

Presence of the Two Viral Pathogens WSSV and MBV in Three Wild Shrimp Species (*Penaeus indicus*, *Metapenaeus ensis* and *Metapenaeus lysianassa*) Cultured in the Mangrove Forest of Ca Mau Province

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Abstract

The aim of this research was to determine the presence of the two viral pathogens White Spot Syndrome Virus (WSSV) and Monodon Baculovirus (MBV) in three wild shrimp species cultured in the mangrove forest of Ca Mau province by using Polymerase Chain Reaction (PCR) and histological techniques. The study also sought to determine the season during which mass mortality occurs more frequently, the correlation between the presence of WSSV and MBV and some main water quality parameters of the mangrove in both mixed and separate models.

The results of this research show that WSSV was simultaneously present in wild shrimp seeds and *Acetes spp.* during January, April, and May while MBV was present all year round. Pathogen was detected more often in the separate model.

WSSV was present in shrimp at harvest in most of the samples, especially in the ones collected during the rainy season and in the middle of the dry season. MBV at harvest appeared throughout the whole year with a higher prevalence during the rainy season.

Mass mortality occurred four times during the sampling period (i.e. January, June, July and October). This phenomenon was associated with the presence of WSSV at high prevalence and intensity.

Some recommendations to reduce the risk factors of mass mortality are also presented in this study.

Introduction

Less than two decades ago, shrimp farming in Mekong Delta showed a rapid increase, when the surface area for shrimp farming increased from 10,000 ha in the early 1980s to 190,000 ha in the early 1990s (Quynh, 1994). Major concerns of such rapid and unregulated expansion of shrimp farming in sensitive coastal environments of Vietnam are the repetition of the widespread loss of mangrove habitat, environmental pollution and consequent decrease in shrimp production (Clough and Johnston, 1998). During the biennium 1994-1995, large-scale mortality of cultured shrimp affected the whole coastal area of the Mekong Delta causing an economic loss of approximately 30 million USD (Tam, 1994). Histopathological examination of moribund shrimp collected during such outbreaks revealed the presence of Monodon Baculovirus (MBV), White Spot Syndrome Virus (WSSV), and other viruses (Hao et al., 1997). The choice of the viral pathogens investigated was due to their high virulence and prevalence in Asian countries. In fact, MBV has been reported to be widely distributed among cultured shrimp populations in India (Panchayuthapani, 1997). MBV infected shrimps were always reported to be infected also with pathogenic *Vibrio spp.*, filamentous bacteria and protozoans (Felix and Devaraj, 1993).

White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) are currently the most serious diseases threatening the shrimp farming industry in Thailand (Boonyaratpalin et al., 1993; Wongteerasupaya et al., 1996). Water and mechanical or biological carriers appear to be the major routes for pond infection. Since infectivity of free WSSV in seawater is quite short (i.e. 3-4 days) the most serious threat seems to come from latent carriers from which the virus can be spread to *P. monodon* by ingestion or cohabitation (Flegel et al., 1997). Moreover, some authors reported that the presence of WSSV does not always lead to disaster (Flegel et al., 1997).

Severe land use conflicts occur in the large intertidal area and almost the entire surface area for shrimp farming is located where the former mangrove forest used to be. The overall goal of the present project is to optimise in a sustainable manner the economic yield from mixed shrimp aquaculture-mangrove forestry farming systems in Ca Mau province (Clough and Johnston, 1998).

This cooperative research project was established and approved by ACIAR with IMS (Australian) and RIA2 (Vietnamese) as the two implementing institutions.

The shrimp disease component is one subproject of Project PN 9412: Mixed Shrimp Farming-Mangrove Forestry Models in the Mekong Delta. The duration of this subproject is one year (May 1997 - May 1998). The main target of this research is the detection by PCR and histological techniques of the year round presence of the two viral pathogens WSSV and MBV in the three main cultured species: *P. indicus*, *Metapenaeus lysianassa* and *M. ensis*.

Materials and Methods

Shrimp sampling

Two different farming systems were placed under study:

- Mixed mangrove forestry - shrimp farming model.
- Separate mangrove forestry- shrimp farming model.

The mixed model consisted of an area where mangrove forestry and shrimp farming activities cohabited. On the contrary, two areas where mangrove forestry (70-80% of total area) and shrimp farming activities (20-30% of total area) were independently carried out constituted the separate model. Pond in both systems consist of a series of long (250-800 m) and wide (3-4 m) canals dug either through mixed or separate (adjacent to the forest).

Shrimp samples were collected from three farms: two farms (one from the mixed model with a pond area of 2.4 ha and a total farm area of 13 ha and one from the separate model with a pond area of 1.1 ha and a total farm size of 10 ha) are located in Tam Giang III Enterprise (latitude 8.8 N, longitude 105 E), and one farm belonging to the mixed model with a pond area of 1.8 ha and a total farm area of 11 ha is situated in 184 Enterprise (latitude 8.5 N longitude 105 E). Both Tam Giang III and 184 are enterprises consisting of extensive farms utilizing two different types of farming systems and located within Ca Mau province of the Mekong Delta of southern Vietnam. Shrimps that grow in ponds are naturally recruited from sluice gate.

Wild stocks of shrimp post-larvae while entering the ponds and harvested shrimps were collected fortnightly by placing a net in front of the main sluice gate. The animals sampled included major shrimp species like *Penaeus indicus*, *Metapenaeus ensis* and *M. lysianassa*. One hundred wild seeds were collected and the whole animals were preserved. From each of the three harvested species, twenty head portions were sampled. About a hundred specimens of *Acetes spp.* were sampled. All samples were fixed in alcohol (95°) for further PCR analysis.

For histopathological observation, 10-20 wild seeds were collected and preserved. From each of the three species harvested, 5-10 animals were dissected and head portions were fixed. Davidson solution was used as fixative for all histology samples.

Shrimp samples were collected also from the surrounding area when mass mortality occurred.

Detection of WSSV and MBV presence

PCR TEST FOR WSSV

PCR was used to determine the presence of WSSV. The 100 wild seeds collected were pooled together before being analysed. A similar procedure was applied to *Acetes spp.* samples. Gills from the head portions collected during each monitoring cycle and mass mortality occurrence were pooled together. A total of about 400 samples were analysed by PCR.

DNA was extracted using the alkaline/SDS boiling method and analysed by PCR following the protocol routinely used in Aquatic Animal Health Research Institute (AAHRI)–Bangkok, Thailand (Charatchakool pers. comm.). Positive samples were divided in three categories depending on the intensity of the bands obtained by electrophoresis. All PCR analyses were conducted at the Department of Biology, College of Natural Science, Ho Chi Minh State University. The results were randomly checked by PCR in AAHRI (Thailand).

HISTOPATHOLOGY OBSERVATION FOR WSSV AND MBV

Histopathology was used to complement the PCR analysis for WSSV and to detect the presence of MBV.

About 550 samples of post-larvae harvested and moribund shrimps were analyzed at the Division of Experimental Biology, Research Institute for Aquaculture No. 2 (Ho Chi Minh City – Vietnam)

Water quality monitoring

Water monitoring lasted from May 1997 to May 1998. During this period, 25 recordings were taken approximately every 15 days simultaneously with shrimp sampling. The 18th sampling cycle was dropped due to the Lunar New Year Holiday.

During each monitoring cycle, water quality data was collected twice a day, i.e. at 6 A.M. and 1 P.M. both in the pond and supply canal. The parameters recorded were pH, secchi depth, salinity, and dissolved oxygen (DO).

A daily record of some parameters (twice a day) was carried out through the cooperation of the owner of the farm (one farm in the separate model, one farm in the mixed model) and the survey team and by supplying the farmer with pH meter, thermometer and secchi disk.

Results and Discussions

Variations of some main water quality parameters

SEPARATE MODEL

Looking at the year round fluctuation of the parameters recorded, it can be concluded that, as expected, both water temperature and salinity gradually decreased in the rainy season and increased during the dry season (Figs. 1 and 2).

Such parameters reached a minimum level of 25°C (February 1998) and 10 ppt (November 1997). Maximum values of temperature and salinity (i.e. 36°C and 36ppt) respectively, were reached in May 1998. Shrimp ponds in Ca Mau are generally shallow, ranging from 69.6 cm to only 28.6 cm, with an overall mean pond depth of 50.5 cm (Clough and Johnston, 1998). This fact

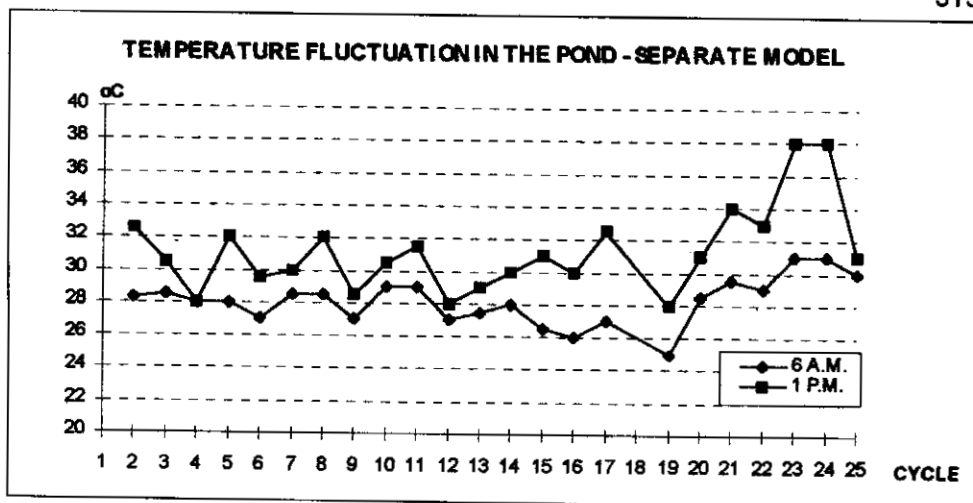


Fig. 1. Temperature fluctuation recorded in the pond in the separate model (mangrove forest and shrimp farming activities were independent).

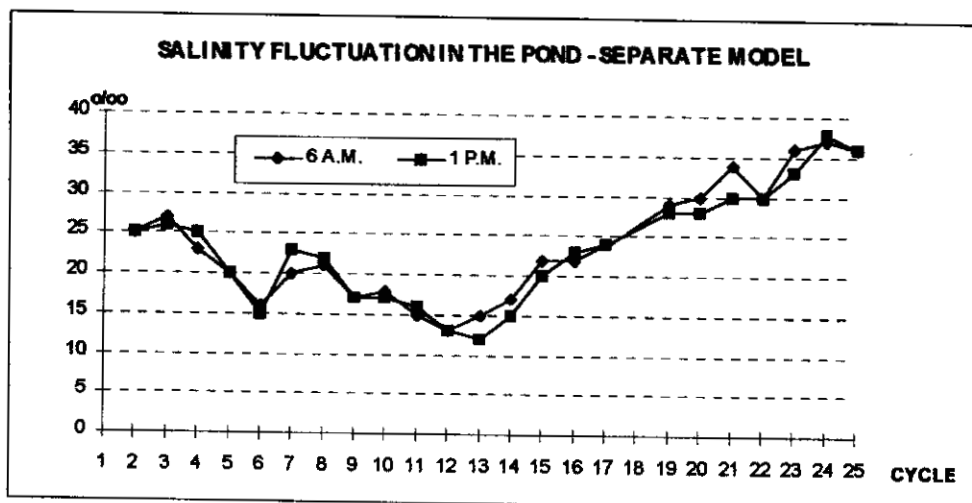


Fig. 2. Salinity fluctuation recorded in the pond in the separate model.

explains the large differences between monitoring cycles and high daily fluctuation of other parameters such as temperature, DO and secchi depth.

These fluctuations occurred both in shrimp ponds and in the water supply canal. However, some differences between the two bodies of water were detected: for example, unlike in the supply canal, salinity in the ponds appeared to be relatively stable.

Secchi depth varied considerably due to the presence of high concentrations of suspended solids. High total suspended solids were recorded also by other authors in both ponds and supply canals and averaged 0.3 g/l and 0.35g/l in ponds and canals respectively (Clough and Johnston, 1998).

In general, DO was relatively low, dropping down in some occasions to 2 ppm (Fig. 3). Oxygen plummets were recorded especially at low tide in the river, probably due to poor tidal flushing. On the contrary, during high tide

DO reached the highest values due to the inflow of fresh seawater (Clough and Johnston, 1998).

As for pH, daily fluctuations were limited (less than 0.5). Of particular interest is the minimum level of pH which is never lower than 7.

Results of our study show that the water monitored in Ca Mau province in the separate model seems to be appropriate for extensive shrimp farming. Clough and Johnston (1998) emphasised the fact that shrimp farms in the province are characterised by acid sulfate soils, which are generally unsuitable for shrimp farming. However, this negative impact usually involves to a greater extent newly built ponds.

MIXED MODEL

Salinity and water temperature in the mixed model varied in a way similar to the separate model. Moreover, it must be noticed that, not only heavy rainfall does cause salinity to drop below the optimal range during the wet season, but freshwater-saltwater layering of the water column also occurs especially in shallow ponds and can be shown by the pond depth-salinity profile reported by Clough and Johnston (1998).

Other parameters such as pH, DO, and secchi depth had higher fluctuations than in the separate model (Figs. 4 and 5). The daily pH fluctuation sometimes reached 0.7. The pH fluctuated markedly over short time periods increasing in one occasion from 5.0 to 5.8 within 1 hr (Clough and Johnston, 1998). Of interest is the fact that pH in both ponds and supply canals was usually higher in the morning than in the afternoon.

The lowest level of DO recorded was about 2ppm. However, other authors studying the same system, recorded in some occasions extremely low DO values, i.e. 0.2-0.65 mg/l (Clough and Johnston, 1998). As for the Secchi depth, it sometimes dropped to 0 cm.

The results from the research of Clough and Johnston (1998) show that there is a high variability in water quality parameters among different positions within the same pond. This fact indicates that water in the pond is poorly mixed and can be attributed to the large pond size and poor design.

Based on the data collected it is evident that water quality is characterised by high fluctuations. The main reasons for such variability are low pond depth, high water leakage from the dike and small surface area. In fact, the authors suggest widening and dredging of the ponds. Pond leakage should also be minimised in order to stabilize and improve the water quality.

Presence of the two viral pathogens WSSV and MBV in the three main shrimp species cultured in the mangrove forest of Ca Mau Province and in *Acetes* spp.

Wild postlarvae have declined considerably in recent years. There are a number of possible reasons that can explain such a decline. These include the loss of postlarvae nursery grounds through mangrove forest destruction, the

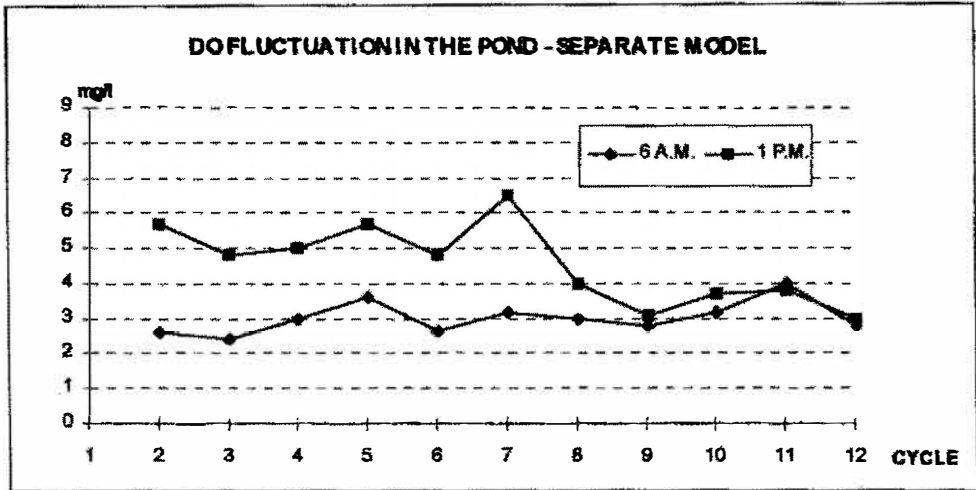


Fig. 3. Dissolved oxygen fluctuation recorded in the pond in the separate model.

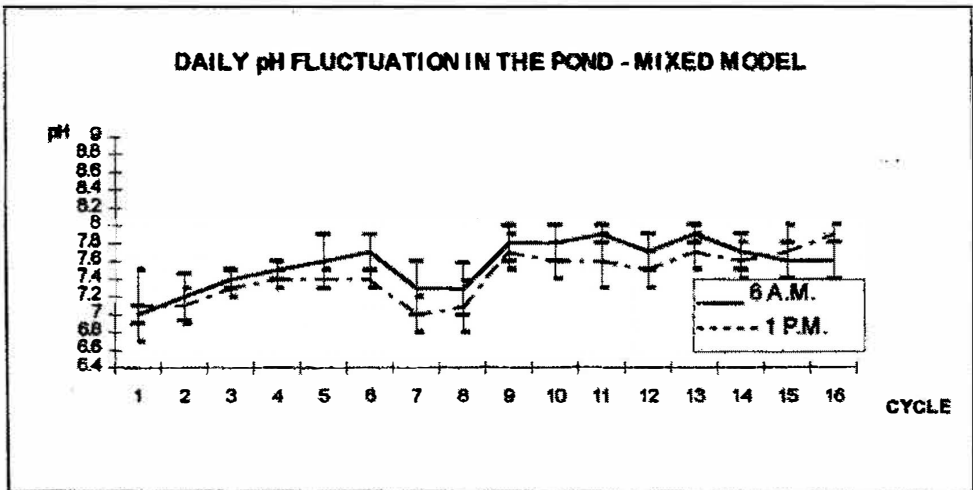


Fig. 4. pH fluctuation recorded in the pond in the mixed model.

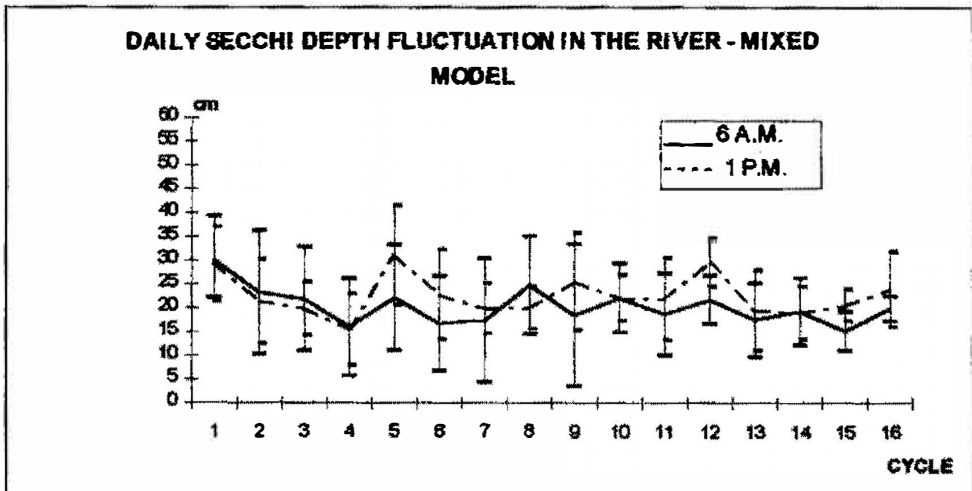


Fig. 5. Secchi depth fluctuation recorded in the pond in the mixed model.

outbreak of fatal disease, the increasing fishing pressure which reduced adult wild stock abundance, and alarmingly high seed losses during harvests. In general, postlarvae recruitment densities in the systems under study were extremely low (Clough and Johnston, 1998).

Looking at the average size of wild shrimp seeds collected (i.e. 13.72 mm), it can be concluded that the majority of wild shrimp seeds recruited into the ponds were juveniles (Clough and Johnston, 1998). In fact, at the time the shrimps entered the pond, their age was approximately 1-2 months. This was due to the fact that, during development the postlarvae must first migrate into the estuaries from offshore spawning grounds before they could be recruited into the ponds (Binh et al., 1997; Clough and Johnston, 1998).

PRESENCE OF WSSV IN THE WILD SHRIMP SEEDS

The results of PCR analysis for WSSV conducted on wild shrimp seeds (Table 1) do not show big differences in the frequency of detection of WSSV in the different culture systems.

WSSV occurrence could be divided into two periods. The first period is at the beginning of the dry season when the water temperature dropped dramatically to the minimum levels of the year (December - January). The second one coincided with the passage from dry to rainy season.

Looking at the results from the mixed model, the presence of WSSV in this system appears to be more frequent. However, the frequency of detection remains at quite low levels.

PRESENCE OF MBV IN WILD SHRIMP SEEDS

The presence of MBV was detected all year round. The results of histopathological analysis show that in the separate model, MBV occurred relatively more frequently (7/12 months) with higher prevalence when compared with the mixed model. In fact in the latter, MBV presence was recorded only for four months and had a prevalence lower than 40% (Table 2).

PRESENCE OF WSSV IN *ACETES SPP.*

WSSV in the *Acetes spp.* was detected in the same period of its occurrence in the wild shrimp seeds (Table 3).

The first period when the virus was found was at the beginning of the dry season (January - February) and was observed only in the separate model. The second period of occurrence was detected at the passage from dry to rainy season (i.e. April - July). WSSV occurrence in this period was observed in both separate and mixed models.

Thus, PCR analysis showed that WSSV occurred in *Acetes spp.* and wild shrimp seeds during the same two periods when postlarvae densities were highest. As for MBV, it was present in the wild seed all year round. These evidences do support the theory of viral contamination during the long migration period and confirmed the function as viral pathogens crustacean carriers

Table 1. Presence of WSSV in wild shrimp seeds.

Month	Model	Prevalence (%)	Intensity of infection (%)*			Method of detection
			+	++	+++	
06/97	Separate	38.5	100			PCR + Histology
	Mixed	19	50	50		PCR + Histology
07/97	Separate	50	100			PCR
	Mixed	100	50		50	PCR
08/97	Separate	0				PCR + Histology
	Mixed	0				PCR + Histology
09/97	Separate	0				PCR + Histology
	Mixed	25	100			PCR
10/97	Separate	0				PCR + Histology
	Mixed	17	50		50	PCR + Histology
11/97	Separate	0				PCR + Histology
	Mixed	8	100			Histology
12/97	Separate	59	88	12		PCR + Histology
	Mixed	43	50	50		PCR + Histology
01/98	Separate	33	100			Histology
	Mixed	8	100			Histology
02/98	Separate	0				PCR + Histology
	Mixed	17		100		Histology
03/98	Separate	0				PCR + Histology
	Mixed	0				Histology
04/98	Separate	50	100			PCR
	Mixed	25		100		PCR
05/98	Separate	0				PCR + Histology
	Mixed	17	50	50		Histology

*%: Percentage of samples falling in the different categories.

Table 2. Presence of MBV in wild shrimp seeds.

Month	Model	Prevalence (%)	Intensity of infection (%)*			Method of detection
			+	++	+++	
06/97	Separate	17	100			Histology
	Mixed	9		100		Histology
07/97	Separate	100	25	50	25	Histology
	Mixed	0				Histology
08/97	Separate	0				Histology
	Mixed	0				Histology
09/97	Separate	30	25	25	50	Histology
	Mixed	40	25	50	25	Histology
10/97	Separate	33		100		Histology
	Mixed	42	80	20		Histology
11/97	Separate	0				Histology
	Mixed	0				Histology
12/97	Separate	50	67	33		Histology
	Mixed	0				Histology
01/98	Separate	0				Histology
	Mixed	37	25	25	50	Histology
02/98	Separate	67		100		Histology
	Mixed	0				Histology
03/98	Separate	17		100		Histology
	Mixed	0				Histology
04/98	Separate	0				Histology
	Mixed	0				Histology
05/98	Separate	0				Histology
	Mixed					Histology

*%: Percentage of samples falling in the different categories.

Table 3. Presence of WSSV in *Acetes spp.*

Month	Model	Prevalence (%)	Intensity of infection (%)*			Method of detection
			+	++	+++	
06/97	Separate	50	100			PCR
	Mixed	50	100			PCR
07/97	Separate	0				PCR
	Mixed	50	100			PCR
08/97	Separate	0				PCR
	Mixed	0				PCR
09/97	Separate	0				PCR
	Mixed	0				PCR
10/97	Separate	0				PCR
	Mixed	0				PCR
11/97	Separate	0				PCR
	Mixed	0				PCR
12/97	Separate	0				PCR
	Mixed	0				PCR
01/98	Separate	50	100			PCR
	Mixed	0				PCR
02/98	Separate	0				PCR
	Mixed	0				PCR
03/98	Separate	0				PCR
	Mixed	0				PCR
04/98	Separate	50	100			PCR
	Mixed	50		100		PCR
05/98	Separate	50		100		PCR
	Mixed	25		100		PCR

*%: Percentage of samples falling in the different categories.

of the three wild shrimp seeds and *Acetes spp.* already suggested by some authors (Flegel et al., 1997).

PRESENCE OF WSSV IN HARVESTED SHRIMP

Annual shrimp yields in the ponds under study were highly variable, ranging from 12 to 1166 kg/ha/year. Average annual yield was 286.04 kg/ha/year (Clough and Johnston, 1998). This value is consistent with the mean shrimp yield of 265.3 kg/ha/year reported by other authors carrying out research in the same systems (Binh et al., 1997).

WSSV in shrimp at harvest was present in several occasions throughout the year, i.e. during 8-9 months over the 12 months period of sampling. The frequency of detection and WSSV prevalence were, however, relatively low. In general, WSSV seemed to make its first appearance during the rainy season (pH, DO fluctuate highly), at the beginning of the dry season (water temperature dramatically drops), and in the middle of the dry season (salinity sharply increases).

No differences in WSSV presence were detected between the two models under study when compared in terms of occurrence period and prevalence in the different harvested shrimp species (Tables 4 and 5).

Table 4. Presence of WSSV in harvested shrimp (separate model).

Month	Model	Prevalence (%)	Intensity of infection (%)*			Method of detection
			+	++	+++	
06/97	<i>P. indicus</i>	75	50	50		PCR + Histology
	<i>M. lysianassa</i>	100	50	50		Histology
	<i>M. ensis</i>	100	50	50		Histology
07/97	<i>P. indicus</i>	50	100			PCR + Histology
	<i>M. lysianassa</i>	50	50		50	PCR + Histology
	<i>M. ensis</i>	50	100			PCR
08/97	<i>P. indicus</i>	50	100			Histology
	<i>M. lysianassa</i>	50			100	PCR
	<i>M. ensis</i>	50	100			Histology
09/97	<i>P. indicus</i>	0				PCR + Histology
	<i>M. lysianassa</i>	50	50	50		PCR + Histology
	<i>M. ensis</i>	50	100			PCR + Histology
10/97	<i>P. indicus</i>	50			100	PCR + Histology
	<i>M. lysianassa</i>	0				PCR + Histology
	<i>M. ensis</i>	50	50		50	PCR + Histology
11/97	<i>P. indicus</i>	50			100	PCR + Histology
	<i>M. lysianassa</i>	75		75	25	PCR + Histology
	<i>M. ensis</i>	50			100	PCR
12/97	<i>P. indicus</i>	50	50		50	PCR + Histology
	<i>M. lysianassa</i>	50	50		50	PCR + Histology
	<i>M. ensis</i>	50	100			Histology
01/98	<i>P. indicus</i>	50	100			Histology
	<i>M. lysianassa</i>	0				PCR + Histology
	<i>M. ensis</i>	0				PCR + Histology
02/98	<i>P. indicus</i>	0				PCR + Histology
	<i>M. lysianassa</i>	0				PCR + Histology
	<i>M. ensis</i>	50	100			Histology
03/98	<i>P. indicus</i>	50	100			Histology
	<i>M. lysianassa</i>	100	50	50		Histology
	<i>M. ensis</i>	100	50	50		Histology
04/98	<i>P. indicus</i>	50			100	Histology
	<i>M. lysianassa</i>	0				PCR + Histology
	<i>M. ensis</i>	50		100		Histology
05/98	<i>P. indicus</i>	0				PCR + Histology
	<i>M. lysianassa</i>	50	100			Histology
	<i>M. ensis</i>	0				PCR + Histology

*%: Percentage of samples falling in the different categories.

Comparing the period of occurrence of WSSV in *Acetes spp.*, wild shrimp seeds and the three species of harvested shrimp, it seems evident that the presence of WSSV in the harvested shrimp is definitely more frequent throughout the year. This phenomenon supports the hypothesis that contamination of the shrimp already present in the pond is carried out by infected wild seeds and *Acetes spp.* during recruitment.

PRESENCE OF MBV IN HARVESTED SHRIMP

From the data collected it appears clear that MBV was present all year round, showing highest prevalence especially in the rainy season and during some months at the beginning of the dry season. Of interest is the fact that, even if the prevalence of MBV in the wild seeds recruited into the pond of the

Table 5. Presence of WSSV in harvested shrimp (mixed model).

Month	Model	Prevalence (%)	Intensity of infection (%)*			Method of detection
			+	++	+++	
06/97	<i>P. indicus</i>	75	50	50		PCR + Histology
	<i>M. lysianassa</i>	75	37	50	13	PCR + Histology
	<i>M. ensis</i>	63	50	50		PCR + Histology
07/97	<i>P. indicus</i>	46	75		25	PCR + Histology
	<i>M. lysianassa</i>	46	100			PCR + Histology
	<i>M. ensis</i>	75	33	67		PCR
08/97	<i>P. indicus</i>	38	22	25	50	PCR + Histology
	<i>M. lysianassa</i>	0				PCR + Histology
	<i>M. ensis</i>	0				PCR + Histology
09/97	<i>P. indicus</i>	38	100			PCR + Histology
	<i>M. lysianassa</i>	25	100			Histology
	<i>M. ensis</i>	50	50	50		PCR + Histology
10/97	<i>P. indicus</i>	50	50		50	PCR
	<i>M. lysianassa</i>	50	50		50	PCR
	<i>M. ensis</i>	25			100	PCR
11/97	<i>P. indicus</i>	67			100	PCR
	<i>M. lysianassa</i>	29	50	50		PCR + Histology
	<i>M. ensis</i>	33	100			PCR
12/97	<i>P. indicus</i>	29	50	50		PCR + Histology
	<i>M. lysianassa</i>	71	75	25		PCR + Histology
	<i>M. ensis</i>	42	25		75	PCR + Histology
01/98	<i>P. indicus</i>	29		100		PCR + Histology
	<i>M. lysianassa</i>	38		50	50	PCR + Histology
	<i>M. ensis</i>	63		75	25	PCR + Histology
02/98	<i>P. indicus</i>	50	100			Histology
	<i>M. lysianassa</i>	0				PCR + Histology
	<i>M. ensis</i>	0				PCR + Histology
03/98	<i>P. indicus</i>	0				PCR + Histology
	<i>M. lysianassa</i>	25	100			PCR + Histology
	<i>M. ensis</i>	0				Histology
04/98	<i>P. indicus</i>	25		100		PCR
	<i>M. lysianassa</i>	25	100			Histology
	<i>M. ensis</i>	50	100			Histology
05/98	<i>P. indicus</i>	0				PCR + Histology
	<i>M. lysianassa</i>	25	100			Histology
	<i>M. ensis</i>	0				PCR + Histology

*%: Percentage of samples falling in the different categories.

separate model is higher than in the mixed model, the presence of MBV in the harvested shrimp showed no significant difference between the two models (Tables 6 and 7).

Prior to 1993, *P. indicus* was apparently the dominant species cultured and fished in Ca Mau province. This situation has drastically changed over the past few years with this species representing only 6.7%-9.7% of the total. At present *M. ensis* and *M. lysianassa* are the dominant species, representing 48-50% and 31-33% of the total species harvested respectively (Clough and Johnston, 1998). In spite of this change in the harvest population characteristics, there are no differences in their infectious percentage and intensity of infection.

Table 6. Presence of MBV in harvested shrimps (separate model).

Month	Model	Prevalence (%)	Intensity of infection (%)*			Method of detection
			+	++	+++	
06/97	<i>P. indicus</i>	100	100			Histology
	<i>M. lysianassa</i>	100		100		Histology
	<i>M. ensis</i>	100		100		Histology
07/97	<i>P. indicus</i>	50		100		Histology
	<i>M. lysianassa</i>	100		100		Histology
	<i>M. ensis</i>	0				Histology
08/97	<i>P. indicus</i>	50		100		Histology
	<i>M. lysianassa</i>	100	50		50	Histology
	<i>M. ensis</i>	100		100		Histology
09/97	<i>P. indicus</i>	100	50	50		Histology
	<i>M. lysianassa</i>	50	100			Histology
	<i>M. ensis</i>	50		100		Histology
10/97	<i>P. indicus</i>	50	100			Histology
	<i>M. lysianassa</i>	50	100			Histology
	<i>M. ensis</i>	100	100			Histology
11/97	<i>P. indicus</i>	100	50	50		Histology
	<i>M. lysianassa</i>	100	100			Histology
	<i>M. ensis</i>	50	100			Histology
12/97	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	50	100			Histology
	<i>M. ensis</i>	0				Histology
01/98	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	50		100		Histology
	<i>M. ensis</i>	50		100		Histology
02/98	<i>P. indicus</i>	100		100		Histology
	<i>M. lysianassa</i>	0				Histology
	<i>M. ensis</i>	0				Histology
03/98	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	0				Histology
	<i>M. ensis</i>	0				Histology
04/98	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	0				Histology
	<i>M. ensis</i>	0				Histology
05/98	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	50	100			Histology
	<i>M. ensis</i>	0				Histology

*%: Percentage of samples falling in the different categories.

PRESENCE OF THE TWO VIRAL PATHOGENS DURING MASS MORTALITY

The relation among seed recruitment, harvest seasons and shrimp mass mortality in the two different models under study is reported in Figure 6.

The Tong season starts in March and ends in July with major harvest occurring in June-July. The Mua season lasts from September until February with major harvests from October to December (Binh and Lin, 1995). On the contrary, Clough and Johnston (1998) affirmed that in the shrimp yields in Ca Mau there appear to be two peaks, i.e. from July to October and March to May. As it has already been explained, wild shrimp seeds carry WSSV during the same period of highest seed recruitment of the Tong season and at the end of the seed recruitment peak of the Mua season.

Table 7. Presence of MBV in harvested shrimp (mixed model).

Month	Model	Prevalence (%)	Intensity of infection (%)*			Method of detection
			+	++	+++	
06/97	<i>P. indicus</i>	25	100			Histology
	<i>M. lysianassa</i>	100	33	67		Histology
	<i>M. ensis</i>	75	67	33		Histology
07/97	<i>P. indicus</i>	50	100			Histology
	<i>M. lysianassa</i>	25		100		Histology
	<i>M. ensis</i>	50	100			Histology
08/97	<i>P. indicus</i>	75	67	33		Histology
	<i>M. lysianassa</i>	75	67	33		Histology
	<i>M. ensis</i>	25		100		Histology
09/97	<i>P. indicus</i>	75	67	33		Histology
	<i>M. lysianassa</i>	67	50		50	Histology
	<i>M. ensis</i>	75	67	33		Histology
10/97	<i>P. indicus</i>	75	67	33		Histology
	<i>M. lysianassa</i>	100	50	50		Histology
	<i>M. ensis</i>	75	67		33	Histology
11/97	<i>P. indicus</i>	67	50	50		Histology
	<i>M. lysianassa</i>	67	50	50		Histology
	<i>M. ensis</i>	67	50	50		Histology
12/97	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	33		100		Histology
	<i>M. ensis</i>	0				Histology
01/98	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	50		100		Histology
	<i>M. ensis</i>	0				Histology
02/98	<i>P. indicus</i>	0				Histology
	<i>M. lysianassa</i>	0				Histology
	<i>M. ensis</i>	0				Histology
03/98	<i>P. indicus</i>	33		100		Histology
	<i>M. lysianassa</i>	0				Histology
	<i>M. ensis</i>	25	100			Histology
04/98	<i>P. indicus</i>	25		100		Histology
	<i>M. lysianassa</i>	25		100		Histology
	<i>M. ensis</i>	25	100			Histology
05/98	<i>P. indicus</i>	75	67	33		Histology
	<i>M. lysianassa</i>	0				Histology
	<i>M. ensis</i>	50	100			Histology

*%: Percentage of samples falling in the different categories.

Mass mortality of cultured shrimps in the mangrove forest was observed to occur simultaneously with the presence of WSSV in wild seeds and to the two peaks of harvest time of the Tong and Mua seasons (Binh and Lin, 1995). Nonetheless, mass mortality also coincided with the two harvest peaks reported by Clough and Johnston (1998), which was at the passage from the Tong to the Mua seasons.

WSSV was always detected in moribund shrimp in ponds where mass mortality occurred, without showing any correlation with the presence of clinical signs (Table 8). There is a clear relationship between the mass mortality and some main environmental parameters. In June (at the beginning of the rainy season) crop failure seems to be associated with the high fluctuation of some main water quality parameters such as pH and DO. Salinity shows a clear stratification after heavy rains (Clough and Johnston, 1998) even if the

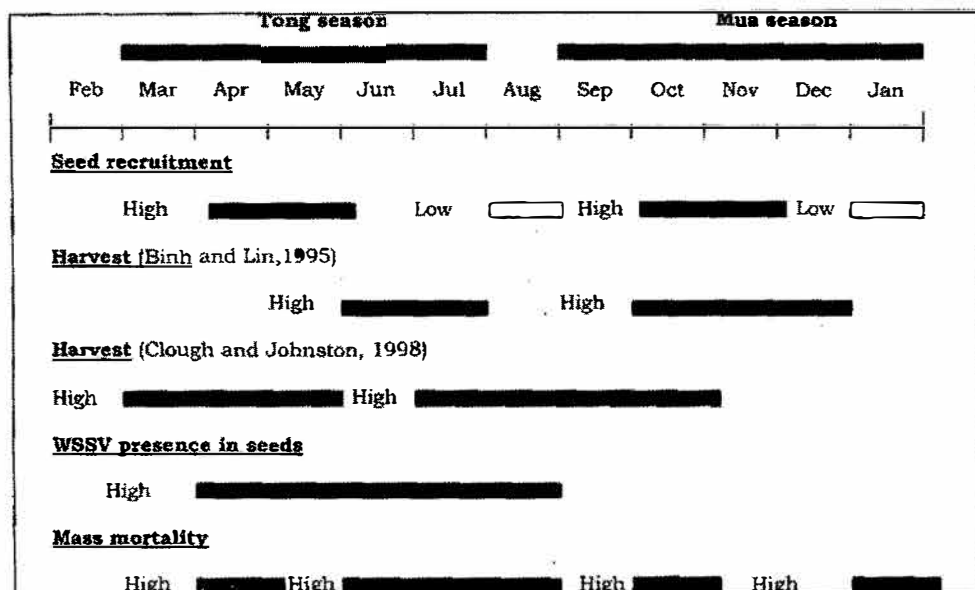


Fig. 6. Relation among seed recruitment, harvest seasons and shrimp mass mortality in the two different models of Ca Mau province (Seed recruitment and harvest conducted all year round for both mixed and separate models. High: acceptable yield of recruited seed or harvested shrimp. Low: unacceptable yield).

Table 8. Presence of WSSV in harvested shrimp during mass mortality.

Cycle	Frequency of detection	Intensity of infection	Species	Model
2 (06/97)	6/7	+	<i>P. indicus</i> , <i>M. lysianassa</i> , <i>M. ensis</i>	Mixed
3 (06/97)	5/9	+ @ +++, mainly +	<i>P. indicus</i> , <i>M. lysianassa</i> , <i>M. ensis</i>	Mixed, separate
5 (07/97)	6/9	+ ● +++, mainly +	<i>P. indicus</i> , <i>M. lysianassa</i> , <i>M. ensis</i>	Mixed, separate
6 (08/97)	2/9	+++	<i>P. indicus</i> , <i>M. lysianassa</i>	Mixed, separate
10 (10/97)	5/9	+++	<i>P. indicus</i> , <i>M. lysianassa</i> , <i>M. ensis</i>	Mixed, separate
16 (01/98)	2/9	+++	<i>M. lysianassa</i>	Mixed
17 (01/98)	3/5	++ @ +++	<i>P. indicus</i> , <i>M. ensis</i>	Mixed
22 (04/98)	1/9	++	<i>P. indicus</i>	Mixed

pond bottom remains at high levels (> 32 ppt). Heavy rains were most common in July, August and October and brought a decrease in salinity. In January and February (at the beginning of dry season) disaster seems to be closely related to low water temperature, high turbidity and the presence of organic pollution coming from the inland flooded area. Based on the presented evidences gathered from this extensive farming system, an appropriate time for pond sanitation and preparation seem to be from May to June and January to February. Moreover, fluctuations of water parameters should be reduced by pond dredging, widening of the surface area and eliminating water leakage.

Moreover, the importance of stabilising water parameters is increased by the low probability of collecting WSSV-free wild seeds, especially during the peaks of seed recruitment. All these factors should be seriously considered in designing a model for sustainable development of this sensitive ecosystem characterised by high potential productivity.

Conclusions

1. Water parameters showed high daily and yearly fluctuations. In fact, both temperature and salinity markedly changed according to the seasonal weather conditions. Other parameters such as pH, DO, and secchi depth showed high variability in both ponds and supplied canals.

2. WSSV was present in the wild shrimp seeds and *Acetes spp.* in the same periods, i.e. beginning of the dry season and passage from dry to rainy season. Such periods closely coincide with the two peaks of seed recruitment during the Tong and Mua seasons.

MBV was present in wild shrimp seeds all year round, showing higher prevalence in the separate model.

3. WSSV was present in the harvested shrimp all year round with higher frequency of detection in the rainy season and at the beginning of the dry season.

MBV was always present throughout the year in the harvested shrimp but viral presence was higher during the rainy season.

There appear to be no differences in prevalence and intensity of infection regardless of the harvest population characteristics, which showed a marked change in recent years.

4. Mass mortality is closely related to the two peaks of harvest and, during the same periods, the presence of WSSV with high intensity of infection was also detected. It appears clear that a correlation of high salinity, low temperature and high fluctuation of other water parameters with large-scale mortality do exist.

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