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ORIGINAL PAPER

Effects of decomposing *Rhizophora apiculata* leaves on larvae of the shrimp *Penaeus monodon*

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Abstract We studied the effects of different concentrations of decomposing *Rhizophora apiculata* leaves and their leachates on larvae of the shrimp *Penaeus monodon* under laboratory conditions. Shrimp mortality was highly dependent on the concentration of oxygen in the water, which in turn was strongly correlated to the amount of decomposing leaves in the same water. Shrimps died after 5 min when placed in water containing the highest concentration of mangrove leachates (15 g l^{-1}) tested in our experiments. Shrimp survival and biomass decreased significantly when the shrimp were cultured at the relatively higher concentrations of leaves and leachates (10 and 15 g l^{-1}); in contrast, moderate amounts of leaves or their leachates (2.5–5 g l^{-1}) had positive effects on shrimps. The survival and biomass of shrimps cultured with plastic leaves was lower than those of shrimps cultured with mangrove leaves, indicating that food derived from mangrove leaves contributed to a higher shrimp survival and biomass. These results have important implications for the culture of shrimps in extensive mangrove-shrimp systems. While litter may promote shrimp production, high leaf concentrations may have negative effects due to the drop in the oxygen concentration. Water circulation may help to prevent low oxygen conditions and reduce local accumulations of mangrove leaves.

Keywords Decomposition · Nitrite · Oxygen · *Penaeus monodon* · *Rhizophora apiculata* leaves · Sulphide · Toxicity

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Introduction

Production in mangrove-shrimp culture systems is relatively low compared to more intensive forms of shrimp farming (Huitric et al. 2002), nevertheless, these culture systems are widely practiced. The growth of shrimps in such mangrove-shrimp systems is predominantly dependent on the availability of food in the ditches. One source of food for all kinds of animals, including shrimps, living in the ditches is fallen mangrove leaves (Roijackers and Nga 2002). In addition, for some of the inhabitants of the ditch, these leaves can function as a good shelter against predation (Primavera 1997). However, while the potential benefits of mangrove leaves for shrimps are undisputed, there are also potentially detrimental effects, such as the release of toxic substances from the leaves during decomposition as well as decreasing oxygen levels in the water and the resulting increase in toxic components such as sulphides, nitrites and ammonium (Nga and Roijackers 2002).

The tiger shrimp, *Penaeus monodon*, is the most important species of penaeid shrimps currently being cultured commercially in Asian countries, especially in Vietnam, where the mangrove-shrimp system has been widely adopted during the past few years. However, the effects of mangrove leaves on shrimp farming have not been well studied. Nga et al. (2005) recently established a positive relation between the input of litter and shrimp production in the ditches. In a practical sense, this result means that an increase in litter input (70% of which consists of leaves) can boost shrimp production – unless the benefits of the presence of leaves are neutralized by the detrimental effects mentioned above. In study reported here, we investigated whether, and in which way, different amounts of mangrove leaf litter (*Rhizophora apiculata* Blume) affect the survival and the growth of larvae of the shrimp *Penaeus monodon* Fabricius under laboratory conditions. Based on our results, we offer advice for improving the management of mangrove-shrimp culture systems.

Materials and methods

Experimental design

Four experiments were performed in the laboratory to test the effects of *Rhizophora apiculata* leaves on the survival and growth of post-larvae *Penaeus monodon*. Table 1 presents a summary of the treatments in each experiment, the experimental parameters and the effects to be studied):

1. Experiment 1 was designed to test the effect of three different rearing systems (biofilter recirculation with aeration, no biofilter recirculation with aeration, and no biofilter recirculation without aeration).
2. Experiment 2 compared the effects of mangrove leaves relative to those of their leachates on the survival and growth of post-larvae *P. monodon* .
3. Experiment 3 investigated the function of decaying leaves as a physical shelter for shrimp. Mangrove leaves were compared with plastic leaves of the same form and quantity.
4. Experiment 4 was designed to exclude the competition factor among shrimp larvae in that individual shrimps were tested for the effects of decomposing mangrove leaves.

Table 1 Summary of the experiments conducted to test the effects of decomposing *Rhizophora apiculata* leaves on larvae of the shrimp *Penaeus monodon*

Experiment no.	Treatments	Water volume (l)	Replicates	Experimental period (days)	Studied effects on shrimps
1	Three rearing systems: -Biofilter, recirculation + aeration; -No biofilter, recirculation + aeration; -No biofilter, recirculation + no aeration. Five levels of leaf leachates for each rearing system: 0, 2.5, 5, 10, and 15 g l^{-1} wet weight	30	4	4	Biofilter recirculation and oxygen
2	Five levels of either leaves or leaf leachates: 0, 2.5, 5, 10, 15 g l^{-1} wet weight	50	4	60	Leaves and leaf leachates
3	Three leaf types: no leaves (control), senescent leaves and plastic leaves. Five leaf concentrations: 0, 2.5, 5, 10, 15 g l^{-1} wet weight	7	4	60	Shelter and release of substances from leaves affecting growth
4	Five leaf concentrations: 0, 2.5, 5, 10, 15 g l^{-1} wet weight	1	20	60	Exclusion of competition

Five concentrations of either leaves or leachates were used in each experiment—0, 2.5, 5, 10 and 15 g l^{-1}. Where applicable, reaeration was by very gentle bubbling.

Preparation of leaves and their leachates

Leaves that were ready to abscise when touched were collected from *R. apiculata* trees growing in 7-year-old stands. These senescent leaves were used directly in all experiments. In order to obtain leachates from the leaves (experiment 2), we incubated the senescent leaves in 500-l tanks of water at a concentration of 15 g senescent leaves per liter (wet weight) for approximately 10 days. The water of these tanks was used as leachates. Lower concentrations of leachates were obtained by diluting this solution. Before being used in experiments (experiment 3), plastic leaves were first washed and then incubated for 30 days in tap water to remove possibly harmful chemical agents. Both natural and plastic leaves were weighed before the experiment.

Rearing systems and chemical analysis of rearing water

Biofilter recirculation and aeration systems were used in experiment 1; reaeration was applied in experiments 3 and 4 by very gentle bubbling. A salinity of 15 ± 1 ppt was maintained during the culture period. In all experiments the water volume was maintained as indicated in Table 1. Temperature, dissolved oxygen (DO), pH and salinity were recorded daily, and total sulphide and nitrite contents were measured weekly according to standard methods (APHA 2000). Biochemical (biological) oxygen demand (BOD) was measured as BOD_5—i.e. a 5-day incubation.

Stocking density, feeding and growth criteria for shrimp

Shrimp larvae (PL: 15–25 mm long) were stocked at a density of 1 l^{-1} in all experiments. Shrimps were fed CP pellets at a level of 10% body weight. Excess feed and shrimp waste were removed every 3 days.

On days 0 and 60 the dry weight (DW) of the shrimps was calculated by determining the change in weight before and after drying at 105°C for 24 h. Survival rates were recorded at the end of the experimental period, except in experiment 1, where it was recorded every 5 min during the first 1 h and then hourly up to the end of the experiment (96 h).

Statistics

Analyses of variances (ANOVA) were performed using the available procedures in the SPSS 10.0 for Windows package (SPSS, Chicago, Ill.). Prior to the one-way ANOVA, variables were tested for normality (Shapiro-Wilk). Post-hoc tests were performed using the Tukey test.

Results

In the first experiment, survival fell off sharply with increasing leaf biomass ($p < 0.05$) although reaeration prevented mortality up to a leaf concentration of

5 g l^{-1}, independent of the biofilter recirculation as long as air (oxygen) was supplied (Fig. 1). The highest shrimp survival occurred at 2.5–5 g l^{-1} leaf or leachate (experiment 2), decreasing significantly ($p < 0.05$) at the higher concentrations (Fig. 2a). Total shrimp biomass was higher when the shrimp were grown in the solutions of leaf leachates than when grown in water containing leaves and tended to decline with increasing leaf biomass (Fig. 2b). The results of experiment 3 revealed that the survival of the shrimps was not significantly different when grown in water containing natural or plastic leaves (Fig. 3a), however the total biomass of the surviving shrimps was lower in the plastic leaf treatment (Fig. 3b). When the shrimp were cultured individually, survival increased in the presence of mangrove leaves (Fig. 4a), reaching a maximum at a mangrove leaf concentration of 10 g l^{-1}. The total biomass of the shrimp larvae was significantly lower at the lower levels of decaying leaves compared to the control (Fig. 4b).

In experiment three, in which the effect of plastic leaves and natural leaves were compared with a control (no leaves) the BOD increased gradually during the incubation period (Fig. 5a). A rapid increase in nitrite was observed for all three series until a maximum was reached in the third week for the control, for all concentrations of plastic leaves and for the two lowest concentrations of mangrove leaves (Fig. 5b). One week later the two higher concentrations of mangrove leaves reached the maximum BOD. The control and all concentrations with plastic leaves maintained these high nitrite levels, but the nitrite level decreased rapidly in all concentrations of natural leaves. The sulphide levels were low in the control series and in all concentrations with plastic leaves (Fig. 5c). However, in the series with natural leaves they increased up to day 28 proportionally to the concentration of the leaves. After day 28 the levels decreased again.

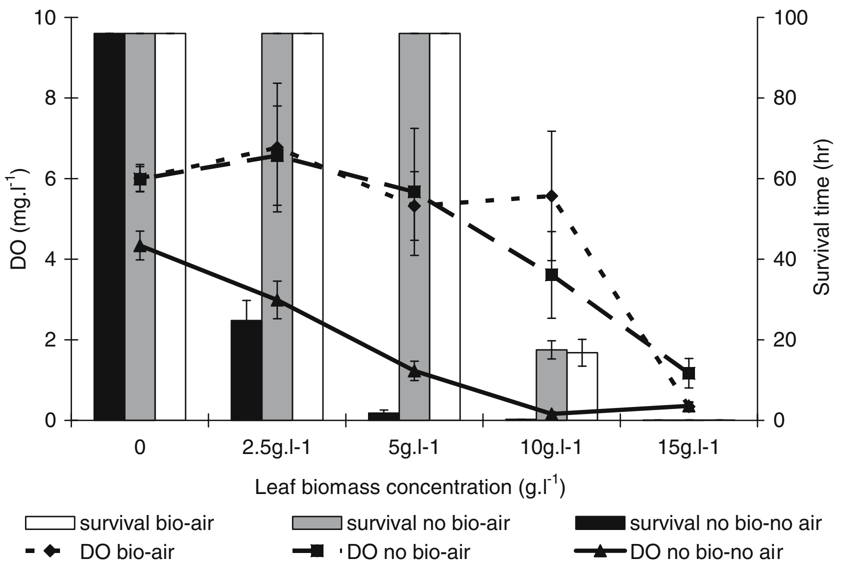


Fig. 1 Survival time of *Penaeus monodon* larvae in three different rearing systems—biofilter-recirculation with aeration (bio-air), no biofilter recirculation with aeration (no bio-air) and no biofilter recirculation without aeration (no bio-no air) tested at different concentrations of decaying *Rhizophora apiculata* leaves. Final concentrations of dissolved oxygen (DO) in the water are shown (line)

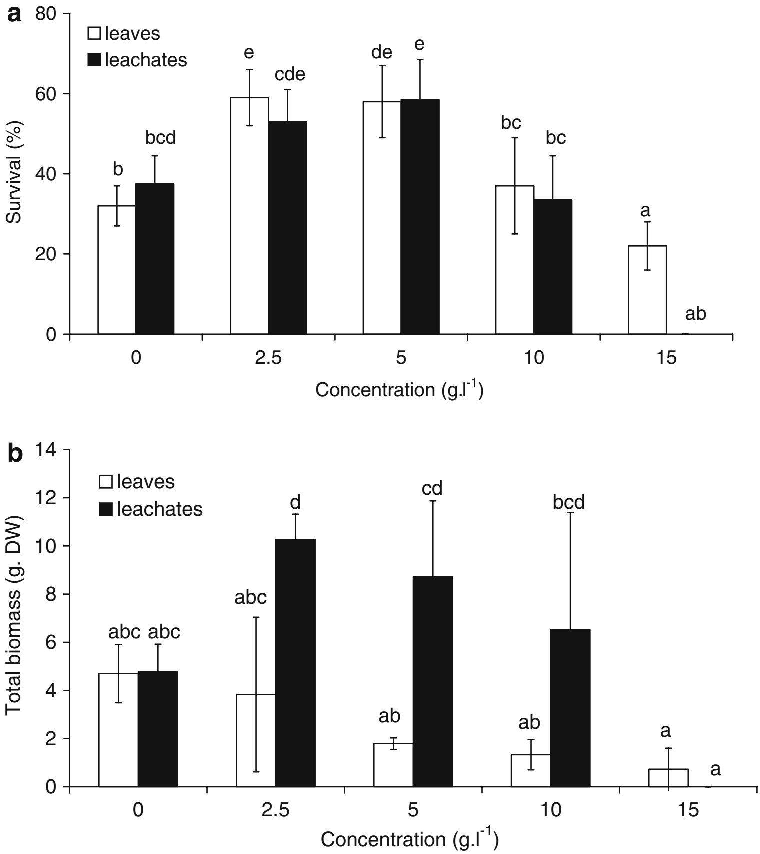
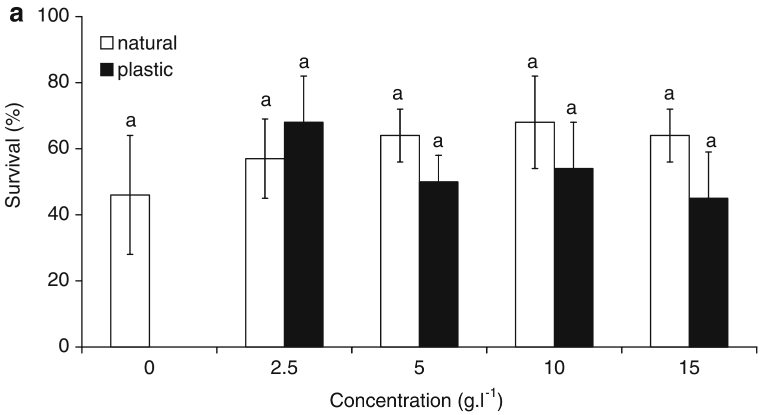


Fig. 2 Survival (**a**) and total biomass (**b**) of *P. monodon* larvae after 60 days when supplied with different concentrations of either decaying *R. apiculata* leaves or leachates from decaying leaves. Each series (leaves and leachates) had their own control. *Different letters above the bars* indicate significant differences ($p < 0.05$)

Discussion

Dissolved oxygen is the most important limiting factor in the intensive cultivation of shrimp species (Rosas et al. 1995), and penaeid shrimps have been shown to be very sensitive to hypoxia (Wu et al. 2002; Rosas et al. 1997, 1999). Allan and Maguire (1991) estimated that the lethal level (96 h LC_{50}) of DO for juvenile *P. monodon* is 0.9 mg l^{-1}. In our experiments (Fig. 1), shrimps died within 10 min in water containing the highest leaf concentrations at a DO level approximating 0 mg l^{-1}. In addition, the lower biomass of shrimps grown on mangrove leaves than those grown on mangrove leaf leachates (Fig. 2) was—apart from the lower nutritional value of the leaves compared to their leachates—probably due to the low oxygen level in the mangrove treatment (1.5–2 mg l^{-1}) than in the leachate treatments (4.5–6 mg l^{-1}). This difference in growth is in line with the studies of Seidman and Lawrence (1985) who reported that when oxygen was below 2 mg l^{-1} the growth rates of *P. monodon* and *P. vannamei* were significantly reduced. Martinez-Cordova et al. (1998) also



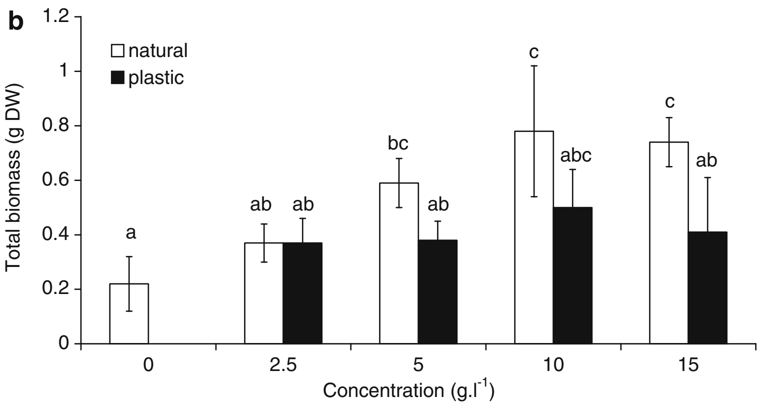


Fig. 3 Survival (**a**) and total biomass (**b**) of *P. monodon* larvae after 60 days of being in direct contact with different concentrations of either decaying *R. apiculata* leaves or plastic leaves. Both the natural and plastic leaves series had the same control. *Different letters above the bars* indicate significant differences ($p < 0.05$)

showed that the average shrimp yield was significantly higher in aerated shrimp ponds (1687–1813 kg ha^{-1}) than in not aerated ponds (1243 kg ha^{-1}). Thus, the supply of DO to a system in which mangrove leaves are decaying is essential, not only for the survival but also for the growth of shrimp larvae.

Although oxygen is clearly important, its low concentration is probably not the only cause of the detrimental effects of high leaf concentrations. We found a strong positive correlation between the sulphide level and the concentration of decomposing mangrove leaves (Fig. 5c; $p < 0.05$; $r = 0.847$). This sulphide clearly originated from the leaves through leaching. Sulphide is toxic to all aerobic organisms (Visman 1996) and plays an important role in several marine environments where many animals are periodically or permanently exposed to high sulphide concentrations (Bagarinao 1992).

In addition to anoxia and high sulphide levels, shrimps are exposed to nitrite resulting largely from their own excretion and nitrogen input from CP pellets (Fast and Lester 1992). While we recorded an increase in nitrite in all series, it was the fastest and highest in the treatments without decaying mangrove leaves (Fig. 5b),

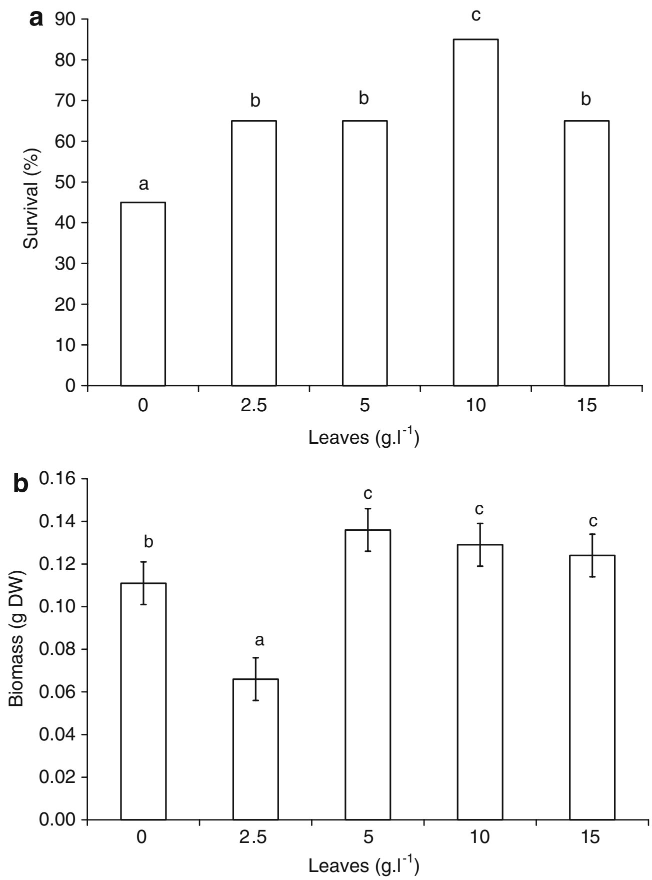


Fig. 4 Survival (**a**) and biomass (**b**) of *P. monodon* larvae incubated with different levels of decaying *R. apiculata* leaves. Competition was excluded. *Different letters above the bars* indicate significant differences ($p < 0.05$)

indicating an ameliorating effect of the leaves on this particular stressor. Nitrite is highly toxic to fish and may be detrimental to shrimps; the concentrations determined in our experiments far exceeded the safe level of nitrite recommended for shrimp growth (<0.5 mg l^{-1}; Chien 1992), although after 1 month the nitrite and sulphide decreased considerably in the treatments with mangrove leaves. The fact that shrimp biomass was significantly higher in the mangrove leaf treatments than in the plastic leaf treatments (Fig. 3b) may well be due to the reduction of nitrite levels in the presence of natural leaves (Fig. 5b). Nitrite is an intermediate product in the microbial nitrification of ammonia to nitrate or in the denitrification of nitrate and is, in general, more toxic than nitrate (Wickins 1976). Therefore, the reduction of nitrite by decomposing leaves may be due to bacterial activities, i.e. uptake, immobilization and mineralization (Robertson 1988; Chale 1993; Tam et al. 1998; Puente et al. 1999). The high C/N ratio in natural leaves thus seems to balance the excessive N input with CP pellets, thereby allowing bacteria to assimilate potentially toxic nitrite.

We have shown that the effects of mangrove leaves on shrimps are rather complex and may work out either positively or negatively. This contrasts with the current

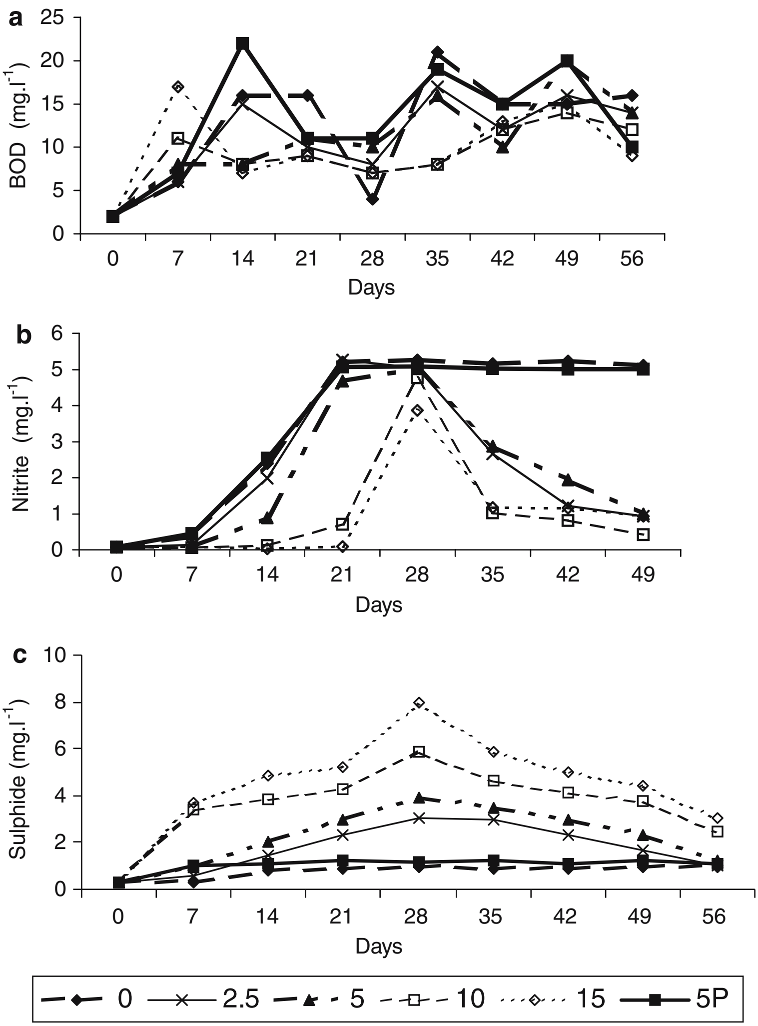


Fig. 5 Concentrations of BOD, nitrite and sulphide in the water for the different treatments (experiment 3). Only one treatment with plastic leaves (*5P*) is shown as all gave the same result

prevailing view which stresses the beneficial effects of mangrove leaves. Without a doubt, the leaves are a source of food and serve as a substratum for attached organisms such as algae, fungi and bacteria, which in turn serve as food for the shrimp (Fell and Masters, 1980; Vijayaraghavan and Wafar 1983; Alongi et al. 1989; Hyde and Lee 1995; Gee and Somerfield 1997; Lee 1999; Zhou 2001). Leaves may also provide hiding places to escape predation which appears to be important for mangrove-associated penaeids (Primavera 1997), just as it is for small fish (Laegdsgaard and Johnson 2001). Overall these positive effects may outweigh the negative effects of anoxia and sulphide in most field situations. A good example is that of the Mekong Delta traditional systems (without mangroves), which have annual yields of 100–400 kg ha^{-1} $year^{-1}$, whereas mangrove-shrimp systems have an annual yield of 100–600 kg ha^{-1} $year^{-1}$ (Johnston et al. 2000).

However, anoxia and increases in sulphide levels are not uncommon in the field (Alongi et al. 1999a, b; McGraw et al. 2001). Our study indicates that production could be higher if the occurrence of such conditions could be reduced. We therefore advice the creation of permanent water exchanges at places where high quantities of mangrove leaves accumulate.

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