Penaeus monodon post-larvae and their interaction with *Rhizophora apiculata*

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Bui Thi Nga

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Chapter 1

INTRODUCTION

1. The Mekong delta

On the world map, Vietnam is located in the Eastern border of the Indochinese peninsular and belongs to the South-East-Asia region (figure 1). The total land area is about 33 million ha, it extends between latitude of 23°22'N- 8°30'N and longtitude of 102°10'E – 109°24'E (Thao, 1997). Therefore, the climatic condition of Vietnam is somehow controlled by continental influences except in the North. About 70% of the territorial mainland consists of hills and mountains. Also large and rather fertile deltas exist, annually deposited by fluvial materials of the Red river system in the North and the Mekong river system in the South. They join with the narrow coastal plains along the Central region. Nowadays, about 55% of the natural land area is used for agriculture (about 7 millions ha), forestry and other specific usages (General Statistics Office, 2001).
The Mekong delta is part of the Mekong River Delta. It covers an area of 3.96 million ha (about 74% of the total area formed by the Mekong river); the rest of the Mekong River Delta (26 %) is located in the Laos, Thailand and Kampuchea. It is the most Southern territory of the country (from 11°N to 8°30'N) and occupies almost the complete down-stream area of the Mekong river with three sides connected to the sea. To the west, the delta borders the Gulf of Thailand, the Eastern and Southern pays join the Eastern Sea and the Northern and North-Eastern part is bonded to Kampuchea and the South-East part of Vietnam (figure 2).

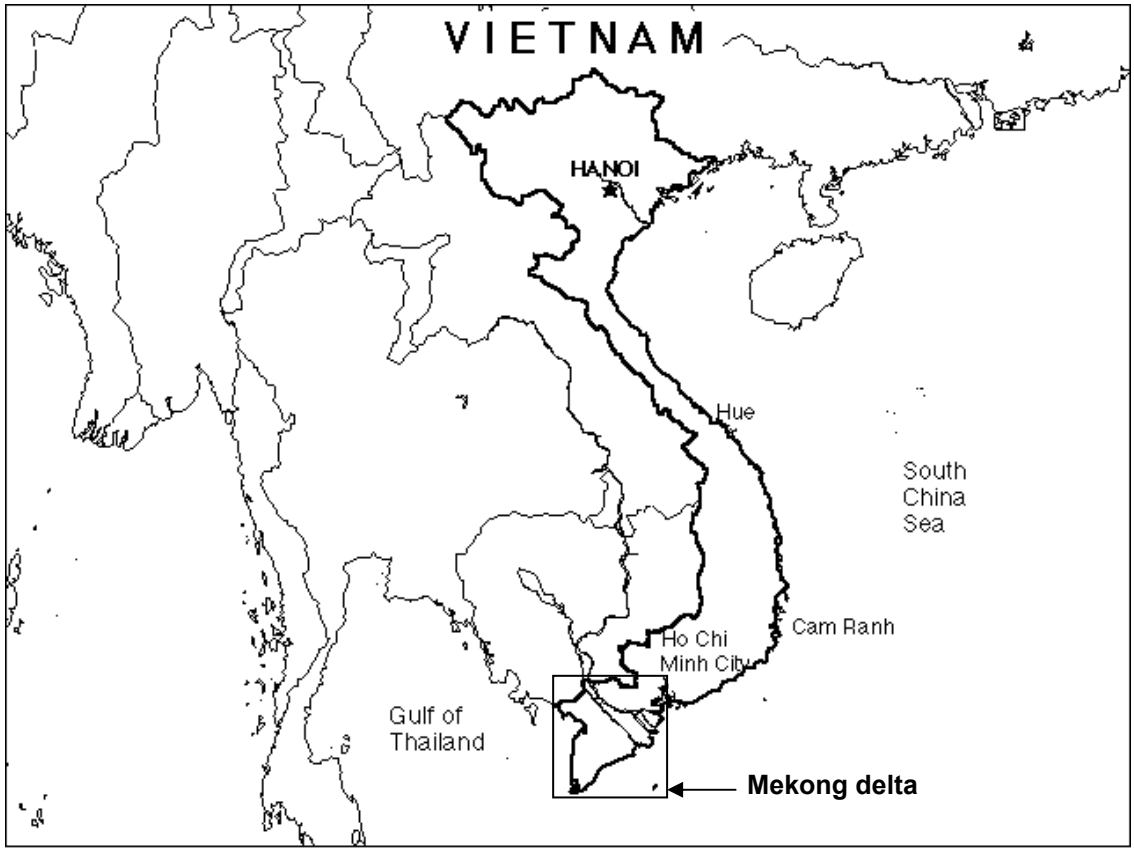

Figure 1. Map of Vietnam and the Mekong delta in South East Asia

The Mekong river is about 4200 km long, and branches into many rivers in the Mekong delta. The Tien and Hau rivers (total about 250 km long) are the most important rivers.

The highest discharge occurs in the flooding period (August - October) and the lowest one in the dry season (March - May). Due to the position and specific characteristics of the Mekong delta, saline water can be periodically intrude the delta during the dry season (about 40 km - 50 km). Nowadays, with the many canals constructed, the canal and river system play a very important role for the agricultural production in the Mekong delta for two reasons: (1) irrigation with a deposit of fluvial material and (2) leaching acidity and salinity (Thao, 1986).

Figure 2. Map of the administrative boundaries of the provinces in the Mekong delta.

The climate is humid tropical and dominated by a monsoon. There are two typical seasons: (1) the rainy season with South-Western winds: May to November and (2) dry season with North-Eastern winds: December to April. Rainfall in the wet season varies from 1200 – 2400 mm. The annual rain in the delta represents about 90 billions m^3 of water (about 1600 mm in the Eastern, 2400 mm in the Western and 1300 mm in the Central area). There are about 107 – 165 rainy days around the year. Evaporation is only 420 mm – 660 mm, dependent on the specific topography of the area. Prolonged and heavy rains, usually associated with water sources from upstream of the Mekong river result in a flooding (usually in August - November) of the delta, with an average flooding depth of 0.8 m – 1.5 m. The relative humidity varies from 79 - 85%; the annual mean temperature is about 26.5 - 27.9 °C with a maximum of 30.9 - 32.7 °C and a minimum of 23.2 - 25.0 °C. Total actual sunshine hours are about 2400 - 2800 hrs. With

these specific climatic characteristics, many areas are lacking fresh water during the dry season. Tidal movements are also important. There are two tidal regimes: (1) the East Sea tides (half-day) with an amplitude of 40 – 50 cm, and (2) the Gulf of Thailand tides (daily) with an amplitude of about 50 cm (Lap et al., 2000).
The Mekong delta comprises 12 provinces, nowadays (figure 2): Ca Mau, Bac Lieu, Soc Trang, Can Tho, An Giang, Kien Giang, Dong Thap, Vinh Long, Tra Vinh, Tien Giang, Ben Tre and Long An. The regional population is about 17 million people (about 20 % of the total population of Vietnam). The delta contributes 47.4% of the national agricultural produce, 50% of the national rice production and 60% of the national fruit production (Can Tho University in 2001). Furthermore, the Mekong delta is by far the most productive area for brackish water aquaculture and freshwater fisheries, representing 67% of Vietnam's total aquaculture and freshwater fisheries production from an area of 301,352 ha (Johnston et al., 2000). Brackish water aquaculture alone has increased from 48,700 ha in 1985 to 237,739 ha in 1994, which is equivalent to a 388 % increase in land area (Lovatelli, 1997).
The Camau peninsula (study area) is located in the southwestern part of the Mekong delta. The main area is covered by coastal plain deposits with scattered marshy deposits. In the southern part of the Camau peninsula mangrove forests expand over a 90 km long and 25 km wide area. In spite of the broad coastal plain, there are no sand dunes or relict beach ridges; however, they are clearly distributed on the Eastern Coastal Area (Lap et al., 2000)

2. The function of mangrove forests

Mangroves are the coastal equivalent of tropical forests. They protect the coasts from erosion caused by hurricanes that periodically scourge these tropical zones. Recent studies have shown that mangrove forests are highly efficient in enhancing the removal of solids and nutrients from sewage or aquaculture effluents (Páez-Osuna et al., 1998). The most common uses of mangroves and their ecosystems are extraction of firewood, material for housing, and more importantly, they serve as spawning, nursery and feeding grounds for many commercially important species of prawn and fish (Lee, 1995; Rasolofo, 1997; Slim et al., 1997; Athithan & Ramadhas, 2000). Recent studies also reported that mangroves enhance the biomass of coral reef fish communities (Mumby et al., 2004). Mangroves provide organic matter and nutrients, i.e. nitrogen and phosphorus to the coastal area through litter fall and decomposition of that litter. The process of litter decomposition involves a complex series of events which includes leaching of water-soluble substances, mechanical abrasion, the colonization by decomposer microbes such as bacteria and fungi (Tam et al., 1998), and the colonization and processing by macroinvertebrates (Zhou, 2001). Aksornkoae (1993) reported that mangrove forests support a high abundance and diverse variety of wildlife being the result of a high rate of leaf fall and a rapid breakdown of detritus. Through mangrove detritus, detritus-based food webs are maintained in the coastal ecosystem and their significance for coastal fisheries has been illustrated in several studies (Alongi, 1990; Alongi et al., 1989;). The role of the mangroves for coastal fisheries must, therefore, be related to the function of mangroves as a food source. Fish production is believed to be dependent on mangrove areas, and the dependence of many penaeid shrimp species on mangroves has also been shown (Christensen, 1978; Barbier, 2000). In fact, the decline in mangrove areas in the Mekong delta (see 3) certainly had an impact on the decrease in coastal fisheries production over the last decades (Graaf and Xuan, 1998).

3. Mangrove forest destruction and shrimp production

At present, mangroves are undergoing a serious process of degradation and disappearance; the reasons are various, but are mostly related to large scale business activities in which the shrimp industry stands out. The establishment of shrimp farms has been the main cause of mangrove loss in many countries over the past 30 years (IUCN, 2002). In Ecuador over 180,000 ha of mangroves were converted into shrimp ponds; in Honduras between 1986 and 1994, over 12,000 ha were destroyed for the construction of shrimp ponds (IUCN, 2002); in the Philippines as much as 50% of mangroves were destroyed for the construction of aquaculture ponds (Primavera, 1995).

Shrimp aquaculture expanded rapidly in Southeast Asia up till the mid-1990s to the extent that land clearing and construction of shrimp ponds in the region is the leading cause of losses of mangrove forests and other forms of coastal deterioration. Between 1980 and 1990, Malaysia lost 12% of its mangrove forest (1.3% annually). In Thailand mangrove forests covered approximately 367,900 ha of the coastal area. These areas decreased yearly; during 1980-1985 about 18,614 ha of the mangrove area was legally relinquished for alternative land-use purposes. The illegal encroachment during the same period was 72,265 ha, most of the encroachment was for shrimp culture on the eastern coast (Aksornkoae, 1993).

Mangrove forests in Vietnam covered about 400,000 ha of which 250,000 ha was situated in the south of Vietnam (Hong and San, 1993). The mangrove forest in the coastal areas of Vietnam suffered severe damage over the past 50 years; it decreased to 290,000 ha due to war, overexploitation of forest timber, firewood and charcoal (Hong, 1996). Since then, the deforestation continued by changing forests into agricultural land and primitive extensive and intensive shrimp farming i.e. about 53,969 ha of forest was left on the Ca Mau peninsula in 1993 (Department of Agriculture, Forestry and Fishery, 1996). Although extension of shrimp farming in mangrove areas occurs at a rapid rate, world shrimp production has leveled off in recent years, as many aquaculture farms have either collapsed or experienced declining yields, For instance, production figures varied dramatically between the various shrimp culture systems with semi-intensive farms producing between 1,000 and 2,000 $kg.ha^{-1}.y^{-1}$, whereas extensive farms produced 100-400 $kg.ha^{-1}.y^{-1}$ in 1996 (Johnston et al., 2000), and 80-250 $kg.ha^{-1}.y^{-1}$ in 2000 (Ministry of Fisheries, 2001). Shrimp farming became unsustainable due to the unplanned development of the shrimp industry, the destruction of mangrove forests and salt marshes, resulting in reduced fish and shellfish catch from the coastal areas, increased salt-water intrusion and increased water pollution (Graaf and Xuan, 1998). Especially in Ca Mau province these problems became prominent in recent years (Hong, 1996).

4. Penaeid shrimps

In the extended mangrove-shrimp culture system we studied, the shrimps arrive in the ditches with the incoming flood waters, and although many species could be involved, the dominant species is *Penaeus monodon* Fabricius, 1798, the tiger shrimp. All penaeid shrimps have a similar life cycle. There are sev en stages in the life cycle of a shrimp (figure 3):

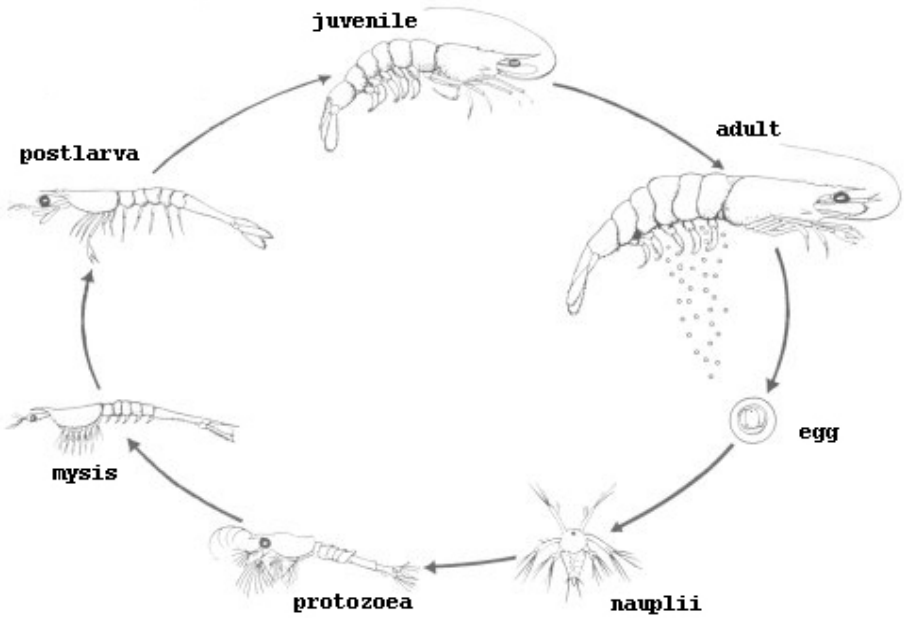

Figure 3. The life cycle of penaeid shrimps (Lee and Williams, 1992).

1. Shrimp eggs (diameter less than 0.4 mm) sink to the bottom at the time of spawning.
2. Five nauplius stages; the first stage is about the size of the egg and succeeding stages are slightly larger.
3. Three protozoeal stages; protozoea have undergone development of their mouth parts and the abdomen has begun to develop.
4. Three mysid stages; mysids have an early development of legs and antennae.
5. Postlarval stage; the walking and swimming legs have developed and the postlarvae appear as miniature shrimps.
6. Juvenile shrimps; growth is rapid and similar to adults.
7. Adults are totally mature to produce sperm and eggs.

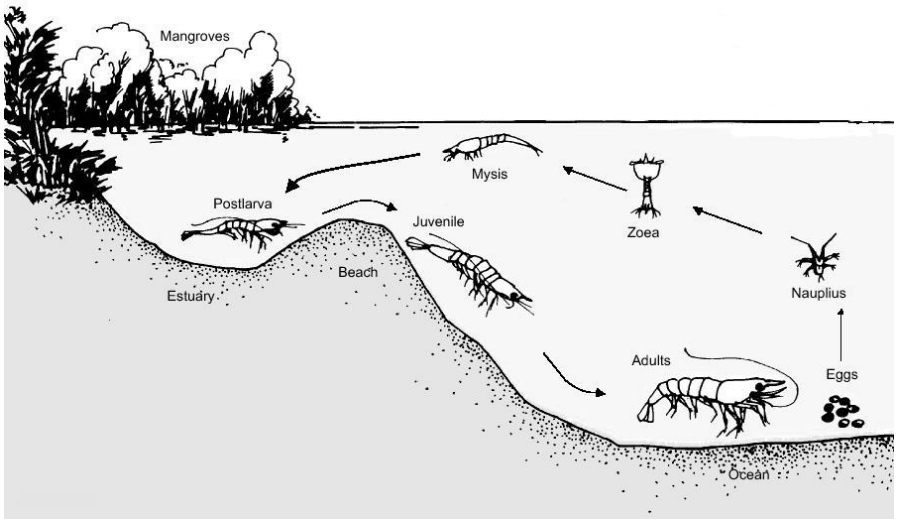

Figure 4. The life cycle of penaeid shrimps in relation to their habitat.

During their development shrimps migrate from the deep open ocean to the shallow estuaries and vice versa (figure 4). Spawning usually occurs in the ocean from near the beaches to several miles offshore. The eggs sink to the ocean floor and develop into nauplius larvae that have a limited swimming ability and usually become a part of the oceanic plankton. These planktonic forms (protozoea) are found in ocean waters. In their nauplius and protozoeal phase algae are the main food source. The mysis phase is also a planktonic life form in the open ocean. During the mysis phase the feeding preference changes from primary herbivorous to carnivorous. The postlarval stage rides the flood tides into the estuaries, apparently becoming active during flood tide and settling to the bottom during ebb tides; they consume phytoplankton, zooplankton, detritus, periphyton and small macro-invertebrates (Lavens and Sorgeloos, 1996). The postlarvae ultimately settle in the upper parts of tidal creeks (mangrove communities). Juveniles typically remain in the marsh creeks and move to the deeper rivers when they become sub-adults. Adults are usually found in the ocean.

5. Shrimp-mangrove culture systems and their effects

Shrimp aquaculture practices vary from traditional extensive culture systems, practiced for centuries, to the super-intensive culturing systems currently being used in Thailand. In traditional extensive systems, relatively large ponds (up to 50 ha, but usually less than 5 ha) are constructed in intertidal (usually mangrove) regions. Wild shrimp fry enters the ponds either during tidal water exchange, or are intentionally collected from the wild and stocked directly to the ponds. Growth of individual shrimp in these systems relies on natural pond fertility: fertilizers and feeds are not generally used. Retention time of the water in the ponds varies, but may be up to two months. Yields of shrimps are low, in the range 100 – 300 $kg.ha^{-1}.y^{1}$ (Huitric et al., 2002). During the past two decades, improvements in extensive shrimp culture systems have led to an increased shrimp production. Among the improvements are liming of ponds prior to stocking to condition the soil, application of organic or inorganic fertilizers to enhance natural food production, increased stocking densities, and removal of pests and predators through chemical techniques.

The Mekong delta of Vietnam is well known as a region rich in aquatic resources. Fisheries and aquaculture are very diversified practices in terms of scales and intensifications, and the coastal area shrimp culture is the major aquaculture practice (Phuong, 2002). The shrimp-mangrove systems were popular in most coastal provinces

in the Mekong delta. In 1997, shrimp farming occupied 186,000 ha, representing extensive, improved extensive, semi-intensive and shrimp-rice culture systems (Christensen et al., 2002). The extensive shrimp culture system resulted in an average low production (250 $kg.ha^{-1}.y^{-1}$) (Alongi et al., 1999).
The mangrove-shrimp culture system is characterized by mangrove stands (in Vietnam mainly *Rhizophora apiculata* Blume 1827), surrounded by ditches in which shrimps are cultured and harvested regularly (figure 5).

Figure 5. A mangrove-shrimp system in the Camau province, Vietnam.

Mangrove leaves fallen in ditches seem likely to have positive effects on shrimp production. First of all they supply nutrients (nitrogen and phosphorus) during decay, thus enhancing algal production (Roijackers and Nga, 2002). Leaves function also as a direct and indirect (the attached communities of algae, protozoa and micro-organisms) food source for all kinds of animals, including shrimps. The presence of leaves in the ditches offers a good shelter against predation. But there are also detrimental effects, such as the release of toxic substances from the leaves and the high oxygen consumption during decomposition. Too low values of dissolved oxygen usually leads to biological stress, which causes low production. In fact, a preliminary investigation on the water quality of a mangrove-shrimp system (Roijacker and Nga, 2002), showed that during certain periods oxygen values were very low near the pond bottom. The low oxygen concentration was especially due to the high SOD (sediment oxygen demand). The SOD in the forest was that high that anaerobic processes prevailed, with toxic endproducts. Figure 6 illustrates the interactions between mangrove leaves and shrimps.
Knowledge of ecological factors controlling pond production is also crucial for a more effective management and sustainability of aquaculture enterprises. The ability to estimate the production capacity of shrimp ponds relies upon knowledge not only of the reproductive and physiological capabilities and tolerance limits of shrimps, but also of system level processes. Insight in and quantifying processes such as rates of natural and supplemented food inputs, internal recycling, and outputs (harvesting, losses via respiration and bacterial production) are needed. To improve a sustainable shrimp production within the mangrove-shrimp systems, research is needed on the key

processes of litter fall and nutrient input from leaf litter to the system and their effects on shrimp growth.

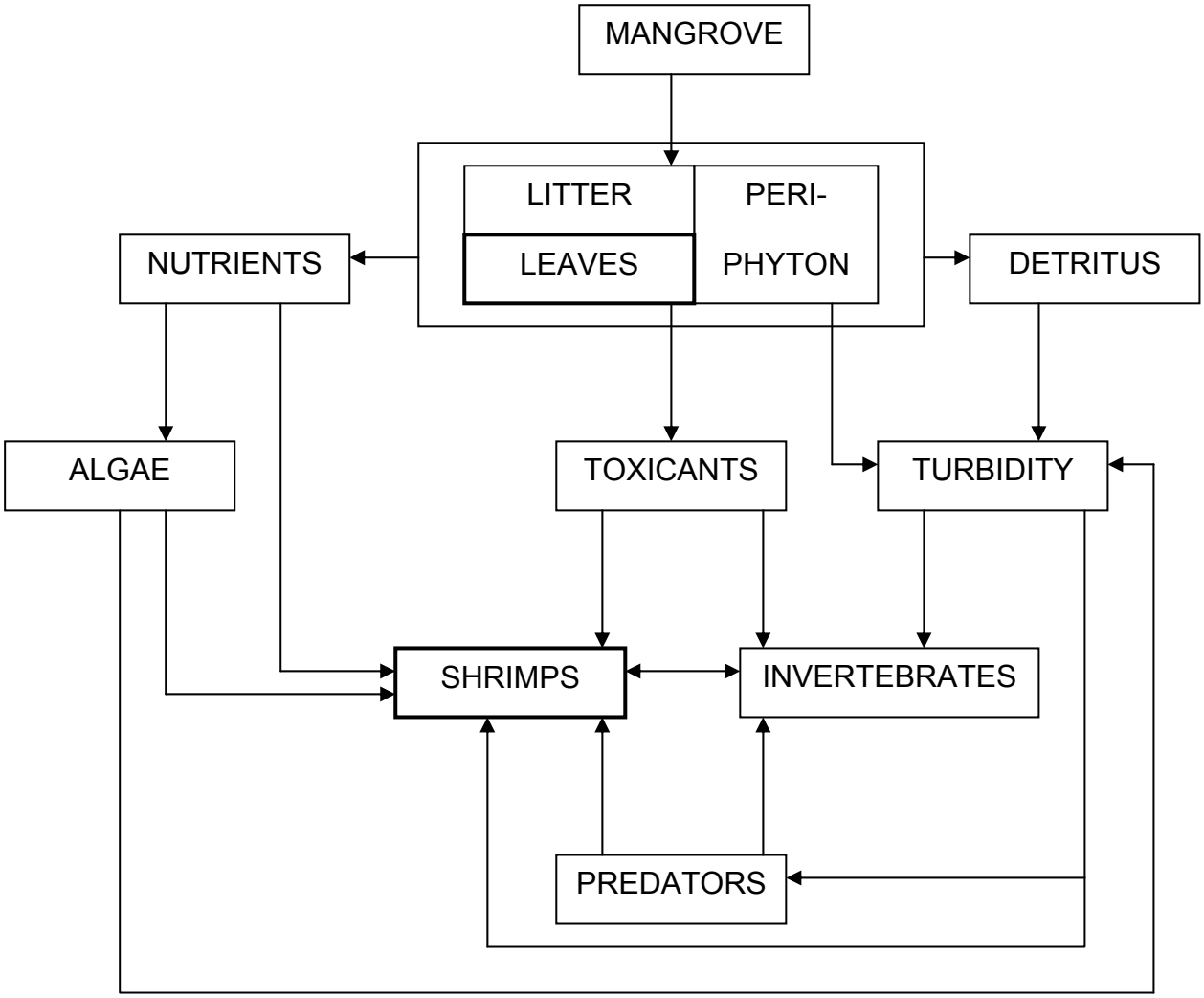

Figure 6. Simplified foodweb in a mangrove-shrimp culture system, illustrating the major relations between (leaf)litter and shrimps.

6. Outline of this thesis

The ways in which mangrove litter may affect shrimp production, coastal resources, and mangrove ecosystem are manyfold (figure 6). However, little is known of the effects of mangrove litter on the survival and growth of shrimps. The work in this thesis concentrates on the litter fall input in mangrove-shrimp systems (chapter 1) and its effects on shrimps.
Chapter 2 deals with litter fall and the input of nutrients in *Rhizophora apiculata* stands of different age. In chapter 3 the decomposition of mangrove leaves as studied both in the field and the laboratory is discussed both as a function of the salinity as well as the seasonality. Effects of decomposing and decomposed leaves with the associated periphyton on the Tiger shrimp, P*enaeus monodon*, are described in chapter 4, and 5. Chapter 6 and 7 concentrate on the effects of shrimp density, crowding chemicals, and

alarm pheromones on shrimp growth. Finally, concluding remarks are given in chapter 8.

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Chapter 2

YOUNG MANGROVE STANDS PRODUCE A LARGE AND HIGH QUALITY LITTER INPUT TO AQUATIC SYSTEMS IN CAMAU PROVINCE, VIETNAM

(B. T. Nga, H. Q. Tinh, D. T. Tam, M. Scheffer and R. Roijackers)

1. Abstract

Mangrove swamps are key ecosystems along the Vietnam coast. Although mangrove litter is thought to represent an important input of organic matter and nutrients to the coastal aquatic systems, the factors determining the quality and size of this litter flux have not been studied so far. We monitored leaf, stipule, twig, and reproductive litter monthly in monocultures of *Rhizophora apiculata* mangrove forests of 7, 11, 17 and 24 years old in the Camau province, Mekong Delta, Vietnam. Litter traps were used to measure litter fall production from June 2001 till May 2002. Total litter fall was in the range of 8.86–14.16 $tDW.ha^{-1}.yr^{-1}$. Leaves were the main component, and represented 70% of litter fall production in all stands. Total litter fall was lower in the older stands but reproductive litter was higher in these stands (17 and 24 years). Biomass of leaf litter was highest between the end of the wet season and the beginning of the dry season. Phosphorus and nitrogen levels were higher in younger than in older stands. Overall, our study indicates that young stands produce the highest input of litter, nitrogen and phosphorus to the surrounding aquatic system. Consequently, these stands may give the largest boost to fisheries

2. Introduction

Mangrove swamps are extremely productive ecosystems, which export organic matter to support a variety of organisms (Odum and Heald, 1975; Lee, 1989). The export of these large amounts of organic material can have substantial effects on food webs in coastal waters (Alongi et al., 1989; Alongi, 1990). In the past decade, mangrove forests have been under severe pressure by a rapidly increasing human population, large scale deforestation practices and conversion of forests into aquaculture farms especially in Southeast Asia. The fast developments in aquaculture have resulted in non-sustainable farming systems (Graaf and Xuan, 1998). Preservation of the mangrove forests is an important issue to keep the coastal ecosystem in balance and to improve the water quality in the coastal areas. Therefore, the need for protection of the coastal forest belt has been an important issue (Loi et al., 2002). In recent years, studies on species classification, tree growth, succession, silviculture, forest utilization, and litter fall of mangroves have been carried out in Vietnam (e.g. Nam and Thuy, 1997; Clough et al., 2000). However, information on litter fall from mangroves in Vietnam is lacking.

The Camau province (Camau Peninsula), situated in the South-Western part of the Mekong Delta has a long shoreline and is the province with the most severe mangrove destruction. Following the cessation of hostilities in Vietnam, mangrove forests in the Camau peninsula, initially recovered as a result of both natural regeneration and manual planting of the preferred forestry species, *Rhizophora apiculata*. However, recent expansion of shrimp culture in the region has now substantially reduced the area of mangroves.

The study described here was carried out as part of a larger study for Integrated Management of Coastal Resources (MHO8-project) in the Mekong Delta, Vietnam, where a number of provincial governments have established shrimp farming-forestry enterprises. In these mixed aquaculture and mangrove forestry systems, shrimp and other cultured species are dependent mostly on the natural food chains for their food supply. It is widely recognized that mangrove derived detritus may be a major source of carbon for estuarine food webs along tropical and subtropical coastlines (e.g. Odum and Heald, 1972; Malley, 1978; Robertson

and Daniel, 1989; Daniel and Robertson, 1990), but data for the systems we studied in Vietnam are lacking. The present research focusses on the question how the dynamics and quality of mangrove litter input to aquatic systems differs between tree stands of different age.

3. Materials and Methods

Description of the research area

Sampling was carried out from June 2001 till May 2002 in monocultures of *Rhizophora apiculata* of respectively 7, 11, 17, and 24 years old. These stands were selected in three mixed shrimp farming-mangrove forestry enterprises: Tam Giang 3, 184, and Kien Vang in Camau province, Mekong Delta, Vietnam (figure 1). The average tree density varied from 7,000-10,000 trees per hectare. The mixed mangrove-shrimp system is characterized by mangrove stands, surrounded by ditches. The area of each of the mangrove stands is 7-12 ha of which approximately 30% are ditches. Average width and depth of these ditches are 7 m and 1 m, respectively. The research area is located at a latitude of about 8º50 N. The climate is humid tropical and dominated by monsoons. Mean annual rainfall was 2400 – 3460 mm over 1996 – 2002. Annually, the temperature is relatively uniform with an average of 27-28^{o}C; the annual sunshine amounts 1918 to 2390 hours and the humidity ranges from 80 – 84% (table 1). During the research period, average rainfall and humidity were the lowest, while temperature and sunshine were the highest recorded over the last five years. The rainy season lasts from May till November, and the rest of the year is the dry season with very little rainfall. During the research period the average rainfall, humidity, temperature, and sunshine were 1756 mm, 83.3%, 27.6^{o}C, and 891 hours respectively in the wet season and 336 mm, 75%, 28.8^{o}C, and 1403 hours in the dry season (table 2). There was a large difference in rainfall between the wet and dry seasons. The rainfall was very low in March and April and in February there was no rain at all.

Table 1. Average annual rainfall, evaporation and temperature in the Camau province, Vietnam (1996-2002).

	1996	1997	1998	1999	2000	2001	2002
temperature (^{o}C)	26.9	27.2	27.9	27	27.3	27.6	27.9
humidity(%)	83.8	83.3	81.25	83.6	82.8	81.5	79.9
sunshine (hrs)	1960.5	2233.3	2232.5	1918.5	2020	2143.7	2391.8
rainfall (mm)	2771.5	2547.9	2595.7	3459.7	2630	2396.5	2329.8

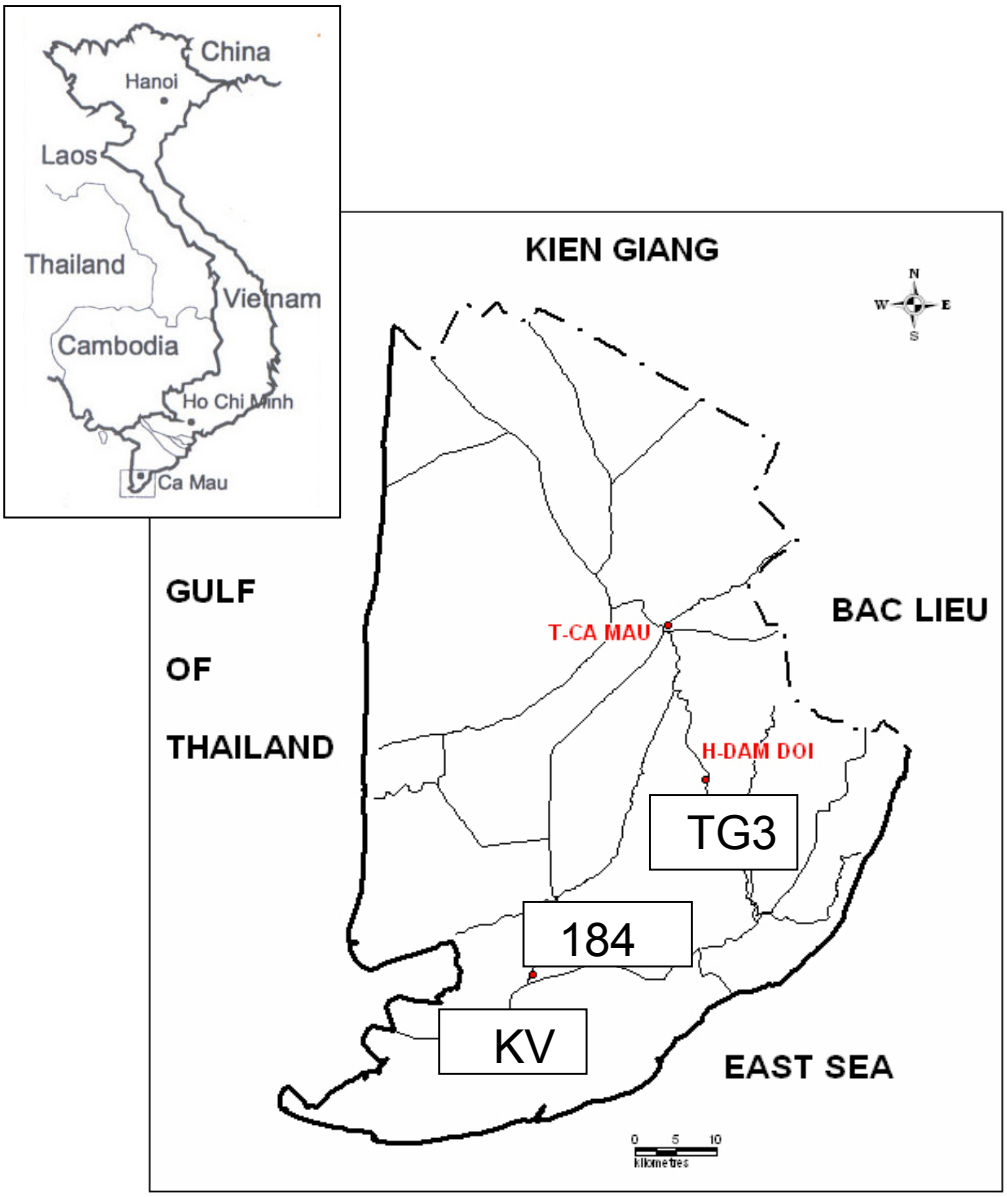

Figure 1. Map of the Camau province, showing the sampling locations.

Table 2. Monthly temperature, humidity, sunshine and rainfall in the Camau province, Vietnam (May, 2001-May, 2002).

	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
temp. (^{o}C)	28.6	27.6	28.6	27.6	27.9	27.2	26.7	26.8	26.3	26.6	27.9	29.7	29.7
humidity (%)	82	85	81	85	84	86	80	77	74	75	74	73	76
sunshine (hrs)	190	114	206	114	171	128	158	219	148	252	291	271	222
rainfall (mm)	237	447	160	287	237	427	198	57	3.5	0	0.6	0.4	274

Sample collection and analyses

Litter traps of 1 m^2 were constructed from nylon nets (1mm mesh size) and positioned in each *Rhizophora apiculata* stand above the highest tidal water level (figure 2). In each stand the content of four traps was collected at monthly intervals for 1 year. The samples were sorted into leaves, stipules, twigs, and reproductive litter. Dry weight (DW) and ashfree dry weight (AFDW) were analyzed for litter samples. From the leaf litter, total nitrogen (TN), total phosphorus (TP), were analyzed directly after sampling. The dry weight was determined by the drying the samples at 105ºC for 24 hours, AFDW was determined by the changing of weight before and after burning at 550^oC during 3 hours. The Kjeldahl method was used for determining TN, colorimetry for determining TP.

Figure 2. Litter trap at the 184 enterprises, Ngoc Hien district, Camau province, Vietnam.

Statistics

All results were analysed using the General Linear Model (GLM – SPSS 10.0 for windows). An Alpha of 0.05 was used as the significance level of Tukey tests.

4. Results

Litter fall

Total litter fall varied from 2.43-3.88 $gDW.m^{-2}.d^{-1}$ and was significantly highest at the 11 year old stand (figure 3). There was no clear seasonal pattern in the total litter fall (figure 4). The oldest stands had a significantly higher proportion of reproductive parts in the litter compared to the younger stands (figure 3). Twig litter fall was relatively low throughout the year, and was signifiantly highest in the

7 and 11 year old stands. About 70% of the total litter was leaf litter (decreasing with increasing age) and stipule, twig and reproductive litter contributed to respectively 8, 12, and 10%. Leaf and stipule litter were significantly highest in the 11 year old stands, and the lowest leaf litter fall was recorded in the oldest stands. The average ashfree dry weight accounted for 90% of the dry weight of the total litter fall. The highest AFDW was recorded in the 7 and 11 year old stands. Litter fall in the Camau forest is high compared to other mangrove systems (table 3).

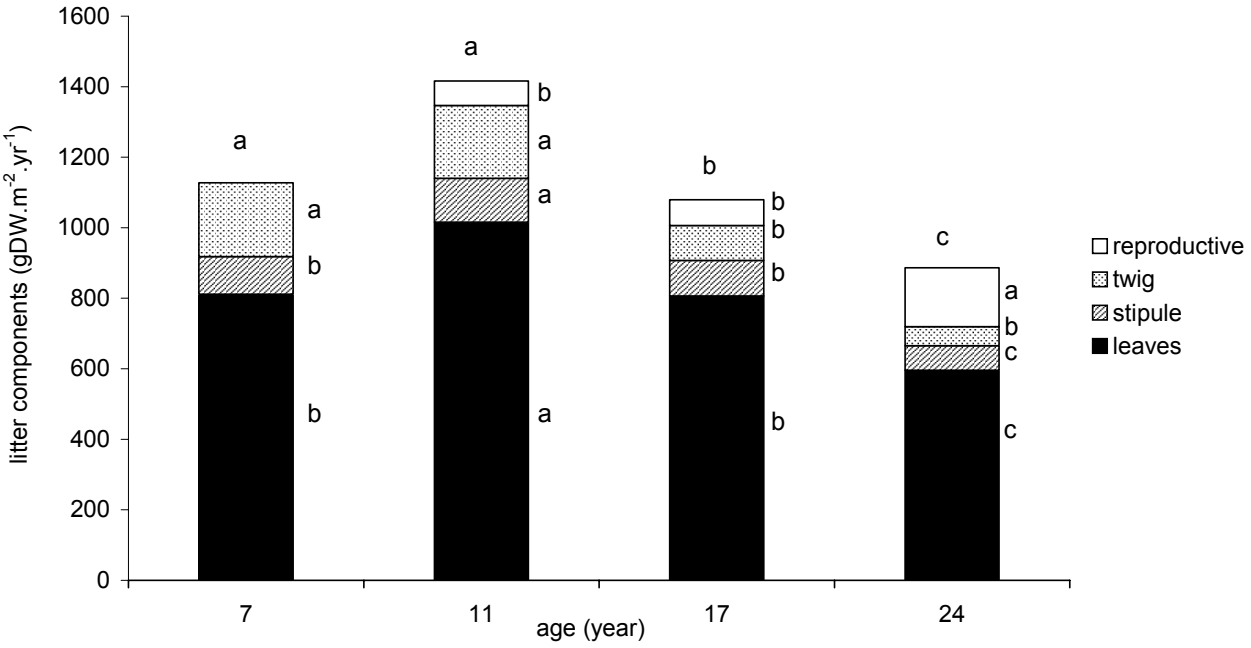

Figure 3. Average biomass ($gDW.m^{-2}yr^{-1}$) of litter fall components from June 2001 till May 2002 in the Camau province, Vietnam. Different letters indicate significant differences between the age classes for the total leaf litter (top) and for the different leaf litter components (next to the bars).

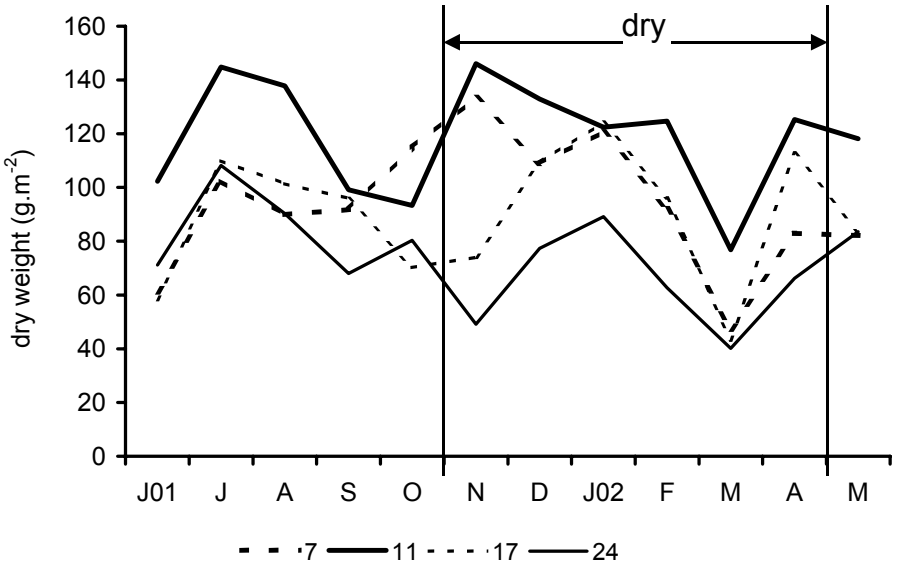

Figure 4. Total litter fall (mgDW.m-2) from June 2001 till May 2002 in the Camau province, Vietnam in 7, 11, 17 and 24 year old stands. The dry period has been indicated.

Table 3. Literature data on total litter fall of Rhizophora spp. mangrove forests around the world (see also Bouillon, 2003).

Species (type of mangal)	Latitude of study area	Total litter ($gDW.m^{-2}.d^{-1}$)	References
Florida: *Rhizophora* spp.	27°41'N	2.21 – 3.50	Pool et al., 1975
Rhizophora spp.	27°41'N	3.10	Dawes et al., 1999
Rhizophora mangle		1.69 2.10	McKee & Faulkner, 2000
Brazil: *Rhizophora* spp.	23°S	2.38	Silva et al., 1998
Papua New Guinea:			
Rhizophora spp.	9°31S	3.91	Leach & Burgin, 1985
Tuvalu: *Rhizophora stylosa*	7°28S	2.12	Woodroffe & Moss, 1984
Hawai: *Rhizophora mangle*		6.90	Cox & Allen, 1999
Mexico: *Rhizophora* spp.		3.40	Day et al., 1987
Ecuador: *Rhizophora* spp.		1.75 - 2.90	Twilley et al., 1986,1997
Australia:*Rhizophora apiculata*		3.10	Bunt, 1982
Rhizophora stylosa		2.55	Duke et al., 1981
Kenya: *Rhizophora mucronata*		2.70	Slimet al.,1996
Rhizophora mucronata		3.22 –4.66	Woitchik et al., 1997
Tanzania: *Rhizophora mucronata*		3.84	Shunula & Whittick, 1999
Fr.Guyana: *Rhizophora* spp.		2.38 – 3.45	Betoulle et al., 2001
India: *Rhizophora apiculata*	6 - 14°N	1.95	Mall et al., 1991
Rhizophora apiculata		3.21 –3.23	Wafar et al., 1997
Thailand: *Rhizophora apiculata*	8°03N	2.70	Christensen, 1978
Rhizophora apiculata	9°97'N	2.43 – 3.54	Aksornkoae, 1993
Rhizophora apiculata	8°03N	3.20	Nielsen & Anderson, 2003
Malaysia:*Rhizophora apiculata*	4°50'N	2.70	Gong et al., 1984
Rhizophora spp.	3°15N	4.32	Sasekumar & Loi, 1983
Vietnam:*Rhizophora apiculata*	8°50'N	2.58 – 5.15	Clough et al., 2000
Rhizophora apiculata	8°50'N	2.43 – 3.88	present study

Nutrients in the leaf litter

The nitrogen and phosphorus contents in the leaf litter as well as the total yearly input of N and P via leaf litter were significantly higher in the younger stands (figure 5). The input of nutrients via the leaf litter showed a clear seasonal pattern in both young and old stands (figure 6a and b).

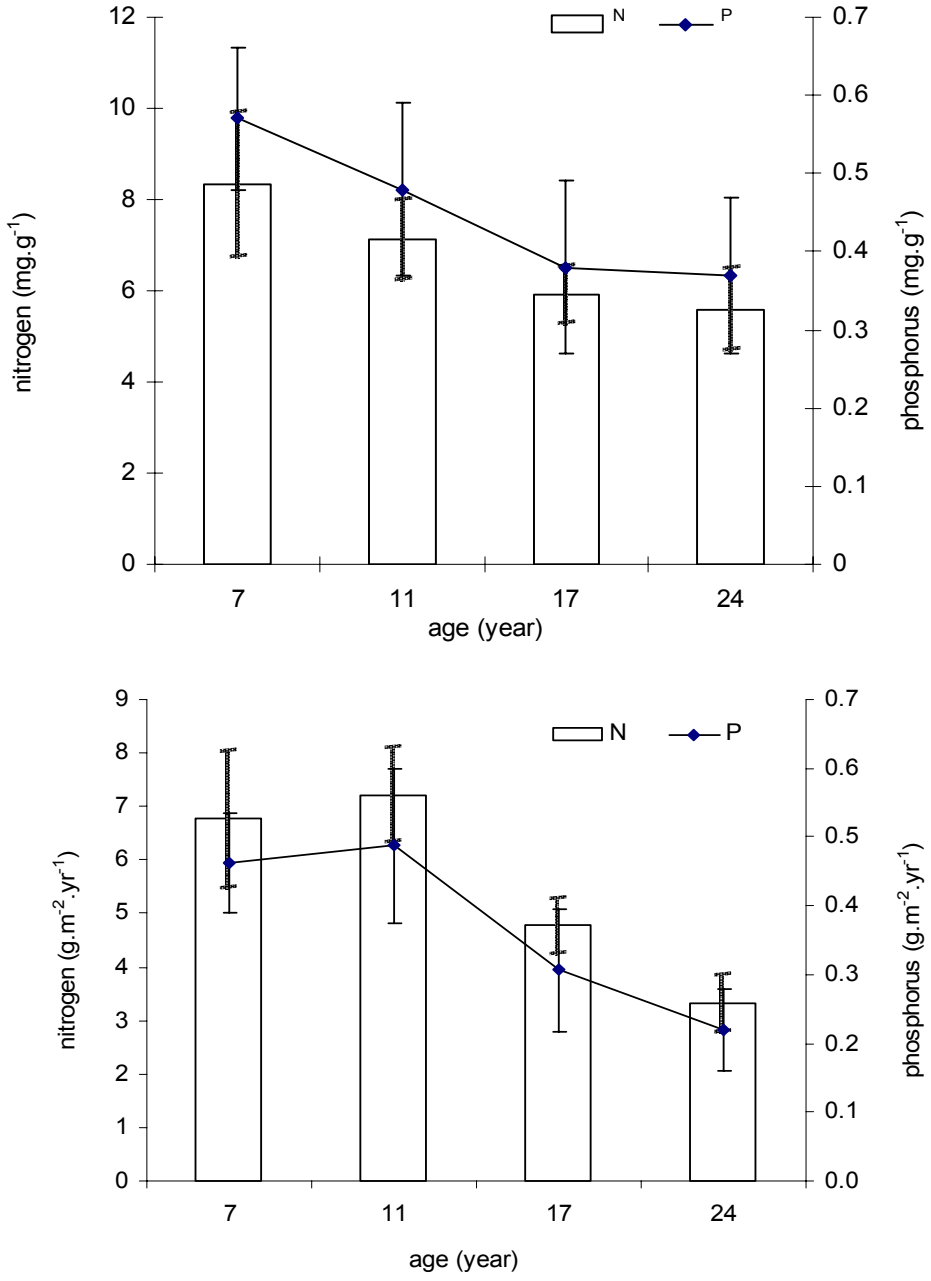

Figure 5. Nitrogen and phosphorus in leaf litter from June till May 2002 in the Camau province, Vietnam (a) average monthly nutrient content in the leaves (mg.g^{-1} DW), (b) total nutrient input through leaf litter (g.m^{-2}.yr^{-1}).

5. Discussion

Litter fall production

Loi et al. (2002) showed that the soils at the sites in the Tam Giang 3 and Kien Vang enterprises are fairly homogeneous with respect to pH, salinity, total-N, total-P, and available-P. Therefore, differences in litter fall of *Rhizophora apiculata* stands, as we found, are directly correlated to the age of the trees. This is in line with findings of Clough (2000) who published similar results for leaves in 6-10 year old stands of *Rhizophora apiculata*, and found a further drop in 36 aged year old stands. This may be due to the more densely leaved canopy in the young stands compared to the more open old stands. Also biological characteristics of the old trees play a role, as these trees produce much woody materials, such as stems, branches and reproductive parts, while the young trees produce more leaves (Hong and San, 1993). Most of the mangrove forests in the Camau had a high density in the young stands (8,000-10,000 trees) and a low density in the old stands (6,000-7,000 trees) because trees in old stands were cut every 5 years (Hong, 1997). Further, Clough et al. (2000) concluded that the leaf area index (LAI) declined as stands age in Camau forest. These certainly affect the litter fall. In conclusion, the decline of litter fall with age seems likely to be causely related to the forest ageing indeed.

Nutrient input through leaf litter

Much of the litter from mangroves can be exported via creeks to adjacent waters (Robertson et al., 1992, Bunt, 1995; Kadlec and Knight, 1996), and the role of this matter in supporting a rich aquatic and benthic food supply for fisheries is well documented (Mackey & Smail, 1997). Nutrients (N, P) derived from mangrove litter may also boost aquatic primary productivity.

Nitrogen and phosphorus levels were considerably higher in the leaf litter of younger stands (7 and 11 years). Mangrove plants in the younger stands are able to take up more nitrogen and phosphorus than those in the older stands (Morrisey et al., 2003), and differences in densities of pneumatophores indicate that root competition is lower in younger stands, leading to a more nutrient-rich leaf tissue. Overall, our results suggest that young stands produce a large quantity of higher quality litter as input to the aquatic system. This may directly or indirectly enhance food availability to the shrimps in younger stands. Indeed, Morrisey et al. (2003) showed that numbers of faunal taxa were higher in younger stands, and numbers of individuals of several taxa were also higher at these sites.

The input of phosphorus and nitrogen peaked during the second part of the wet period and again during the first part of the dry period. This input of nutrients (and organic matter) to the aquatic systems surrounding the mangrove forests is a potential boost to the shrimp production. This can be either directly through the high amount of prime quality food, or indirectly via algae, fungi and bacteria. In fact, Johnston et al. (2000) found that shrimp yields peaked between July-October (1996/97) in the Ca Mau province (figure 6c). We suggest that there may be a direct relationship between the input of nutrients via leaf litter and dynamics of shrimp production. The shrimp peak followed the N and P input peaks in the

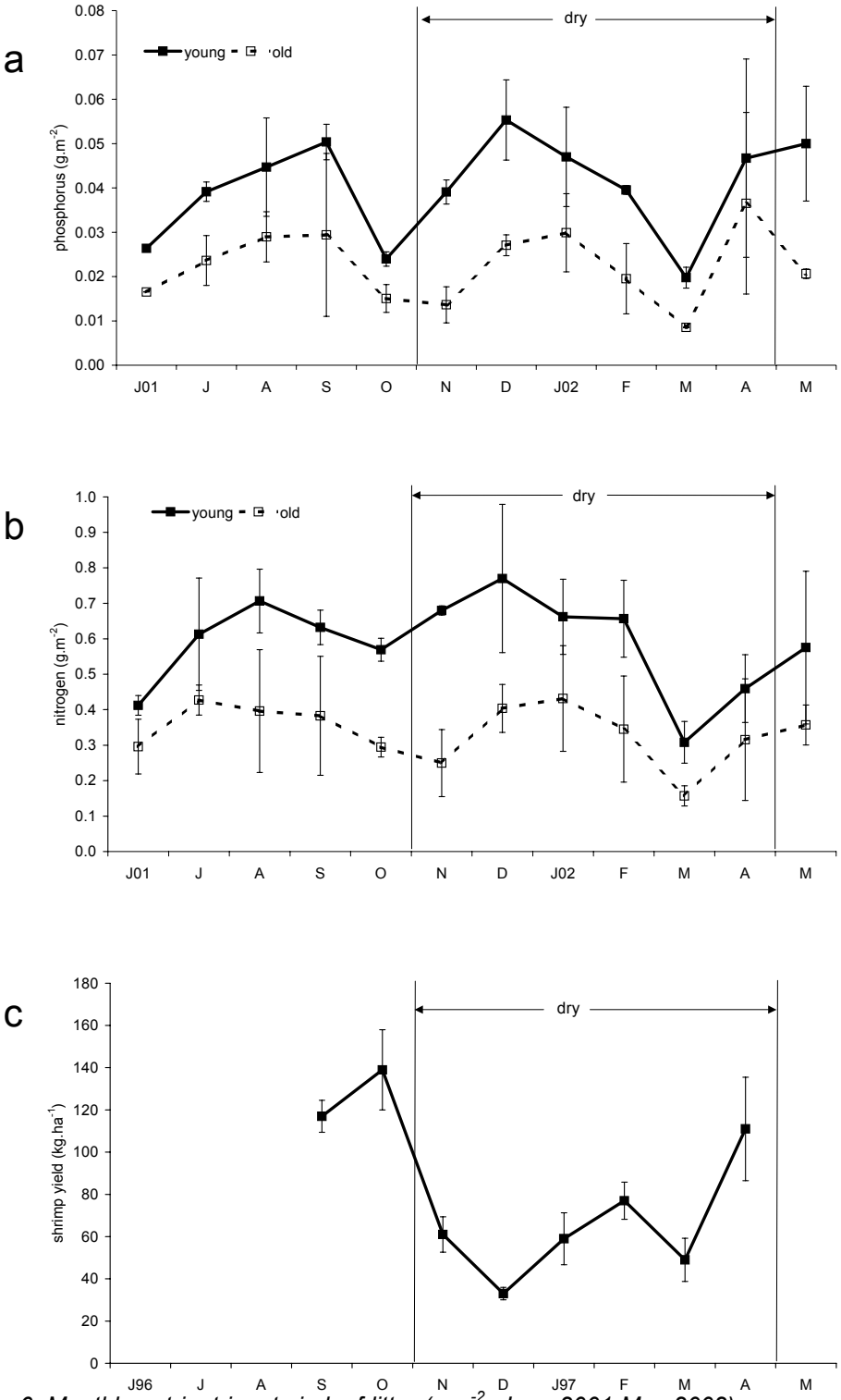

Figure 6. Monthly nutrient input via leaf litter (g.m^{-2}; June 2001-May 2002) compared with shrimp yields (kg.ha^{-1}) in the Camau province, Vietnam.
a. phosphorus (this study), b. nitrogen (this study), c. shrimp yield (kg.ha^{-1}; from Johnston et al., 2000)

wet period when litter derived material may directly be washed into the ditches and become available to the foodwebs. In the subsequent dry period, the material will tend to stay on the mangrove soil for longer and only gradually becomes available to the ditches.

Our results suggest that litter of younger mangrove stands serves as a significant food source for aquaculture and fisheries in coastal areas. As the Mekong Delta is the most important commercial fishery area in Vietnam, local government and managers should focus on preservation and plantation of mangrove system in the region.

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Chapter 3

THE DECOMPOSITION AND NUTRIENTS RELEASE OF *Rhizophora apiculata* LEAVES IN THE CAMAU PROVINCE, MEKONG DELTA, VIETNAM

(B. T. Nga, D.T. Tam, M. Scheffer and R. Roijackers)

1. Abstract

Decomposition and the release of nutrients (organic matter, nitrogen, and phosphorus) of *Rhizophora apiculata* leaves were examined in the field and under laboratory conditions. The dynamics of leaf decomposition in the field and the laboratory were similar in that the decomposition rate was highest at 30 days of incubation, thereafter decreased more gradual. The decomposition rate was significantly higher at low (5 ppt) than at high salinities (15, 25 and 35 ppt). Also the release of organic matter was significantly higher in the 5 ppt treatment than in the other treatments (0, 15, 25, and 35 ppt). There was no clear difference between the dry and wet season with respect to the release of organic matter nor phosphorus nor nitrogen. However phosphorus and nitrogen releases tended to be higher in the wet season than in the dry season. The decomposition rate was always highest when the leaves were completely submersed in the ditches of the mangrove-shrimp systems than when placed on land between the mangrove roots. The increase of nitrogen and phosphorus concentrations in decomposing mangrove leaves suggests an increase in food quality over the first half year. In this way leaf litter decomposition produces the essential nutrients (nitrogen and phosphorus) to support the aquatic organisms in mangrove-shrimp system, thus boosting shrimp production.

2. Introduction

Mangroves can be considered as open ecosystems which are important in providing energy and matter to estuarine and coastal systems via litter fall and decomposition (Mackey and Smail, 1996). The decomposition of mangrove litter, primarily as leaves, contributes with a large quantity of nutrients to the adjacent waters and sediments (Benner et al., 1986; Tam et al., 1990). Only a small percentage of decaying leaves is consumed directly by terrestrial grazing animals (Lee et al., 1990), whereas mangrove detritus constitutes a large organic matter reservoir potentially available to the estuarine food web (Benner & Hodson 1985; Tam et al., 1990).

Laegdsgaard & Johnson (2001) revealed that the role of the mangrove habitat for small juvenile fish is that they provide maximum food availability and nutrients to the aquatic ecosystem. Besides, mangrove communities are net exporters of organic material (Day et al., 1987) serving as nurseries and habitats for commercial fisheries (Tomlinson, 1986).

Decomposition involves three processes: fragmentation (caused by abiotic factors), leaching (loss of the chemical structure) and decay through microbial activity (Stewart & Davies 1989; Robertson et al., 1992). The decomposition of leaf litter and its accompanied release of nutrients are an essential function of the mangrove swamps (Holmer and Olsen, 2002). It is through the decomposition processes that nutrients i.e. nitrogen and phosphorus are released to the estuarine ecosystem and open ocean. However, the timing of release of energy and organic matter from mangrove ecosystems depends on the decomposition rates of mangrove litter, which in turn depend on the degree and frequency of tidal inundation, oxygen availability, temperature, mangrove species type, and the presence or absence of litter-consuming fauna within the forests (Benner & Hodson, 1985; Twilley et al., 1986; Steinke and Ward, 1987; Robertson, 1988; Chale 1993; Mackey & Smail, 1996). Also salinity is very important in affecting the decomposition rate (Steinke and Charles, 1986; Nga and Roijackers, 2002) .There have been many studies of mangrove litter decomposition in subtropical

regions (Lu and Lin 1990; Twilley et al., 1997; Tam et al., 1990, 1998). Recent studies showed that the decomposition rate of mangrove leaves differ among species (Mfilinge et al., 2002; Bouillon, 2003). However, information on nutrient release and supply of organic matter from decomposing mangrove leaves are not well known. Therefore, the present study aimed at investigating factors affecting on dynamics of decomposition and nutrient release from *Rhizophora apiculata* leaves.

3. Materials and methods

Experimental design and sample collection

To study the decomposition of *Rhizophora apiculata* leaves in the laboratory, senescent *R. apiculata* leaves were picked from 7 years old trees. These leaves were taken back to the laboratory within 12 hours. Approximately 30 g of the air-dried leaves were placed into a ceramic tank of 10 liters. The water was taken from the mangroves-shrimp system, and diluted to create a salinity range of 0, 5, 15, 25 and 35 ppt. The experiment was arranged in a Completely Randomized Design with four replicates for each treatment. There was a series with aeration and one without aeration. Samples from the tanks were taken at 0, 30, 90, 180, 270, and 360 days. Temperature and salinity were measured daily. Evaporation from the tanks was compensated for.

Decomposition of *Rhizophora apiculata* leaves in the field was studied in both the dry and the wet seasons (2001- 2002) in the Kien Vang enterprise, Ngoc Hien district, Ca Mau province, Viet Nam. The Ca Mau province (Camau Peninsula) is situated in the south west part of the Mekong Delta, and has a long shoreline. This region is characterised by two typical seasonal patterns, i.e. the clearly defined wet season (from May till November) with strong rainfall and high temperature (27.6 ^{o}C); and the dry season (December till April) with a lower temperature (27.1 ^{o}C) and very little rainfall. Mangrove forests in the Camau peninsula have been initially recovered as a result of both natural regeneration and manual planting of the preferred forestry species *Rhizophora apiculata*. Large areas are used for the combined mangrove-shrimp culture. The research area covered 10.7 ha of which 1.9 ha were ditches. The depth of these ditches varied (1.2 – 1.5 m); the average width was about 12 m. Dikes surrounded the shrimp pond. The water in system was exchanged every 15 days, normally at the 13^{th} – 17^{th} and 27^{th} – 3^{th} of the lunar calendar.

Senescent leaves of *Rhizophora apiculata* were collected from the trees of 7 year old stand. Approximately 50 g of the air-dried leaves were placed in fine cloth bags (20 cm x 27 cm) with a mesh size of 0.015 mm. The cloth bags were put in baskets which were covered by a plastic net with a mesh size of 0.4 mm. The baskets were placed near the bottom of the ditches and near the root system of *Rhizophora apiculata* trees in mangrove-shrimp system. After 0, 30, 60, 90, 120 and 150 days of incubation, three baskets with decaying leaves were retrieved for further analysis.

Sample analysis

Residual leaf fragments were carefully collected from the cloth bags and tanks, washed gently in order to remove all the attached materials on the leaves. These leaves were divided into two equal parts; one part was dried at 60 ^{o}C for 4-5 days subsequently grinded and analysed for total nitrogen, total phosphorus, sodium, potassium, calcium, and magnesium according to standard methods. The dry weight was determined by drying the remaining leaves of the second part at 105 ^{o}C for 24 hours; ash weight was analysed after ashing at 550 ^{o}C for 3 hours.

Decay model and nutrient release calculation

An exponential function of the type $W_t = W_o \times e^{-kt}$ (Olson, 1963) was used for determing the decomposition rate (k); in which W_t is the residual dry weight after t days, W_o is the initial dry weight, k is the decomposition rate (day^{-1}), and t is the incubation time (day).

The nutrient release ($mg.g^{-1}.d^{-1}$) was calculated as follows:

$NUTRIENT_{t\ (released)} = (DW_0 * NUTRIENT_0) - (DW_t * NUTRIENT_t)$; in which DW_0 is the initial dry weight of leaf litter, DW_t is the residual dry weight at t days. $NUTRIENT_0$ is the initial nutrient content, $NUTRIENT_t$ is the residual nutrient content at t days, and t is the incubation time (day).

Statistical analysis

Analyses of Variances (ANOVA) were performed using the available procedures in the SPSS 10.0 for Windows package. Prior to ANOVA, variables were tested for normality. Post-hoc tests were performed using the Tukey test.

4. Results

The leaf decomposition dynamics in the field and the laboratory were similar in that the decomposition rate was highest at 30 days of incubation, and decreased rapidly during the next month after which the decrease was more gradual (figure 1). There was an optimum of decomposition rate at 5 ppt both in aerated and non-aerated situations (p=0.001 and p=0.000 respectively; figure 1a, 1b). The release of organic matter was significantly highest at 5 ppt, but there were no significant differences in the release of nitrogen and phosphorus (table 1). The average and maximal decomposition rates were significantly higher at the low salinities (p=0.005 and p=0.004 respectively) than at the higher salinities (corresponding the wet and the dry season respectively; figure 2).

The decomposition rate at the end of the incubation period was not significantly different between the dry and the wet seasons (figure 1c), this was also true for the release of organic matter, phosphorus and nitrogen. However, the phosphorus and nitrogen release tended to be higher in the wet season than in the dry season (table 2). The decomposition was always highest at the bottom and there was a significant difference between the decomposition rate near the bottom of the ditches and that near the roots of mangrove stands (p=0.000; fig 1c).

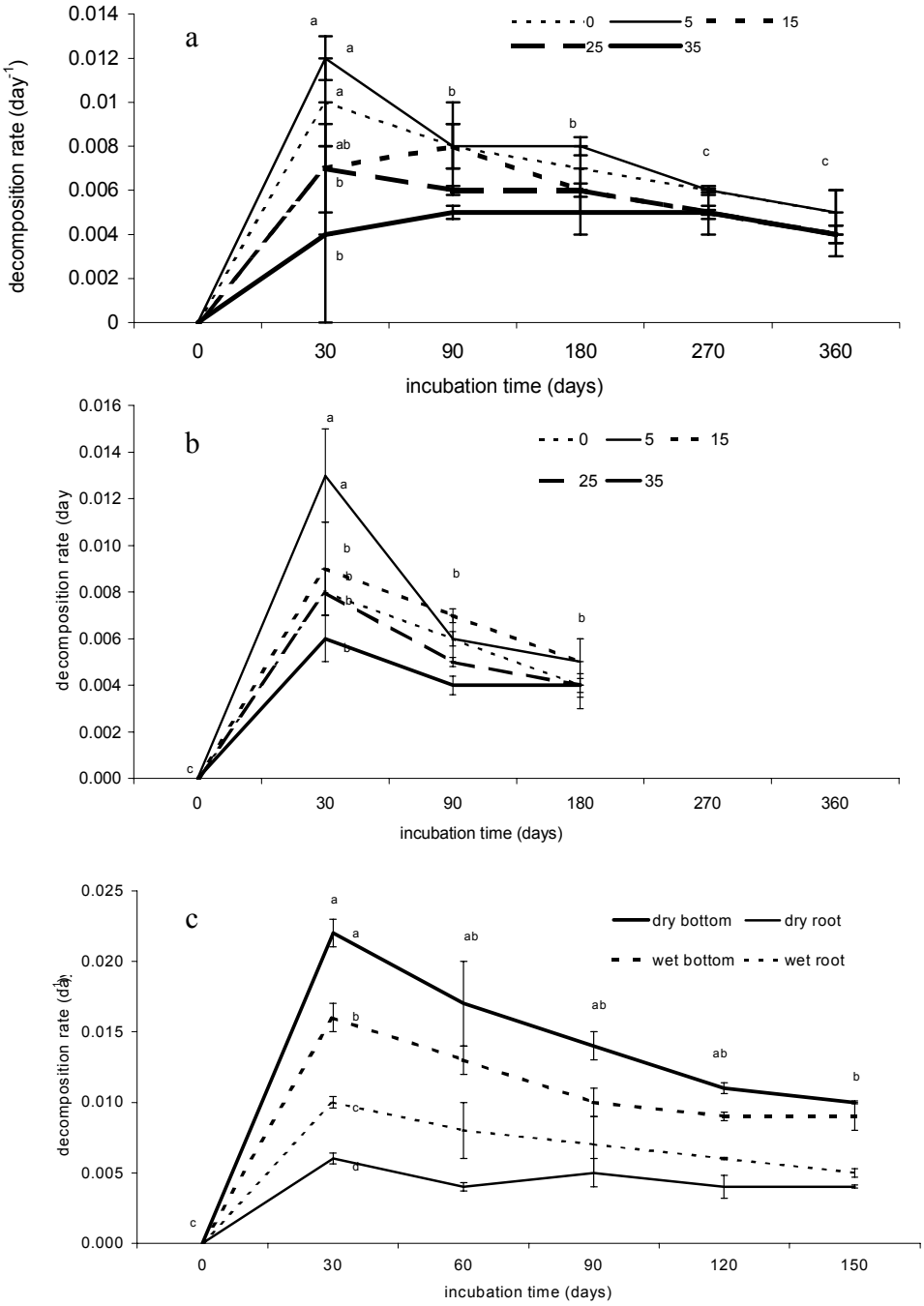

Figure 1 Decomposition rate of Rhizophora apiculata leaves in salinity with aeration (a), without aeration (b), and in seasonality (dry, wet) and location (submerged, in air) (c). Different letters above the bars indicate significant differences (p<0.05)

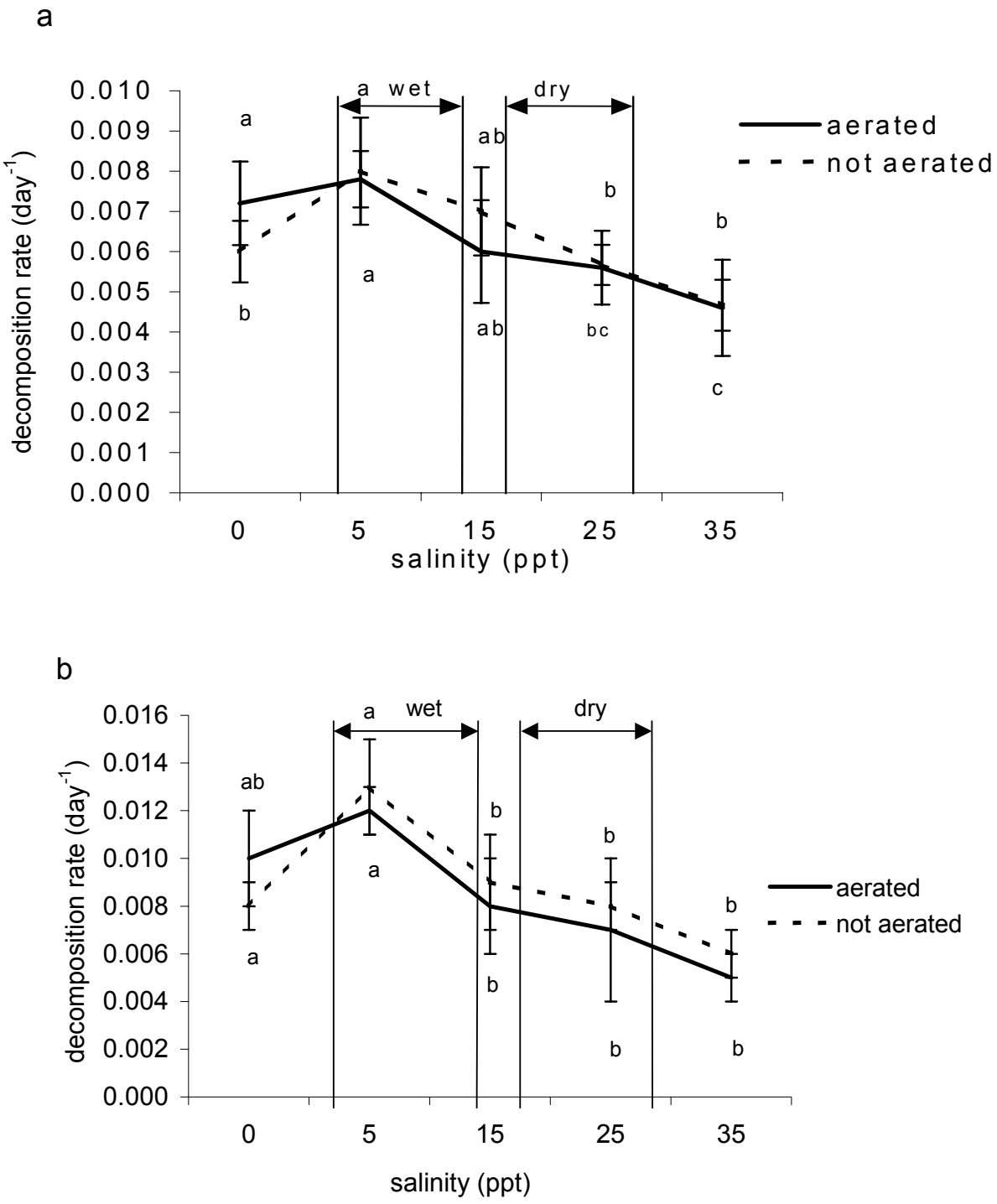

Figure 2 Average decomposition rate (a) and maximal decomposition rate of Rhizophora apiculata leaves (b) in different salinities. Different letters above the bars indicate significant differences ($p<0.05$)

Table 1. Release of nutrients from decomposing Rhizophora apiculata leaves in the laboratory ($mg.g^{-1}.d^{-1}$).

Salinity	organic matter	nitrogen	phosphorus
0	52.5 [a]	- 0.012 [a]	0.0012 [a]
5	82 [b]	- 0.015 [a]	0.0012 [a]
15	57.3 [a]	- 0.011 [a]	0.0013 [a]
25	55.1 [a]	- 0.013 [a]	0.0014 [a]
35	57.8 [a]	- 0.010 [a]	0.0015 [a]

Our results indicate that the phosphorus concentration in decomposing leaves increased significantly during the decomposition period both in the dry and wet seasons (p=0.000; figure 3a). The phosphorus concentration was significantly higher in the dry season than in the wet season after 120 days of incubation (figure 3a). The nitrogen concentration tended to be higher in the dry season than in the wet season, and was significantly higher in the end than in the beginning of incubation in both the dry and wet season (p=0.000; figure 3b).

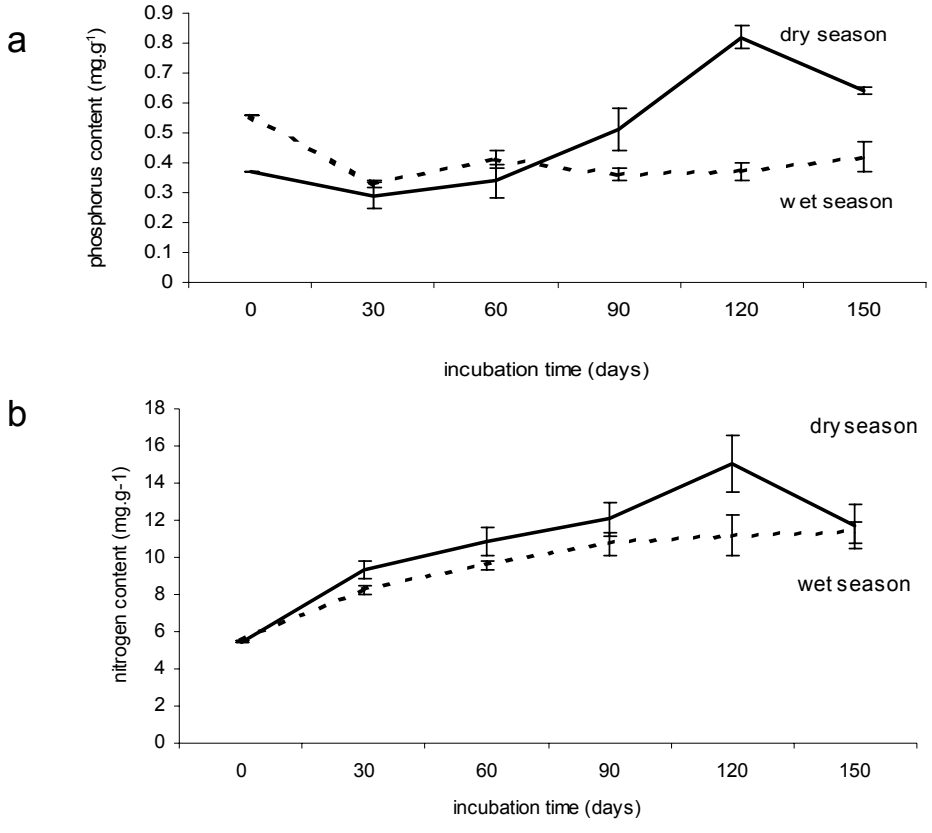

Figure 3 Nitrogen and phosphorus retentions in decomposing leaves in the Camau province, Vietnam. Different letters above the bars indicate significant differences (p<0.05)

Table 2 Release of nutrients from decomposing Rhizophora apiculata leaves in the field ($mg.g^{-1}.d^{-1}$).

	organic matter	nitrogen	phosphorus
Dry season	95.2 [a]	0.014 [a]	0.001 [a]
Wet season	93.9 [a]	0.019 [a]	0.003 [a]

Using an average leaf litter fall of 2.21 $g.m^{-2}.d^{-1}$ (Nga et al., in prep), the total amount of nutrients released in the Ca Mau *Rhizophora apiculata* forests of 7 years old was higher in the wet season than in the dry season (p=0.001; table 3).

Table 3. Organic matter and nutrient release from average mangrove leaf litter (2.21 $g.m^{-2}.d^{-1}$) at 7 year old stands in Ca Mau province, Vietnam.

Components	Average nutrient release in dry season ($mg.g^{-1}.d^{-1}$)	Average nutrient release in wet season ($mg.g^{-1}.d^{-1}$)	Nutrient release in Ca Mau forest in dry season ($mg.m^{-2}.d^{-1}$)	Nutrient release in Ca Mau forest in wet season ($mg.m^{-2}.d^{-1}$)
Organic matter	95.7 [a]	94.2 [a]	211.6 [a]	208.2 [a]
Nitrogen	0.014 [a]	0.019 [a]	0.031 [a]	0.042 [a]
Phosphorus	0.001 [a]	0.003 [a]	0.003 [a]	0.007 [a]
Potassium	0.054 [a]	0.062 [b]	0.118 [a]	0.136 [b]
Sodium	0.157 [a]	0.180 [b]	0.347 [a]	0.398 [b]
Calcium	0.027 [a]	0.078 [b]	0.061 [a]	0.171 [b]
Magnesium	0.024 [a]	0.027 [a]	0.054 [a]	0.059 [a]

5. Discussion

In the decomposition proces several factors play a key role, i.e. temperature, moisture and salinity. Our study allowed for a closer look at these factors.
With respect to the temperature it is found that mangrove leaf litter decomposition rates are higher in forests at the lower latitudes (Bouillon, 2003). This probably reflects a dependency of the decomposition rate on temperature, and a consistently higher temperature throughout the year at the lower latitudes results in an increased decomposition (Mackey & Smail, 1996). Temperature may be acting through its effects on the population level of microbial decomposers or invertebrate shredders; i.e. in the tropics, with their higher temperatures, decomposers and shredder populations will be active throughout the year (Steinke & Charles, 1986). Mackey & Smail (1996) illustrated the effect of temperature on a local scale in a south-east Queensland forest by measuring higher decomposition rates in the summer compared to the winter. In our study the decomposition rate near the bottom of the ditches was significant higher in

the dry season than in the wet season, probably due to the higher temperatures (dry season: 27.7 - 29.7^{o}C; wet season: 27.2 - 28.6^{o}C). Furthermore, the oxygen concentration in the ditches was higher in the dry season, and mangrove leaves decomposition was faster in the aerobic conditions (Mall et all., 1991; Mfilinge et al., 2002).

The effect of moisture on the decomposition rate was illustrated by Twilley et al. (1986) and Flores-Verdugo et al. (1987), who observed higher decomposition rates in the rainy season. This is in line with our finding that the decomposition rate (near the roots of mangrove trees) was higher in the wet season than in the dry season. Furthermore, we found that the decomposition rate was higher when the leaves were continuous submerged (bottom of the ditches) than only occasionally submerged (near the roots) at high water levels and during heavy rainfall. The moisty environment increases the capability of microbial decomposers to utilise detritus and its greater availability may have also led to a higher decomposition rate (Cundell, 1979; Fell & Masters, 1980; Woitchik et al., 1997, Dick et al., 2000). The high decomposition rate in the wet season may enhance the availability of nutrients to aquatic organisms. In fact, shrimp yields were significantly higher in the wet season than in the dry season ($F=15.1$, $p=0.001$) at the Tam Giang and 184 farms in Camau province (Johnston et al., 2000).
Low salinity levels also facilitate decomposition (Steinke & Charles, 1986; Mall et al., 1991). In our study, the decomposition rate of *Rhizophora apiculata* leaves was dependent on salinity. A higher decomposition rate was recorded in the lower salinity ranges reaching an optimum at 5 ppt. Fungi are important decomposers in the decomposition process that usually starts with colonization on dead material (Townsend et al., 2000). Very few studies have investigated the effects of salinity on the mycota in mangrove forests (Hyde & Lee, 1995), however, Hyde (1992) concluded that the distribution of fungi was probably limited by periods of higher salinity, and higher salinity ranges may inhibit the activity of fungi. In the Camau forests, we recorded a water salinity ranging from 4 - 9 ppt and 17- 25 ppt in the wet and the dry season respectively. The higher decomposition rates in the rainy season seems likely to be related to low salinities during that season.

Increases in nutrient concentration in residual decomposing leaves occurred for phosphorus and nitrogen. Whether nutrients are released or absorbed during decomposition is the net result of mineralization and the import and export of nutrients through animal activity, translocation in fungal and abiotic processes (O'Connell, 1988). Our study showed that after a peak increase at 120 days, the nitrogen concentration declined gradually. An increase followed by a decrease in nitrogen content during decomposition of mangrove leaf litter is commonly reported (Steinke et al., 1993; Lee, 1989; Tam et al., 1990). The increase in nitrogen level could be a combination of the presence of nitrogen compounds which are not easily leached and microbial immobilization of nutrients by bacteria and fungi colonizing the residual leaves (Robertson 1988; Chale, 1993). The

microbial immobilized nitrogen is then mineralized and released to the surrounding environment leading to a decrease in nitrogen content. The increase of nitrogen and phosphorus concentrations in decomposing leaves could result in a higher food quality, thus making it more attractive to aquatic organisms. This was reported by Zhou (2001) who revealed that meiofauna colonization on mangrove leaves varied over the decaying time: no colonization in the early stages of decomposition (1-10 days), followed by a transitional period (10-30 days) and, finally, a high colonization in the later stages (30-60 days). This suggests that such meiofauna can utilize not only periphyton but also bacteria, invertebrates and organic matter developing during leaf litter decomposition, and thus becoming themselves also a prime food source for other aquatic animals in mangrove-shrimp systems, especially for shrimps. Therefore, the continuous increase of N and P concentrations during the decomposition period may support the aquatic organisms, particularly shrimps, in the mangrove-shrimp system as well as the detritus-based food web (figure 4).
Furthermore, through the decomposition process nitrogen and phosphorus may become available to sediments where these nutrients will be absorbed by *Rhizophora apiculata* (Steinke et al., 1993; Lu & Lin, 1990). This suggests that recycling of nutrients as nitrogen and phosphorus is an important aspect for sustainable management of the mangrove-shrimp system and the mangroves ecosystem itself.

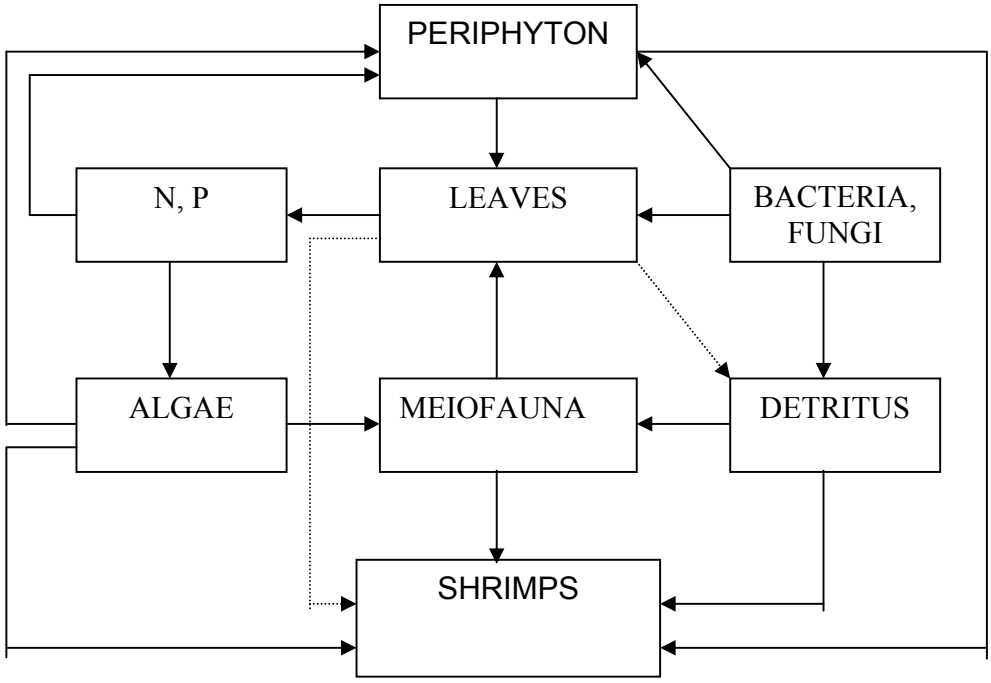

Figure 4 The detritus-based food web in mangrove-shrimp system in Camau province, Vietnam.

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Chapter 4

EFFECTS OF DECOMPOSING *Rhizophora apiculata* LEAVES ON POST- LARVAE OF THE SHRIMP *Penaeus monodon*

(B.T. Nga, T.T. Nghia, V.N. Ut, M. Scheffer and R. Roijackers)

1. Abstract

We studied the effects of different concentrations of decomposing *Rhizophora apiculata* leaves and their extracts on larvae of the shrimp *Penaeus monodon* under laboratory conditions. Shrimp mortality depended highly on the oxygen concentration, which was strongly correlated to the amount of decomposing leaves. Shrimps died after five minutes at the highest concentration in mangrove extracts (15 $g.l^{-1}$). Both leaves and their extracts decreased the shrimp survival and biomass significantly at the higher concentrations. By contrast moderate amounts of leaves or their extracts had positive effects on shrimps. Survival and biomass of the shrimps when together with plastic leaves was lower than for shrimps with mangrove leaves. This indicated 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 of oxygen concentration. Water circulation may help to prevent low oxygen conditions and reduce local accumulation of mangrove leaves.

2. Introduction

Mangrove forests are known as one of the best nurseries for aquatic larvae and juveniles and provides a rich food supply for fisheries (Weinstein and Brooks, 1983; Wright, 1986; Robertson and Duke, 1987; Little et al., 1988; Zhou, 2001). Mangroves provide litter which is a source of organic matter, and nutrients in coastal waters. Detritus from mangrove litter is a major energy source for most marine benthic systems (Findlay and Tonore, 1982). For example, many bottom-feeding and filter-feeding finfish, shellfish and copepods have been reported to utilize decomposed mangrove litter as a prime food source (Rao and Nair, 1984; Athithan and Ramadhas, 2000). It has been demonstrated that 1 ha of mangrove forest may support 100-1000 $kg.yr^{-1}$ of marine fish and shrimp catch (Hambrey, 1996).

In mangrove-shrimp systems, most of the growth of shrimp is dependent on the available food in the ditches; mangrove leaves fallen in ditches where shrimps live may function as a source of food for all kind of animals, including shrimps (Roijackers and Nga, 2002). The presence of leaves in the ditches could offer a good shelter against predation (Primavera, 1997). Apart from these potential benefits of mangrove leaves for shrimps, 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 was practiced abundantly in recent years. However, the effects of mangrove leaves on shrimp have not been well studied. In the present study, we investigated whether and in which way the different amounts of mangrove leaves (*Rhizophora apiculata*) affect the mortality and the growth of larvae of the shrimp *Penaeus monodon*.

3. Materials and Methods

Experimental design

Four experiments were performed at the laboratory to test the effects of *Rhizophora apiculata* leaves on survival and growth of *Penaeus monodon* post larvae (see table 1 for an overview):

1. In the first experiment, we tested the effect of three different rearing systems (biofilter recirculation with aeration, no biofilter recirculation with aeration, and no biofilter recirculation without aeration).
2. In the second experiment, we compared the effects of mangrove leaves to that of their extracts.
3. Experiment 3 aimed to investigate 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. In the final experiment, individual shrimps were tested for the effects of decomposing mangrove leaves to exclude competition among shrimp larvae.

Five concentrations of either mangrove leaves or extracts were used in each experiment

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

no	*Treatments*	*water volume (l)*	*replicates*	*exp. 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 extracts 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 extracts: 0, 2.5, 5, 10, 15 $g.l^{-1}$ wet weight	50	4	60	leaves and leaf extracts
3	Three leaf types: no leaves, 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 growth affecting substances from leaves
4	five leaf concentrations: 0, 2.5, 5, 10, 15 $g.l^{-1}$ wet weight	1	20	60	exclusion of competition

Preparation of leaves and their extract

Leaves that were ready to abscise when touched were collected from *Rhizophora apiculata* trees in stands of 7 years old. These senescent leaves were incubated at the laboratory in 500-liter tanks with a concentration of 15 $g.l^{-1}$ (wet weight) for approximately 10 days. Lower concentrations of leaf extracts were obtained by diluting the solution. Before being used the plastic leaves were washed and incubated for 30 days in tap water to remove possibly harmful chemical agents.

Rearing systems and chemical of rearing water

Biofilter recirculation and aeration systems were used in experiment 1. Aeration was applied in experiment 3 and 4 . 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 NO_2^- were measured weekly.

Stocking density, feeding and growth criteria for shrimp

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

On days 0, 30 and 60 the dry weight (DW) of the shrimps was measured. DW of the shrimps was calculated by the change in weight before and after drying at 105^oC for 24 hours. Survival was recorded at the end of experimental period except in experiment 1, where it was recorded every five minutes during the first 1 hour and after that hourly up to the end of the experiment (96 hours).

Statistics

Analyses of Variances (ANOVA) were performed using the available procedures in the SPSS 10.0 for Windows package. Prior to ANOVA, variables were tested for normality. Post-hoc tests were performed using the Tukey test.

4. Results

In our first experiment, survival decreased steeply with leaf biomass (p= 0.000) 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 (figure 1).

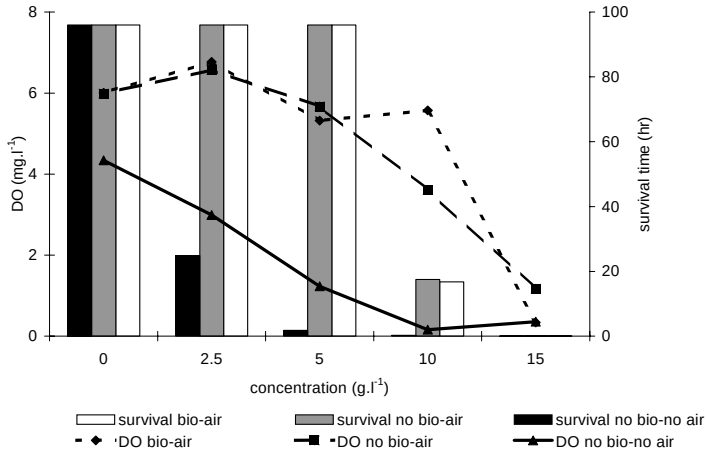

Figure 1. Survival time of Penaeus monodon larvae in three different rearing systems, i.e. 1) biofilter-recirculation with aeration (bio-air), 2) no biofilter recirculation with aeration (no bio-air) and 3) no biofilter recirculation without aeration (no bio-no air)) tested at different concentrations of decaying Rhizophora apiculata leaves. Final concentrations of oxygen (DO) in the water have been indicated (line).

In the second experiment, both leaves and their extracts produced the highest shrimp survival at 5 g.l^{-1} and a significantly (p =0.000) strong decrease in survival at the higher concentrations (figure 2a); the shrimp biomass was higher when grown on leaf extracts, than when grown on leaves. In fact, shrimp biomass tended to decline with increasing leaf biomass (figure 2b).

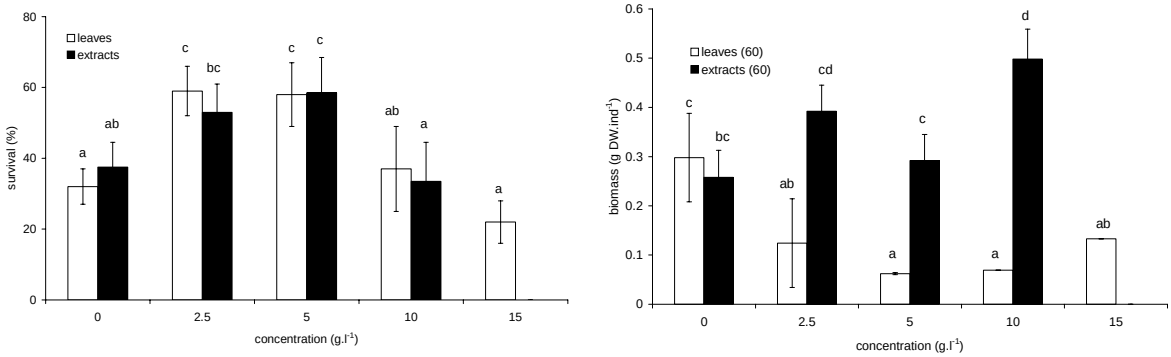

Figure 2. Survival (left) and biomass (right) of Penaeus monodon post-larvae after 60 days, supplied with different concentrations from either decaying Rhizophora apiculata leaves or extracts from decaying leaves. Different letters above the bars indicate significant differences (p < 0.05)

Our third experiment revealed that the survival of shrimps was not significant different between the natural and plastic leaves (figure 3a); the biomass of the surviving shrimps, however, was significantly lower (p=0.000) in the plastic leaf treatment (figure 3b).

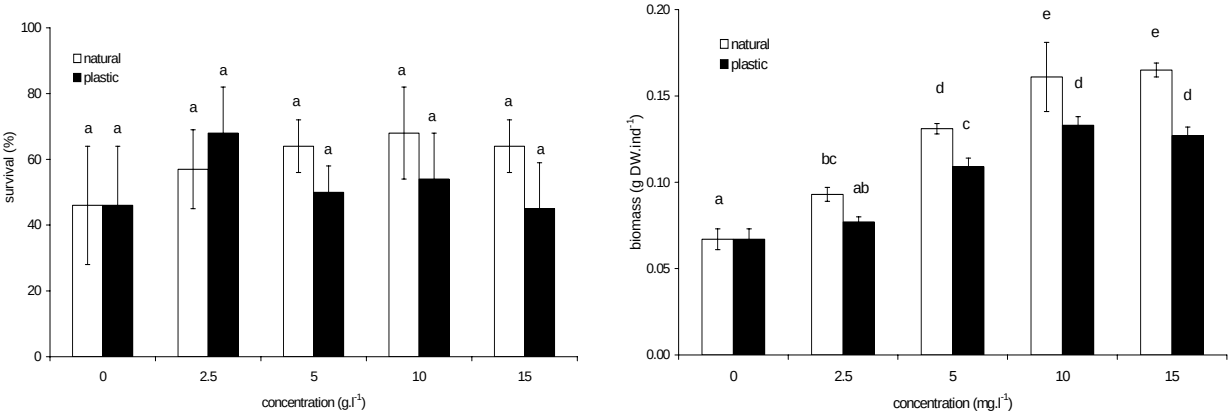

Figure 3. Survival (left) and biomass (right) of Penaeus monodon post-larvae after 60 days in direct contact with different concentrations of decaying Rhizophora apiculata leaves or artificial leaves. Different letters above the bars indicate significant differences (p < 0.05).

When cultured individually the survival of shrimps increased in the presence of mangrove leaves (figure 4a), reaching a maximum at a mangrove leaf concentration of 10 g.l^{-1}. The biomass of the shrimp post-larvae was significantly lower at the lower levels of decaying leaves compared to the control (figure 4b).

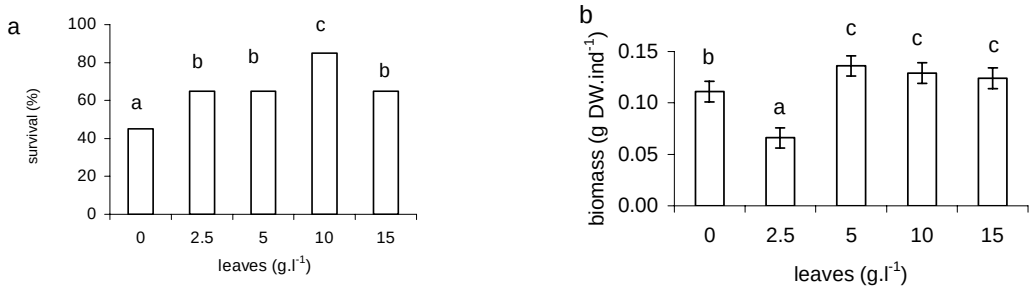

Figure 4. Survival (left) and biomass (right) of Penaeus monodon post-larvae incubated with different levels of decaying Rhizophora apiculata leaves. Competition was excluded. Different letters above the bars indicate significant differences ($p < 0.05$).

The BOD increased gradually during the incubation period (experiment 3; figure 5a). In all series a rapid increase in nitrite was observed until a maximum was reached in the third week for the control, all series with plastic leaves and the two series with the lowest concentration of mangrove leaves (figure 5b). One week later the other series reached their maximum. The control and all series with plastic leaves kept these high nitrite levels, but in the series with natural leaves the nitrite level decreased rapidly. The sulphide levels were low in the control series and all series with plastic leaves (figure 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.

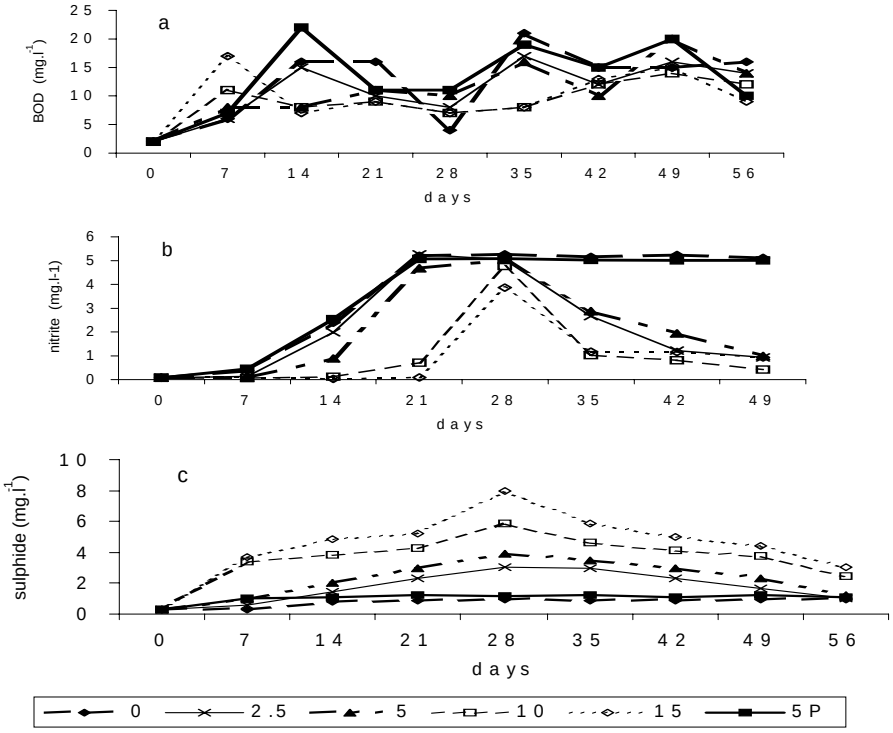

Figure 5. Concentrations of BOD, nitrite and sulphide in the water for the different treatments (0, 2.5, 5, 10, 15 g.$^{l-1}$ leaves). Only one treatment with artificial leaves (5P) has been shown as all gave the same result.

5. Discussion

Dissolved oxygen (DO) is the most important limiting factor in the intensive cultivation of shrimp species (Rosas et al., 1995). Penaeid shrimps have been shown to be 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 *Penaeus monodon* was 0.9 $mg.l^{-1}$. In our experiments shrimp post-larvae died within 10 minutes at the highest leaf concentrations with a DO level of approximately 0 $mg.l^{-1}$. Also, the fact that the biomass of shrimps grown on mangrove leaves was lower than the biomass of those grown on mangrove leaf extracts, was probably due to the low oxygen level in the mangrove treatment (1.5 - 2 $mg.l^{-1}$) as compared to that in extract treatments (4.5 - 6 $mg.l^{-1}$). The observed difference in growth is in line with the studies of Seidman and Lawrence (1985) who reported that when oxgen was below 2 $mg.l^{-1}$ the growth rates of *Penaeus monodon* and *Penaeus vannamei* were significantly reduced. Also, Martinez-Cordova et al. (1998) showed that the average shrimp yield was significantly higher in aerated shrimp ponds (1687-1813 $kg.ha^{-1}$) compared to not aerated ponds (1243 $kg.ha^{-1}$). Thus, the supply of dissolved oxygen (DO) to a system in which mangrove leaves are decaying is essential not only for the survival but also for the growth of shrimp post-larvae.

Although oxygen is clearly important it 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 ($p=0.000$; $R=0.847$). This sulphide clearly originated from the leaves through leaching. Sulphide is toxic to all aerobic organisms (Visman,1996), and plays a large 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). We recorded an increase in nitrite, which was fastest and highest in the treatments without decaying mangrove leaves indicating an ameliorating effect of the leaves in our experiments on this particularly stressor. Nitrite is highly toxic to fish and could be detrimental to shrimps; concentrations in our experiments far exceeded the safe level of nitrite recommended for shrimp growth (<0.5 $mg.l^{-1}$, Chien, 1992), although after one month the nitrite and sulphide decreased considerably in the treatments with mangrove leaves. The fact that shrimp biomass was significantly higher for the mangrove leaf treatments compared to the plastic leaf treatments, may well be due to the reduction of nitrite levels in the presence of real leaves (figure 5b).

Nitrite is the intermediate product in the microbial nitrification of ammonia to nitrate or in the denitrification of nitrate and 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). In a way

the high C/N ratio in natural leaves thus seems to balance the excessive N input with CP pellets, allowing bacteria to assimilate potentially toxic nitrite.
Our results show that the effects of mangrove leaves on shrimps are rather complex and may work out either positively or negatively (figure 6). This contrasts with the dominant view which stresses benificial effects of mangrove leaves. Indeed the leaves are a source of food and serve as a substratum for attached organisms such as algae, fungi and bacteria which also serve as food (Fell and Masters, 1980; Vijayaraghavan and Wafar, 1983; Alongi et al., 1989; Hyde and Lee, 1995; Michael-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. For instance, in the Mekong Delta traditional systems (without mangroves) give annual yields of 100-400 $kg.ha^{-1}.yr^{-1}$, whereas mangrove-shrimp systems give an annual yield of 100-600 $kg.ha^{-1}.yr^{-1}$ (Johnston et al., 2000).

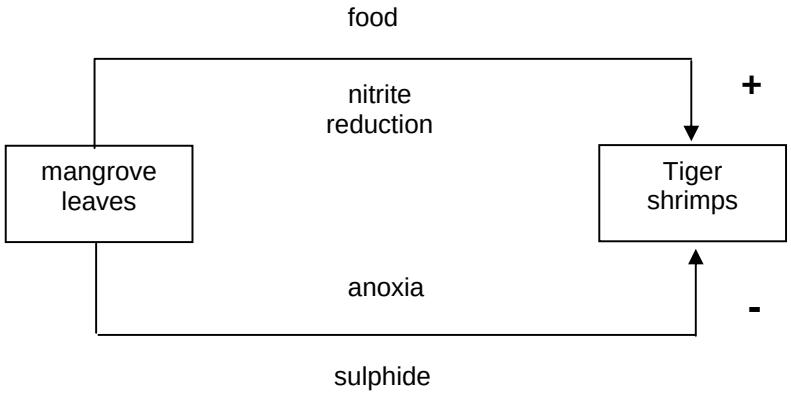

Figure 6. Effects of mangrove leaves on shrimps; while anoxia and sulphide have negative effects on shrimps, positive effects (food and nitrite reduction) outweigh the negative effects.

Still anoxia and rise of sulphide levels are not uncommon in the field (Alongi et al., 1999a, b; McGraw et al., 2001). Our results suggest that production could be higher if occurrence of such conditions could be reduced. It seems, therefore, a good idea to create a permanent water exchange at places where high quantities of mangrove leaves accumulate.

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Chapter 4

EFFECTS OF DECOMPOSING *Rhizophora apiculata* LEAVES ON POST- LARVAE OF THE SHRIMP *Penaeus monodon*

(B.T. Nga, T.T. Nghia, V.N. Ut, M. Scheffer and R. Roijackers)

1. Abstract

We studied the effects of different concentrations of decomposing *Rhizophora apiculata* leaves and their extracts on larvae of the shrimp *Penaeus monodon* under laboratory conditions. Shrimp mortality depended highly on the oxygen concentration, which was strongly correlated to the amount of decomposing leaves. Shrimps died after five minutes at the highest concentration in mangrove extracts (15 $g.l^{-1}$). Both leaves and their extracts decreased the shrimp survival and biomass significantly at the higher concentrations. By contrast moderate amounts of leaves or their extracts had positive effects on shrimps. Survival and biomass of the shrimps when together with plastic leaves was lower than for shrimps with mangrove leaves. This indicated 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 of oxygen concentration. Water circulation may help to prevent low oxygen conditions and reduce local accumulation of mangrove leaves.

2. Introduction

Mangrove forests are known as one of the best nurseries for aquatic larvae and juveniles and provides a rich food supply for fisheries (Weinstein and Brooks, 1983; Wright, 1986; Robertson and Duke, 1987; Little et al., 1988; Zhou, 2001). Mangroves provide litter which is a source of organic matter, and nutrients in coastal waters. Detritus from mangrove litter is a major energy source for most marine benthic systems (Findlay and Tonore, 1982). For example, many bottom-feeding and filter-feeding finfish, shellfish and copepods have been reported to utilize decomposed mangrove litter as a prime food source (Rao and Nair, 1984; Athithan and Ramadhas, 2000). It has been demonstrated that 1 ha of mangrove forest may support 100-1000 $kg.yr^{-1}$ of marine fish and shrimp catch (Hambrey, 1996).

In mangrove-shrimp systems, most of the growth of shrimp is dependent on the available food in the ditches; mangrove leaves fallen in ditches where shrimps live may function as a source of food for all kind of animals, including shrimps (Roijackers and Nga, 2002). The presence of leaves in the ditches could offer a good shelter against predation (Primavera, 1997). Apart from these potential benefits of mangrove leaves for shrimps, 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 was practiced abundantly in recent years. However, the effects of mangrove leaves on shrimp have not been well studied. In the present study, we investigated whether and in which way the different amounts of mangrove leaves (*Rhizophora apiculata*) affect the mortality and the growth of larvae of the shrimp *Penaeus monodon*.

3. Materials and Methods

Experimental design

Four experiments were performed at the laboratory to test the effects of *Rhizophora apiculata* leaves on survival and growth of *Penaeus monodon* post larvae (see table 1 for an overview):

1. In the first experiment, we tested the effect of three different rearing systems (biofilter recirculation with aeration, no biofilter recirculation with aeration, and no biofilter recirculation without aeration).
2. In the second experiment, we compared the effects of mangrove leaves to that of their extracts.
3. Experiment 3 aimed to investigate 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. In the final experiment, individual shrimps were tested for the effects of decomposing mangrove leaves to exclude competition among shrimp larvae.

Five concentrations of either mangrove leaves or extracts were used in each experiment

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

no	*treatments*	*water volume (l)*	*replicates*	*exp. 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 extracts 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 extracts: 0, 2.5, 5, 10, 15 $g.l^{-1}$ wet weight	50	4	60	leaves and leaf extracts
3	Three leaf types: no leaves, 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 growth affecting substances from leaves
4	five leaf concentrations: 0, 2.5, 5, 10, 15 $g.l^{-1}$ wet weight	1	20	60	exclusion of competition

Preparation of leaves and their extract

Leaves that were ready to abscise when touched were collected from *Rhizophora apiculata* trees in stands of 7 years old. These senescent leaves were incubated at the laboratory in 500-liter tanks with a concentration of 15 $g.l^{-1}$ (wet weight) for approximately 10 days. Lower concentrations of leaf extracts were obtained by diluting the solution. Before being used the plastic leaves were washed and incubated for 30 days in tap water to remove possibly harmful chemical agents.

Rearing systems and chemical of rearing water

Biofilter recirculation and aeration systems were used in experiment 1. Aeration was applied in experiment 3 and 4 . 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 NO_2^- were measured weekly.

Stocking density, feeding and growth criteria for shrimp

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

On days 0, 30 and 60 the dry weight (DW) of the shrimps was measured. DW of the shrimps was calculated by the change in weight before and after drying at 105^oC for 24 hours. Survival was recorded at the end of experimental period except in experiment 1, where it was recorded every five minutes during the first 1 hour and after that hourly up to the end of the experiment (96 hours).

Statistics

Analyses of Variances (ANOVA) were performed using the available procedures in the SPSS 10.0 for Windows package. Prior to ANOVA, variables were tested for normality. Post-hoc tests were performed using the Tukey test.

4. Results

In our first experiment, survival decreased steeply with leaf biomass (p= 0.000) 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 (figure 1).

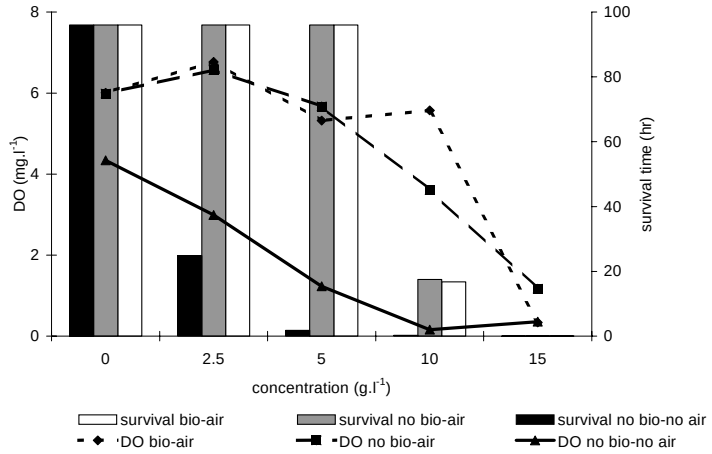

Figure 1. Survival time of Penaeus monodon larvae in three different rearing systems, i.e. 1) biofilter-recirculation with aeration (bio-air), 2) no biofilter recirculation with aeration (no bio-air) and 3) no biofilter recirculation without aeration (no bio-no air)) tested at different concentrations of decaying Rhizophora apiculata leaves. Final concentrations of oxygen (DO) in the water have been indicated (line).

In the second experiment, both leaves and their extracts produced the highest shrimp survival at 5 g.l^{-1} and a significantly (p =0.000) strong decrease in survival at the higher concentrations (figure 2a); the shrimp biomass was higher when grown on leaf extracts, than when grown on leaves. In fact, shrimp biomass tended to decline with increasing leaf biomass (figure 2b).

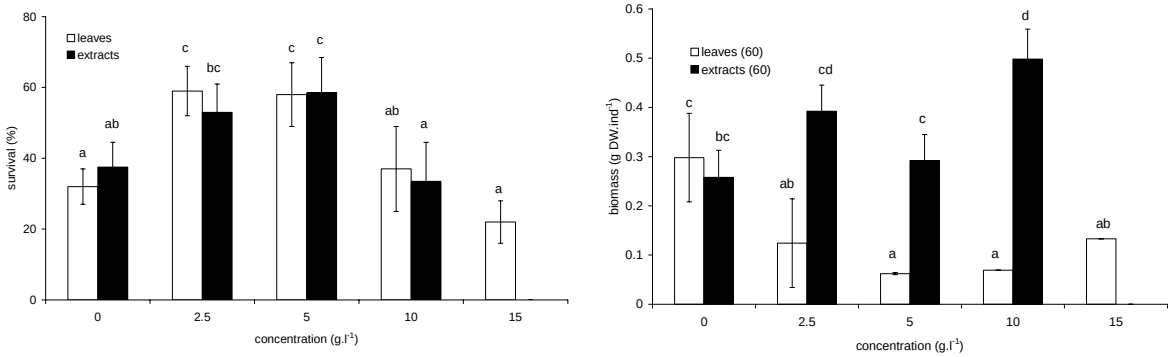

Figure 2. Survival (left) and biomass (right) of Penaeus monodon post-larvae after 60 days, supplied with different concentrations from either decaying Rhizophora apiculata leaves or extracts from decaying leaves. Different letters above the bars indicate significant differences (p < 0.05)

Our third experiment revealed that the survival of shrimps was not significant different between the natural and plastic leaves (figure 3a); the biomass of the surviving shrimps, however, was significantly lower (p=0.000) in the plastic leaf treatment (figure 3b).

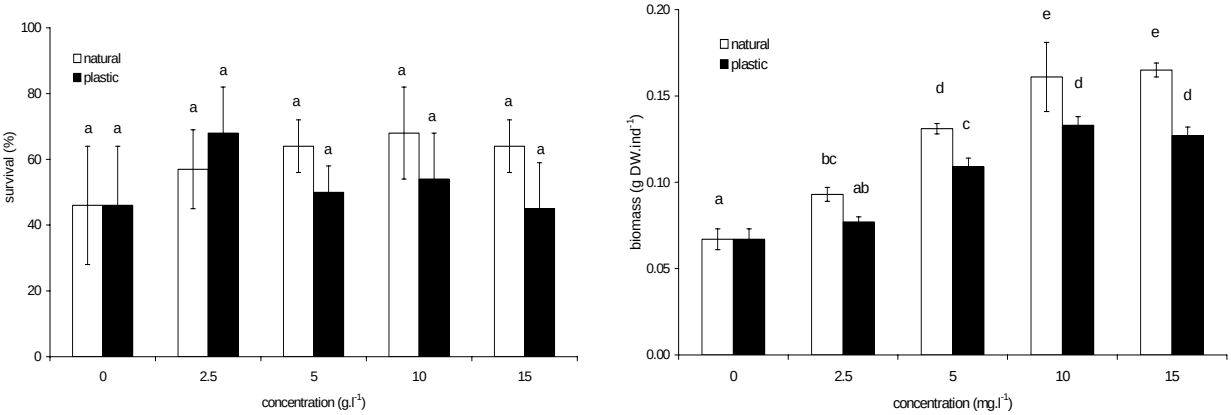

Figure 3. Survival (left) and biomass (right) of Penaeus monodon post-larvae after 60 days in direct contact with different concentrations of decaying Rhizophora apiculata leaves or artificial leaves. Different letters above the bars indicate significant differences (p < 0.05).

When cultured individually the survival of shrimps increased in the presence of mangrove leaves (figure 4a), reaching a maximum at a mangrove leaf concentration of 10 g.l^{-1}. The biomass of the shrimp post-larvae was significantly lower at the lower levels of decaying leaves compared to the control (figure 4b).

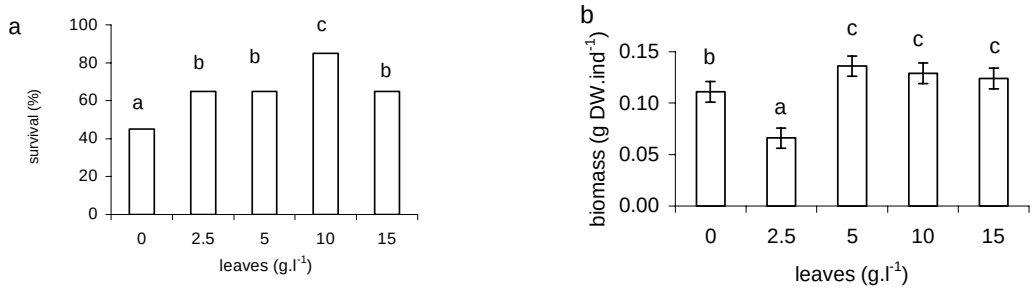

Figure 4. Survival (left) and biomass (right) of Penaeus monodon post-larvae incubated with different levels of decaying Rhizophora apiculata leaves. Competition was excluded. Different letters above the bars indicate significant differences (p < 0.05).

The BOD increased gradually during the incubation period (experiment 3; figure 5a). In all series a rapid increase in nitrite was observed until a maximum was reached in the third week for the control, all series with plastic leaves and the two series with the lowest concentration of mangrove leaves (figure 5b). One week later the other series reached their maximum. The control and all series with plastic leaves kept these high nitrite levels, but in the series with natural leaves the nitrite level decreased rapidly. The sulphide levels were low in the control series and all series with plastic leaves (figure 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.

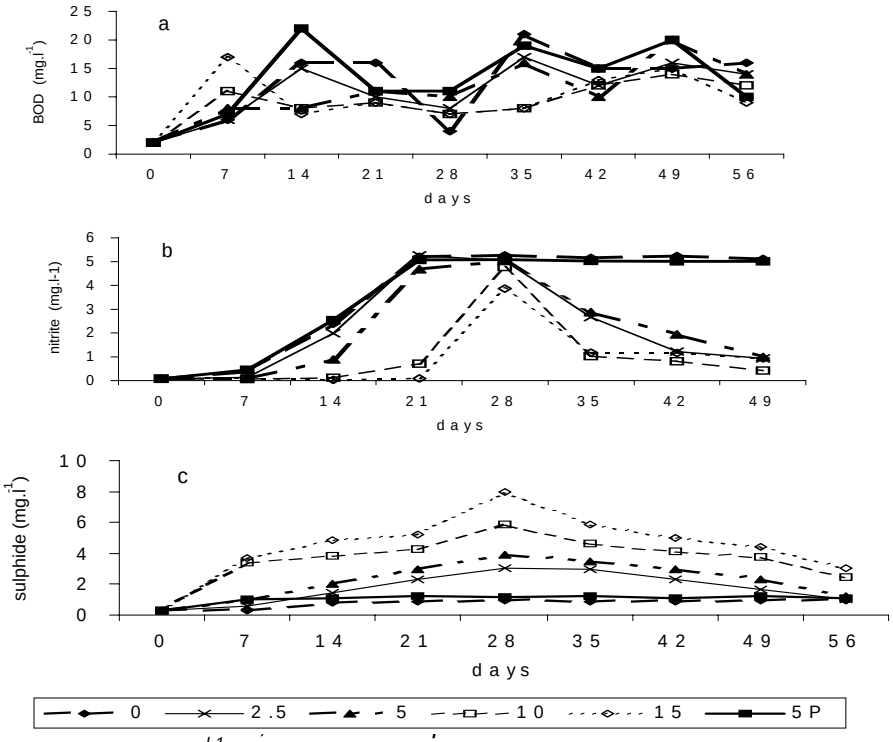

Figure 5 ents (0, 2.5, 5, 10, 15 g.l-1 leaves). Only one treatment with artificial leaves (5P) has been shown as all gave the same result.

5. Discussion

Dissolved oxygen (DO) is the most important limiting factor in the intensive cultivation of shrimp species (Rosas et al., 1995). Penaeid shrimps have been shown to be 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 *Penaeus monodon* was 0.9 $mg.l^{-1}$. In our experiments shrimp post-larvae died within 10 minutes at the highest leaf concentrations with a DO level of approximately 0 $mg.l^{-1}$. Also, the fact that the biomass of shrimps grown on mangrove leaves was lower than the biomass of those grown on mangrove leaf extracts, was probably due to the low oxygen level in the mangrove treatment (1.5 - 2 $mg.l^{-1}$) as compared to that in extract treatments (4.5 - 6 $mg.l^{-1}$). The observed difference in growth is in line with the studies of Seidman and Lawrence (1985) who reported that when oxgen was below 2 $mg.l^{-1}$ the growth rates of *Penaeus monodon* and *Penaeus vannamei* were significantly reduced. Also, Martinez-Cordova et al. (1998) showed that the average shrimp yield was significantly higher in aerated shrimp ponds (1687-1813 $kg.ha^{-1}$) compared to not aerated ponds (1243 $kg.ha^{-1}$). Thus, the supply of dissolved oxygen (DO) to a system in which mangrove leaves are decaying is essential not only for the survival but also for the growth of shrimp post-larvae.

Although oxygen is clearly important it 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 ($p=0.000$; $R=0.847$). This sulphide clearly originated from the leaves through leaching. Sulphide is toxic to all aerobic organisms (Visman,1996), and plays a large 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). We recorded an increase in nitrite, which was fastest and highest in the treatments without decaying mangrove leaves indicating an ameliorating effect of the leaves in our experiments on this particularly stressor. Nitrite is highly toxic to fish and could be detrimental to shrimps; concentrations in our experiments far exceeded the safe level of nitrite recommended for shrimp growth (<0.5 $mg.l^{-1}$, Chien, 1992), although after one month the nitrite and sulphide decreased considerably in the treatments with mangrove leaves. The fact that shrimp biomass was significantly higher for the mangrove leaf treatments compared to the plastic leaf treatments, may well be due to the reduction of nitrite levels in the presence of real leaves (figure 5b).

Nitrite is the intermediate product in the microbial nitrification of ammonia to nitrate or in the denitrification of nitrate and 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). In a way

the high C/N ratio in natural leaves thus seems to balance the excessive N input with CP pellets, allowing bacteria to assimilate potentially toxic nitrite.
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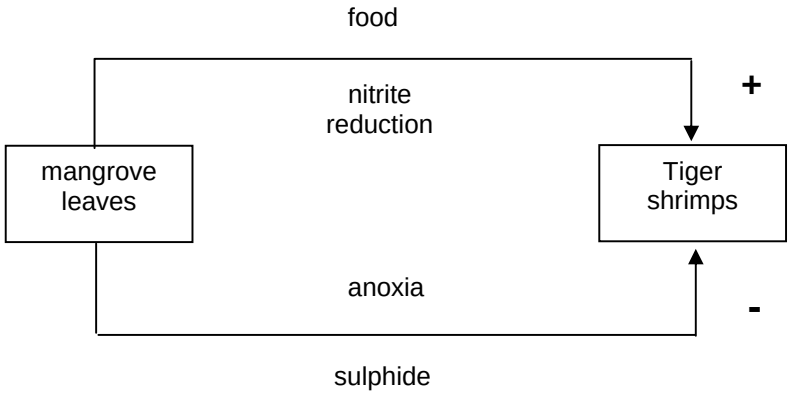

Figure 6. Effects of mangrove leaves on shrimps; while anoxia and sulphide have negative effects on shrimps, positive effects (food and nitrite reduction) outweigh the negative effects.

Still anoxia and rise of sulphide levels are not uncommon in the field (Alongi et al., 1999a, b; McGraw et al., 2001). Our results suggest that production could be higher if occurrence of such conditions could be reduced. It seems, therefore, a good idea to create a permanent water exchange at places where high quantities of mangrove leaves accumulate.

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Chapter 5

SURVIVAL AND GROWTH OF TIGER SHRIMP POST- LARVAE, *Penaeus monodon*, ON MANGROVE LEAVES AND ASSOCIATED PERIPHYTON

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1. Abstract

Many aquaculture studies have been performed to assess the dietary needs of the black tiger shrimp *Penaeus monodon*. However, the importance of periphyton associated on mangrove leaves in the feeding of these shrimps is poorly understood. Three laboratory experiments were performed in which different food items derived from mangrove leaves were offered to postlarval *P. monodon* shrimps to investigate the importance of mangrove leaves and periphyton for the survival and growth of these shrimps.

The present study clearly showed that postlarval tiger shrimp survived and grew best on CP pellets but also that mangrove leaves with or without periphyton were a better food source than solely periphyton. The results also demonstrate that growth of postlarval shrimps was significantly lower on mangrove leaves without periphyton than on leaves with dead or living periphyton. The role of food quality, size of food, and selective feeding by the shrimps are discussed.

2. Introduction

Periphyton, the entire complex of all sessile biota attached to the substratum plus associated detritus, contributes considerably to the primary and secondary production in aquatic ecosystems (Danilov and Ekelund 2001). Various aquaculture studies showed that increased periphyton biomass can significantly increase fish production (Norberg 1999; Wahab et al. 1999). This idea of periphyton-based aquaculture originates from traditional fishing methods (Welcomme 1972; Wahab and Kibria 1994; Shankar et al. 1998) and the communities of microorganisms that colonize plants, stones and other substrates are exploited in various traditional fisheries (Legendre et al., 1989; MacGrory and Williams, 1996). Tree branches or leaves are usually placed in shallow open waters to attract fish and enhance productivity (Azim et al. 2002a). In presence of plant material and associated periphyton, many fish species show higher food intake per unit time per fish (Dempster et al. 1993) and it is assumed that this higher production is due to grazing on the periphyton community (Azim et al. 2002a; b; c; 2003). Recently, the addition of substrates to enhance periphyton production in aquaculture ponds has been explored in order to investigate whether reduction of feeding inputs and thus operating costs can be obtained (Wahab et al. 1999).

Several species of shrimp move into mangrove forests when the forests are inundated by flood tides (Vance et al. 2002). Mangrove forests support a characteristic fauna that include crabs, penaeid shrimp juveniles, and various molluscs. Furthermore, mangrove leaf litter promotes meiofaunal diversity (Gee and Sommerfield 1997) and macrobenthic colonization (Lee 1999). The species *Penaeus monodon* Fabricius, 1798 is a typical inhabitant of mangrove ecosystems (Primavera and Lebata 1995) and its dependence on mangrove forests is assumed to be due to sheltering during their juvenile stages (MacNae 1974; Laegdsgaard and Johnson 2001). Furthermore, mangrove detritus may serve as an important food source of juvenile shrimps (Chong et al. 2001). According to Dittel et al. (1997) mangrove detritus had a positive effect on the growth of postlarvae of *Penaeus vannamei* but the contribution of mangrove sources to the food webs of juvenile shrimps appears to be limited to a very small spatial scales (Loneragan et al. 1997).

Studies on freshwater macroinvertebrates show that periphyton is an important food source. This is the case for grazing species (e.g. Cattaneo and Mousseau 1995; Feminella and Hawkins 1995), but also for species feeding on coarse organic matter (e.g. Graça et al. 2001). For example, the detritivorous isopod

Asellus aquaticus and the amphipod *Gammarus pulex* fed preferentially on conditioned rather than on unconditioned leaf material (Graça et al. 1993). Some studies on shrimp feeding showed that decomposing mangrove leaves stimulated the growth of *Penaeus indicus* (e.g. Ramesh and Kathiresan 1992; Athithan and Ramadhas 2000). Many aquaculture studies have been performed to assess the dietary needs of *P. monodon* (e.g. Akiyama et al. 1992; Chen 1998). Some of these studies showed that postlarval *P. monodon* grew well when fed algae (e.g. D'souze and Loneragan 1999; D'souza et al. 2002), bacteria (e.g. Mohamed 1996; Rengpipat et al. 1998; Al Azad et al. 2002) or 'lablab' a microbenthic complex consisting of blue-green algae, diatoms and other small plants and animals (e.g. Apud 1988). However, the importance of periphyton associated on mangrove leaves in the feeding of *P. monodon* remains rather unclear.

The objective of the present study is to investigate the impact of periphyton associated with mangrove leaves on the survival and growth of the tiger shrimp *P. monodon*. The hypothesis that periphyton has a positive effect on the growth of this shrimp species is tested. Three laboratory experiments were performed in which different food items derived from mangrove leaves were offered to postlarval *P. monodon* shrimps to investigate the importance of mangrove leaves and periphyton for the survival and growth of these shrimps.

3. Material and methods

Three experiments were conducted at the laboratories of the Can Tho University, Vietnam, to analyze survival and growth of the shrimp *Penaeus monodon* post larvae (PL) fed with different mangrove food items. Mangrove leaves that were ready to abscise when touched were collected by hand from 7 years old *Rhizophora apiculata* Blume stands in Ca Mau forest (latitude about 8^{o} 50 N). The collected leaves were taken to the laboratory within 12 hours and incubated in the tanks directly.

In all experiments, CP pellets (obtained from a local manufacturer) and *R. apiculata* leaves were offered as food. CP pellets contained 30% nitrogen and mangrove leaves 1%. Shrimps were fed CP pellets at al level of 10% body weight and mangrove leaves at a level of 300% body weight.

In all experiments, a constant water volume was maintained during the experimental period with a salinity of 15±1 ppt. All systems were aerated and temperature, pH, salinity, and dissolved oxygen were recorded daily, whereas biological oxygen demand, nitrite, nitrate, ammonium, and sulphide were measured weekly, except for experiment 2 where measurements were done at the beginning and at the end of the experiment.

<u>Experiment 1: Shrimp survival and growth on four mangrove food items</u>

In this experiment, shrimps were offered different food items derived from incubated mangrove leaves. Senescent mangrove leaves were divided in 5 portions and each portion (approximately 20 kg) was placed in a 500 L tank (Ø 1.09 m, height 1.1 m) filled with seawater for a period of 60 days prior to the start of the experiment. After incubation, leaves were treated differently to obtain different food sources for the shrimps (Table 1). The food sources were obtained as follows. Living periphyton (LP) was obtained by carefully removing the periphyton layer of the mangrove leaves. Dead periphyton plus leaves (DP+L) was obtained by freezing mangrove leaves for 3 days at a temperature of -10ºC.

For living periphyton plus leaves (LP+L) incubated leaves were used. Leaves without periphyton (L) were obtained by carefully removing the periphyton layer on the leaves. In the treatment without leaves (CP), only CP pellets were offered.
The experiment was performed in tanks (volume 500 L) with biofilter-recirculation and lasted for 60 days. In total, 20 tanks were used because for each treatment there were four replicates. Starting with 45 g mangrove leaves on day 0, the amount of leaves offered to the shrimps was doubled every 3.5 days up to day 21. Thereafter they were fed with 2880 g leaves on a weekly basis. Food items were added 3 times every day (morning, afternoon and evening) and food wastes were removed weekly.
Shrimp larvae (PL 15-25) (initial average length of 11.4 mm and initial average DW of 1.5 mg) were stocked at a density of 1 L^{-1} and growth was measured through changes in dry weight. Dry weight (105 ^{o}C for 24h) of the shrimps was measured on day 0, 30, and 60 by weighting 30 specimen per treatment. Survival was recorded at the end of the experiment.

Experiment 2: Shrimp survival and growth on grinded mangrove leaves

In this experiment mangrove leaves were grinded before they were offered as food. The grinded leaves were offered in different combinations with CP pellets (Table 2).
Senescent leaves (approximately 11 kg) were either incubated for 60 days in 500 L tanks (as in experiment 1) or in the field. In the field incubation, leaves were placed in plastic baskets that were covered by a plastic net with a mesh size of 0.4 mm. These baskets were placed in the ditches of a mangrove-shrimp system at a depth of 10-15 cm below the water surface. After incubation the leaves were dried at 60^{o}C for 72-96 hours until constant weight. Thereafter, leaves were grinded and this mangrove powder was mixed with cellulose (1% added as binder) and pressed mechanically into pellets. These pellets of grinded leaves together with CP-pellets were offered to the shrimps. The amount of food offered to the shrimps was doubled every 3.5 days.
The experiment lasted 21 days and was performed in 10 L ceramic tanks with 7 L water. There were four replicates for each treatment. During the experiment water volume was maintained constant. Shrimp larvae (PL 15-25) (initial average length 9.01 mm, initial DW 0.5 mg) were stocked at a density of 1 L^{-1}. Dry weight (105 ^{o}C for 24h) of the shrimps was measured prior to stocking (30 specimens) and at the end of the experiment. Growth of shrimps was measured through changes in DW and survival was recorded at the end of the experiment.

Experiment 3: Effects of mangrove leaves on periphyton and shrimps

The third experiment focused on the effect of mangrove leaves on the growth of periphyton and on survival and growth of shrimps. Again different combinations are tested (table 3). Senescent mangrove leaves (3 kg) were divided in groups of 10, 20, and 30 g L^{-1} and incubated in the field for 30 days, following the same methodology as in experiment 2. After incubation leaves were taken from the field within 2 hours and put directly into the vessels.
The experiment lasted 28 days and was performed in 1.5 L plastic vessels with 1 L water. One shrimp larva (PL 15-25) (initial average length 11.4 mm, initial DW 1.1 mg) was stocked in each vessel. In total, there were 4 series of all treatments. Each week 1 series was removed and analyzed. Dry weight (105 ^{o}C for 24h) of the shrimps was measured prior to stocking (30 specimens) and each week (on days 7, 14, 21 and 28). Growth of shrimps was measured through changes in DW and survival was recorded at the end of the experiment.

Periphyton biomass was measured weekly (on days 0, 7, 14, 21, and 28). Per treatment, mangrove leaves from 3 vessels were collected and immediately washed gently in 200 ml distilled water to remove all the attached material. The resulting solution was filtered over a glass fibre filter (47 µm diameter). Dry weight (105^{o}C, 24h) and ash free dry weight (550ºC, 3h) were determined.

Statistical analyses

Analyses of Variances (ANOVA) were performed to test differences between means. Repeated measures ANOVA were used in case data were available from measurements over time. Prior to ANOVA, variables were tested for normality. All analyses were performed with the SPSS 10.0 for Windows package.

Table 1. Food sources used in experiment 1.

treatment	food source			
	periphyton		mangrove leaves	CP-pellets
	living	dead		
LP	X			
DP+L		X	X	
LP+L	X		X	
L			X	
CP				X

LP=living periphyton; DP+L=dead periphyton plus mangrove leaves; LP+L=living periphyton plus mangrove leaves; L=mangrove leaves without periphyton; CP= CP pellets.

Table 2. Food sources offered to the shrimp in experiment 2. The percentages show the amount of food item included in the diet based on shrimp body weight. For example, for the treatment CP-CPF 5% of shrimp body weight comes from CP pellets and 150% of shrimp body weight comes from pellets made of field incubated mangrove leaves.

treatment	food source		
	CP pellets	grinded leaves	
		field incubated	lab incubated
CP	5%		
2CP	10%		
CP-CPF	5%	150%	
CP-CPL	5%		150%
CPF		300%	
CPL			300%

CP=CP pellet; CPF=pellets made from field incubated mangrove leaves; CPL=pellets made from laboratory incubated mangrove leaves.

Table 3. Food sources offered to shrimps in experiment 3.

treatment	food source				
	no food	CP	mangrove leaves		
			10 g L^{-1}	20 g L^{-1}	30 g L^{-1}
WF	X				
CP		X			
ML10			X		
ML20				X	
ML30					X

WF=without food; CP=CP pellets; ML10=mangrove leaves 10 g L^{-1}; ML20=mangrove leaves 20 g L^{-1}; ML30=mangrove leaves 30 g L^{-1}.

4. Results

Experiment 1: Shrimp survival and growth on four mangrove food items

Mean survival of shrimps was low ranging from 2 to 35% (Figure 1a) and after logarithmic transformation survival differed significantly ($F_{4,15}$=28.556, p=0.000) among the treatments. Post-hoc test showed that survival was significantly higher in the treatment with CP-pellets, survival was significantly lower in the treatment with solely periphyton, and the treatments in which mangrove leaves were offered were not significantly different from each other. Individual shrimp biomass (Figure 1b) was significantly different between the treatments after 30 days ($F_{4,15}$=14.877, p=0.00) and after 60 days ($F_{4,15}$=200.444, p=0.000). Post-hoc tests showed that the CP treatment resulted in significantly higher biomass, that biomass was significantly lower for solely periphyton treatment, and that after 60 days shrimp biomass in treatment with solely living mangrove leaves was significantly lower than in the treatments with mangrove leaves and periphyton (dead or alive).

Total biomass after 60 days (Figure 1c) differed significantly between the treatments ($F_{4,15}$=117.428, p=0.000). The treatment with solely periphyton had the lowest total biomass and the treatment with CP-pellets resulted in the highest total biomass. Post-hoc tests showed that these two treatments differed significantly from each other and from the treatments in which mangrove leaves were used.

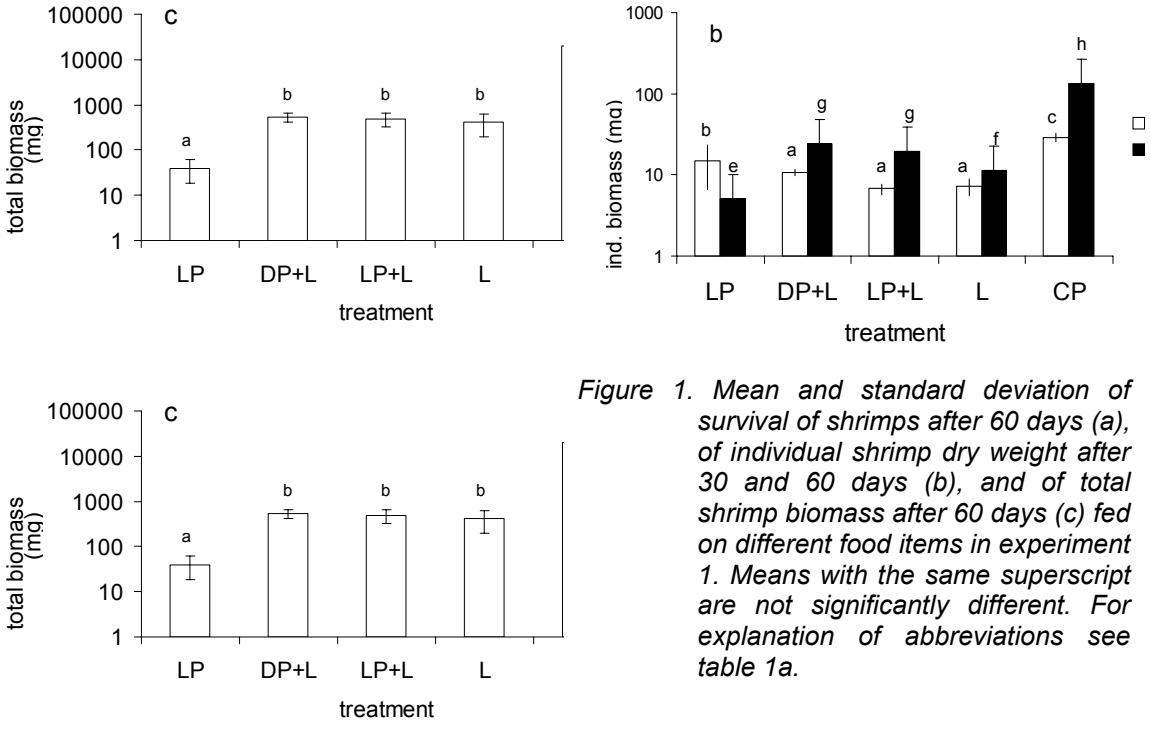

Figure 1. Mean and standard deviation of survival of shrimps after 60 days (a), of individual shrimp dry weight after 30 and 60 days (b), and of total shrimp biomass after 60 days (c) fed on different food items in experiment 1. Means with the same superscript are not significantly different. For explanation of abbreviations see table 1a.

The treatments with mangrove leaves had higher ammonium and nitrate concentrations than the treatment with solely periphyton (Table 4). However, the CP treatment had even higher values for these variables. Furthermore, the biological oxygen demand in the CP treatment was twice as high as in the other treatments.

Table 4. Mean (and standard deviation) of the abiotic conditions during experiment 1.

Variable		Treatment				
	Initial	LP	DP+L	LP+L	L	CP
Temp (ºC)	27.2	27.1 (0.2)	27.1 (0.2)	27.1 (0.1)	27.2 (0.1)	27.1 (0.2)
DO[1] (mg L^{-1})	6.7	6.8 (0.1)	6.2 (0.3)	6.3 (0.3)	6.4 (0.2)	6.3 (0.2)
pH (-)	8.2	8.2 (0.0)	8.2 (0.0)	8.2 (0.0)	8.2 (0.)	8.2 (0.0)
BOD[2] (mg L^{-1})	3.0	4.3 (0.8)	5.0 (1.3)	5.0 (1.0)	4.4 (0.7)	10.2 (3.6)
NH_4 (mg L^{-1})	0.06	0.08 (0.01)	0.15 (0.05)	0.18 (0.08)	0.16 (0.07)	0.34 (0.22)
NO_2^- (mg L^{-1})	0.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.03 (0.02)
NO_3^- (mg L^{-1})	0.05	0.06 (0.01)	0.11 (0.04)	0.10 (0.04)	0.09 (0.03)	0.24 (0.17)
S^{2-} (mg L^{-1})	0.01	0.01 (0.00)	0.02 (0.00)	0.02 (0.00)	0.01 (0.00)	0.03 (0.01)

1: dissolved oxygen
2: biological oxygen demand

Experiment 2: Shrimp survival and growth on grinded mangrove leaves

Survival of shrimps (Figure 2a) ranged between 10 and 90 %. ANOVA with post-hoc tests showed that survival in the treatments CPF and CPL was significantly lower than in the other treatments ($F_{5,16}$=7.490, p=0.001). Logarithmically transformed individual dry weight (Figure 2b) differed significantly between treatments ($F_{5,16}$=10.391, p=0.000) with shrimp dry weight significantly lower in the treatments CPL and CPF than in the treatments where CP pellets were combined with mangrove leaves. The treatments CP and 2CP did not differ significantly from each other nor from the other treatments where CP was added. Dry weight of shrimps fed on pellets made from mangrove leaves in combination with CP showed larger standard deviations than the treatments with solely CP (Figure 2b). ANOVA showed that these differences were indeed significant ($F_{1,4}$=31.589, p=0.005). Logarithmically transformed total biomass (figure 2c) showed significant differences (ANOVA: $F_{5,16}$=32.403, p=0.000) with the low biomass in CPL and CPF being different from the others.

Table 5 shows the values for the measured abiotic variables at the end of the experiment. Biological oxygen demand at the end of the experiment was significantly higher for the treatments with conditioned mangrove leaves ($F_{1,4}$=7.778, p=0.049. Treatments with only CP pellets had significant higher NO_2 values ($F_{1,4}$=11738.671, p=0.000) and significant lower sulphide values ($F_{1,4}$=23.443, p=0.008).

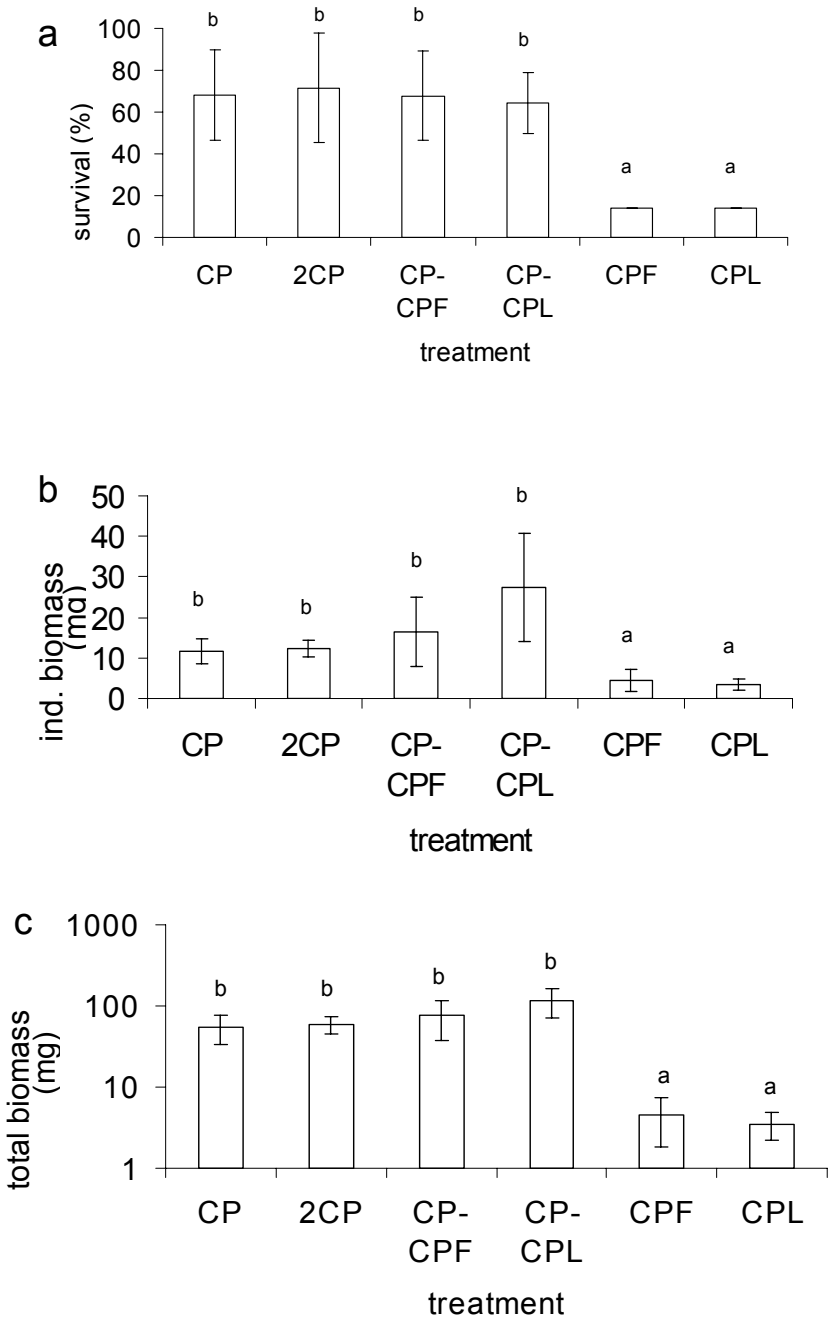

Figure 2. Mean and standard deviation of survival of shrimps (a), of individual dry weight (b), and of total biomass (c) fed on different types of pellets. Means with the same superscript are not significantly different. For explanation of abbreviations see table 1b.

Table 5. Values of the abiotic variables at the beginning and at the end of experiment 2.

Variable		Treatment					
	Initial	CP	2CP	CP-CPF	CP-CPL	CPF	CPL
Temp (ºC)	26.7	28.4	27.4	29.2	29.1	28.9	28.6
DO^1 (mg L^{-1})	6.6	5.9	6.2	5.6	4.8	3.6	5.2
pH (-)	8.3	8.2	8.2	8.3	8.3	8.0	8.4
BOD^2 (mg L^{-1})	6.0	6.0	9.0	19.0	30.0	31.0	47.0
NH_4 (mg L^{-1})	0.05	0.20	0.29	1.08	1.85	0.07	0.22
NO_2^- (mg L^{-1})	0.00	1.69	1.71	0.04	0.06	0.05	0.09
NO_3^- (mg L^{-1})	0.04	0.51	0.70	0.35	0.68	0.69	1.08
S^{2-} (mg L^{-1})	0.01	1.17	0.02	2.41	2.52	2.48	3.06

1: dissolved oxygen
2: biological oxygen demand

Experiment 3: Effects of mangrove leaves on periphyton and shrimps

The abiotic conditions were similar for the different treatments, except for the higher ammonium, nitrite, and nitrate concentrations in the CP pellet treatment (Table 6). Ammonium, nitrite, and nitrate concentrations in the CP treatment had a different pattern over time (Figure 3). Ammonium increased during the first week and decreased thereafter. The decrease in ammonium coincided with the increase in nitrite. The increase in the nitrate concentration is rather small during the first 3 weeks, but showed a large increase in the fourth week.

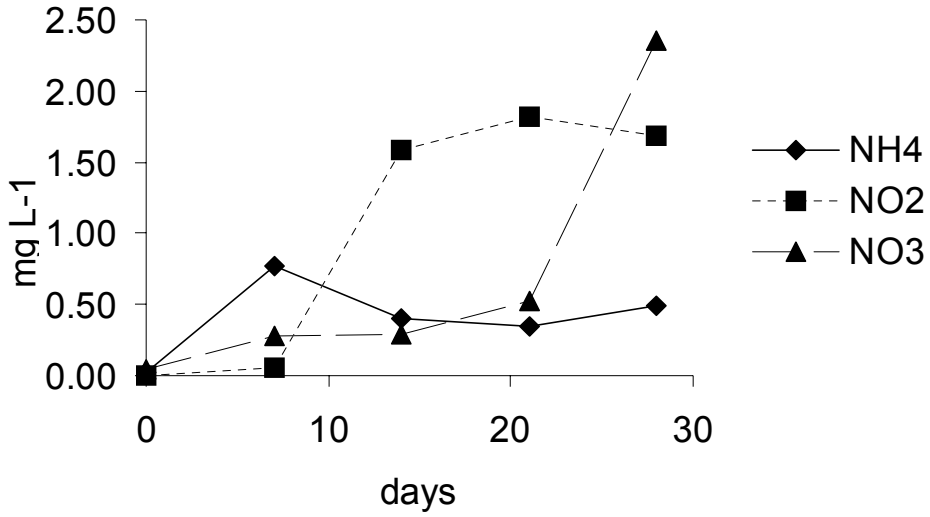

Figure 3. Ammonium, nitrite, and nitrate concentrations as a function of time for the treatment with CP pellets in experiment 3.

Survival ranged between 70 and 90% except for the treatment with no food where no specimens survived after two weeks. Repeated measures ANOVA without the treatment with no food showed that there were significant differences in shrimp growth (Figure 4) between the food items ($F_{3,19}$=36.397, p=0.000). Post-hoc tests showed that growth on CP pellets remained behind in the first weeks but was largest after three weeks (Figure 4). Shrimp growth in treatment with 30 g mangrove per L was not significantly different from the treatment with only CP in the fourth week but differed significantly from the treatments with 10 and 20 g L^{-1}.

Periphyton biomass on mangrove leaves decreased during the experiment from 12 to 6 mg g^{-1} leaf (Figure 5). The pattern is similar for all three treatments.

5. Discussion

The present study clearly showed that postlarval tiger shrimp survived and grew best on CP pellets but also that mangrove leaves with or without periphyton were a better food source than solely periphyton. The results also demonstrate that growth of postlarval shrimps was significantly lower on mangrove leaves without periphyton than on leaves with dead or living periphyton. The latter result is in line with observations on freshwater macroinvertebrates that prefer conditioned over unconditioned leaves (Graça et al. 1993; Nolen and Pearson 1993).

Furthermore, based on stable carbon isotope ratios in a riverine mangrove in Guimaras, central Philippines,

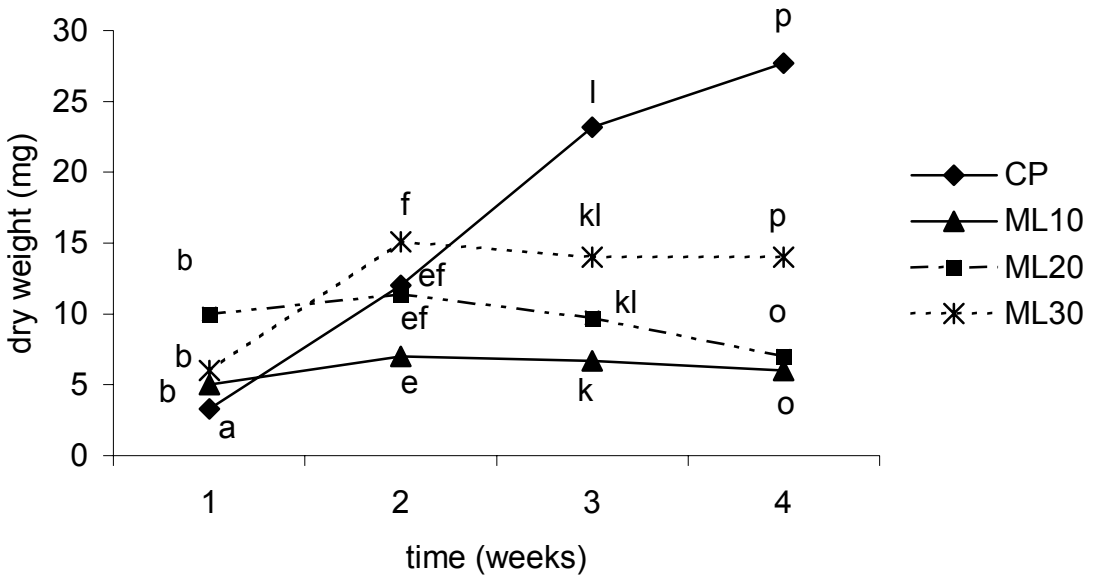

Figure 4. Shrimp dry weight as function of time for the different treatments of experiment 3. Means from the same week with the same superscript are not significantly different when logarithmically transformed.

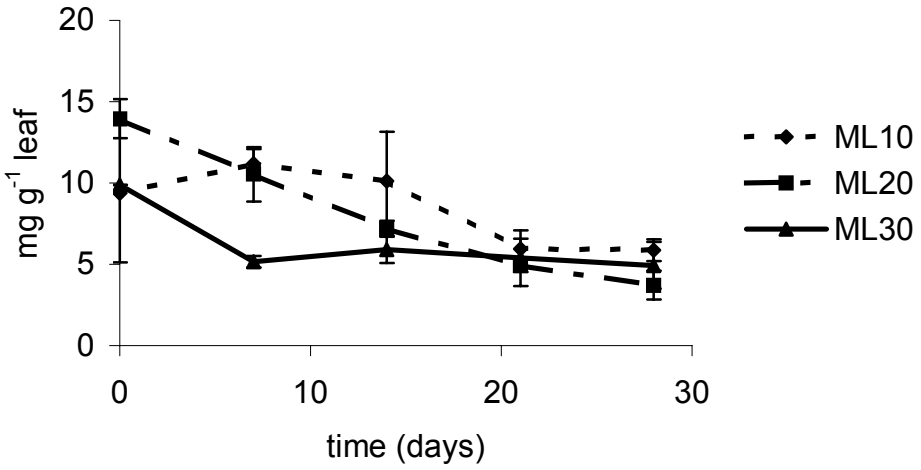

Figure 5. Periphyton biomass on mangrove leaves in experiment 3 as a function of time.

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. This may explain the better performance of the shrimps on mangrove leaves with than without periphyton. The present study clearly showed that mangrove leaves are of major importance for shrimp growth and additional growth can be obtained when mangrove leaves are covered with a periphyton layer. Therefore, the results support the hypothesis that periphyton had a positive effect on growth of shrimp *P. monodon*.

Survival of shrimps in the present study showed large variation among the experiments. Survival rates presented in literature show also variability, for

example, Bautista et al. (2003) had survival rates higher than 70 % whereas Wei (1998) reported low values. Vogt et al. (1985) observed that postlarvae of *P. monodon* were able to maximally survive a period of 15 days of starvation. Therefore, no survival at all after four weeks in treatments without food could be expected. However, survival of shrimps was low in all treatments of experiment 1. Quality and quantity of available food affects survival of cultured shrimps and inadequate nutrition is one of the main limitations for aquaculture (Vogt et al. 1985). However, CP pellets were also used as food for shrimps in the first experiment and these pellets are regarded as high quality food. Therefore, it is not likely that high mortality was due to low food quality alone. A possible explanation might be the vulnerability of penaeid shrimps to handling (Olin and Fast 1992) and failure to maintain optimal conditions during each step in culturing can result in low survival. Furthermore, the experimental set-up might have caused too much stress for the shrimps although the equipment used is common practice in culturing shrimps. For example, 500 L tanks with a bare bottom were used in experiment 1 but it has been observed that *P. monodon* prefers soft silty mud bottoms and dense vegetation cover (Wakwabi 1988) and that habitat complexity (e.g. Jayasundera et al. 1999) and the presence of places to shelter (e.g. Subramaniam 1990; Primavera and Lebata 1995) are important for *P. monodon*.

In the present study feeding of *P. monodon* PL on CP pellets resulted in higher biomass than when offering mangrove leaves as food. A possible explanation for the observed difference might be the size of the offered food. In contrast to mangrove leaves, CP pellets are small (Ø 0.01-1 mm) and this size is more or less comparable to the size of the mouth of the postlarval shrimp and thus it is much easier for the shrimps to pass it to their mouth by using their pereiopods and maxillipeds (Baily-Brock and Moss 1992). Some studies demonstrated that detritus, which has a similar size as CP pellets, was present in the gut-content of postlarval shrimps (e.g. Bombeo et al. 1993). Therefore, shrimps do not need to invest much energy to take up these particles in comparison to mangrove leaves. However, the second experiment in this study showed that pellets made from incubated mangrove leaves gave a much lower growth than CP pellets. Therefore, it seems unlikely that the size of food particles is responsible for the observed growth differences in the first experiment.
Another reason for the observed differences in shrimp growth between mangrove leaves and CP pellets might be the inhibition of shrimp growth due to mangrove leaves. This inhibition is probably due to the release of degradation products (Cundell et al. 1979; Robertson 1988) and lasts for approximately four weeks (Nga et al. in prep). In the present study, mangrove leaves were incubated for 60 days before they were offered as food to the shrimps and therefore it is unlikely that growth inhibition took place.
The nutritional value of CP pellets used in this study is assumed to be higher than that of mangrove leaves due to its high content of nitrogen (30% versus 1%). Growth of shrimps is largely determined by the quality of the available food source (Vogt et al. 1985). Therefore, it seems likely that differences in nutritional quality resulted in differences in growth between CP pellets and mangrove leaves.

The results of the third experiment showed that incubation of mangrove leaves for a period of 30 days resulted in a periphyton layer with a biomass of 10 to 14 mg g^{-1} mangrove leaf. A reduction of approximately 50% occurred during the experiment and this was independent from the densities of mangrove leaves. Shrimp grew better when offered mangrove leaves in a concentration of 30 g L^{-1} than 10 or 20 g L^{-1}. Although periphyton biomass, expressed as mg g^{-1} leaf, did not differ between the treatments, more biomass was in total available in the treatment with 30 g L^{-1}. Furthermore, juvenile prawn like *P. monodon* is depended on mangrove forest and the use of mangrove structures by these juvenile shrimp as refuge from predation is known (Subramaniam 1990; Primavera and Lebata 1995). Effective provision of shelter depends not only on structure type and density but also on the behaviour of predator and prey as well (Primavera 1997). Therefore, it seems likely that the observed higher shrimp growth is not only due to more available food but also to the impact of other factors like the presence of suitable places to shelter.

Pellets made fully from grinded mangrove leaves incubated either in the field or the laboratory had a lower shrimp survival and growth in comparison to pellets made from mangrove leaves in combination with CP pellets. Addition of grinded mangrove leaves to CP pellets on the other hand, did not change shrimp survival and growth in comparison to CP pellets alone. Interestingly, pellets made from grinded mangrove leaves and CP resulted in significant higher variation in individual shrimp weight than CP pellets alone. Therefore, within a treatment with CP and grinded mangrove leaves, some specimen grew faster than others. All factors were similar for all replicates and thus it seems likely that it is related to the offered food or to the food selection of the shrimps. Shrimps locate their food with cuticular chemosensory setae that are concentrated at the anterior end of the body (Van de Braak 2002). The rather low proportion of inorganic particles in the gut of shrimp larvae (e.g. Bombeo 1993; Moorthy and Altaff 2002) is a support for the selective feeding on organic particles by shrimp larvae. Focken et al. (1998) found that the proportion of plant tissue and pellet material in samples of “lablab” was much lower than in the guts of shrimps indicating that these items were selectively taken by shrimps. When shrimps are able to discriminate between high and low quality food particles then the variation between pellets made from mangrove leaves plus CP and pellets made from solely CP should be in the same order of magnitude. However, this was not the case in the present study, and therefore, it seems questionable whether shrimp larvae discriminate between different organic sources.

In all three experiments conducted in this study, increased concentrations of nitrite were observed when feeding the shrimps with solely artificial CP pellets. It is well known that metabolism of postlarvae shrimps requires oxygen and releases CO_2 and NH_3 (Olin and Fast 1992). Ammonia excreted by shrimps can be converted into nitrite and nitrate by nitrogen converting bacteria (Gupta et al. 2001). The measured ammonia and nitrite concentrations in the experiments were lower than the ‘safe’ concentrations (e.g. Chen and Lei 1990; Chen and Chen 1992). Shishehchian et al. (1999) observed that nitrogenous excretion was predominant in shrimps fed with artificial diets and live food like algae and chironomids, despite a high protein content. The present study showed that adding conditioned mangrove leaves to artificial pellets may pose less adverse

effects on water quality compared to CP pellets only with respect to nitrogen but on the other hand may result in increased H_2S concentrations.

In conclusion, this study shows that shrimps feeding on mangrove leaves grow better when these leaves are covered by a periphyton layer. CP pellets were a better food source than mangrove leaves probably due to the higher nutritional values of CP. Pellets made of a combination of grinded mangrove leaves and CP pellets showed higher variation in individual dry weight. Higher density of decomposing mangrove leaves offer more food and more places to shelter resulting in a better growth of *P. monodon* PL.

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Chapter 6

CHEMICAL AND PHYSICAL EFFECTS OF CROWDING ON GROWTH AND SURVIVAL OF *PENAEUS MONODON* FABRICIUS POST-LARVAE

(B. T. Nga, M. Lürling, E.T.H.M. Peeters, R. Roijackers, M. Scheffer and T. T. Nghia)

1. Abstract

The hypothesis that crowding effects through physical and/or chemical interference may be an important factor in lowering the chance of survival and reducing growth of *Penaeus monodon* post-larvae under high stocking densities was tested. To separate physical interference from chemically-exerted effects, two-stage systems were used in which shrimps were cultured at different densities (stage 1) and water from these being supplied to individually kept *P. monodon* (stage 2). Stocking density significantly affected *P. monodon* survival, body-size and dry weights over a 4 weeks experimental period. Physical interference stress and cannibalism could be excluded as causal factors meaning that the negative impact of crowding on shrimp growth and survival was due to some chemical compounds or other water quality variables. Among these pH, temperature, salinity, dissolved oxygen, chlorine, nitrite and nitrate appeared of minor influence. In contrast, ammonia toxicity could not be excluded as the causal factor for the observed mortality and reduced growth of *P. monodon* post-larvae in our experiments.

2. Introduction

In many tropical countries, farming of penaeid shrimp has become an important aquaculture activity and has the potential of becoming a sustainable industry (Primavera, 1993). In South-East Asia, and especially in the brackish Mekong Delta of Vietnam, where extensive and intensive farming has become an important economic activity, the most widely cultured penaeid shrimp is the giant tiger prawn *Penaeus monodon*, (Brennan et al., 2000). In 1997, about 150,000 tonnes of shrimp were produced in this region (Josupeit, 1999). Despite the initial success and good economic prospects, rapid growth of shrimp farming coincided with various problems in recent years, such as viral diseases (Flegel, 1997), poor management and environmental damage that all may corrode the sustainability of shrimp aquaculture (Primavera, 1998).

One of the major goals of shrimp farming is to optimize production in order to meet the world markets demand. Where from an economical point of view one would like to stock ponds as dense as possible, several studies have shown that, in general, increased shrimp stocking densities resulted in higher mortality and lower feeding efficiency of *P. monodon* (e.g. Martin et al., 1998; Ray and Chien, 1992; Tseng et al., 1998). A straightforward explanation is that excretory products, feces and unconsumed food items accumulate in the culture water causing toxic effects on shrimps (e.g. Ray and Chien, 1992). However, also in indoor re-circulating systems in which high water quality was maintained, high densities of *P. monodon* may experience stress leading to lower growth and survival (Tseng et al., 1998). The latter suggests that also other causal factors, such as competition for resources and cannibalism (e.g. Piyatiratitivorakul et al., 2001), might account for the observed effects under high stocking densities. The general picture emerging from all studies is that crowding conditions may create a hostile environment through strong competition for resources, chemical interactions, mechanical/physical interference and even cannibalism. In the current study we tested the hypothesis that crowding effects through physical and/or chemical interference may be an important factor in lowering the chance of survival and reducing growth of *Penaeus monodon* post-larvae under high stocking densities. To separate physical interference from chemically-exerted effects, two-stage systems were used in which shrimps were cultured at different

densities (stage 1) and water from these being supplied to individually kept *P. monodon* (stage 2).

3. Materials and methods

Experimental design

Two related experiments were performed simultaneously under controlled conditions to investigate the effects of different shrimp densities and crowding chemicals on growth and survival of *Penaeus monodon*. The experiments were run in a two-stage system (figure 1) consisting of spherical plastic vessels (19-cm height and 11.7-cm diameter) that had been soaked and washed in water for 30 days prior to use. In the first stage, shrimps were reared at different stocking densities, whereas in the second stage, only one shrimp was stocked per vessel that received water from the corresponding first stage vessels (figure 1). All rearing vessels were oxygenated during the entire experimental period of 4 weeks and their water volumes kept constant at one liter per vessel.

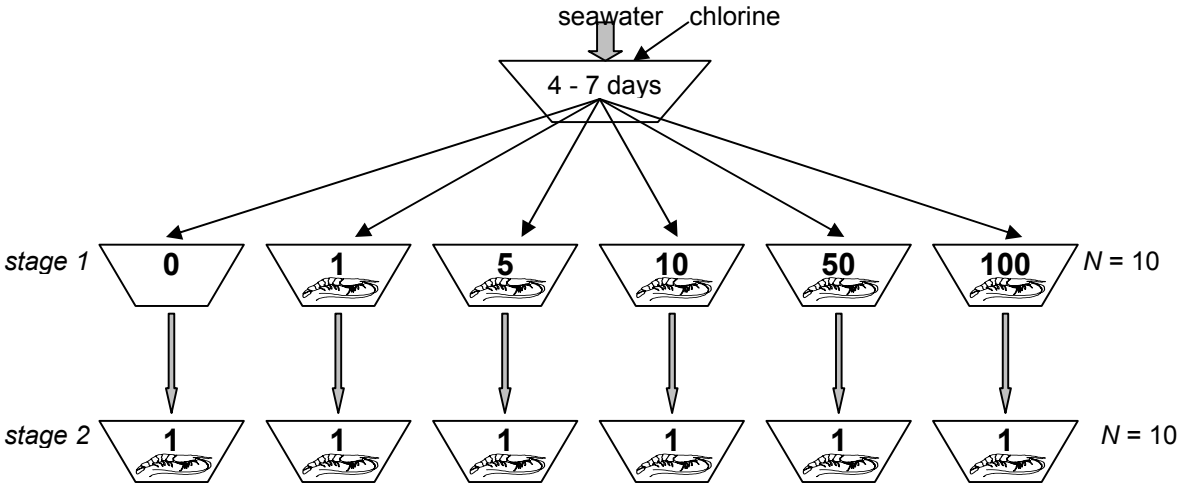

Figure 1. Experimental design: Seawater is treated with 20-30 ppm chlorine, settled for 4-7 days, diluted to 15 ppt salinity and then supplied to vessels containing different densities of P. monodon post-larvae (stage 1; 0 to 100 post-larvae l^{-1}). Daily 700 ml of fresh seawater is added to each vessel of 1 liter; the one-day old "crowding water" is supplied to individual P. monodon post-larvae in the second stage (stage 2). Each control and treatment series consist of ten replicates.

Stage one: crowding

P. monodon post larvae (PL 15-25, i.e. 15-25 days after hatching, with an average length of 9.9 mm and dry weight of 7 mg) were stocked at densities of 1, 5, 10, 50 and 100 specimens per liter in water with a salinity of 15 (± 1) ppt. This water was prepared from seawater with a salinity of 25 - 35 ppt taken from the mangrove area at Camau forest (Mekong Delta, Vietnam). The seawater was chlorinated (20-30 ppm active chlorine), settled for 4 to 7 days to remove chlorine and then diluted with tap-water to obtain the required salinity of 15 ppt. Ten replicates per density were used yielding 5 × 10 = 50 experimental units in stage 1. In addition, ten vessels without any shrimps provided the needed controls for

the second stage. Treatments were randomly distributed over the experimental vessels. Daily the shrimps were fed with CP (Crude Protein, approximately 30% nitrogen) pellets at a level of 10% of their body weight. A salinity of 15 (± 1) ppt was maintained throughout the experimental period of 28 days. Excess food and shrimp waste were removed daily and 70% (v/v) of the water was refreshed, i.e. 700 ml of each vessel. This water that had been in each vessel for 24 h was not wasted, but used in stage 2.

Stage two: crowding chemicals

The second stage was started 24 hours after stage 1 and was aimed for testing the reaction of shrimp to crowding chemicals released from the different shrimp densities in stage 1. Hereto, one *P. monodon* post larva was stocked per experimental vessel. Daily 70% (v/v) of the water in these vessels was replaced by 700 ml of water from corresponding vessels of stage 1. This resulted in 5 treatments (i.e. water from the stage one 1, 5, 10, 50, 100 shrimp l^{-1} vessels) with 10 replicates each and an additional control that received daily clean water (0 shrimp l^{-1}). The latter control was comparable to the 1 shrimp treatment in stage one.

Analyses

Shrimp measurements

Prior to being stocked in culture vessels, shrimps were measured for their length and weight (DW). Dry weights were determined after drying for 24 h at 105°C using a 4 decimal precision balance. Initially and at the end of the experimental period, the protein content of the shrimps was determined following the Kjeldalh method.

The number of surviving animals was recorded at the end of the experiment, i.e. after 28 days, their body length measured and dry weight determined.

Water measurements

Daily temperature, pH, salinity, and dissolved oxygen were recorded using a YSI-556-MPS oximeter. Weekly nitrogen (NO_2^-, NO_3^-, and NH_4^+) was measured following standard titration methods.

Statistical analysis

The effects of crowding and chemical interactions on body-sizes and dry weight between the two stages of the experiment were statistically compared using two-way analysis of variance (ANOVA). The ANOVA was run in the statistical tool pack SPSS® version 10.1.0 (SPSS Science, Briljant Software & Techniek, Mijdrecht, The Netherlands), with the experiment (crowding and crowding water) and density as fixed factors. Significantly differences (at the 95% confidence level) were distinguished using Tukey's post-hoc comparison test. Effects within each stage of the experiment were analyzed using a one-way ANOVA. Survival between the two stages was compared running a *t*-test over the regression lines of survival against density.

4. Results

Survival

Survival (S) was highest at the lowest shrimp densities (Shrimp) in the first stage of the experimental set-up and gradually decreased with increasing stocking densities figure 2). The decrease could be described by a linear relationship: S = 71.5 - 0.615 × Shrimp (r^2_{adj} = 0.878; P = 0.012; n = 5). A similar pattern was observed in the second stage of the experimental set-up, where individual shrimp were exposed to water from first stage vessels with varying shrimp densities (figure 2). Also the linear relation between survival and crowding water (CrW) appeared significant: S = 71.4 - 0.591 × CrW (r^2_{adj} = 0.800; P = 0.010; n = 6). However, there was no difference between both regression lines (t-test; t = -0.14; P = 0.446).

Body length

Final body lengths of shrimps were log-transformed prior to statistical analysis in order to fulfill assumptions of homogeneity in variance. A Tukey test following a one-way ANOVA for final body lengths of animals in different treatments from the first stage ($F_{4,42}$ = 3.83; P = 0.010) yielded two homogenous groups: 1) 1, 5 and 10 prawns per liter, and 2) 5, 10, 50 and 100 prawns per liter (figure 3). For the second stage, in the treatments that received water from the 100 prawns per liter vessels only one animal survived; hence, one-way ANOVA ($F_{5,27}$ = 4.58; P = 0.004) could not be followed by a post-hoc comparison test. Omitting the one 100 prawn value

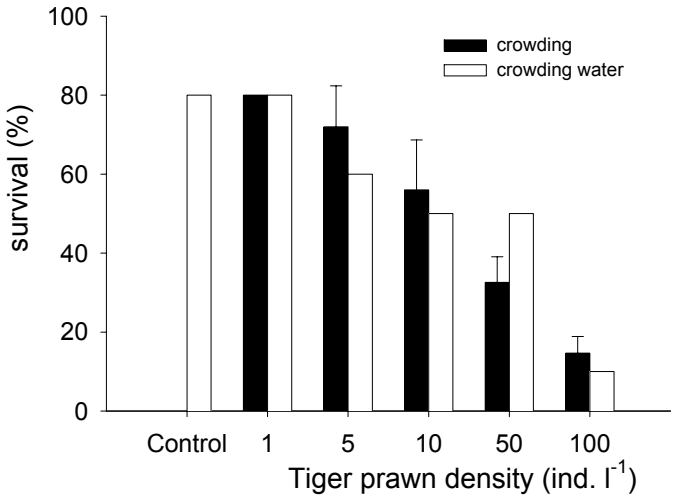

Figure 2. Percentage survival of P. monodon post-larvae reared for 4 weeks at different densities (filled bars; crowding) and in water from these different crowding densities (open bars; crowding water). Error bars indicate one standard deviation (N = 10).

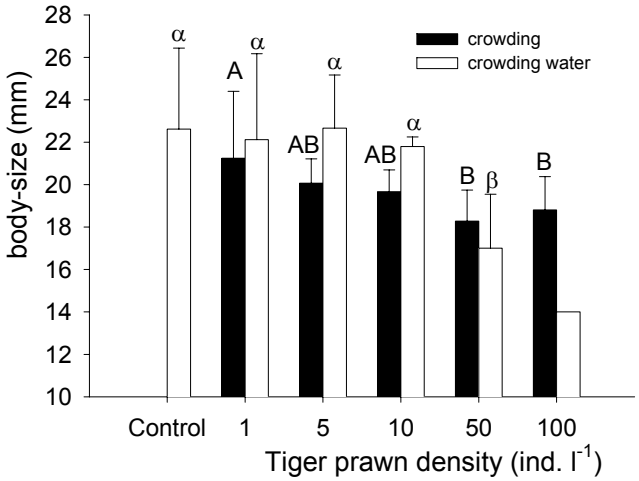

Figure 3. Body-size (mm) of P. monodon post-larvae reared for 4 weeks at different densities (filled bars; crowding) and in water from these different crowding densities (open bars; crowding water). Error bars indicate one standard deviation (N = 10). Different symbols above bars (A,B and α,β) indicate significantly different groups (Tukey test; P < 0.05).

revealed significant differences among body-sizes of prawns ($F_{4,27}$ = 3.76; P = 0.015) and Tukey yielded two homogenous groups: 1) 0, 1, 5 and 10 prawns per liter, and 2) 50 prawns per liter (figure 3).
Assumptions for a two-way ANOVA could not be fulfilled; hence the results only give an indication. The outcome yielded an indication for similar body lengths of animals between the two stages ($F_{1,62}$ = 0.52; P = 0.473), different body lengths at different densities ($F_{4,62}$ = 12.0; P < 0.001), and a stage × density interaction ($F_{4,62}$ = 4.02; P = 0.006) indicating the density effect could differ between the two stages.

Dry weight

The similar problem as for body lengths was encountered for a two-way ANOVA on dry weights; the indications, however, pointed in the same direction. A one-way ANOVA indicated similar ($F_{4,42}$ = 1.69; P = 0.171) final dry weights of animals reared in the first stage (figure 4). Dry weights of animals reared in the second stage was log-transformed prior to statistical analysis in order to fulfill assumptions of homogeneity in variance. As for body lengths, one-way ANOVA indicated significant differences ($F_{5,27}$ = 6.10; P = 0.001), but the one sample point of the “100 shrimp water” treatment had to be omitted to allow any post-hoc comparison. The resulting ANOVA indicated significant differences among dry weights of prawns ($F_{4,27}$ = 3.76; P = 0.015) and Tukey post-hoc test yielded two homogenous groups: 1) 0, 1, 5 and 10 prawns per liter, and 2) 50 prawns per liter (figure 4).

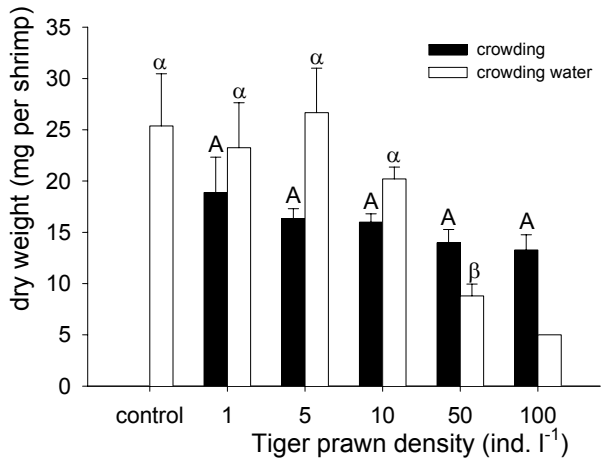

Figure 4. Dry weight (mg) of P. monodon post-larvae reared for 4 weeks at different densities (filled bars; crowding) and in water from these different crowding densities (open bars; crowding water). Error bars indicate one standard deviation (N = 10). Similar symbols above bars (A and α,β) indicate homogeneous groups that are not significantly different at the 95% level (Tukey test).

Shrimp protein content

The protein content of *P. monodon* grown in the first stage at different densities had increased from almost 70% of DW at the start of the experiment to 73 – 78 % after 4 weeks (figure 5). The highest protein content was found in animals at lowest density, and an almost significant relation of decreasing protein levels with increasing densities (r^2_{adj} = 0.628; P = 0.069; n = 5).

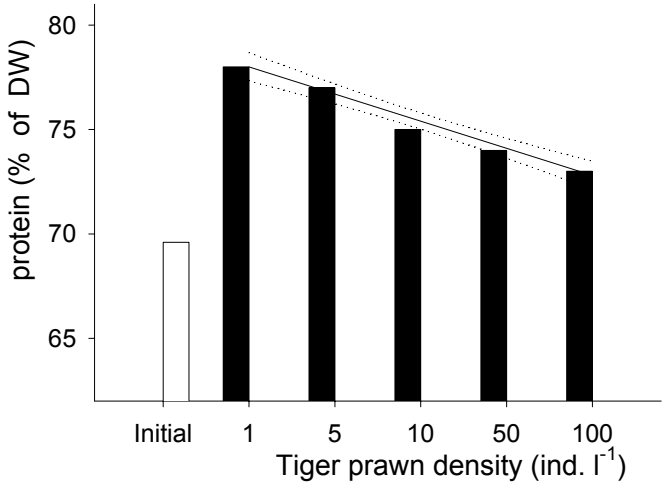

Figure 5. Protein content as percentage of the dry weight of P. monodon post-larvae reared for 4 weeks at different densities (1 to 100 post-larvae l^{-1}). The open bar represents the protein content at the start of the experiment. The solid line represents linear regression (Protein = 76.5 – 0.042 × density, r^2_{adj} = 0.628), dotted lines the 95% confidence intervals.

Water samples

The dissolved oxygen concentration, temperature, and pH of cultured water were similar among treatments and between the two-stages and well within the range to sustain shrimp growth (Table 1). In contrast, nitrogen compounds showed considerable variations in time and among treatments (figure 6). Ammonium increased in the first two weeks and was proportional to the stocking densities reaching a maximum concentration of 8.5-mg l^{-1}. Nitrite reached a maximum of 1.7-mg l^{-1}after 1 to 2 weeks and was highest in the 5 and 10 shrimp per liter treatments in stage 1 and in the water from 10, 50 and 100 shrimp per liter treatments in stage 2. Nitrate reached a maximum of 2.1-mg l^{-1} after 4 weeks (figure 6). Combining the results from both stages and running regression analysis of shrimp survival after 4 weeks against average nitrogen compound concentrations yielded no significant relations for nitrite (r^2_{adj} = 0.104; P = 0.176; n = 11) and nitrate (r^2_{adj} = 0.226; P = 0.079; n = 11). However, for survival and the mean ammonium concentration a highly significant relation was found (r^2_{adj} = 0.806; P < 0.001; n = 11). For body length at the end of the experimental period, regression analysis yielded no significant relation with nitrite (r^2_{adj} = 0.106; P = 0.173; n = 11), but significant relationships with nitrate (r^2_{adj} = 0.370; P = 0.028; n = 11) and ammonium (r^2_{adj} = 0.713; P < 0.001; n = 11). Finally, dry weight of *P. monodon* post-larvae at the end of the experimental period was not correlated with nitrite (r^2_{adj} = 0.090; P = 0.192; n = 11), almost significantly with nitrate (r^2_{adj} = 0.279; P = 0.055; n = 11), and highly significant with ammonium (r^2_{adj} = 0.699; P < 0.001; n = 11).

Table 1. Mean values for the daily measured water quality parameters dissolved oxygen (DO, mg l^{-1}), temperature (temp. °C) and pH at different P. monodon post-larval densities (shr. l^{-1}) in stage 1 (crowding) and stage 2 (crowding water) of the experimental set-up.

	stage 1: crowding				stage 2: crowding water		
shr. l^{-1}	DO	temp.	pH	shr.l^{-1}	DO	temp.	pH
1	6.8 (0.2)	27.3 (0.5)	7.9 (0.1)	*Ctrl*	6.7 (0.3)	27.2 (0.8)	7.9 (0.1)
5	6.4 (0.3)	27.8 (0.6)	7.9 (0.1)	*1*	6.7 (0.4)	27.1 (0.8)	7.9 (0.1)
10	6.2 (0.4)	28.3 (0.6)	7.8 (0.1)	*5*	6.6 (0.4)	27.0 (0.8)	7.9 (0.1)
50	5.9 (0.6)	27.7 (0.6)	7.8 (0.2)	*10*	6.8 (0.3)	26.8 (0.8)	7.8 (0.1)
100	6.0 (0.6)	27.4 (0.7)	7.8 (0.1)	*50*	6.5 (0.5)	26.7 (0.8)	7.9 (0.1)
				100	6.9 (0.3)	26.4 (0.8)	8.0 (0.2)

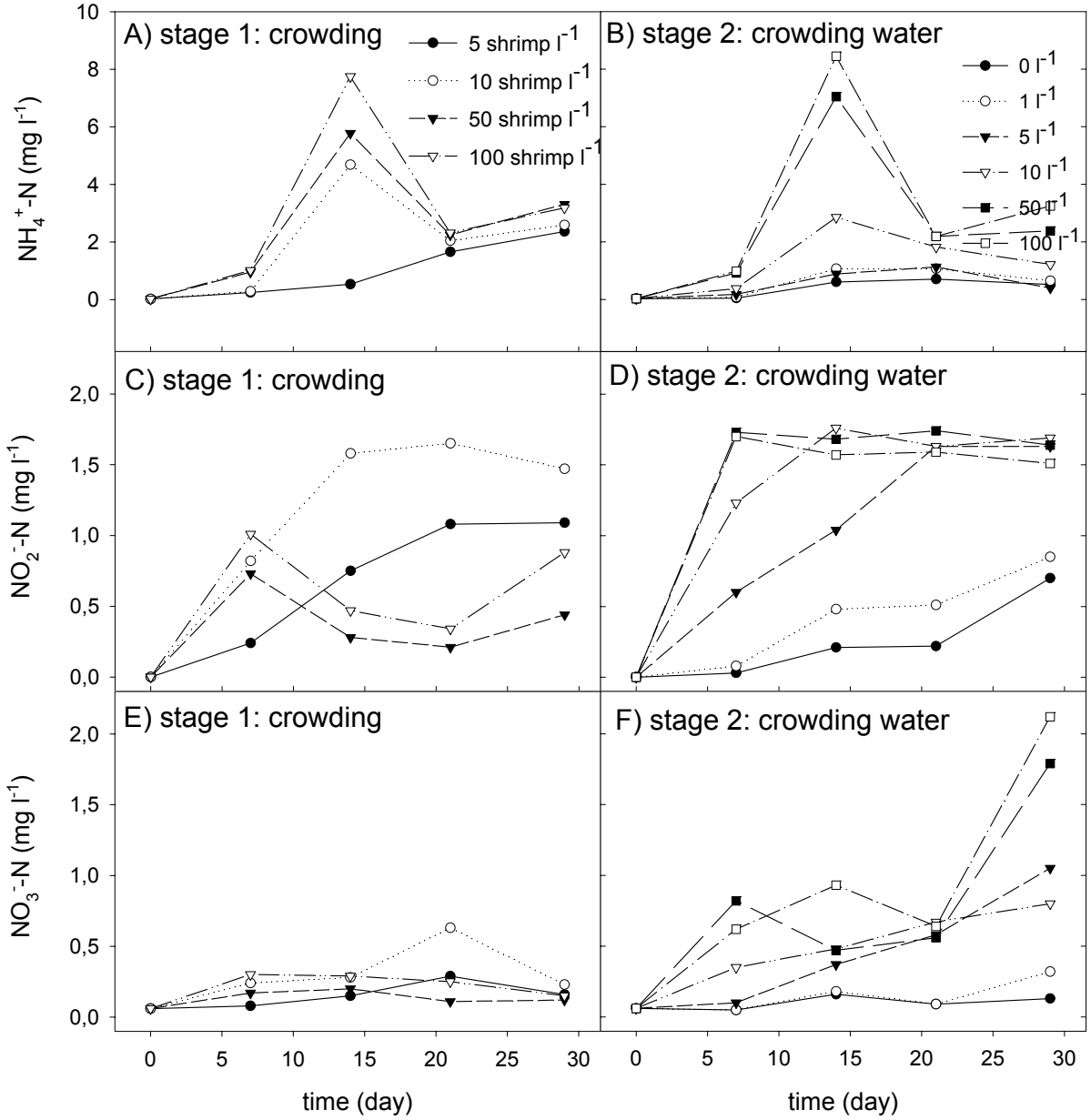

Figure 6. Course of nitrogenous compounds ammonia (upper panels A and B), nitrite (middle panels C and D) and nitrate (lower panels E and F) during four weeks of rearing P. monodon post-larvae at different densities (stage 1: crowding) and of individual larvae in water from stage 1 (stage 2: crowding water).

5. Discussion

Growth and survival

The results of the current study are in line with previous studies in which higher stocking densities were found to depress shrimp growth and survival (e.g. Allan and Maguire, 1992a; Rasheed and Bull, 1992; Martin et al., 1998). Our experimental design allowed a separation of mechanical/physical crowding interference (stage 1) and chemical effects (stage 2) and the results obtained were in favour of the hypothesis that the negative impact of crowding on shrimp growth and survival was due to chemical compounds.

Survival was significantly affected by stocking densities in our study with the highest survival at the lowest stocking densities. Several studies have reported similar observations for example for *P. monodon* PL35 (Ray and Chien, 1992), for *P. stylirostris* PL17 (Martin et al., 1998), *P. indicus* P1 to PL1 larvae (Emmerson and Andrews, 1981) and for different families of juvenile *P. japonicus* (Coman et al., 2004). In contrast, Allan and Maguire (1992a) found no effect of stocking density on survival of juvenile *P. monodon*. They did, however, observe a decline in growth as densities increased. Although crowding effects on survival appear not always as consistent, the vast majority of the studies have pointed out an inverse relationship between density and survival. More consensus is found on the inverse relation between shrimp density and growth (e.g. Allan and Maguire, 1992a; Rasheed and Bull, 1992; Martin et al., 1998; Tseng et al., 1998; Coman et al., 2004). Emmerson and Andrews (1981) determined incipient limiting stocking densities (ILSD) above which growth was significantly reduced to be 100 larvae l^{-1} for P1 to M2 *P. monodon* and 50 l^{-1} for M3 and PL1. The ILSD values of 10 post-larvae l^{-1} in the current study were somewhat lower, but in agreement with the observed decrease in ILSD as organisms grow (Emmerson and Andrews, 1981). The latter authors suggested a reduction in living space to be a causal factor, which could act through physical interference and altered behaviour resulting in lower feeding efficiency (Rasheed and Bull, 1992). Other studies could not attribute the negative impact of crowding to water quality parameters and suggested either conditions at the sediment-water interface (Martin et al., 1998) or high-density stress to be involved (Tseng et al., 1998). Because in our experiment the effects of crowding traveled on to the second stage, in which single post-larvae were exposed to water from different crowding conditions, no sediment was present and waste removed daily, such physical interference stress and sediment related toxicity can be excluded to be of major influence. Moreover, for the post-larvae used and under the experimental conditions employed cannibalism could be excluded as a causal factor too, but may still be a significant source of mortality in older animals (e.g. Piyatiratitivorakul et al., 2001).

Effects of pH, salinity, dissolved oxygen and chlorine

The result that the observed negative impact of crowding on shrimp growth and survival was due to chemical compounds finds support from numerous studies in which several water quality parameters have been found to affect growth and survival of penaeid shrimps. Among these are water quality parameters such as

pH, temperature, dissolved oxygen, salinity, chlorine, nitrate, nitrite and ammonia.
In the current study, the water quality parameters pH, temperature and dissolved oxygen showed very little variation and were similar among treatments. Moreover, pH, oxygen and salinity were all within the range of optimal conditions for *P. monodon* culture as summarized by NACA (1994) with an optimal pH of 7.5 to 8.0, salinity between 10 and 20 ppt and oxygen ranging from 3.8 to 5.0 mg l^{-1}. Noor-Hamid et al. (1994) suggested a pH range of 7.5 – 8.0 to be suitable for larval hatchery and rearing. The average pH of 7.9 found in our experiments is well within the suggested range meaning that any detrimental impact of pH can be excluded. This is corroborated by the study of Allan and Maguire (1992b), in which no mortality at all was observed in juvenile *P. monodon* that had been reared for 23 days at a pH of 7.8.
The salinity level of 15‰ employed in our study is well within the tolerance range of *P. monodon* and several studies have revealed 100% survival of *P. monodon* at salinity of 15‰ (e.g. Allan and Maguire, 1992b; Tsai and Chen 2002). Also growth of *P. monodon* reared for 23 days at 15‰ and 30‰ was reported to be similar (Allan and Maguire, 1992b). Moreover, because salinity levels were similar among treatments in our experiment, we do not expect salinity a major factor in mortality and growth inhibition.
Dissolved oxygen concentrations in our experiment never reached critical levels due to the gentle aeration by air-stone diffusers. In stage 1, the lowest value measured was 3.8-mg l^{-1} with in total four out of 150 measurements being below 5-mg l^{-1}, the rest above it, while in stage 2, all of the dissolved oxygen measurements yielded values above 5 mg l^{-1}. These values are well above the 1.2-mg l^{-1} reported to depress growth (Seidman and Lawrence, 1985) or the 96-h LC50 of 0.9-mg l^{-1} (Allan and Maguire, 1991). In addition, *P. monodon* are very resilient to short-term oxygen stress and even daily 12 h exposure to 0.5-0.6 mg l^{-1} oxygen had no effect on shrimp growth over a 21 day period (Allan and Maguire, 1991). Also prolonged periods at oxygen-levels as low as 2.2-mg l^{-1} had no effect on *P. monodon* survival and growth (Allan et al., 1990). Therefore, also dissolved oxygen can be excluded as a stress factor in our experiment.
Although 20-30 ppm active chlorine was used to disinfect the seawater from pathogenic organisms, and 24-h LC50 values of 0.9 mg active chlorine l^{-1} for *P. monodon* PL18 have been found (Lin and Lin, 2002), the settling period of 4 – 7 days prior to use of water in the experiment was sufficient to reduce the chlorine to below detectable levels.

Effect of nitrite
The nitrogen-compounds measured in the water showed more variation in time and between treatments than the above mentioned water quality parameters. Nitrite concentrations remained below 2 mg l^{-1} in all treatments, which is below the concentration of 6 mg l^{-1} reported to affect growth in *P. indicus* (Wickins, 1976). In two other penaeid species, 20 days exposure to 4.8 mg l^{-1} NO_2^-- N did not affect growth (Wickins, 1976). Hence, no detrimental effect of NO_2^-- N on growth is expected, which is supported by the absence of any correlation

between the measured average nitrite concentrations and final body-sizes and dry weights of *P. monodon* post-larvae. This is further corroborated by the finding that NO_2^-- N concentrations in the 5 and 10 shrimp l^{-1} treatments of the first stage were as high as in the higher density treatments of stage 2, but that growth was similar to that in controls (*see* figure 6). For seven species of penaeid shrimps, Wickins (1976) found a 48-h LC50 value of 170 mg NO_2^-- N l^{-1}, which is close to the 48-h LC50 value of 193 mg NO_2^-- N l^{-1} found for *P. monodon* adolescents (Chen et al., 1990). In general, tolerance to nitrogenous compounds increases with age (Chin and Chen, 1987; Chen et al., 1990), which also applies to the nitrite species (Chen and Chin, 1988). The 96-h LC50 value of 13.6 mg NO_2^-- N l^{-1} found for *P. monodon* PL6 (Chen and Chin, 1988) is still much higher than the maximum values of 1.65 and 1.76 mg NO_2^-- N l^{-1} in stage 1 and 2, respectively. Based on all studies mentioned above and the finding that no relation between survival and mean NO_2^-- N concentrations was found in the current study, we reject nitrite as an important factor in growth inhibition and mortality of *P. monodon* in our experiment.

Effect of nitrate

Nitrite is the intermediate product in the microbial (*Nitrosomonas* and *Nitrobacter*) nitrification of ammonia to nitrate or in the denitrification of nitrate and in general considered more toxic than nitrate (e.g. Wickins, 1976). Indeed, growth of *P. monodon* was not affected by exposure to concentrations over 200 mg NO_3^-- N l^{-1} and estimated LC50 values for nitrate were 20 times higher than those for nitrite (Wickins, 1976). Although we observed a statistically significant relation between average nitrate concentrations and final post-larval body-size and an almost significant relation between nitrate and dry weights, the regression models only explained about 37% and 28% of the variation, respectively. A possible explanation is that the nitrate concentrations follow those of ammonium, the major excretory product, as a result of the nitrification process (Ray and Chien, 1992), causing an apparent correlation with post-larval body length and dry weights. Working on *P. monodon* juveniles in seawater of 15‰ (as in the present study), Tsai and Chen (2002) found a 96-h LC50 of 1450 mg NO_3^-- N l^{-1}. In contrast, Muir et al. (1991) reported an effect on survival of *P. monodon* zoeae at 0.23 mg NO_3^-- N l^{-1}. Based on the majority of the literature studies as well as the lack of any relation between survival and mean nitrate concentrations in the present study we eliminate nitrate as an important factor in shrimp growth and survival in our experiment.

Effect of ammonia

The highest ammonia concentrations were observed after two weeks in treatments with the highest densities with a maximum of 7.8 mg l^{-1} and 8.5 mg l^{-1} in stage 1 and 2, respectively. These values are in good agreement with those found in outdoor tanks (5 mg l^{-1}, Smith et al., 2002) and in *P. monodon* grow out systems with frequent water exchange where ammonia increased up to 6.5 mg/l (Chen and Tu, 1991). The increase in ammonia concentrations with higher densities of shrimps is a direct consequence of ammonia being the major

excretory product of shrimp (e.g. Burford and Williams, 2001). The decrease after two weeks can be explained from death of animals in the high-density treatments, lowering the population density and thereby the overall excretion of ammonia. Ammonia toxicity, especially by the unionized species NH_3, is one of the common causes of shrimp death and thus high concentrations pose a severe threat to the animals (e.g. Noor-Hamid et al., 1994). As for other nitrogenous compounds, tolerance of larval *P. monodon* to ammonia increases from nauplius to postlarval stages, which is reflected in 24-h LC50 values of ammonia for nauplius, zoea, mysis and postlarva of 6.0, 8.5, 24.0 and 52.1 mg l^{-1} (Chin and Chen, 1987). The toxicity of ammonia not only depends on age of *Penaeus*, but is also influenced by environmental factors, such as salinity and pH, and exposure time.

The iso-osmotic point of *P. monodon* is equivalent to 25 ‰ (Ferraris et al., 1986) meaning that the hypo-osmotic environment of 15 ‰ employed in the current study could result in higher susceptibility to ammonia. This is expected based on previous studies in which hypo-osmotic rearing conditions resulted in less tolerance of penaeid shrimps to ammonia (Chen and Lin, 1991; 1992) and nitrate (Tsai and Chen, 2002). The effect of pH on ammonia toxicity most probably is directly related to the effect pH has on speciation. Higher pH will result in increased concentrations of unionized ammonia and thereby in increased toxicity (Noor-Hamid et al., 1994). Toxicity of ammonia to *P. monodon* postlarvae increased with exposure time with 24-h, 48-h, 72-h and 96-h LC50 values of 52.1, 27.7, 17.1 and 11.5-mg ammonia l^{-1} (Chin and Chen, 1987). Similar observations have been made for other penaeid shrimp juveniles (Chen and Lin, 1991; 1992) or for *P. monodon* juveniles and other nitrogenous compounds (Chin and Chen, 1988; Tsai and Chen, 2002). The relation between LC50 values and exposure time found by Chin and Chen (1987) can be expressed by an exponential decay function: $LC50 = 7.30 + 97.4 \times \exp(-0.78 \times \text{time})$ (r^2_{adj} = 0.999). Applying this function and taking into account that shrimps may be more susceptible at lower salinity levels, thereby assuming 50% lower LC50; yields an estimated LC50 value of 4.1-mg l^{-1} after one-week exposure. The same value of 4.1 mg ammonia l^{-1} was found to be the maximum acceptable level, i.e. that which reduced growth of juvenile *P. monodon* by 5 % over 3 weeks, but was determined at a much higher salinity of 36‰ (Allan et al., 1990). In *P. japonicus*, 5-mg ammonia l^{-1} was sufficient to cause significant reduction in shrimp weight and length (Chen and Kou, 1992). Because ammonia concentrations in the 50 and 100 postlarva l^{-1} treatments exceeded the value of 4.1 mg l^{-1} and a highly significant relation was found between mortality and mean ammonia concentrations, ammonia toxicity can not be excluded as the causal factor for the observed mortality in our experiments.

However, the correlation found between mortality and mean ammonia concentrations needs experimental confirmation, as we can not exclude potential other excretory products to be involved. For example, crowding chemicals such as alarm pheromones may be involved. Such crowding chemicals have been detected in crustaceans ranging from zooplankton (*Daphnia*; Pijanowska, 1997), amphipods (*Gammarus*; Wisenden et al., 2001), and crayfish (*Orconectes*;

Hazlett, 1994) to crabs (*Heterozius*; Hazlett and Mclay, 2000). These conspecific cues may not only affect behaviour, but could also reduce growth and survival and in the case of *Daphnia* ammonia and urea have been excluded as the causal excretory products (Lürling et al., 2003). Therefore, further studies, under identical experimental conditions, with controlled ammonia concentrations and with water from injured shrimps will reveal whether alarm substances or ammonia can be excluded as causal factors for growth inhibition or not.

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Chapter 7

THE EFFECTS OF CRUSHED CONSPECIFICS ON GROWTH AND SURVIVAL OF *PENAEUS MONODON* FABRICIUS POST-LARVAE

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1. Abstract

The hypothesis was tested that *Penaeus monodon* post-larvae will show a reduction in their growth when exposed to crushed conspecifics, which was done by exposing individual *P. monodon* post-larvae during four weeks to a gradient from 0 to 100 crushed conspecifics per liter. Both the dry weight (48.5 $\pm$ 7.2 mg) and the body size (28.0 $\pm$ 1.3 mm) of experimental animals exposed to 1 macerated conspecific per liter were significantly ($P \leq 0.011$) higher than those of animals in controls and treatments with 5 and 10 crushed conspecifics (25.6 $\pm$ 3.2 mg; 23.4 $\pm$ 0.5 mm). Unexpectedly, all animals died within one week when exposed to 70 and 100 crushed conspecifics per liter, and after three and four weeks when exposed to 50 and 30 crushed conspecifics per liter, respectively. Exposure duration affected mortality and LC50 values decreased from 60 crushed shrimp per liter to 13 per liter from one to four weeks' exposure, respectively.

The survival of *P. monodon* post-larvae was negatively correlated to pH, BOD, ammonia and nitrate meaning that an increase in these water quality variables coincided with a reduced survival. In conclusion, low dose of crushed conspecifics has a stimulatory effect on *P. monodon* post-larvae, as larvae were heavier, larger and more aggressive, whereas high doses cause high mortality.

2. Introduction

Penaeid shrimp farming has become a significant aquacultural activity in many countries in the tropics. In Viet Nam, as in the most of Southeast Asia, the tiger prawn *Penaeus monodon* is the most widely cultured species. In both extensive and intensive farming shrimps are often stocked at high densities to optimize production, but farmers face the risk of considerable shrimp mortality. In general, high shrimp stocking densities result in higher mortality, lower feeding efficiency and reduced growth of *P. monodon* (e.g. Allan and Maguire, 1992a; Martin et al., 1998; Rasheed and Bull, 1992; Ray and Chien, 1992; Tseng et al., 1998). Apparently, such high shrimp densities or crowding conditions may create a hostile environment. There is, however, much less consensus on the causal factors and several causes for the negative effects of high shrimp density on shrimp fitness have been proposed. First, high-density stockings may adversely influence water quality. Ammonia being the major excretory product of shrimp will increase with higher densities of shrimps (e.g. Burford and Williams, 2001) and high concentrations pose a severe threat to the animals (Chin and Chen, 1987; Noor-Hamid et al., 1994). The same holds true for nitrite, the intermediate product in the microbial nitrification of ammonia to nitrate, that may have a strong negative impact on shrimp growth and survival (Chen and Chin, 1988; Wickins, 1976). Second, a reduction in living space could affect shrimp growth and survival too (Emmerson and Andrews, 1981), which could act through physical interference and altered behaviour resulting in lower feeding efficiency (Rasheed and Bull, 1992). Third, conditions at the sediment-water interface where the animals live may become hazardous (Martin et al., 1998). The accumulation of feces and non-consumed food may lead to low-oxygen conditions and anaerobic decomposition at the bottom (Funge-Smith and Briggs, 1998). Finally, in the case where sediment-toxicity or water-quality effects could be excluded undefined high-density stress has been proposed (Tseng et al., 1998). However, of the many factors that can influence shrimp growth, development and survival, chemical cues released from injured con-specifics has attracted no attention.

Penaeid shrimp are on the menu of many predators, mainly fish (Robertson, 1988; Minello et al., 1989), but also cannibalism may occur (Piyatiratitivorakul et al., 2001). During a successful predatory attack prey body fluids are released that may convey information of the imminent danger to conspecifics. The conspecifics may use these injury-released chemical cues to minimize their probability of being eaten through activation of anti-predator responses (e.g. Wisenden, 2000). Such responses are widespread among aquatic taxa ranging from protozoa to amphibia and fish (Chivers and Smith, 1998; Tollrian and Harvell, 1999), and have been reported in crustacea as well. For example, exposure to macerated conspecifics caused an inhibition in feeding in the smooth pebble crab *Philyra laevis* (McKillup and McKillup, 1992) and avoidance behaviour in juvenile blue crabs (Diaz et al, 1999; 2003). Alarm cues reduced the activity of amphipod crustaceans in the genus *Gammarus* (Williams and Moore, 1985; Wudkevich et al., 1997; Wisenden et al., 2001). In the freshwater crustacean *Daphnia magna*, exposure to crushed conspecifics resulted not only in behavioural anti-predatory changes (Pijanowska, 1997), but also in decreased somatic growth and reproduction (Pijanowska and Kowalczewski, 1997).
Inasmuch the potential use of chemical information released from injured conspecifics has been an untouched area in shrimp research so far, we tested the hypothesis that *Penaeus monodon* post-larvae will show a reduction in their growth when exposed to crushed conspecifics. This hypothesis was tested by exposing individual *P. monodon* post-larvae to a gradient from 0 to 100 crushed conspecifics per liter.

3. Materials and methods

Experimental design

The effect of crushed *Penaeus monodon* post-larvae on growth and survival of individual *P. monodon* post-larvae was performed under controlled conditions at the laboratories of Can Tho University, Vietnam. The experiment was conducted in spherical plastic vessels (19-cm height and 11.7-cm diameter) that had been soaked and washed in water for 30 days prior to use. All vessel contained water with a salinity of 15 (± 1) ppt. This water was prepared from seawater with a salinity of 25 - 35 ppt taken from the mangrove area at Ca Mau forest (8°50N, Mekong Delta, Vietnam). The seawater was chlorinated (20-30 ppm active chlorine), settled for 4 to 7 days to remove chlorine and then diluted with tap-water to obtain the required salinity of 15 ppt.
P. monodon post larvae (PL 15-25, i.e. 15-25 days after hatching, with an average length of 9.9 mm and dry weight of 7 mg) were stocked individually in the rearing vessels with a constant water volume of one liter per vessel. The cues from crushed conspecifics were prepared at concentrations of 0, 1, 5, 10, 30, 50, 70 and 100 *P. monodon* post larvae per liter. Ten replicates per crushed concentration were used yielding 8 (concentrations) × 10 (replicates) = 80 experimental units. Daily the shrimps were fed with CP (Crude Protein, approximately 30% nitrogen, derived from a local manufacturer) pellets at a level of 10% of their body weight. Excess food and shrimp waste was removed daily and the water was refreshed in each vessel. Daily conspecifics were crushed

using a mortar and pestle, and diluted to the final volume of one liter in the corresponding rearing vessels. All rearing vessels were aerated during the entire experimental period of 4 weeks and their water volumes kept constant at.

Analyses and measurements

Prior to being stocked in culture vessels, shrimps were measured for their length and weight (DW). Dry weights were determined after drying for 24 h at 105°C using a 4 decimal precision balance. Initially and at the end of the experimental period, the protein content of the shrimps was determined following the Kjeldalh method.

The number of surviving animals was recorded at the end of the experiment, i.e. after 28 days, their body length measured and dry weight determined.

Of each vessel, daily the temperature, pH, salinity, and dissolved oxygen were recorded using a YSI-556-MPS oximeter, whereas nitrogen (NO_2^-, NO_3^-, and NH_4^+) and biological oxygen demand were measured weekly following standard titration methods.

The effects of crushed conspecifics on final *P. monodon* body size and dry weight was statistically compared using one-way analysis of variance (ANOVA). The ANOVA was run in the statistical tool pack SPSS® version 10.1.0 (SPSS Science, Briljant Software & Techniek, Mijdrecht, The Netherlands), with the crushed concentration (0 – 100 shrimp l^{-1}) as the fixed factor. Significantly differences (at the 95% confidence level) were distinguished using Tukey's post-hoc comparison test. Median lethal concentrations (LC_{50} values) were determined fitting the logistic function $y = \frac{a}{1+\left(\frac{x}{x_0}\right)^b}$, where *y* is the survival, *a* = the survival in the controls, *b* = a slope parameter, *x* = the crushed conspecific concentration (shrimps l^{-1}) and x_0 = the LC_{50} value (shrimps l^{-1}).

4. Results

Survival

Post-larvae exhibited excellent survival at the lowest crushed conspecific concentrations (i.e. 0 and 1 crushed post-larva l^{-1}) throughout the entire experimental period. The first three weeks all experimental animals survived, while only two died in each treatment in the fourth week (figure 1). In contrast, in the highest crushed conspecific treatment (100 crushed post-larva l^{-1}) all animals died within the first week; in the second highest (70 crushed post-larva l^{-1}) all except one animal had died within one week. In the 50 and 30 crushed post-larva l^{-1} treatments all animals had died after 3 and 4 weeks, respectively (figure 1), whereas in the 10 and 5 crushed post-larva l^{-1} treatments 60 and 70% of the animals survived until the end of the experiment.

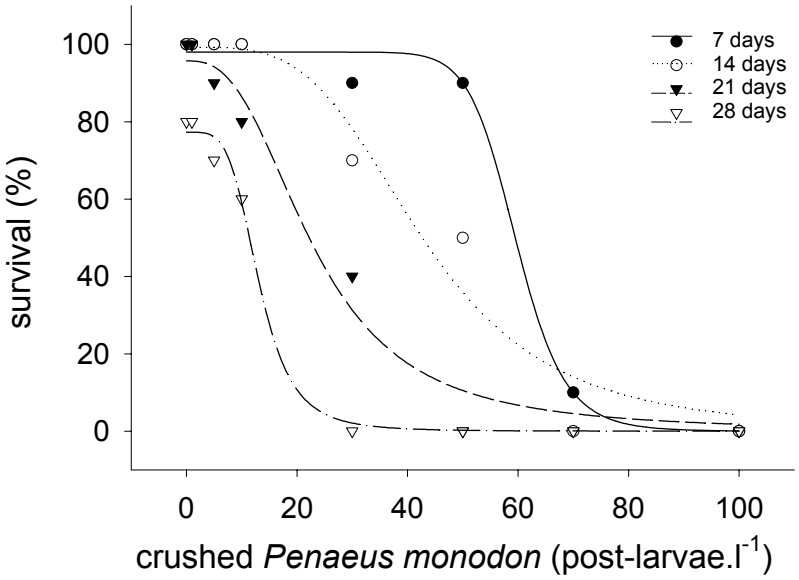

Figure 1. Weekly percentage survival of P. monodon post-larvae reared for 4 weeks at different concentrations of crushed conspecifics (0-100 l^{-1}). Lines represent non-linear regression analysis, logistic function).

The crushed conspecific concentration at which 50% of the experimental animals died (LC_{50}-value) depended on the exposure duration and decreased with prolonged exposure from about 60 crushed post-larva l^{-1} for one week exposure to 13 crushed post-larva l^{-1} at four weeks exposure (Table 1).

Table 1. Median lethal concentrations (LC_{50}) of crushed conspecifics to Penaeus monodon post-larvae reared individually for 28 days at different concentrations of crushed conspecifics for four consecutive weeks. Included are r^2 of logistic regression analyses and standard errors (SE) of estimated LC_{50}.

weeks	LC_{50}	SE	r^2
1	59.7	1.6	0.994
2	42.9	4.1	0.964
3	23.0	3.5	0.980
4	13.1	1.3	0.995

Body length

Because in the high crushed conspecific treatments (30, 50, 70 and 100 crushed post-larva l^{-1}) all animals had died before the end of the experiment, these treatments were excluded from statistical analysis of the effects on body length. For the remaining treatments, one-way ANOVA ($F_{3,25}$ = 4.54; P = 0.011) indicated significantly different body sizes of post-larvae and Tukey's post-hoc test revealed two homogenous groups: 1) 0, 5 and 10 crushed post-larva l^{-1} and 2) 1 and 5 crushed post-larva l^{-1} (figure 2). *P. monodon* reared in the presence of

cues from one conspecific per liter were significantly larger than those reared in the absence of such stimuli.

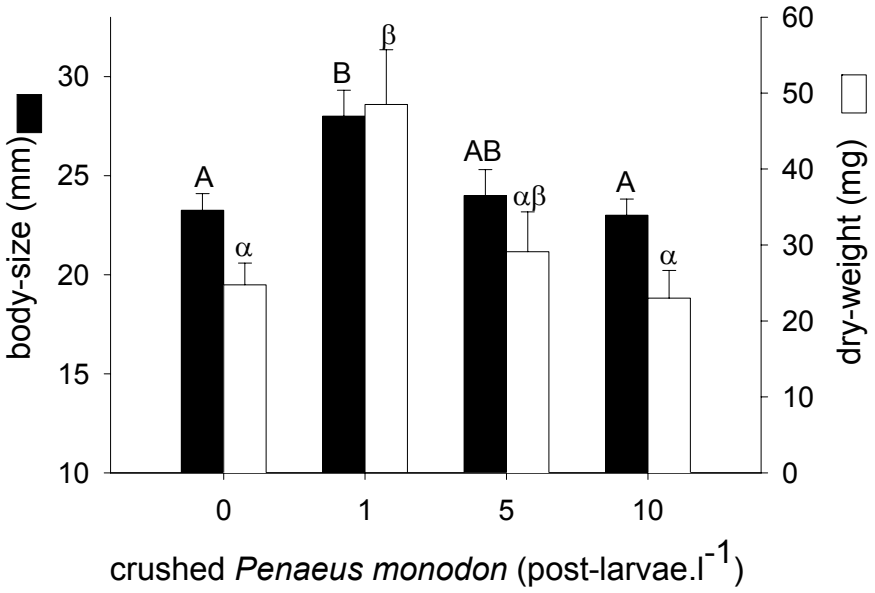

Figure 2. Body size (mm; filled bars) and dry weight (mg; open bars) of P. monodon post-larvae reared for 4 weeks at different concentrations of crushed conspecifics (0, 1, 5 and 10 l^{-1}). Error bars indicate one standard deviation (N = 8, 7, 6 and 6 respectively). Different symbols above bars (A,B and α,β) indicate significantly different groups (Tukey test; P < 0.05).

Dry weight

A one-way ANOVA on final dry weights of *P. monodon* post-larvae reared for 4 weeks at different crushed conspecific concentrations indicated significant differences ($F_{3,25}$ = 5.25; P = 0.006). As for body size the Tukey's post-hoc test revealed two homogenous groups: 1) 0, 5 and 10 crushed post-larva l^{-1} and 2) 1 and 5 crushed post-larva l^{-1} (figure 2). *P. monodon* reared in the presence of cues from one conspecific per liter were significantly heavier than those reared in the absence of such stimuli. Moreover, it was noted that post-larvae in the 1 crushed conspecific l^{-1} treatments were more aggressive in comparison to the other treatments and that their antennules were extremely well developed.

Shrimp protein content

The protein content of *P. monodon* post-larvae grown at different crushed conspecific concentrations showed minor deviations (figure 3). The protein content in controls had remained around 70% of DW, and only in the 1 crushed conspecific l^{-1} treatment at after 4 weeks protein content had slightly increased to 74% (figure 3).

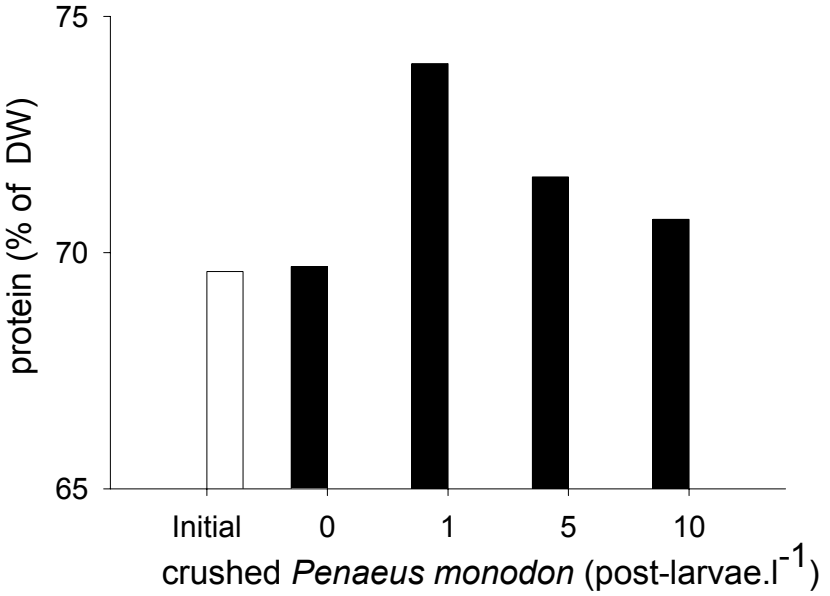

Figure 3. Protein content as percentage of the dry weight of P. monodon post-larvae reared for 4 weeks at different concentrations of crushed conspecifics (0, 1, 5 and 10 l^{-1}). The open bar represents the protein content at the start of the experiment (Initial).

Water quality variables

The dissolved oxygen concentration was similar among treatments (one-way ANOVA $F_{7,179}$ = 1.85; P = 0.081) and on average 6.6 (± 0.3) mg l^{-1} (figure 4). However, temperature ($F_{7,179}$ = 4.45; P < 0.001) appeared significantly different among treatments. Post-hoc comparison revealed three homogenous groups: 1) 0 – 50 crushed shrimp l^{-1}, 2) 0, 1, 10, 50 and 100 crushed shrimp l^{-1}, and 3) 50, 70 and 100 crushed shrimp l^{-1}. Temperature in the 70 and 100 crushed shrimp l^{-1} appeared significantly higher than those in the 5 and 30 crushed shrimp l^{-1} treatments. This can, however, be explained from a time effect as temperature showed a gradual decrease of about 0.08°C per day during the course of the experiment (figure 4) and measurements in the high crushed treatments were terminated three weeks prior to those in the low crushed shrimp treatments. Also for pH a one-way ANOVA indicated significant differences among treatments ($F_{7,179}$ = 24.6; P < 0.001). Two homogenous groups were detected: 1) 0 – 10 crushed shrimp l^{-1} and 2) 30 – 100 crushed shrimp l^{-1}. Albeit statistically significant, differences in mean pH-values among treatments were small and varied between 7.9 and 8.2, with individual measurements varying between 7.7 and 8.4 (figure 4).

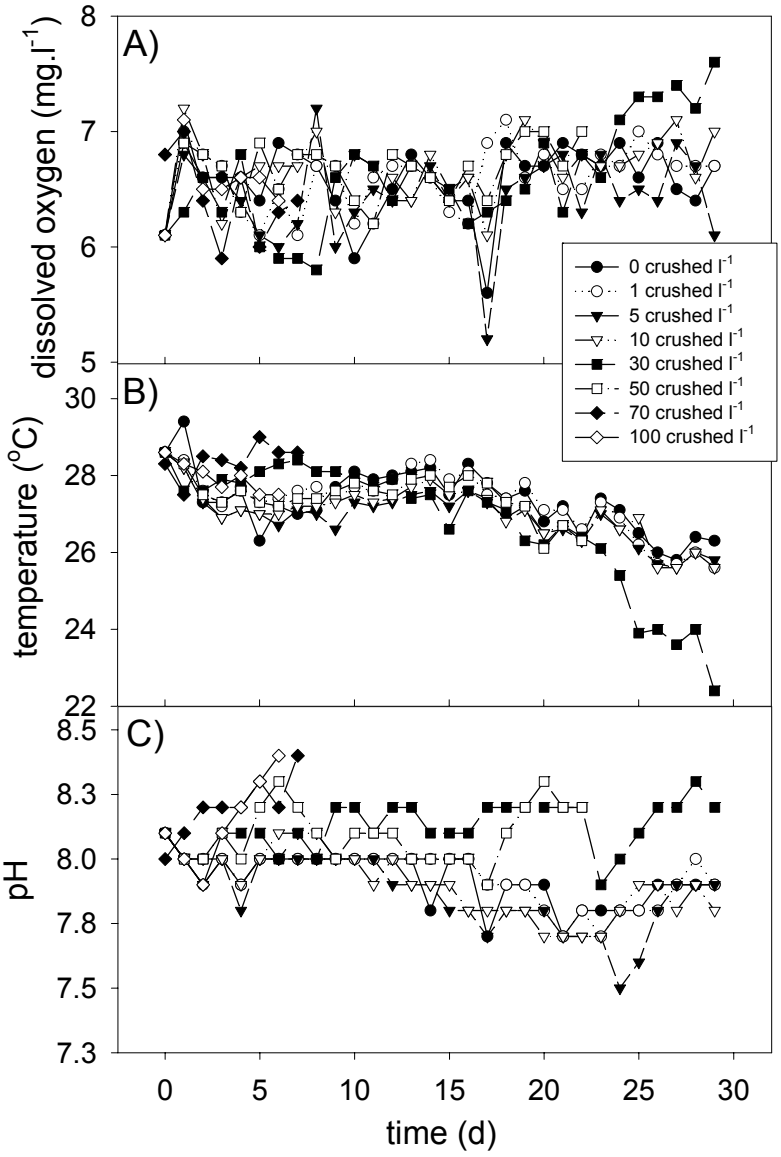

Figure 4. Course of the water quality variables dissolved oxygen (mg l^{-1}; upper panel), temperature (°C; middle panel) and pH (lower panel) during four weeks in rearing vessels with individual P. monodon post-larvae at different concentrations of crushed conspecifics (0, 1, 5 and 10 l^{-1}).

The biological oxygen demand showed a rather erratic pattern, but increased in all treatments during the course of the experiment (figure 5). In general, the increase in BOD was lowest in the low crushed shrimp treatments (from 7.5 to about 14-mg l^{-1}) and highest in the high number of crushed shrimp treatments (from 7.5 to about 50-mg l^{-1}).

Ammonia concentrations in the 0 to 10 crushed shrimp l^{-1} did not exceed 1.5-mg l^{-1} during the course of the experiment, whereas the 70 and 100 crushed shrimp l^{-1} treatments already after one week had ammonia concentrations exceeding 3

mg l^{-1} (figure 5). The highest ammonia concentrations were measured in the 30 and 50 crushed shrimp l^{-1} treatments, with 7.7 and 8.5-mg l^{-1}, respectively. Nitrite concentrations remained below 1.8-mg l^{-1} in the 0, 1, 30, 70 and 100 crushed shrimp l^{-1} treatments, but values of 3.2, 5.1 and 5.9-mg l^{-1} were measured in the 5, 10 and 50 crushed shrimp l^{-1} treatments, respectively (figure 5). Nitrate concentrations were all below 2.3-mg l^{-1}, and in all but one case below 1.6-mg l^{-1} (figure 5).
Correlation analysis (Pearson correlation) showed that *P. monodon* survival was negatively correlated to pH, BOD, ammonia and nitrate meaning that an increase in these water quality variables coincided with a reduced survival (Table 2).

Table 2. Pearson correlation (** $P \leq 0.01$; * $P \leq 0.05$) of Penaeus monodon post-larval survival (Surv.) and measured water quality variables dissolved oxygen (DO, mg l^{-1}), temperature (Temp.,°C), pH, biological oxygen demand (BOD, mg l^{-1}), ammonia (NH_4, mg l^{-1}), nitrite (NO_2, mg l^{-1}) and nitrate (mg l^{-1}).

	surv.	DO	temp	pH	BOD	NH_4	NO_2	NO_3
surv.	----							
DO	-0.333	----						
temp	0.267	-0.680**	----					
pH	-0.491*	0.014	0.223	----				
BOD	-0.581**	0.031	-0.298	0.436*	----			
NH_4	-0.660**	0.023	-0.112	0.636**	0.870**	----		
NO_2	-0.168	0.258	-0.285	-0.323	0.279	0.044	----	
NO_3	-0.716**	0.342	-0.245	-0.031	0.290	0.247	0.378	----

5. Discussion

Growth and chemical cues

The results of the current study support the hypothesis that *Penaeus monodon* post-larvae show changes in their growth when exposed to crushed conspecifics. However, both the rapid and strong mortality at high doses of crushed conspecifcs as the stimulatory effect at the lowest dose of 1 crushed shrimp l^{-1} were unexpected.
Numerous studies have examined the effect of alarm cues released from injured (crushed) aquatic organisms on conspecifics, albeit mainly on behavioural changes (e.g. Borowsky, 1985; Chivers and Smith, 1998; Ichinose, 2002; Wisenden et al., 1999; 2001). The general picture emerging from those studies is that exposure to crushed conspecifics resulted in a reduced activity. A reduced activity may be beneficial to reduce the risk of being eaten by predators, but generally such reactions come with costs (Harvell 1990). Indeed, alarm cues reduced growth in the crustacean *Daphnia magna* (Pijanowska and Kowalczewski, 1997) and in mayfly and tadpoles alarm cues decreased foraging activity leading to a reduced growth (Skelly & Werner 1990, Peckarsky *et al*. 1993). Based on these studies we expected that exposure to crushed conspecific

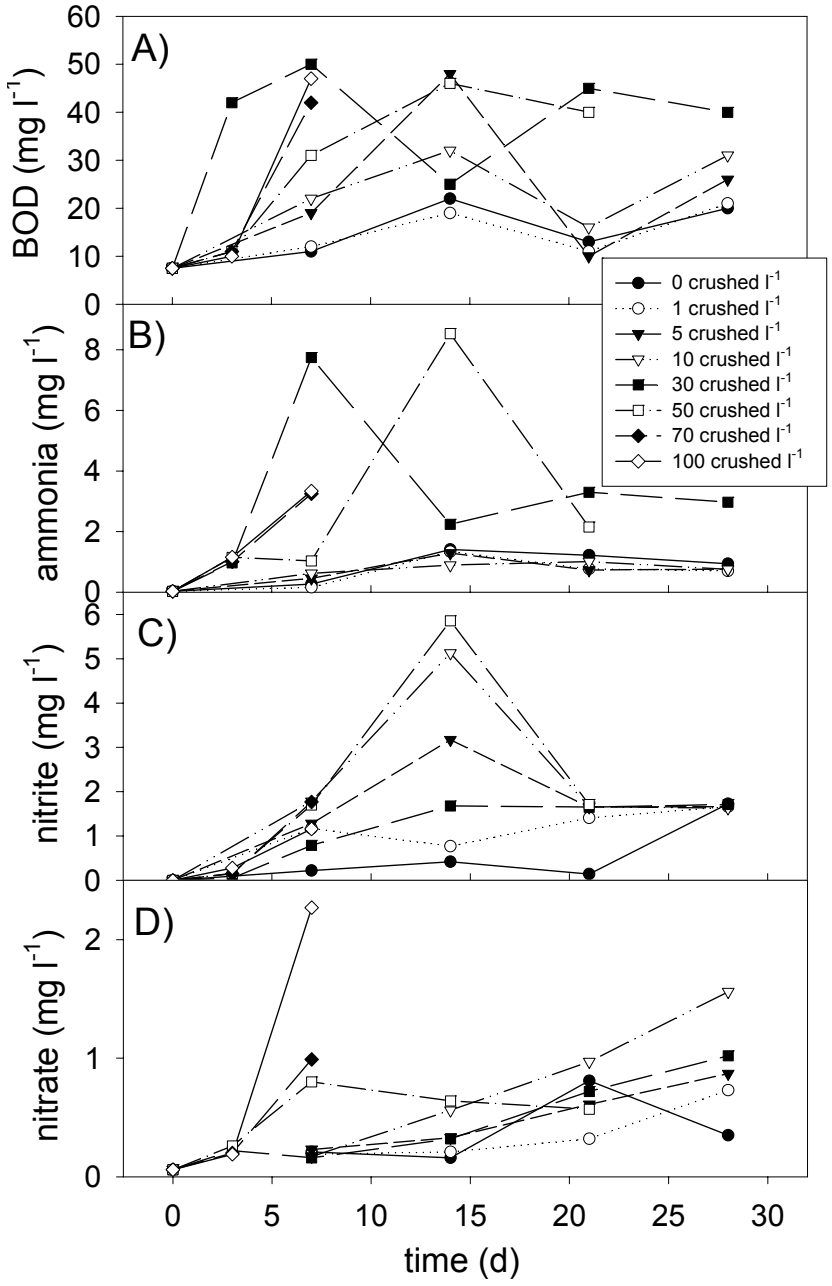

Figure 5. Water quality variables biological oxygen demand (BOD, mg l^{-1}; panel A), ammonia (mg l^{-1}; panel B), nitrite (mg l^{-1}; panel C) and nitrate (mg l^{-1}; panel D) during four weeks in rearing vessels with individual P. monodon post-larave at different concentrations of crushed conspecifics (0, 1, 5 and 10 l^{-1}).

organisms would reduce (feeding) activity in *P. monodon* and that the reduced resource acquisition would in turn affect growth. In contrast to our expectation, compared to controls, growth (based on final body size and dry weight) was not reduced in the 5 and 10 crushed post-larvae l^{-1} treatments, but even significantly promoted in the 1 crushed post-larvae l^{-1} treatment (*see* figure 2). Also protein content was somewhat enhanced in this low dose crushed shrimp treatment (1

post-larva l^{-1}). The stimulatory effect at low dose and the lethal effect at high dose strongly suggests hormesis, of which thousands examples in toxicological studies exist (Calabrese and Baldwin, 2003).
Although predator avoidance may result in reduced survival as has been found in snails (Rigby and Jokela, 2000), mortality as high as the observed 100% was not expected. Such a reaction can not be explained as being beneficial in an alarm cue – predator avoidance context, but probably reflects strong toxicosis. The stimulatory effect on growth at low dose of crushed shrimp coincided with more aggression in experimental animals compared to the other treatments, which indicates behavioural changes as well. Because of this more aggressive behaviour, effects of exposure to low dose of crushed shrimp on population growth of *P. monodon* can not be predicted beforehand and certainly needs further experimental exploration to investigate whether conspecific cues can be used to enhance yield.
The unexpected stimulatory effect at the lowest dose of 1 crushed shrimp l^{-1} in combination with observed behavioural changes, strongly supports the hypothesis that *P. monodon* is capable of detecting and responding to chemical changes in its environment. As the shrimps are challenged to survive in rather unpredictable or variable environments this may force them to track environmental changes and respond appropriately to increase their fitness. A general feature of shrimp is that they are equipped with chemosensory sensilla mostly located on the antennules. These chemosensory organs allow shrimp to use chemical information for evaluation of their habitat (e.g. Mead et al., 2003), which, in fact for *P. monodon* post-larvae may be the most important way of gathering information as they often live in turbid environments such as the mangroves in the Mekong Delta. Indeed, chemical cues are especially useful in turbid aquatic environments due to solution and dispersal properties and are widely used by aquatic organisms ranging from algae to fish (e.g. Tollrian & Harvell, 1999; Wisenden, 2000). Several studies have shown that shrimps may be attracted to extracts from crab (Carr, 1978; Carr et al., 1984), to a betaine-based product of natural marine extracts and a mixture of amino acids (Costero and Meyers, 1993) and to shrimp-head offal extract (Holland and Borski, 1993). The latter finding is of particular interest, because if the lowest dose of crushed shrimp in our experiment would stimulate food search behaviour, thereby increasing the encounter rate of feed, it could lead to the observed stimulatory effect compared to controls. The absence of a stimulatory effect at higher doses are in line with the observations of Carr (1978) who found a bell-shaped dose-response relationship for feeding response to crab extract.

Survival and water quality

The survival of *P. monodon* post-larvae appeared negatively correlated to pH, BOD, ammonia and nitrate meaning that an increase in these water quality variables coincided with a reduced survival. Although the pH in the high dose treatments was significantly higher than in the low dose treatments, differences in average pH-values were marginal (between 7.9 and 8.2) and all measured pH-values were within the range of 7.7 and 8.4. These upper values are slightly

higher than the range of 7.5 – 8.0 suggested to be suitable for larval hatchery and rearing (NACA, 1994; Noor-Hamid et al., 1994). However, a 100% survival of *P. japonicus* juveniles reared for 60 days at a pH of 8.21 has been found (Chen and Kou, 1992), whereas Martin et al. (1998) observed 94% survival of *P. stylirostris* at pH of 8.26 (± 0.17). Moreover, Thakur and Lin (2003) reported between 50 and 78% survival of *P. monodon* in pH ranging from 7.0 up to 9.4. Hence, we exclude pH as the causal factor for the observed mortality, which in fact is supported by the result of similar pH values in 30, 50, 70 and 100 crushed post-larvae l^{-1} treatments, but strongly deviating effects on survival of the experimental animals in those treatments (*see* figure 1).

The BOD reflects potential organic decomposition that could lead to a hazardous environment for shrimps through low dissolved oxygen (Allan and Maguire, 1991) and a potential interaction with ammonia (Allan et al., 1990). Dissolved oxygen concentrations in our experiment, however, never reached critical levels due to the gentle aeration by air-stone diffusers. The significant negative correlation between BOD and survival is probably the result of an apparent correlation, because more crushed shrimps imply a higher BOD, but also a higher dose of the unknown toxic compounds.

Despite the negative correlation between nitrate and survival, nitrate concentrations were all below 2.3-mg l^{-1}, which is far below the 96-h LC50 of 1450 mg NO_3^-- N l^{-1} found for *P. monodon* juveniles in seawater of 15‰ (Tsai and Chen, 2002).

Therefore, we also exclude nitrate as an important factor in shrimp survival in our experiment. The other nitrogen species that showed a negative correlation with survival, ammonia, is one of the common causes of shrimp death and thus high concentrations pose a severe threat to the animals (e.g. Noor-Hamid et al., 1994). Toxicity of ammonia to *P. monodon* post-larvae has been found to increase with exposure time yielding 24-h, 48-h, 72-h and 96-h LC50 values of 52.1, 27.7, 17.1 and 11.5-mg ammonia l^{-1} (Chin and Chen, 1987). This relation between LC50 values and exposure time found by Chin and Chen (1987) can be expressed by an exponential decay function: $LC50 = 7.30 + 97.4 \times \exp(-0.78 \times \text{time})$ ($r^2_{adj} = 0.999$). Applying this function to the exposure duration employed in our study yields estimated LC50 values of 7.7-mg l^{-1} ammonia after one-week exposure to 7.2-mg l^{-1} for a four weeks exposure. Our experiment was conducted at a salinity of 15‰, which is below the iso-osmotic point of *P. monodon* (25‰ *cf.* Ferraris et al., 1986). This may lead to lower LC50 values than the estimated ones as hypo-osmotic rearing conditions may result in less tolerance of penaeid shrimps to ammonia (Chen and Lin, 1991; 1992). However, our data do not support this, as in the two highest doses (70 and 100 crushed shrimp l^{-1}) ammonia concentrations were 3.3-mg l^{-1} and mortality 90% and 100%, respectively, while in the intermediate 30 crushed shrimp l^{-1} treatment ammonia concentration was 7.7-mg l^{-1}, but mortality only 10%. Inasmuch our data do not convincingly support ammonia to be the major cause of the observed mortality, we conclude the whole mixture of body fluids or non-determined components of it to be toxic to *P. monodon*.

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SUMMARY

In recent years, expansion of shrimp aquaculture in Vietnam has brought considerable financial benefits to farmers and local communities. In the coastal provinces in the Mekong Delta, brackish shrimp aquaculture is the major economy activity. Extensive shrimp-mangrove culture systems are popularly practiced here. Although the average shrimp production is low, due to over-exploitation and destruction of mangrove forests and salt marshes, these systems are of special interest in view of the problems of sustainability of intensive aquaculture (Naylor et al. 2000 Nature 405: 1017-1024). Several studies demonstrated that mangrove swamps are highly productive ecosystems providing food, shelter and nurseries for various aquatic organisms, many of which are commercially important. The tiger shrimp, *Penaeus monodon*, is a clear example in this case. Natural shrimp production in these areas is believed to depend to a large extend on the presence of mangroves. However, the complex of mechanisms through which mangroves affect shrimp production is still poorly understood. The work in this thesis is an attempt to unravel some of the key-processes involved. It confirms the picture that mangrove litter represents a formidable input of organic material and nutrients into the aquatic system, and reveals how this input may have positive as well as negative effects on growth and survival of post-larval shrimp.

Mangrove stands of different age have been studied for one year with respect to their litter fall and nutrient input (chapter 2). Litter fall consisted for 70% of leaf litter and organic matter accounted for 90% of the dry weight. Litter fall declined with the age of the mangrove stands, and also nitrogen and phosphorus levels were considerably higher in the leaf litter of younger stands (7 and 11 years) as compared to the older stands (up to 24 years). Thus, both the amount and the quality of litter input to the aquatic systems are highest in younger mangrove stands.

As a next step key factors affecting the decomposition of mangrove leaves were analyzed (chapter 3). Decomposition rates tended to be highest at lower salinities, and reached an optimum at 5 ‰. The decomposition rates were also highest in the wet season, and this may well be due to relatively low salinities in this period. Wet season salinity in the Camau area was in the range of 4 – 9 ‰, close to the optimum for decomposition derived from laboratory experiments. Our studies also indicated an effect of humidity per se. We found that the decomposition rate was higher for leaves submerged in the ditches, than for leaves incubated near the roots of mangrove stands in the open air, where decomposition rates were higher in the wet than in the dry season. We also analyzed the dynamics of nutrient concentrations in decomposing litter. Nitrogen and phosphorus levels in decomposing leaves increased during the decomposition period. This enrichment indicates an increase of food quality over the first period of decomposition .

The following chapters show that the effects of decomposing mangrove leaves on shrimps can be positive but also negative (Chapter 4 and 5). The amount of decomposing leaves appeared key. At high concentrations of leaves negative effects prevailed. These effects were probably due to the release of nitrite and sulphide, and a decrease in dissolved oxygen concentration. On the positive side, mangrove moderate concentrations of leaves promoted growth of *Penaeus monodon* post-larvae, and apparently served as a shelter and as a food source. The fact that micro-organisms growing on the leaves, rather than the leaf material itself may be important as food was illustrated by the result that shrimps feeding on mangrove leaves grew better when a periphyton layer covered these leaves (chapter 5). A somehow surprising positive effect of leaves was the apparent prevention of excessive concentrations of ammonium and nitrite. The results suggest that adding conditioned

mangrove leaves might ameliorate negative effects of high protein pellets on the water quality. The high C/N-ratio of leaves tends to balance the stochiometry of the system which may otherwise be dominated by the excessive N-input through CP pellets.

In the final chapters the interaction among the shrimp larvae themselves, i.e. the effects of stocking density and the release of crowding chemicals and possible alarm pheromones on the shrimp populations are addressed (chapter 6, 7). A strong effect of crowding on shrimp growth and survival was shown. Physical interference stress and cannibalism could be excluded as causal factors. It was thus clear that the effects were caused by other water quality variables. Temperature, pH, salinity, dissolved oxygen, chlorine, nitrite and nitrate appeared of minor influence. However, ammonia toxicity could not be excluded as the causal factor for the observed mortality and reduced growth of *P. monodon* post-larvae in our experiments. On the other hand, alarm cues, as released by crushed conspecifics had negative effects on post-larval survival at high concentrations (100, 70, 50 and 30 crushed shrimps.l^{-1}). Surprisingly, low concentrations of crushed conspecifics (1 crushed shrimp.l^{-1}) were shown to have rather stimulatory effects on body size and dry weight.

Put in an applied perspective, this study suggests simple ways to improve the management of mangrove-shrimp systems. Clearly, mangrove leaves can promote the survival and growth of shrimp post-larvae. However, at high leaf concentrations negative effects may prevail related to a drop in dissolved oxygen and the release of sulphide. A straightforward way to ameliorate such negative effects may be to increase the water flow. This will reduce the risk of local anoxia, and may help spreading the litter over the area, thus avoiding accumulation of these leaves at some sites. The reduction of potentially toxic nitrite and ammonium concentrations by decomposing leaves suggests that mangrove leaves may serve as a useful complement to CP pellets in semi-natural production systems.

TÓM TẮT

Trong những năm gần đây, việc mở rộng diện tích nuôi tôm ở Việt Nam đã mang lại lợi nhuận đáng kể cho người dân và cộng đồng địa phương. Nuôi tôm nước lợ là hoạt động kinh tế chủ yếu của các tỉnh ven biển Đồng Bằng Sông Cửu Long, nơi mà hệ thống nuôi quảng canh tôm-rừng đã được áp dụng rộng rãi. Mặc dù sản lượng bình quân thấp, nhưng hệ thống này đã được quan tâm đặc biệt trên quan điểm bền vững của nghề nuôi thuỷ sản (Naylor et al. 2000 Nature 405: 1017-1024). Một số nghiên cứu trước đây cho thấy rằng rừng ngập mặn là hệ sinh thái có tiềm năng sản xuất lớn, là nơi cung cấp thức ăn, trú ẩn và nuôi dưỡng nhiều sinh vật thuỷ sinh, trong đó có nhiều loài có giá trị thương phẩm cao. Tôm sú, *Penaeus monodon* là loài tôm có giá trị kinh tế cao, được nuôi phổ biến trong hệ thống tôm-rừng. Sản lượng tôm tự nhiên trong vùng ven biển chắc chắn lệ thuộc vào diện tích rừng ngập mặn. Tuy nhiên, chưa có nhiều nghiên cứu tổng hợp về các cơ chế ảnh hưởng của rừng ngập mặn đối với năng suất tôm. Mục tiêu của luận án là xác định một số tiến trình chủ yếu có liên quan đến vấn đề nêu trên. Kết quả nghiên cứu cho thấy vật rụng từ rừng ngập mặn cung cấp lượng lớn chất hữu cơ và dưỡng chất cho thuỷ vực, và các chất này có ảnh hưởng có lợi hay bất lợi như thế nào đối với sự sinh trưởng và phát triển của tôm sú *Penaeus monodon*.

Nghiên cứu được thực hiện trong vòng một năm để theo dõi về sự cung cấp vật rụng và dưỡng chất ở các cấp độ tuổi khác nhau của rừng ngập mặn (chương 2). Vật rụng có khoảng 70% là lá rụng, và chất hữu cơ chiếm khoảng 90% tính theo trọng lượng khô. Vật rụng giảm theo tuổi của cây rừng, và hàm lượng đạm và lân cao hơn đáng kể ở khu rừng có độ tuổi nhỏ (7 và 11 tuổi) so với khu rừng có độ tuổi lớn hơn (17 và 24 tuổi). Do đó, ở các khu rừng ngập mặn có độ tuổi nhỏ cung cấp vật rụng với số lượng và chất lượng cao nhất cho thuỷ vực .

Các yếu tố chính ảnh hưởng trên sự phân hủy của lá đước được trình bày trong chương 3. Kết quả cho thấy tốc độ phân hủy của lá đước càng cao khi nước ngâm lá có độ mặn càng thấp, và cao tối đa ở nồng độ muối là 5 phần ngàn. Tốc độ phân hủy cao nhất vào mùa mưa, vì mùa mưa nước có độ mặn tương đối thấp. Vào mùa mưa, độ mặn của nước trong ao nuôi tôm- rừng kết hợp ở Cà Mau biến động trong khoảng 4-9 phần ngàn, độ mặn này phù hợp với độ mặn tối hảo cho sự phân hủy của lá đước trong điều kiện phòng thí nghiệm. Ngoài ra tốc độ phân hủy của lá đước cũng bị ảnh hưởng bởi độ ẩm; tốc độ phân hủy cao hơn có ý nghĩa đối với lá đước được ngâm ủ gần đáy ao so với lá ngâm ủ gần rễ của cây đước, và tốc độ phân hủy của lá đước gần rễ cao hơn vào mùa mưa so với mùa nắng. Động thái của các chất dinh dưỡng trong lá đước phân hủy cũng được theo dõi, hàm lượng đạm và lân trong lá đước phân hủy gia tăng trong suốt giai đoạn phân hủy. Chính sự gia tăng này đã làm tăng chất lượng thức ăn cho sinh vật thuỷ sinh ở thời kỳ đầu của quá trình phân hủy.

Trong chương 4 và 5 tập trung thảo luận về ảnh hưởng của lá đước phân hủy trên tôm sú *Penaeus monodon*. Lượng lá phân hủy là vấn đề đáng quan tâm nhất, vì lượng lá nhiều gây ảnh hưởng bất lợi cho tôm do bởi sự phân huỷ của lá làm giảm nồng độ oxy hoà tan và gia tăng sulphide. Ở lượng lá trung bình giúp kích thích sự tăng trưởng của tôm sú, và lá đước phân hủy là nơi trú ẩn và cung cấp thức ăn giàu dưỡng chất cho tôm. Thực tế những sinh vật bám trên lá đước chính là nguồn thức ăn quan trọng cho tôm, bởi vì khi cho tôm ăn lá đước có sự hiện diện của sinh vật bám trên lá, tôm tăng trưởng tốt hơn khi cho ăn với lá đước không có sinh vật bám. Ảnh hưởng gây ít nhiều ngạc nhiên của lá đước phân hủy là ngăn cản sự dư thừa đạm trong quá trình nuôi tôm. Từ kết quả này, chúng tôi có đề nghị là trong quá trình nuôi tôm việc cung cấp lá đước với số lượng cân đối trong điều kiện cho phép, có thể làm giảm bớt ảnh hưởng bất lợi của thức ăn nhân tạo có hàm lượng đạm cao đối với chất lượng nước nuôi tôm. Tỉ lệ C/N cao của lá đước có khuynh hướng cân bằng hoá học trong hệ thống nuôi có sự thừa đạm do thức ăn nhân tạo gây ra.

Trong hai chương cuối, chúng tôi nghiên cứu mối quan hệ qua lại giữa tôm giống cùng loài, ví dụ như ảnh hưởng của mật độ nuôi, chất hoá học bầy đàn, và chất dẫn dụ nguy hiểm trên quần thể tôm (chương 6 và 7). Ảnh hưởng của mật độ nuôi trên sự sinh trưởng và tỉ lệ sống của tôm là quan trọng nhất. Chúng tôi không tìm thấy ảnh hưởng bất lợi về mặt cơ học và sự ăn nhau của tôm. Ảnh hưởng của chất lượng nước nuôi đối với tôm cũng được quan sát. Kết quả cho thấy ảnh hưởng của nhiệt độ, độ pH, độ mặn, oxy hoà tan, clorine, đạm dạng NO_2^-, NO_3^- là không đáng kể. Tuy nhiên, độc tính của đạm amôn có thể là yếu tố gây chết và giảm tăng trưởng cho tôm giống *Penaeus monodon.* Mặt khác, chúng tôi tìm thấy ngưỡng gây chết ở các mật độ 100, 70, 50, và 30 cá thể trong một lít nước nghiền tôm giống cùng loài, và tôm chết càng nhanh ở mật độ tôm càng cao. Rất thú vị, nồng độ nước nghiền thấp (1 con tôm trong 1 lít) giúp tăng trọng lượng và kích thước của tôm thí nghiệm.

Để áp dụng trong thực tế, chúng tôi đề xuất vài biện pháp đơn giản để cải thiện việc quản lý hệ thống nuôi tôm-rừng. Rõ ràng lá đước có thể làm tăng sinh trưởng và sự sống của tôm sú. Tuy nhiên, ở nồng độ lá đước cao sẽ gây tác hại đối với tôm do bởi sự phân huỷ lá làm giảm nhanh nồng độ oxy hoà tan và tăng sulphide. Một cách làm dễ áp dụng để cải thiện ảnh hưởng bất lợi này là tăng dòng chảy của nước trong ao nuôi. Điều này giúp giảm tác hại do thiếu oxy tại chổ, và tạo ra sự phân bố vật rụng khắp trong ao, do vậy tránh được sự tích tụ lá đước quá nhiều ở một nơi. Sử dụng lá đước đang phân hủy như là nguồn thức ăn bổ sung cùng với thức ăn nhân tạo (CP) nhằm để làm giảm tiềm năng gây độc khi thức ăn CP được sử dụng khá nhiều trong hệ thống nuôi bán tự nhiên.

SAMENVATTING

In de kustprovincies in de Mekong Delta is de brakwatercultuur van garnalen de belangrijkste economische activiteit. Het kweken van garnalen heeft de boeren en de lokale gemeenschappen in Vietnam de laatste jaren aanzienlijke inkomsten gebracht. De extensieve garnaal-mangrove kweeksystemen zijn in deze regio het populairste. En alhoewel de gemiddelde garnalenproductie laag is, als gevolg van overexploitatie en de vernietiging van mangrovebossen en kwelders, zijn deze kweeksystemen vooral van belang vanuit het oogpunt van duurzaamheid van de intensieve aquacultuur (Naylor et al., 200 Nature 405: 1017-1024). Verschillende studies hebben aangetoond dat mangrovemoerassen uitermate productieve ecosystemen zijn, die voorzien in voedsel, schuilplaatsen en broedkamers voor allerlei aquatische organismen, waaronder veel met een hoge economische waarde. De tijgergarnaal, *Penaeus monodon*, is hiervan een duidelijk voorbeeld. Men neemt aan dat de naturulijke garnalenproductie in deze regio in hoge mate afhangt van de aanwezigheid van mangroves. Echter, het complex van mechanismen, waarmee mangroves de productie van garnalen beïnvloeden, is nog steeds niet goed doorgrond. Het werk in dit proefschrift is een poging om sommige van de sleutelprocessen in deze te ontrafelen. Het bevestigt het beeld dat mangrovestrooisel een enorme aanvoer van organisch materiaal en voedingsstoffen naar het aquatisch systeem verzorgt, en laat zien hoe deze aanvoer zowel positieve als negatieve effecten kan hebben op de groei en overleving van post-larven van de garnaal.

Gedurende een jaar is de aanvoer van strooisel en voedingsstoffen in mangrovebossen van verschillende ouderdom bestudeerd (hoofdstuk 2). Strooisel bestond voor 70 % uit bladafval en het gehalte aan organische stoffen was 90 % van het drooggewicht. De hoeveelheid strooisel nam af met toenemende ouderdom van de mangroves en ook de stikstof- en fosforgehalten waren aanzienlijk hoger in het bladafval van de jongere mangroves (7 en 11 jaar oud) vergeleken met die van de oudere mangroves (tot 24 jaar). Bijgevolg is in zowel kwalitatieve als kwantitatieve zin de aanvoer van strooisel in de aquatische systemen het hoogste in jonge mangrovebossen.

Vervolgens zijn de sleutelfactoren geanalyseerd die de afbraak van mangrovebladeren bepalen (hoofdstuk 3). Afbraaksnelheden lijken het hoogste te zijn bij de lagere saliniteiten en bereiken een optimum bij 5 ‰. De afbraaksnelheden waren ook het hoogst gedurende de regenperiode en dit kan zeker het gevolg zijn van de relatief lage saliniteiten gedurende deze periode. De saliniteit in de Camau lag in de regenperiode in de range van 4-9 ‰, dicht bij het optimum voor de decompositie, zoals afgeleid uit laboratoriumproeven. Deze studie toonde tevens een rechtstreeks effect aan van de humiditeit. De afbraaksnelheid was hoger voor bladeren die in de sloten waren geïncubeerd, dan wanneer ze bij de luchtwortels van de mangroves, in de open lucht, waren geïncubeerd, waar overigens de afbraaksnelheden hoger waren in de regenperiode dan in de droge periode. Ook is de dynamiek van concentraties aan voedingsstoffen in rottende bladeren gevolgd. Stikstof- en fosforgehalten in rottende bladeren namen toe

gedurende de afbraakperiode. Deze verrijking geeft aan dat er een toename in voedselkwaliteit optrad gedurende de eerste periode van afbraak.

Het effect van rottende mangrovebladeren op garnalen kan zowel positief als negatief zijn (hoofdstuk 4 en 5). De hoeveelheid rottende bladeren bleek bepalend te zijn. Bij hoge concentraties van bladeren overheersten de negatieve effecten. Deze effecten waren waarschijnlijk het gevolg van het vrijkomen van nitriet en sulfiden, alsook een verlaging van de hoeveelheid opgelost zuurstof. Daartegenover staat dat niet al te hoge concentraties van bladeren de groei van *Penaeus monodon* postlarven stimuleerden en zij dienden blijkbaar als schuilplaats en als voedsel. Het feit dat de micro-organismen, die op de bladeren groeiden en niet de bladeren zelf, als voedsel belangrijk zijn werd geïllustreerd doordat garnalen die op mangrovebladeren foerageerden beter groeiden wanneeer er een laag perifyton op deze bladeren was (hoofdstuk 5). Een ietwat verrassend positief effect van bladeren was het klaarblijkelijke voorkòmen van excessieve concentraties ammonium en nitriet. De resultaten suggereerden dat het toevoegen van geconditioneerde mangrovebladeren de negatieve effecten van CP-pellets met hoge gehalten aan eiwitten op de waterkwaliteit dempten. De hoge C/N-ratio van bladeren lijkt de stochiometrie van het systeem in balans te brengen, welke anders gedomineerd zou worden door de excessieve aanvoer van stikstof middels CP-pellets.

In het laatste hoofdstuk wordt ingegaan op de interactie tussen de garnalenlarven zelf, zoals de effecten van populatiedichtheden en het afgeven van chemische signalen (crowding) en mogelijke alarmferomonen op de garnalenpopulaties (hoofdstuk 6, 7). Een sterk effect van crowding op de groei en overleving van garnalen werd aangetoond. Fysische interferentie stress en canibalisme konden als oorzakelijke factoren uitgeschakeld worden. Het was dus duidelijk dat de effecten veroorzaakt werden door waterkwaliteitsvariabelen. Temperatuur, pH, saliniteit, opgelost zuurstof, chloride, nitriet en nitraat bleken van weinig invloed te zijn. Echter, ammonia toxiciteit kon niet uitgesloten worden als oorzaak van de waargenomen mortaliteit en gereduceerde groei van *P. monodon* post-larven. Aan de andere kant hadden alarmsignalen, vrijgegeven door hoge concentraties vermalen soortgenoten (100, 70, 50 en 30 vermalen garnalen.l^{-1}) negatieve effecten op de overleving van post-larven. Verrassend genoeg bleken lage concentraties vermalen soortgenoten (1 vermalen garnaal.l^{-1}) juist stimulerende effecten te hebben op lichaamsgrootte en drooggewicht.

In een toegepast perspectief geplaatst, geeft deze studie eenvoudige manieren om het beheer van mangrove-garnaal systemen te verbeteren. Het is duidelijk dat mangrovebladeren de overleving en groei van post-larven van garnalen bevorderen. Echter, bij hoge concentraties aan bladeren gaan negatieve effecten overheersen, samenhangend met een daling van het zuurstofgehalte en het vrijkomen van sulfiden. Een simpele manier om dergelijke negatieve effecten te verminderen kan het verhogen van de doorstroming zijn. Dit zal het risico op plaatselijke anoxia verminderen en kan helpen het strooisel over het gebied te verspreiden, daarmee de opeenhoping van deze bladeren op sommige plaatsen vermijdend. De reductie van de

potentieel giftige hoeveelheden nitriet en ammonium door rottende bladeren suggereert dat mangrovebladeren als een nuttige aanvulling van de CP-pellets in half-natuurlijke productiesystemen kunnen dienen.

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