



FEATURE ARTICLE

# Sunlight and sediment improve the environment of a litter biofilm-based shrimp culture system

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**ABSTRACT:** In silvofishery, where shrimp culture is integrated with mangrove trees, the mangrove leaf litter may modify the environment in these culture systems. This study tested the potential of submerged leaf litter of *Rhizophora mucronata* and associated biofilm in providing a favorable environment for post-larval shrimp *Penaeus monodon*. Litter decomposition and assembly of microalgae and epifauna were assessed under exposure to sunlight or shade, and presence or absence of sediment. Litter incubated with sediment and exposed to sunlight was rapidly decomposed and supported the highest biomass and diversity of microalgae and epifauna. The litter also supported the highest abundance of diatoms, polychaetes and nematodes during the 4<sup>th</sup> week of decomposition. *Cyanobacteria* of the genus *Microcystis* dominated litter incubated without sediment, in sunlight, after decomposition for 5 wk. Under shaded conditions, diatoms of the genus *Navicula* and the *Cyanobacteria Anabaena* spp. and *Oscillatoria* spp. continued to grow at high total ammonium nitrogen, low dissolved oxygen, low temperature and low pH. Our study illustrates synergy between sediment and direct sunlight in promoting diversity of microalgae and polychaetes (of dietary benefit to shrimps), inhibiting growth of *Cyanobacteria* and maintaining water quality at levels favorable to culture of post-larval shrimp. Our findings support 4 practices for a healthy environment in fish ponds: (1) locating ecological shrimp culture in less forested areas, (2) promoting sediment conditions in artificial shrimp culture systems, (3) exposing litter-derived biofilm within ponds to sunlight and incubating with sediment to maintain favorable water quality and control *Cyanobacteria* blooms, and (4) minimizing the use of pond liners and related sludge removal.

**KEY WORDS:** Shrimp culture · Sunlight · Sediment · Mangrove litter · Microalgae · Epifauna · Biofilm



Modified shrimp pond in a mangrove mudflat at Mtwapa creek, Kenya coast, equipped with natural sediment and exposed to sunlight

Photo credit: C. Gatune, Karatina University, Kenya

## INTRODUCTION

The importance of decomposing mangrove leaf litter in providing natural food to post-larvae of penaeid shrimps lies mainly in the associated periphytic biofilm (Gatune et al. 2012). The biofilm consists of phyto- and zoobiota which is mediated by a microbial loop (Burford et al. 2003, Azim & Wahab 2005, Pascal et al. 2008). Our previous studies revealed that the biofilm developing on decomposing litter possesses potential and direct nutritive value to post-larval shrimp (Gatune et al. 2012, 2014a, 2014b). These studies also identified strategic intervention timelines in managing mangrove litter falling into silvo-shrimp aquaculture systems. Silvo-aquaculture is an ecological shrimp culture system where the shrimp diet is naturally derived from a mangrove

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ecosystem (Primavera 1998). Ecological shrimp aquaculture in mangrove systems has gained environmental support because of its tendency to conserve the mangrove forest (Primavera 1998, Fitzgerald 2000) by rearing fish without clearing mangrove trees (Primavera 1998). This integrated approach implies that the shrimp culture system constantly intercepts mangrove litter fall, which proceeds to decompose under the influence of the microbiota present in the biofilm (Benner & Hodson 1985, Rajendran & Kathiresan 2007). The mangrove trees also shade the shrimp culture system from direct sunlight either partially or completely. Since mangrove leaf litter falling into the water eventually sinks to the bottom layers, the microalgae accumulating at the water surface or other suspended particles in the water column further shield the litter from direct sunlight. 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 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 needs to reduce aquaculture activities in mangrove systems, shrimp culture practices are being pushed further inland. However, shrimp aquaculture further inland lacks the natural services from mangrove-based estuarine ecosystems (Kautsky et al. 2001). This has caused an increase in shrimp culture systems that have adopted artificial substrates to mimic nature in promoting proliferation of natural food sources. One example is the use of leaf litter to promote proliferation of natural biota for maintaining water quality (Bratvold & Browdy 2001, Otoshi et al. 2006, Asaduzzaman et al. 2008). Hypothetically, if the artificial substrate used is decomposing mangrove leaf litter, the growth and composition of biofilm on the decomposing leaf litter substrate may depend on the extent of exposure to sunlight and sediment.

It is essential that ecological shrimp culture systems, whether in mangroves or further inland, support phyto- and zoobiota for maintaining water quality and providing a quality food source for post-larval shrimp (Burford 1997, Bratvold & Browdy 2001, Thompson et al. 2002). Thus it is important to assess the potential of decomposing mangrove leaf litter to support biofilm contributing to the natural

diet of post-larval shrimp when the environmental conditions are modified. In this study, we hypothesized that water quality and the value of the natural food provided by the biofilm associated with decomposing mangrove leaf litter depends on exposure to direct sunlight and sediment. Environmental factors investigated included light intensity, water transparency, temperature, dissolved oxygen, pH, salinity and total ammonium nitrogen (TAN). The assembly of the major taxa of microalgae and invertebrate fauna colonizing the decomposing mangrove leaf litter of *Rhizophora mucronata* was also investigated. Once isolated and identified, the potential application of the various microalgae and fauna in providing a natural diet to post-larval shrimp *Penaeus monodon* was assessed by comparing with the natural food types commonly used in shrimp aquaculture.

## MATERIALS AND METHODS

### Study site

The study was carried out in an integrated mangrove and shrimp culture farm at Mtwapa Creek, northern coastal region of Kenya (3° 57' S, 39° 42' E). The project area is characterized by a reforested mangrove forest dominated by *Rhizophora mucronata*.

### Experimental set-up

Senescent mangrove leaf litter (hereinafter mangrove leaf litter), which had just turned yellow-brown and dropped from the trees, were carefully selected to include leaf litter of similar surface area. The leaf litter was then incubated (see 'Litter decomposition' for details) in pond water inoculated into circular mesocosms, i.e. open-top 200 l tanks of 0.75 m water depth, at a litter loading density not exceeding 1 g l<sup>-1</sup> (Hai & Yakupitiyage 2005). Tank water was slowly replenished (to avoid dislodging the biofilm) daily at a rate of 25%. The tanks were not stocked with shrimps, to prevent grazing on biofilm, and were set to provide 4 treatments, with each treatment consisting of 3 replicates as follows: direct sunlight with sediment (LS), direct sunlight without sediment (LX), shaded with sediment (DS) and shaded without sediment (DX). The shaded tanks were placed under the canopy of a thick mangrove forest to prevent exposure to direct sunlight. The sunlit tanks were placed in an area without mangrove trees with direct exposure to sunlight but in close proximity to the shaded

tanks. The sediment treatments consisted of a 5 cm layer of sediment obtained from the bottom of a pond which had previously been used to culture shrimps. The sediment was not given any special treatment to exclude natural microbiota.

### Litter decomposition

Senescent mangrove leaf litter was dried in the shade to a constant weight and incubated by suspending it 3 cm above the bottom of the tank for a period of 7 wk. Leaves were suspended vertically and above the sediment or bottom to allow an all-round uniform development of biofilm as the leaf litter decomposed. Three decomposing leaves, which had previously been marked and initial weight taken, were sampled weekly and pooled from each replicate tank. Biofilm on the sampled leaf litter was gently washed off with distilled water and pooled. The biofilm and the decomposing leaves were oven-dried at 70°C to a constant weight and weighed. Litter decomposition was recorded as percentage weight loss of the pooled leaves after removal of the biofilm as follows:

$$\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (1)$$

### Microalgae abundance and taxa composition

Mangrove leaf litter was sampled from each tank weekly in triplicates by pooling 3 decomposing leaves per replicate. 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). Microalgae were classified and counted in the laboratory by first diluting each replicate 5–10 times. Five sub-replicates of 0.02 ml were then examined under an inverted microscope.

### Epifauna abundance and taxa composition

Epifauna included both meiofauna (metazoans that can pass through a 1 mm sieve and are retained on a 38 µm sieve) and macrofauna (organisms retained on 1 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 1 mm and 38 µm mesh size sieves. Sieved fauna was gently washed from the 38 µm sieve with a soft spray of filtered fresh water, 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 densities were standardized towards leaf surface (i.e. per cm<sup>2</sup>).

### Species diversity and evenness of main microalgae and epifauna groups

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

$$H' = - \sum_{i=1}^n P_i \cdot \ln P_i \quad (2)$$

$$EH = \frac{H'}{\ln S} \quad (3)$$

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

### Microalgae 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 leaves with a known volume of filtered seawater and were GF/F filtered (0.45 µm mesh, 47 mm diameter). Phytopigments were extracted from the collected biofilm after adding 10 ml 90% acetone to the lyophilized GF/F filters at 4°C in the dark and the supernatant was analyzed for chlorophyll *a* according to the protocol of Granger & Lizumi (2001).

## Environmental factors

Light intensity and water transparency were recorded daily at noon, using a Hanna HI97500 lux meter and Secchi disk. The quality of the mid-water was monitored by weekly measurements of temperature, dissolved oxygen, pH, salinity and TAN. Temperature, dissolved oxygen and pH were measured using meters, salinity was measured using a refractometer, and TAN was analyzed in the laboratory according to Eaton et al. (2005). Un-ionized ammonia ( $\text{NH}_3$ ) toxicity was assessed using the TAN ( $\text{NH}_4\text{-N}$ ) to ammonia-nitrogen ( $\text{NH}_3\text{-N}$ ) (pH, temperature and salinity-thermodynamic dependent) ratio as described by Spotte & Adams (1983).

## Data analysis

Repeated-measures ANOVA was performed with the software Statistica 7. Time was the repeated measure, with sunlight and sediment treatments as fixed factors. This statistical method was selected because leaf litter and water quality parameters were repeatedly sampled from the same tanks at each sampling time. Therefore the sampling dates were dependent on each other. All data were checked for normality and variance homogeneity requirements for parametric analysis. Data that did not meet normality requirements after being transformed were analyzed nonparametrically following Kruskal-Wallis ANOVA and median test. Multidimensional scaling and 2-way ANOSIM using Primer 6.0 software (Clarke & Gorley 2006) were used to compare similarity in species distribution. The Shannon-Wiener index was used to estimate the diversity and evenness of the community of the microalgae and epifauna colonizing the decomposing mangrove litter at the different conditions of sunlight and sediment.

## RESULTS

### Litter decomposition

Mangrove leaf litter incubated with sediment and exposed to direct sunlight recorded the highest percentage weight loss (mean  $\pm$  SD:  $70.3 \pm 2.3\%$ ) during the 7<sup>th</sup> week of decomposition, and was significantly more decomposed compared to litter incubated without sediment and in the

shade (Tukey's HSD,  $p < 0.01$ ) (Fig. 1). Litter incubated in sunlight without sediment recorded the lowest mean ( $\pm$ SD) weight loss ( $42.5 \pm 4.2\%$ ), and did not significantly differ from litter incubated in the shade, independent of the presence or absence of sediment (Tukey's HSD,  $p > 0.05$ ).

### Microalgae biomass

Decomposing mangrove leaf litter exposed to sunlight supported significantly higher biomass of microalgae compared to litter decomposing in the shade (Tukey's HSD,  $p < 0.001$ ). Compared to sediment, the light effect was the determining factor ( $p < 0.05$ ), and the disparity between the sunlit and shaded treatments during the 4<sup>th</sup> week of leaf litter decomposition greatly influenced the statistical results (sunlit:  $92.82 \pm 61.53 \mu\text{g l}^{-1}$  chl *a*,  $N = 3$ ; shaded:  $2.98 \pm 1.38 \mu\text{g l}^{-1}$  chl *a*,  $N = 3$ ; mean  $\pm$  SD) (Fig. 2).

### Microalgae taxonomic composition

Nine major taxa of microalgae were identified as abundant in the periphytic biofilm present on decomposing mangrove leaf litter: diatoms, *Cyanobacteria*, dinoflagellates, Zygnemophyceae, Chlorophyceae, Chrysophyceae, flagellates, Euglenozoa and Cocolithales. Diatoms dominated the microalgae community on leaf litter decomposed for a period of  $< 6$  wk, especially during the 4<sup>th</sup> week when the highest average ( $\pm$ SD) abundance of  $1.5 \times 10^4 \pm 7.3 \times 10^3$  cells  $\text{ml}^{-1}$  was recorded in leaf litter decomposing under direct sunlight in the presence of sediment (Fig. 3).

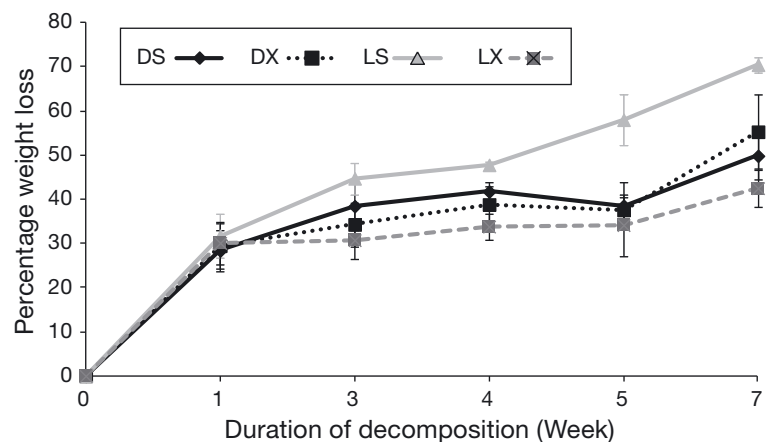


Fig. 1. Decomposition of mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX). Error bars represent SD

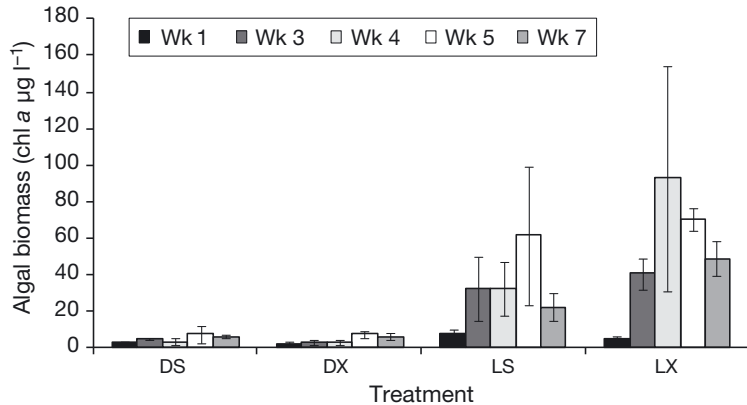


Fig. 2. Biomass of microalgae in biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different time intervals and different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX). Error bars represent SD

Leaf litter decomposing under sunlight in absence of sediment supported the highest abundance of *Cyanobacteria* ( $3.3 \times 10^8 \pm 2.8 \times 10^8$  cells ml<sup>-1</sup>, mean  $\pm$  SE) during the 7<sup>th</sup> week of decomposition. However, the abundance of microalgae on leaf litter decomposed for <6 wk was not significantly different in all treatments ( $p > 0.05$ ), except for the treatment exposed to sunlight in the absence of sediment, which differed significantly during the 7<sup>th</sup> week of decomposition (Tukey's HSD,  $p < 0.01$ ).

### Microalgae species diversity

Among the 9 major taxa of microalgae, 23 species were identified, including 6 diatom species, 5 *Cyanobacteria* species, 5 dinoflagellate species, 1 Zygnemophyceae species, 3 Chlorophyceae species, 1 Chrysochlorophyceae species, 1 flagellate species and 1 Coccolithales species. The diatoms *Navicula* sp. and *Pleurosigma* sp. were the most predominant species. *Navicula* sp. dominated litter decomposing in the shade, with a proportion of 85% and 79% in the presence and absence of sediment, respectively. Among the *Cyanobacteria*, *Microcystis* sp. dominated the decomposing litter exposed to sunlight in the absence of sediment, reaching a proportional cover of 99.9% during the 7<sup>th</sup> week. Also among *Cyanobacteria*, *Anabaena* sp. dominated the litter incubated in the shade without sediment, while *Oscillatoria* sp. was dominant in litter incubated in the shade in the presence of sediment. The highest microalgae diversity was recorded in decomposing mangrove litter exposed to sunlight in the presence of sediment ( $H' = 1.08$ ), concomitantly with the high-

est species evenness (EH = 0.092) (Table 1). The microalgae community differed significantly between all treatments (2-way crossed ANOSIM: light and sediment,  $R = 0.362$ ,  $p = 0.002$ ; and week,  $R = 0.414$ ,  $p = 0.001$ ), especially due to the light effect (2-way crossed ANOSIM:  $R = 0.463$ ,  $p = 0.003$ ) (Fig. 4a).

### Epifauna abundance and diversity

Eighteen major taxa of epifauna 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 decomposing mangrove leaf litter differed significantly between different treatments across the week groups, especially due to the effect of light (2-way crossed ANOSIM:  $R = 0.426$ – $0.704$ ,  $p = 0.001$ ) (Fig. 4b). Decomposing mangrove litter exposed to sunlight supported significantly higher abundance of epifauna compared to litter incubated in the shade ( $p = 0.002$ ). The abundance of epifauna in the shade increased after the 4<sup>th</sup> week of litter decomposition. During Weeks 5 and 7, decomposing litter exposed to sunlight in the presence of sediment supported the highest mean abundance of epifauna ( $2.2 \pm 1.1$  ind. cm<sup>-2</sup>, mean  $\pm$  SD), which significantly differed (Tukey's HSD,  $p < 0.05$ ) from litter incubated in the shade, especially with sediment, which supported the lowest abundance of epifauna ( $0.4 \pm 0.3$  ind. cm<sup>-2</sup>) (Fig. 5).

Rotifera and Copepoda dominated the shaded mangrove litter decomposing in the absence of sediment, with a proportion of 34% and 55%, respectively whereas Polychaeta preferred conditions receiving direct sunlight. Nematoda preferred conditions with sediment. Litter decomposing in the presence of sediment supported the highest diversity and an evenly distributed assembly of the different classes of epifauna, regardless of light conditions (Table 2).

### Environmental factors

A significantly higher light intensity was recorded for the sunlight treatments ( $97\,053 \pm 7093$  lux, mean  $\pm$  SD) than the shaded treatments ( $2224 \pm 703$  lux) ( $p < 0.05$ ). All treatments recorded water transparency of

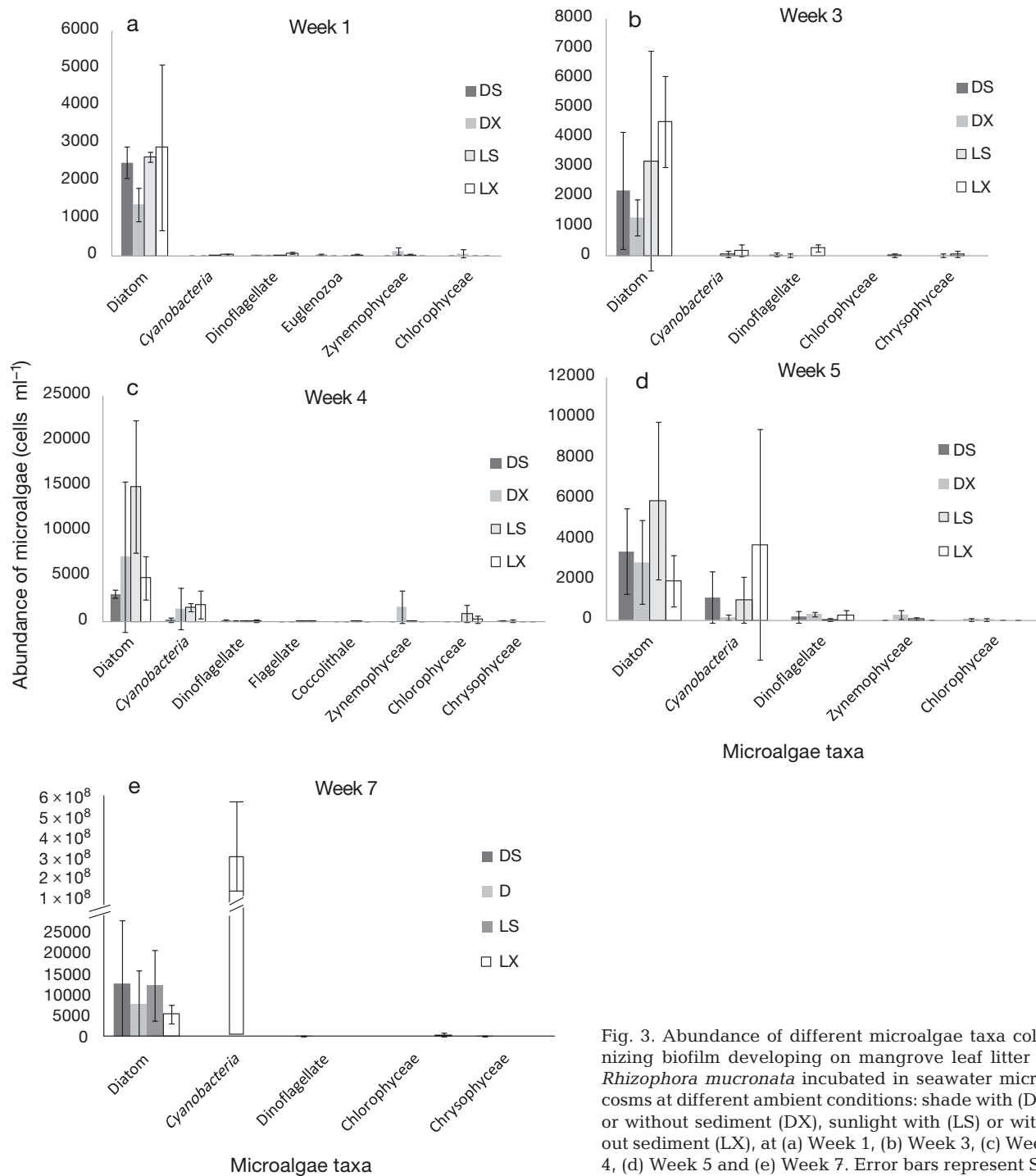


Fig. 3. Abundance of different microalgae taxa colonizing biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX), at (a) Week 1, (b) Week 3, (c) Week 4, (d) Week 5 and (e) Week 7. Error bars represent SD

not less than the set-up water depth of 0.75 m. The water in the tanks supporting decomposing mangrove litter exposed to sunlight had high dissolved oxygen, temperature and pH, but low levels of TAN. The concentration of dissolved oxygen in the sunlight treatments ranged between  $6.5 \pm 0.8$  and  $3.0 \pm 0.5$  mg l<sup>-1</sup> (mean  $\pm$  SD), whereas it was significantly lower ( $p <$

0.05) in the shade treatments, ranging between  $2.9 \pm 0.4$  and  $0.2 \pm 0.1$  mg l<sup>-1</sup> (Fig. 6a). Although there was no statistical difference in terms of temperature ( $p > 0.05$ ) and pH ( $p > 0.05$ ) between the sunlight and shade treatments, temperature and pH were generally low in the shade treatments (Fig. 6b,c). The lowest pH ( $5.4 \pm 0.1$ ) was recorded in the shaded tanks without

Table 1. Proportional assemblage of microalgae in biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX); and species diversity and evenness. Microalgae data are percentages calculated from all weeks pooled together

		DS	DX	LS	LX
<b>Microalgae taxa</b>					
Diatoms	<i>Guinardia</i> sp.	0.0	0.0	0.0	$3 \times 10^{-5}$
	<i>Nitzschia</i> sp.	0.0	0.04	0.0	$3 \times 10^{-5}$
	<i>Navicula</i> sp.	84.95	78.57	65.87	0.004
	<i>Rhizosolenia</i> sp.	0.0	0.0	0.0	0.0
	<i>Pleurosigma</i> sp.	5.81	1.46	20.66	$9 \times 10^{-5}$
	<i>Coscinodiscus</i> sp.	0.89	0.46	0.08	$8 \times 10^{-5}$
	<i>Fragilaria</i> sp.	0.30	0.0	0.0	0.0
Cyanobacteria	<i>Striatella unipunctata</i> sp.	0.15	0.0	0.0	0.0
	<i>Anabaena</i> sp.	0.15	5.93	3.15	$5.1 \times 10^{-4}$
	<i>Pseudo-anabaena</i> sp.	0.0	0.0	0.24	0.0
	<i>Oscillatoria</i> sp.	4.17	0.29	0.65	0.0
	<i>Microcystis</i> sp.	0.0	2.46	6.21	99.99
Dinoflagellates	<i>Spirulina</i> sp.	1.49	0.72	0.40	$1.3 \times 10^{-4}$
	<i>Gyrodinium</i> sp.	0.0	0.0	0.08	0.0
	<i>Proto-peridinium</i> sp.	1.34	1.32	0.08	$8 \times 10^{-5}$
	<i>Prorocentrum</i> sp.	0.15	0.14	0.0	0.0
	<i>Ostreopsis</i> sp.	0.0	0.14	0.0	$5 \times 10^{-5}$
Flagellates	<i>Peridinium</i> sp.	0.15	0.0	0.0	0.0
	<i>Choanoflagellate</i> sp.	0.0	0.0	0.08	$1 \times 10^{-5}$
Coccolithales	<i>Coccolithus</i> sp.	0.0	0.0	0.08	0.0
Zygnemophyceae	<i>Cosmarium</i> sp.	0.0	8.04	0.24	0.0
Chlorophyceae	<i>Scenedesmus</i> sp.	0.0	0.14	0.0	$1 \times 10^{-5}$
	<i>Dunaliella</i> sp.	0.15	0.0	2.02	$7 \times 10^{-5}$
	<i>Chloromonas</i> sp.	0.0	0.0	0.0	$8 \times 10^{-5}$
Chrysophyceae	<i>Dinobryon</i> sp.	0.30	0.29	0.16	0.0
<b>Diversity and evenness</b>					
Diversity index ( $H'$ )		0.6824	0.8953	1.0841	0.0007
Species evenness (EH)		0.0614	0.0803	0.0924	$3.1 \times 10^{-5}$

sediment during the 4<sup>th</sup> week of litter decomposition. During the same period, the tank without sediment had an increase in TAN, which progressed to a much higher concentration of  $0.0078 \pm 0.0055$  mg l<sup>-1</sup> (mean  $\pm$  SD) during the 5<sup>th</sup> week of decomposition. However, the highest levels of TAN ( $0.038 \pm 0.006$  mg l<sup>-1</sup>) were recorded in the shade treatments with sediment during the 1<sup>st</sup> week of litter decomposition, but then declined over time (Fig. 6d).

## DISCUSSION

### Litter decomposition

The ecological approach of shrimp aquaculture in mangrove forests implies that mangrove leaf litter falls into the shrimp pond, and plays a potentially important function in the ecological processes within the pond. These processes may be limited by the exposure of decomposing mangrove leaf litter to sunlight due to shading by mangrove trees and contact with sediment.

In our present study, we observed that mangrove leaf litter

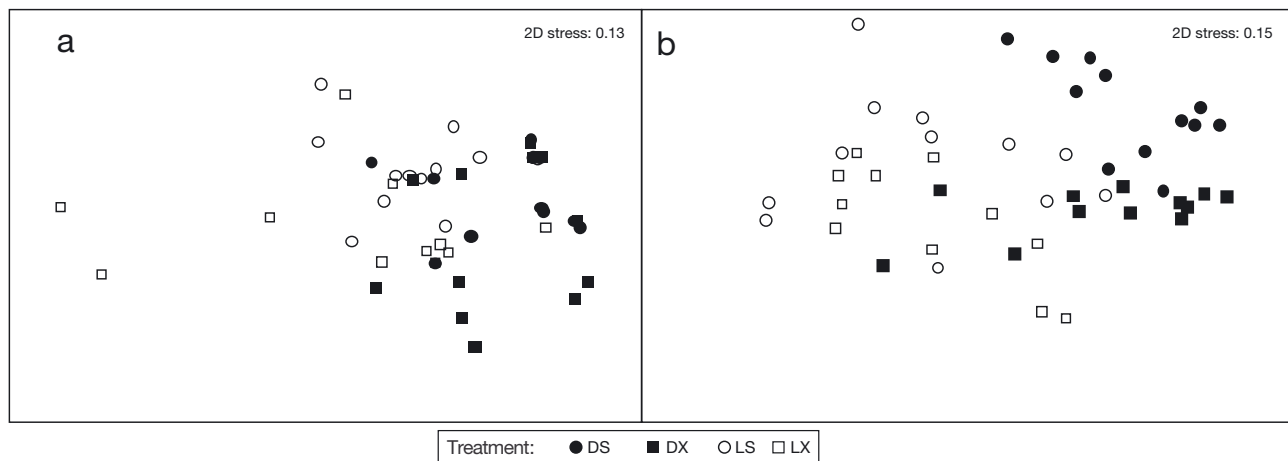


Fig. 4. Multidimensional scaling plot of similarity in (a) microalgae and (b) epifauna colonizing biofilm on decomposing mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX)

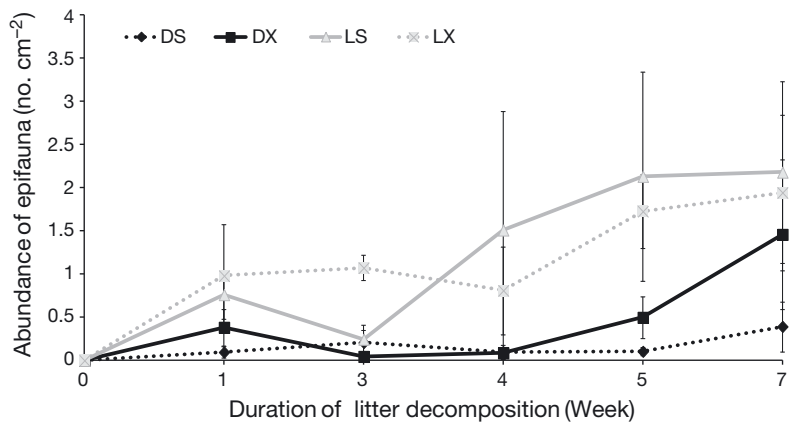


Fig. 5. Abundance of epifauna in biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX). Error bars represent SD

Table 2. Proportional assemblage of epifauna on biofilm developing on mangrove leaf litter of *Rhizophora mucronata* incubated in seawater microcosms at different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX); and species diversity and evenness. Epifauna data are percentages calculated from all weeks pooled together

	DS	DX	LS	LX
<b>Epifauna taxa</b>				
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
Mysida	0.06	0.00	0.00	0.00
Ostracoda	0.25	0.19	0.28	0.09
Bivalvia	0.00	0.00	0.01	0.00
<i>Oikopleura</i>	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
<b>Diversity and evenness</b>				
Diversity index ( $H'$ )	1.59	1.09	1.58	1.38
Species evenness (EH)	0.21	0.13	0.17	0.15

exposed to sunlight in the presence of sediment degraded faster than litter decomposing in the shade. Although sunlight has been observed to directly enhance the rate of decomposition of plant leaf litter through photodegradation (Gallo et al. 2009), other studies have observed decomposition as being mediated by associated macrobiota (Franken et al. 2005),

microbiota (Francoeur et al. 2006) and adsorption of nutrients by sediment (Avnimelech & Gad 2003).

In our study, litter exposed to sunlight seem to be characterized by a higher abundance of microalgae and epifauna compared to litter incubated in the shade. 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 microbial communities associated with natural decaying plant litter in wetlands. Sediment also has a significant tendency in promoting proliferation of microfauna (Avnimelech & Gad 2003).

By stimulating microbial activities, exposure to sunlight and sediment is therefore an important physical parameter influencing the microbially mediated decomposition of mangrove leaf litter in ecological shrimp culture systems. The effect of sunlight on abundance and activities of the epifauna in litter decomposition is an indirect process, as explained by Franken et al. (2005). Light intensity first influences algal biomass, density and composition, which in turn positively influence the growth of epifauna, some of which are efficient shredders of the decomposing leaf litter.

The higher decomposition of mangrove leaf litter exposed to sunlight and in the presence of sediment suggests a synergistic effect of light, sediment and the associated organisms in enhancing the decomposition of submerged mangrove leaf litter. According to Avnimelech & Gad (2003), the concentrations of nutrients, including organic carbon compounds, in the shrimp pond bottom soil are higher than those found in the water column. Hence the bottom becomes the favorable site for microbial development due to the availability of organic matter. In the present study, the sediment was not sterilized. The rapid decomposition of mangrove leaf litter incubated with sediment may be 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 sunlight in the absence of sediment seems to have decomposed slowly, defying the potential photodegradation influences of sunlight. The possible influence of the pre-



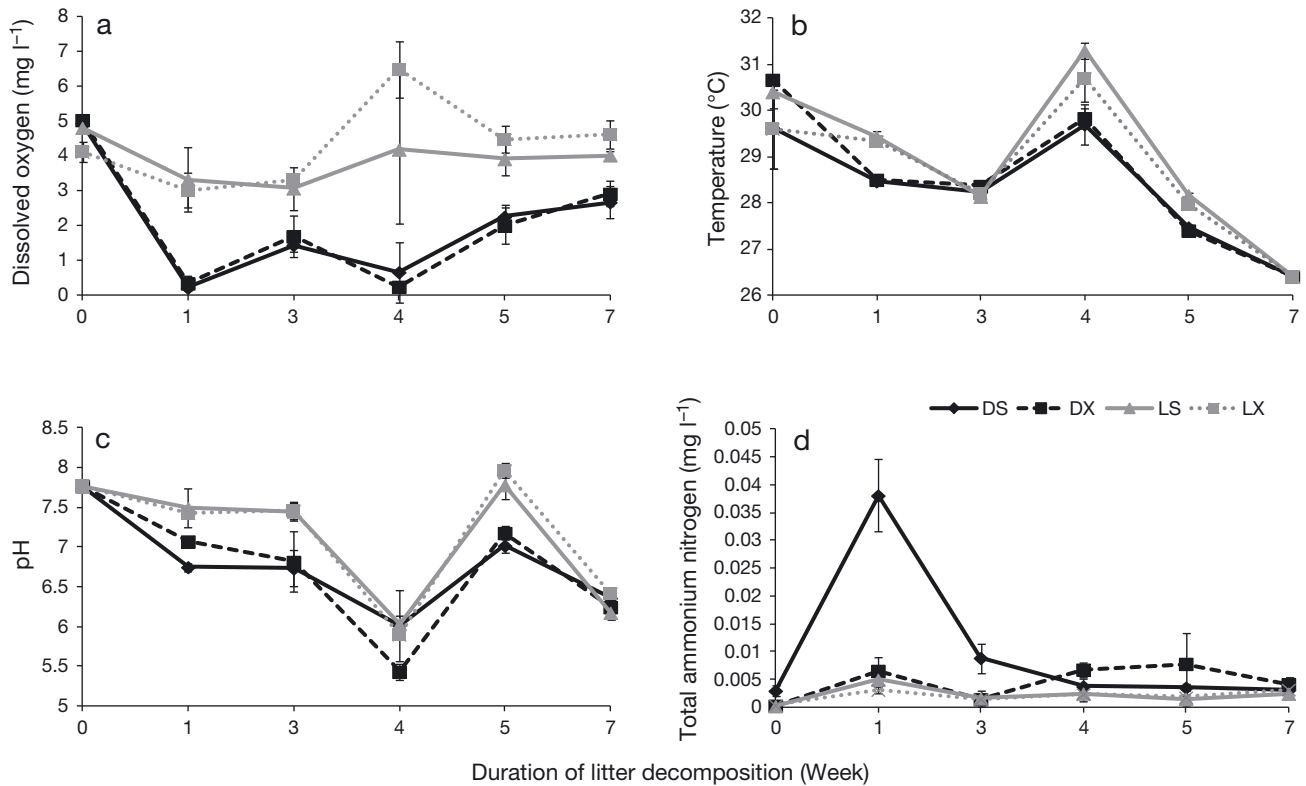


Fig. 6. Water quality parameters (a: dissolved oxygen, b: temperature, c: pH, d: total ammonium nitrogen) of seawater in microcosms with decomposing mangrove leaf litter of *Rhizophora mucronata* at different ambient conditions: shade with (DS) or without sediment (DX), sunlight with (LS) or without sediment (LX). Error bars represent SD

dominant *Cyanobacteria* on the slow decomposition 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 biochemical interaction of the *Cyanobacteria* with the various decomposition processes of mangrove leaf litter in the absence of sediment.

#### Effect of direct sunlight on natural food supply

The exposure of pond water to sunlight is crucial in promoting primary production which is the baseline for energy transfer to higher trophic levels in the pond food web. The relevance of this pathway depends on the nutritional value of the primary producer to the immediate (primary) or secondary consumers. In our present study, decomposing mangrove litter exposed to sunlight supported the largest biomass of microalgae compared to litter decomposing in the shade. This was expected, since sunlight is essential for photosynthesis and thus for primary productivity. The synergy in the combination of sunlight and sediment

in promoting the diversity of epifauna was clearly demonstrated. This ecological condition is beneficial in promoting natural food sources in shrimp culture systems. The absence of sediment seems to favor proliferation of a dominant species of microalgae. This could be a result of a modification of environmental conditions. Sediment therefore seems to complement sunlight in the shrimp pond food web, which therefore suggests it is an important condition in maintaining the ecological stability of the shrimp culture system.

The dominating presence of polychaetes on litter decomposing in sunlight suggests the importance of placing shrimp culture systems outside mangrove canopy to promote growth of polychaetes, which are a potential natural food for post-larvae of penaeid shrimps (Nunes & Parsons 2000). In addition, the importance of sediment was observed in the improved proliferation of diatoms, especially *Navicula* and *Pleurosigma* spp., which are of nutritional importance in supporting ecological shrimp aquaculture (Bombero-Tuburan et al. 1993, Abu Hena & Hishamuddin 2012). Litter decomposing in the absence of sediment supported only *Cyanobacteria*, 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 *Anabaena*, *Anabaenopsis*, *Nostoc* and *Planktothrix* (*Oscillatoria*) (Amado & Monserrat 2010), and other bioactive metabolites with potential to degrade the nutritional status of aquaculture species (inhibitors of proteases and grazer deterrents) (Schrader et al. 1998, Smith et al. 2008).

Diatoms, especially *Navicula* spp., continued to grow in the shade, although in lower abundances compared to sunlight conditions, and could be the reason behind the continued growth of copepods on litter decomposing in the shade. The diatoms *Navicula* and *Nitzschia* spp. are capable of growing in the shade, since they can adopt a heterotrophic mode of feeding (Lewin 1953, Admiraal & Peletier 1979). The *Cyanobacteria* *Anabaena* and *Oscillatoria* were also found to thrive on litter decomposing in the shade. The filamentous nitrogen-fixing *A. variabilis* is capable of heterotrophic growth in complete darkness (Mannan & Pakrasi 1993). Nevertheless, our present study revealed reduced abundance of *Cyanobacteria* on litter decomposing in the presence of sediment, as further demonstrated by elevated growth of *Cyanobacteria* in bare sunlight treatments during the 7<sup>th</sup> week of litter decomposition. This observation elucidates the importance of coupling sediment and sunlight in enabling the decomposing mangrove litter to support the growth of high-quality food microalgae in ecological shrimp culture systems. Application of substrates to promote growth of biofilm in shrimp culture systems without sediment should therefore be approached with caution, since the low food-quality *Cyanobacteria* may dominate. Sediment play an important role in providing a whole range of nutrients required for the growth of different types of microalgae (Avnimelech & Gad 2003). The concentrations of nutrients in sediment are higher than those recorded in pond water (Avnimelech & Gad 2003). Sediment accumulated in shrimp culture systems is typically highly enriched in nitrogen and phosphorous (Boyd et al. 1994), which are important nutrients in supporting proliferation of microalgae.

### Effect of sunlight on environmental conditions

Sunlight is capable of influencing the dynamics of various water quality parameters and therefore plays an important role in determining the ecological health of a shrimp pond in a mangrove forest. In the present study, tanks with decomposing litter in the shade were low in dissolved oxygen compared to those exposed to sunlight. Sunlight promotes photo-

synthesis, whereby CO<sub>2</sub> is absorbed and oxygen is released as a result (Dawes 1998). This process can oxygenate the sediment at the pond bottom (Avnimelech & Gad 2003). According to Avnimelech & Gad (2003), algae produce oxygen during the day, leading to oxygen enrichment of the water column, especially the top layer. 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 microalgae in promoting oxygen in water depends on the type of algae. *Cyanobacteria* is not a preferred food by epifauna and shrimp (Preston et al. 1998). This microalgae is therefore likely to bloom in the biofilm and then collapse. Subsequent decomposition of dead *Cyanobacteria* by bacteria would consume a lot of dissolved oxygen from the water. On the other hand, diatoms are preferred food for epifauna and shrimp (Brown & Jeffrey 1995, Borowitzka 1997, De Troch et al. 2010). Diatoms would therefore not tend to bloom in the biofilm if grazing by the epifauna is adequate. Epifauna and shrimp feeding on diatoms would consume less oxygen than bacteria, resulting in 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* would not.

In the absence of adequate sunlight, the bottom sediment and decomposing mangrove litter provide large amounts of organic matter, which are favorable conditions for microbial development (Avnimelech & Gad 2003). Bacteria consume large amounts of oxygen (Avnimelech & Gad 2003), and with no oxygen production due to the absence of photosynthetic activity, the water and the sediment become anoxic. In our present study, litter decomposing in the shade in the presence of sediment was characterized by relatively higher TAN, especially during the early stages of decomposition as observed in Weeks 1 and 3, and low dissolved oxygen levels. In case of depletion of oxygen, denitrification occurs in the organic matter, with nitrate as an electron acceptor (Reddy et al. 1986). 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 sulfate is used as an electron acceptor and sulfide is released (Chien 1992). The released sulfide inhibits nitrification (Joye & Hallibaugh 1995). These reactions could have largely contributed to the increased levels of TAN in the tanks with mangrove leaf litter decomposing in the shade, even more than in the presence of sediment.

### Potential toxicity to post-larval shrimp

The water quality in shaded tanks has the potential to build a toxic environment for macroinvertebrates, including post-larval shrimp. When oxygen is depleted, other terminal electron acceptors such as nitrate, iron, manganese, sulfate and CO<sub>2</sub> 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, un-ionized ammonia, reduced divalent manganese, hydrogen sulfide, organic acids and methane (Nix & Ingols 1981, Chien 1992, Boyd 1998, Avnimelech & Gad 2003). Another important reaction under anaerobic conditions is the fermentation of organic substrates by fermenting bacteria, releasing reduced acids, alcohols, CO<sub>2</sub> and hydrogen, which produce offensive odors, and some are toxic to shrimp (Moriarty 1997). The potential to produce reduced acids, CO<sub>2</sub>, hydrogen sulfides and hydrogen ions could have contributed to the reduced levels of pH in the tanks with mangrove litter decomposing in the shade.

Litter decomposing in the shade was characterized by pH values as low as 5.4. Low pH (<6.0) can stress shrimp and cause limited calcification and poor survival (Kater et al. 2006). It increases nitrite toxicity to fish and has similar effects on shrimp (Kater et al. 2006). The combined toxicity of both TAN (NH<sub>4</sub>-N) and ammonia-nitrogen (NH<sub>3</sub>-N) occurs at pH < 8.3 (Kater et al. 2006). Un-ionized ammonia (NH<sub>3</sub>) is the more toxic form of ammonia to post-larvae of penaeid shrimps and occurs at elevated temperature and pH > 8.3 (Chien 1992). The safe level of un-ionized ammonia for post-larvae of *Penaeus monodon* aged 6 to 25 d is 0.1 mg l<sup>-1</sup> (Chien 1992) or 1.11 mg l<sup>-1</sup> TAN at pH 8.2, 29.5°C and a salinity of 34 (Spotte & Adams 1983). In our experiment, the levels of TAN were lower than the allowable upper limit. However, the slightly higher TAN levels observed in the shaded treatments, especially with sediment, suggest an increased potential of TAN toxicity if the incidence of sunlight is limited. Low pH levels as observed in the shaded tanks can increase the fraction of un-ionized

hydrogen sulfide (H<sub>2</sub>S), which is toxic to post-larval shrimp (Chien 1992).

The above observations demonstrate the importance of exposing decomposing mangrove leaf litter to sunlight and sediment, especially in situations where ecological shrimp culture systems may be under a dense canopy of 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 1 g l<sup>-1</sup>, of mangrove litter could play an important role in promoting shrimp growth and survival in aerobic conditions (Hai & Yakupitiyage 2005). In this respect, our study demonstrates the additional advantage of sunlight and sediment in enhancing the proliferation of high-quality natural food from the decomposing mangrove leaf litter.

### CONCLUSION

This study highlights the synergistic effect between sediment and sunlight in maintaining a high diversity of microalgae and good water quality favorable for the culture of post-larval shrimp. In the presence of sunlight, a lack of sediment enhances the growth of *Cyanobacteria* (*Microcystis* spp.) on mangrove litter decomposed for a period >5 wk. However, shading cannot be relied upon as a deterrent, since *Cyanobacteria* (especially *Anabaena* spp. and *Oscillatoria* spp.) may continue to colonize mangrove litter decomposing in the shade with considerable success. Furthermore, shading of decomposing mangrove litter inhibits growth of polychaetes, which are potential food for post-larval shrimp.

This study supports promoting an environmentally friendly system of culturing shrimps in less forested areas. In case a situation arises where leaf litter must be used to promote biofilm as natural food for shrimp, then complete shading of the culture system should be avoided. This study demonstrated synergy in the combination of sunlight and sediment in promoting the proliferation of high-quality natural food for penaeid shrimps, moderating water quality and controlling blooms of *Cyanobacteria*. The findings may also provide an important economic insight into the design and management of liner-based shrimp culture systems. The synergy between sunlight and sediment, in maintaining a stable environment, can be employed to limit the use of pond liners and reduce the frequency of removing sludge which tends to accumulate at the bottom of lined ponds for the culture of both crustaceans and finfish.

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