Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. . Mar. Ecol. Prog. Ser. 227, 187-194.

FAO/NACA, 2012. Farming the Waters for People and Food. In: Subasinghe, R.P., Arthur, J.R., Bartley, D.M., De Silva, S.S., Halwart, M., Hishamunda, N., Mohan, C.V.,

Sorgeloos, P. (Eds.), Proceedings of the Global Conference on Aquaculture 2010, Phuket, Thailand, pp. 22–25

FID, 1989. Annual fisheries report for Malindi district. Kenya Fisheries Department. Ministry of Regional Development.

FID, 1999. Annual fisheries report for Malindi district. Kenya Fisheries Department, Ministry of Tourism and Wildlife.

Fitzgerald Jr., W.J., 2000. Integrated mangrove forest and aquaculture systems in Indonesia. In: Primavera, J.H., Garcia, L.M.B., Castranos, M.T., Surtida, M.B. (Eds.), Mangrove-Friendly Aquaculture. SEAFDEC, pp. 21-34.

Focken, U., Groth, A., Coloso, R.M., Becker, M., 1998. Contribution of natural food and supplemental feed to the gut content of *Penaeus monodon* Fabricus in a semi-intensive pond system in the Philippines. Aquaculture 164, 105-116.

Folken, C., Kautisky, N., Berg, H., Jansson, A., Larsson, J., Troell, M., 1998. The ecological foot print concept for sustainable seafood production- a review. Ecol. Appl. 8, 63-71.

Francoeur, S.N., Schaecher, M., Neely, R.K., Kuehn, K.A., 2006. Periphytic photosynthetic stimulation of extracellular enzyme activity in aquatic microbial communities associated with decaying typha litter. Microb. Ecol. 52, 662-669.

Franken, R.J.M., Waluto, B., Peeters, E.T.H.M., Gardeniers, J.J.P., Beijer, J.A.J., Scheffer, M., 2005. Growth of shredders on leaf litter biofilms: the effect of light intensity. FreshWater Biol. 50, 459-466.

Fry, B., 2008. Stable isotope ecology. Springer, New York, 21-39 pp.

Fry, B., Ewel, K.C., 2003. Using stable isotopes in mangrove fisheries research- a review and outlook. Isot. Environ. Healt. S. 39, 191-196.

Gallo, M.E., Porras-Alfaro, A., Odenbach, K.J., Sinsabaugh, R.L., 2009. Photoacceleration of plant litter decomposition in an arid environment. Soil Biol. Biochem. 41, 1433-1441.

Gatune, C., Vanreusel, A., Ruwa, R., Bossier, P., De troch, M., PhD Thesis Chapter 4. Effect of micro-algae and epifauna biofilm on decomposing mangrove litter on the growth and survival of shrimp post larvae of *Penaeus monodon*, Faculty of Science, Marine Biology Section. Ghent University, Ghent.

Gatune, C., Vanreusel, A., Cnudde, C., Ruwa, R., Bossier, P., De troch, M., 2012. Decomposing mangrove litter supports a microbial biofilm with potential nutritive value to penaeid shrimp post larvae. J. Exp. Mar. Biol. Ecol. 426-427, 28-38.

Gearing, J.N., 1991. The study of diet and trophic relationships through natural abundance 13C. In: Coleman, D.C., Fry, B. (Eds.), Carbon isotope techniques. Academic Press, London, pp. 201-218.

Gee, J.M., Somerfield, P.J., 1997. Do mangrove diversity and leaf litter decay promote meiofaunal diversity? J. Exp. Mar. Biol. Ecol. 218, 13-33.

GOK, 2006a. Status of the impact of the prawn fishery in Malindi-Ungwana bays: Implications for management and conservation. Fisheries Department. Ministry of Livestock and Fisheries Development, Nairobi, Kenya.

GOK, 2006b. Status and impact of the prawn fishery in Malindi-Ungwana bays, Implications for Management and Conservation. Ministry of livestock and fisheries development, Nairobi.

Gonzalez-Farias, F., Mee, L.D., 1988. Effect of humic-like substances on biodegradation rate of detritus. J. Exp. Mar. Biol. Ecol. 119, 1-13.

Granger, S., Lizumi, H., 2001. Water quality measurement methods for seagrass habitat. In: Short, F.T., Coles, R.G. (Eds.), Global seagrass research methods. Elservier Science BV, Amsterdam, pp. 402-404.

Guary, J.C., Kayama, M., Murakami, Y., Ceccaldi, H.J., 1976. The effects of a fat-free diet and compounded diets supplemented with various oils on molt, growth and fatty acid composition of prawn, *Penaeus japonicus* Bate. Aquaculture 7, 245-254.

Hai, T.N., Yakupitiyage, A., 2005. The effects of the decomposition of mangrove leaf litter on water quality, growth and survival of black tiger shrimp (*Penaeus monodon* Fabricius, 1798). Aquaculture 250, 700-712.

Hall, R.O., Meyer, J.L., 1998. The trophic significance of bacteria in a detritus-based stream food web. Ecology 79, 1995-2012.

Hamilton, L., Dixon, J., Miller, G., 1989. Mangrove: an undervalued resource of the land and the sea. Ocean Yearbook 8, 254-288.

Hargreaves, J.A., 2006. Photosynthetic suspended-growth systems in aquaculture. Aquacult. Eng. 34, 344-363.

Hari, B., Madhusoodana, K., Varghese, J.T., Schrama, J.W., Verdegem, M.C.J., 2004. Effect of carbohydrate addition on production in intensive shrimp culture systems. Aquaculture 241, 179-194.

Hari, B., Kurup, B.M., Varghese, J.T., Schrama, J.W., Verdegem, M.C.J., 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 252, 248-263.

Hepher, B., 1988. Nutrition of pond fishes. Cambridge Univ. Press, Cambridge, N.Y, USA, 388 pp.

Hernes, P.J., Benner, R., Cowie, G.L., Goni, M.A., Bergamaschi, B.A., Hedges, J.L., 2001. Tannin diagenesis in mangrove leaves from a tropical estuary; a novel molecular approach. Geochim. Cosmochim. Acta. 65, 3109-3122.

Herrera-Siveira, J.A., Ramirez-Ramirez, J., 1996. Effects of natural phenolic material (tannin) on phytoplankton growth. Limno. Oceangr. 41, 1018-1023.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook of ingredients for aquaculture feeds. Kluwer Academic Publishers, Dordrecht, the Netherlands, 624 pp.

Hill, B.J., Wassenberg, T.J., 1987. Feeding behaviour of adult tiger prawns, *Penaeus esculentus*, under laboratory conditions. Aust. J. Mar. Freshw. Res. 38, 183-190.

Jory, D.E., Cabrera, T.R., Dugger, D.M., Fegan, D., Lee, P.G., Lawrence, A.L., Jackson, A.L., McIntosh, R.P., Castaneda, J., 2001. A global review of shrimp feed management: status and perspectives. In: Browdy, C.L., Jory, D.E. (Eds.), The new wave, proceedings of the special session on sustainable shrimp culture: Aquaculture 2001. The World Aquaculture Society, Baton Rouge, USA, pp. 104-152.

Joye, S.B., Hallibaugh, J.T., 1995. influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. Science 270, 623-625.

Kairo, J.G., Abuodha, P.A.W., 2001. Human induced stress on mangrove swamps along the Kenyan coast. Hydrobiologia 458, 255-265.

Kanazawa, A., Teshima, S., Tokiwa, S., 1977. Nutritional requirements of prawn.VII. Effect of dietary lipids on growth. B. Jpn. Soc. Sci. Fish. 43, 894-856.

Kanazawa, A., Teshima, S., Endo, M., 1979. Requirements of prawn *Penaeus japonicus* for essential fatty acids, Memoirs of Faculty of Fisheries. Kagoshima University, pp. 27-33.

Kanazawa, A., Teshima, S., Endo, M., Kayama, M., 1978. Effects of eicosapentaenoic acid on growth and fatty acid composition of the prawn *Penaeus japonicus*, Memoirs of Faculty of Fisheries. Kagoshima University, pp. 35-40.

Kandil, F.E., Grace, M.H., Seigler, D.S., Cheeseman, J.M., 2004. Polyphenolic in *Rhizophora mangle* L. leaves and their changes during leaf development and senescence. Trees 18, 518-528.

Kater, B.J., Dubbeldam, M., Jaap, F., Postma, 2006. Ammonium Toxicity at High pH in a Marine Bioassay Using *Corophium volutator*. Arch. Environ. Con.Tox. 51, 347-351.

Kathiresan, K., Rajendran, N., 2007. Microbial flora associated with submerged mangrove leaf litter in India. Rev. Biol. Trop. 55, 393-400.

Kautisky, N., Folke, C., Ronnback, P., Troell, M., Beveridge, M., Primavera, J., 2001. Aquaculture. Encyclopedia of Biodiversity 1, 185-198.

Kautsky, N., Ronnback, P., Tidengren, M., Troell, M., 2000. Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture 191, 145-161.

Kautsky, N., Berg, H., Folke, C., Larsson, J., Troell, M., 1997. Ecological foot print as a means for the assessment of resource use and devolpoment limitations in shrimp and *Tilapia* aquaculture. Aquac. Res. 28, 753-766.

Kelly, J.R., Scheibling, R.E., 2012. Fatty acids as dietary tracers in benthic food webs. Mar. Ecol. Prog. Ser. 446, 1-22.

Keshavanath, P., Gangadhar, B., 2005a. Research on periphyton-based aquaculture in India. In: Azim, M.E., Verdegem, M.C.J., Van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton and ecology, exploitation and management. CABI Publishing, Wallingford, pp. 223-236.

Keshavanath, P., Gangadhar, B., 2005b. Research on periphyton-based aquaculture in India In: Azim, M.E., Verdegem, M.C.J., Van Dam, A.A., Beveridge, M.C.M. (Eds.), Periphyton and Ecology, Exploitation and Management. CABI Publishing, Wallingford, pp. 223-236.

Larsson, J., Folke, C., Kautisky, N., 1994. Ecological limitations and appropriations of ecosystem support by shrimp farming in Columbia. Environ. Manage. 18, 663-676.

Lavens, P., Sorgeloos, P., 1996. Manual on the production and use of live food for aquaculture. FAO fisheries technical paper, 361, pp. 295.

Le Bitoux, J.F., 1985. Report on analysis of shrimp culture trials, Development of Coastal Aquaculture Phase II. FAO corporate document repository KEN/80/018.

Lee, S.Y., 1999. The Effect of Mangrove Leaf Litter Enrichment on Macrobenthic Colonization of Defaunated Sandy Substrates. Estuar. Coast. Shelf Sci. 49, 703-712.

Lee, S.Y., Meziane, T., 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: Tracer transfer. J. Exp. Mar. Biol. Ecol. 336, 42-53.

Lewin, J.C., 1953. Heterotrophy in Diatoms. J. gen. Microbiol. 9, 305-313.

Liang, Y., Mai, K., 2005. Effect of growth phase on the fatty acid compositions of four species of marine diatoms. J. Ocean Univ. China 4, 157-162.

Lin, C.K., 1989. Prawn culture in Taiwan, what went wrong? World Aquaculture 20, 19-20.

Lin, Y.M., Sternberg, L.S.L., 2007. Nitrogen and Phosphorus dynamics and nutrient resorption of *Rhizophora mangle* leaves in South Florida, USA. Bull. Mar. Sci. 80, 159-169.

Lin, Y.M., Liu, J.W., Xiang, P., Lin, Z.H., Sternberg, L., Da, S.L., 2007. Tannins and nitrogen dynamics in mangrove leaves at different age and decay stages (Jiulong River Estuary, China). Hydrobiologia 583, 285-295.

Loneragan, N.R., Bunn, S.E., Kellaway, D.M., 1997. Are mangroves and seagrasses sources of organic carbon for penaeid prawns in a tropical Australian estuary? A multiple stable isotope study. Mar. Biol. 130, 289-300.

Lovett, D.L., Felder, D.L., 1990a. Ontogenetic change in digestive enzyme activity of larval and post larval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidea). Biol. Bull. 178, 144-159.

Lovett, D.L., Felder, D.L., 1990b. Ontogenetic change in enzyme distribution and midgut function in development stages of *Penaeus setiferus* (Crustacea, Decapoda, Penaeidea). Biol. Bull. 178, 160-174.

Mandal, S., Ghosh, K., 2010. Inhibitory effect of *Pistia* tannin on digestive enzymes of Indian major carps: an in vitro study. Fish Physiol. Biochem. 36, 1171-1180.

Mannan, R.M., Pakrasi, H.B., 1993. Dark heterotrophic growth conditions result in an increase in the content of photosystem II units in the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. Plant Physiol. 103, 971-977.

Marguillier, S., Van Der Velde, G., Dehairs, F., Hemminga, M.A., Rajagopal, S., 1997. Trophic relationships in an interlinked mangrove-sea grass ecosystem as traced by δ13C and δ15N. Mar. Ecol. Prog. Ser. 151, 115-121.

Mazumder, D., Saintilan, N., 2010. Mangrove Leaves are Not an Important Source of Dietary Carbon and Nitrogen for Crabs in Temperate Australian Mangroves. Wetlands 30, 375-380.

Meunpol, O., Meijing, P., Piyatiratitivorakul, S., 2005. Maturation diet based on fatty acid content for male *Penaeus monodon (Fabricius)* broodstock. Aquac. Res. 36, 1216-1225.

Mfilinge, P.L., Meziane, T., Bachok, Z., Tsuchiya, M., 2003. Fatty acids in decomposing mangrove leaves: microbial activity, decay and nutritional quality. Mar. Ecol. Prog. Ser. 265, 97-105.

Mohamed, K.S., 1996. Heterotrophic marine bacteria as supplementary feed for larval *Penaeus monodon*. Naga 19 23-26.

Mohan, P.C., Rao, R.G., Dehairs, F., 1997. Role of Godavari mangroves (India) in the production and survival of prawn larvae. Hydrobiologia 358, 317-320.

Moorthy, M.S., Altaff, K., 2002. Role of natural productivity in modified extensive shrimp pond growing *Penaeus monodon* (Penaeidea, Crustacea). Indian J. Mar. Sci. 31, 195-200.

Morales, C.E., 1987a. Carbon and nitrogen content of copepod feacal pellets: effect of food concentration and feeding behavior. Mar. Ecol. Prog. Ser. 36, 107-114.

Morales, C.E., 1987b. Carbon and nitrogen content of copepod faecal pellets: effect of food concentrations and feeding behavior. Marine Ecology Progress Series 36, 107-114.

Moriarty, J.W., 1997. The role of micro-organisms in aquaculture ponds. Aquaculture 151, 333-349.

Moss, S.M., 2004. Biotechnology- aquaculture interface. The site of maximum impact workshop. Biosecure zero-exchange systems for intensive shrimp culture: Past experiences, biocomplexity challenges, future opportunities. The oceanic Institute, Waimanalo, Hawaii, pp. 11.

Naylor, R.L., Goldberg, R.J., Primavera, J.H., Kautisky, N., Beveridge, C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017-1024.

Nga, B.T., Roijackers, R., Lürling, M., Nghia, T.T., Ut, V.N., Khoa, T.M., Scheffer, M., Peeters, E.T.H.M., 2004. Post larvae of the shrimp *Penaeus monodon* and their interaction with *Rhizophora apiculata*. Wageningen University, Netherlands.

Nix, J., Ingols, R., 1981. Oxidized manganese from hypolimnetic water as a possible cause of trout mortality in hatcheries. Prog. Fish-Cult. 43, 32-36.

NRC, 2011. Nutrient requirements of fish and shrimp. National Research Council. The National Academic Press, Washington, D.C, 376 pp.

Nunes, A.J.P., Parsons, G.J., 2000. Effects of the Southern brown shrimp, *Penaeus subtilis*, predation and artificial feeding on the population dynamics of benthic polychaetes in tropical pond enclosures. Aquaculture 183, 125-147.

O'Brien, C.J., 1994. Population dynamics of juvenile tiger prawns, *Penaeus esculentus*, in South Queensland, Australia. Mar. Ecol. Prog. Ser. 104, 247-256.

Ochoa- Solano, J.L., Olmos-Soto, J., 2006. The functional property of *Bacillus* for shrimp feeds. Food Microbiol. 23, 519-525.

Otoshi, C.A., Montgomery, A.D., Matsuda, E.M., Moss, S.M., 2006. Effects of Artificial Substrate and water Source on Growth of Juvenile Pacific White Shrimp, *Litopenaeus vannamei*. J. World Aquac. Soc. 37, 210-213.

Ouisse, V., Riera, P., Migné, A., Leroux, C., Davoult, D., 2012. Food web analysis in intertidal *Zostera marina* and *Zostera noltii* communities in winter and summer Mar. Biol. 159, 165-175.

Parker, P.L., Anderson, R.K., Lawrence, A.L., 1989. A $\delta^{13}C$ and $\delta^{15}N$ tracer study of nutrition in Aquaculture: Penaeus vannamei in a pond grow-out system. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds.), Stable Isotopes ion Ecological Research. Springer-Verlag, New York, pp. 288-303.

Parker, P.L., Anderson, R.K., Lawrence, A.L., 1991. Stable isotope methodology for evaluation of nutritional requirements of shrimp. In: De Loach, P.F., Dougherty, W.J., Davidson, M.A. (Eds.), Frontiers of shrimp research. Elsevier, Amsterdam, pp. 157-171.

Parnell, A., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning Using stable isotopes: coping with too much variation. PlosOne 5, 72-96.

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in Aquatic Ecosystems. Springer, Dordrecht, pp. 309-326.

Parrish, C.C., French, V.M., Whiticar, M.J., 2012. Lipid class and fatty acid composition of copepods (*Calanus finmarchicus, C. glacialis, Pseudocalanus sp., Tisbe furcata* and *Nitokra lacustris*) fed various combinations of autotrophic and heterotrophic protists. J. Plankton Res. 34, 356-375.

Parrish, C.C., Abrajano, T.A., Budge, S.M., Helleur, R.J., Hudson, E.D., Pulchan, K., Ramos, C., 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky, P. (Ed.), The handbook of environmental chemistry, Part D, Marine Chemistry. Springer, Berlin, Heidelberg, pp. 193-233.

Pascal, P., Dupuy, C., Richard, P., Rzeznik-Orignac, J., Niquil, N., 2008. Bacterivory of a mudflat nematode community under different environmental conditions. Mar. Biol. 154, 671-682.

Phillips, M.J., Kwei Lin, C., Beveridge, M.C.M., 1993. Shrimp culture and the environment: Lessons from the world's most rapidly expanding warmwater aquaculture sector. In: Pullin, R.S.V., Rosenthal, H., Maclean, J.I. (Eds.), Environmental and aquaculture in developing countries. International centre for living aquatic resources management, Eschborn, Federal Republic of Germany, pp. 171-197.

Preston, N.P., Burford, M.A., Stenzel, D.J., 1998. Effects of *Trichodesmium spp.* blooms on penaeid prawn larvae. Mar. Biol. 131, 671-679.

Preston, N.P., Burford, M.A., Coman, F.E., Rothlisberg, P.C., 1992. Natural diet of larval *Penaeus merguiensis* (Decapoda: Penaeidae) and its effect on survival. Mar. Biol. 113, 181-191.

Primavera, J.H., 1996. Stable carbon and nitrogen isotope ratios of penaeid juveniles and primary producers in a riverine mangrove in Guimaras, Philippines. Bull. Mar. Sci. 58, 675-683.

Primavera, J.H., 1998. Tropical shrimp farming and its sustainability. In: De Silva, S. (Ed.), Tropical Mariculture. Academic Press, New York, pp. 257-289.

Primavera, J.H., 2006. Overcoming the impacts of aquaculture on the coastal zone. Ocean Coast. Manage. 49, 531-545.

Primavera, J.H., Lavilla-Pitogo, C.R., Ladja, J.M., dela Pena, M.R., 1993. A survey of chemical and biological products used in intensive prawn farms in the Philippines. Mar. Pollut. Bull. 26 35-40.

Rajendran, N., Kathiresan, K., 2000a. Biochemical changes in decomposing leaves of mangroves. Chem. Ecol. 17, 91-102.

Rajendran, N., Kathiresan, K., 2000b. Biochemical changes in decomposing leaves of mangroves. Chem. Ecol. 17, 91-102.

Rajendran, N., Kathiresan, K., 2004. How to increase juvenile shrimps in mangrove waters. Wetlands Ecol. Manage. 12, 179-188.

Rajendran, N., Kathiresan, K., 2006. Microbial flora associated with submerged mangrove leaf litter in India. Rev. Biol. Trop. 55, 393-400.

Rajendran, N., Matsuda, O., Imamura, N., Urushigwa, Y., 1995. Microbial community structure analysis of euxinic sediments using phospholipids fatty acids biomarkers. J. Oceanogr. 51, 21-38.

Rasowo, J., 1992. Mariculture development in Kenya: Alternatives to siting ponds in the mangrove ecosystem. Hydrobiologia 247, 209-214.

Read, G.H.L., 1981. The response of *Penaeus indicus* (Crustacean : Penaeid) to purified and compounded field of varying fatty acid composition. Aquaculture 24, 245-256.

Reddy, K.R., Feijtel, T.C., Patrick Jr, W.H., 1986. effect of soil redox conditions on microbial oxidation of organic matter. In: Chen, Y., Avnimelech, Y. (Eds.), The Role of Organic Matter in Modern Agriculture. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp. 117-156.

Renaud, S.M., Zhou, H.C., Parry, D.L., Thinh, L.-V., Woo, K.C., 1995. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone T.ISO). J. Appl. Phycol. 7, 595-602.

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S., Menasveta, P., 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture 167, 301-313.

Ribeiro, F.A.L.T., Jones, D.A., 2000. Growth and ontogenetic change in the activities of digestive enzymes in *Fennero Penaeus indicus* postlarvae. Aquacult. Nutr. 6, 53-64.

Ronnback, P., 2001. Shrimp aquaculture-State of the art. Swedish EIA Centre, Report 1. Swedish University of Agricultural Sciences, Uppsala.

Ronnback, P., Macia, A., Almqvist, G., Schultz, L., Troell, M., 2002. Do Penaeid shrimps have a preference for mangrove habitats? Distribution pattern analysis for on Inhaca island, Mozambique. Estuar. Coast. Shelf Sci. 55 427-436.

Rothlisberg, P.C., 1998. Aspects of Penaeid biology and ecology relevance to aquaculture: a review. Aquaculture 164, 49-65.

Sandifer, P.A., Joseph, J.D., 1976. Growth responses and fatty acid composition of juvenile prawns (Macrobrachium rosenbergir) fed a prepared ration augmented with shrimp head oil. Aquaculture 8, 128-138.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry 30, 3875-3883.

Schneider, O., Sereti, V., Eding, E.H., Verreth, J.A.J., 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. Aquacult. Eng. 32, 379-401.

Schrader, K.K., de Regt, M.Q., Tidwell, P.R., Tucker, C.S., Duke, S.O., 1998a. Selective Growth Inhibition of the Musty-Odor Producing Cyanobacterium *Oscillatoria* cf. *chalybea* by Natural Compounds. Bull. Environ. Contam. Toxicol. 60, 651-658.

Schrader, K.K., de Regt, M.Q., Tidwell, P.D., Tucker, C.S., Duke, S.O., 1998b. Compounds with selective toxicity towards the off-flavor metabolite-producing cyanobacterium *Oscillatoria* cf. *chalybea*. Aquaculture 163, 85-99.

Schryver, P.D., Crab, R., Defoirdt, T., Boon, N., Verstraete, W., 2008. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture 277, 125-137.

Schwamborn, R., Ekau, W., Voss, M., Saint-Paul, U., 2002. How important are mangroves as a carbon source for decapods crustacean larvae in a tropical estuary. Mar. Ecol. Prog. Ser. 229, 195-205.

Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J., p.623.

Shi-Yen, S., 1998. Nutrient requirements of penaeid shrimps. Aquaculture 164, 77-93.

Smetacek, V.S., 1985. Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. Mar. Biol. 84, 239-251.

Smith, J.L., Boyer, G.L., Zimba, P.V., 2008. A review of cyanobacterial odorous and bioactive metabolites: Impacts and management alternatives in aquaculture. Aquaculture 280, 5-20.

Somerfield, P.J., Gee, J.M., Aryuthaka, C., 1998. Meiofaunal communities in a malaysian mangrove forest. J. Mar. Biol. Ass. U.K 78, 717-732.

Sorgeloos, P., Lavens, P., 2000. Experiences on importance of diet for Shrimp post larvae quality. Aquaculture 191, 169-176.

Spalding, M., Blasco, F., Field, C., 1997. World Mangrove Atlas, Okinawa International Society for Mangrove Ecosystems, 178 pp.

Spalding, M., Kainuma, M., Collins, L., 2010. World atlas of mangroves. Earthscan, London, 319 pp.

Spellerberg, I.F., Fedor, P.J., 2003. A tribute to Claude Shannon (1916-2001) and a plea for more rigorous use of species richness, species diversity and the ' Shannon-Wiener' Index. Global Ecology and Biogeography 12, 177-179.

Spotte, S., Adams, G., 1983. Estimation of the allowable upper limit of ammonia in saline waters. Mar. Ecol. Prog. Ser. 10, 207-210.

Stonner, A.W., Zimmerman, R.J., 1988. Food pathways associated with penaeid shrimps in a mangrove fringed estuary. Fish. B-NOAA 86, 543-551.

Tacon, A.C.G., 1998. In: Fraser, S. (Ed.), International Aquafeed Directory Turret, Middlesex, UK, pp. 5-37.

Tacon, A.G.J., 1996a. Feed formulation and evaluation for semi-intensive culture of fishes and shrimps in the tropics. In: Santiago, C.B., Coloso, R.M., Millamena, O.M., Borlongan, I.G. (Eds.), Feeds for Small-Scale Aquaculture. Proceedings of the National Seminar-Workshop on Fish Nutrition and Feeds. SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 29-43.

Tacon, A.G.J., 1996b. Feeding tomorrow's fish. World Aquacult. 27, 20-32.

Taylor, J., Parkes, R.J., 1983. The cellular fatty acids of the sulphate-reducing bacteria *Desulfobacter* sp., *Desulfobulbus* sp. and *Desulfovibrio desulfuricans*. J. gen. Microbiol. 129, 3303-3309.

Temmerman, R., Scheirlinck, I., Huys, G., Swings, J., 2003. Culture-Independent analysis of probiotic products by denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 69, 220-226.

Thompson, F., P, Abreu, P.C., Wasielesky, W., 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203, 263-278.

Tremblay, L., Benner, R., 2006. Microbial contributions to N-immobilization and organic matter preservation in decaying plant detritus. Geochim. Cosmochim. Acta. 70, 133-146.

Tsuchiya, M., Mfilinge, P.L., Meziane, T., Bachok, Z., 2005. Total lipids and fatty acid classes in decomposing mangrove leaves of *Bruguira gymnorrhiza* and *Kandelia candel*: Significance with respect to lipid input. J. Oceanogr. 61, 613-622.

Twilley, R.R., Pelegri, S.P., 1998. Heterotrophic nitrogen fixation (acetylene reduction) dering leaf-litter decomposition of two mangrove species from South Florida, USA. Mar. Biol. 131, 53-61.

Van Hoorde, K., Verstraete, T., Vandamme, P., Huys, G., 2008. Diversity of lactic acid bacteria in two Flemish artisan raw milk Gauda-type cheeses. Food Microbiol. 25, 929-935.

Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in d15N and d13C trophic fractionation: Implications for aquatic food web studies

Limno. Oceangr. 46, 2061-2066.

Vogt, G., Starch, V., Quinitio, E.T., Pascual, F.P., 1985. Midgut glad as monitor organ for the nutritional value of diets in *Penaeus monodon* (Decapoda). Aquaculture 48, 1-12.

Wakwabi, E.O., Jaccarini, V., 1993. The distribution and abundance of planktonic penaeid larvae in Tudor creek, Mombasa, Kenya. Hydrobiologia 264, 185-192.

Watanabe, T., 2002. Strategies for further development of aquatic feeds. Fisheries Sci. 68, 242-252.

Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish. A review. Aquaculture 34, 115-143.

Wilson, J.G., 2002. Productivity, fisheries and aquaculture in temperate estuaries. Estuar. Coast. Shelf Sci. 55, 953-967.

Woitchik, A.F., Ohawa, B., Kazungu, J.M., Rao, R.G., Goeyens, L., Dehairs, F., 1997. Nitrogen enrichment during decomposition of mangrove leaf litter in an East African coastal lagoon (Kenya): Relative importance of biological nitrogen fixation. Biochemistry 39, 15-35.

Wouters, R., Lavens, P., Nieto, J., Sorgeloos, P., 2001. Penaeid shrimp nutrition. An updated review on research and development. Aquaculture 202, 1-21.

YiMing, L., Sternberg, L., 2007. Nitrogen and Phosphorous dynamics and nutrient resorption of *Rhizophora mangle* leaves in South Florida, USA. Bull. Mar. Sci. 80, 159-169.

Ying, L., Kang-sen, M., Shi-chun, S., 2000. Total lipid and fatty acid composition of eight strains of marine diatoms. Chin. J. Oceanol. Limn. 18, 345-349.

9 Appendices

9.1 **Appendix 1:** Univariate tests of significance for the percentage weight loss of the decomposing leaf litter relative to the week, light and sediment conditions.

	SS	Degr. of Freedom	MS	F	p
week	3622.42	3	1207.47	52.799	0.000000
Light	147.40	1	147.40	6.445	0.016186
sediment	790.56	1	790.56	34.569	0.000002
week*Light	100.09	3	33.36	1.459	0.244208
week*sediment	260.77	3	86.92	3.801	0.019438
Light*sediment	897.62	1	897.62	39.250	0.000001
week*Light*sediment	414.94	3	138.31	6.048	0.002206
Error	731.82	32	22.87		

9.2 **Appendix 2:** Univariate tests of significance for the log transformed total abundance of micro-algae on the decomposing mangrove leaf litter relative to the week, light and sediment conditions.

	SS	Degr. of	MS	F	p
Intercept	740.7864	1	740.7864	2561.433	0
Week	15.5831	3	5.1944	17.961	0.000001
Light	6.3	1	6.3	21.784	0.000052
Sediment	2.1161	1	2.1161	7.317	0.010855
Week*Light	6.9072	3	2.3024	7.961	0.000419
Week*Sediment	7.3785	3	2.4595	8.504	0.000269
Light*Sediment	2.3889	1	2.3889	8.26	0.007145
Week*Light*Sediment	8.6162	3	2.8721	9.931	0.000088
Error	9.2546	32	0.2892		

9.3 **Appendix 3:** Univariate tests of significance for the log transformed abundance of Diatoms on the decomposing mangrove leaf litter relative to the week, light and sediment conditions.

	SS	Degr. of	MS	F	p
Week	1.9076	3	0.6359	4.064	0.014862
Light	0.5048	1	0.5048	3.227	0.081897
Sediment	0.371	1	0.371	2.371	0.133443
Week*Light	0.2371	3	0.079	0.505	0.681466
Week*Sediment	0.2556	3	0.0852	0.544	0.655417
Light*Sediment	0.0246	1	0.0246	0.157	0.694153
Week*Light*Sediment	0.4493	3	0.1498	0.957	0.424771
Error	5.0068	32	0.1565		

REPUBLIC OF KENYA