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Major Viral Diseases of *Penaeus monodon* in Taiwan

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ABSTRACT

In Taiwan, the shrimp farming industry has relied mainly on intensive and semi-intensive culture systems. While these high density and monoculture systems can significantly increase production per unit area, however, they have deviated from a "natural" ecosystem for shrimp. As a result, they are apt to create physiological stresses. Under such conditions, when pathogens are present in the culture systems, the onset of the disease is often rapid, and the consequent lethality is remarkable. In the last decade, outbreaks of infectious diseases have caused significant losses in this shrimp culture industry and hampered its development. Of all the known shrimp pathogens, the viruses have had and continue to have the most serious impact on the shrimp farming industry. This paper uses our recent survey data to focus on the question of viral infection of cultured shrimp in Taiwan. The effects that some of the more serious viruses can have on shrimp populations are discussed with emphasis mostly on the shrimp themselves—brooders and offspring—rather than the economic impact. We also review the work of several research groups on white spot syndrome virus, work that has provided tools and knowledge to combat the virus, and suggest future directions of research that may be useful in the effort to develop a sustainable shrimp industry.

Key words: *Penaeus monodon, Viral diseases, Taiwan*

INTRODUCTION

The black tiger shrimp *Penaeus monodon* is the most common cultured penaeid in Asia. In Taiwan *P. monodon* has a culture history spanning three decades, and it continues to be the major culture species. The artificial propagation of this species was first established in 1968 (Liao *et al.*, 1969). *P. monodon* production surged, especially in the 1980's, and peaked at 95,000 metric tons in 1987 (Liao *et al.,* 1992). Late in that same year, however, mass mortalities struck the *P. monodon* culture farms, and by 1988 the annual production had plunged 70%. This sudden, massive reduction in yield came unexpectedly, and puzzled many observers in Taiwan and other countries (Liao, 1989). Even now not everyone agrees on the causes, although poor management and planning were doubtless partly to blame, the baculovirus MBV (*Penaeus monodon*-type baculovirus) has also been implicated (Chen *et al.,* 1989; Liao *et al.,* 1990; Liao *et al.,* 1992; Annie and Lucien, 1993; Flegel, 1997). Surprisingly though, in the years following the initial outbreak, MBV was suddenly no longer widely found in cultured *P. monodon*, but the yield of *P. monodon* did not recover as expected. Then, in 1993, the extremely virulent agent white spot syndrome virus (WSSV) was first officially reported in Taiwan. There was no dramatic decline in productivity between 92 and 93, but even so, we

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suspect that WSSV was probably a major factor in holding cultured *P. monodon* productivity below 1985 levels (Fig. 1).

On the other hand, since MBV was proposed as the cause of the catastrophic mortalities of *P. monodon* in Taiwan in 1987/88, many farmers switched from *P. monodon* to kuruma shrimp *P. japonicus* because of its resistance to MBV (Fukuda *et al.,* 1988). Kuruma shrimp soon became a popular species in northern Taiwan. Most of these shrimp were raised in culture ponds that had originally been used to culture *P. monodon*. The productivity increased yearly and peaked at 11,460 metric tons in 1991.

Unfortunately, at this time a new disease, the explosive epidemic disease of prawn (EEDS; the disease now known as WSS), began to be observed in mainland China. This disease was caused by a more dangerous virus, *i.e.,* the virus now known as WSSV (or hypodermal and hematopoietic necrosis baculovirus, HHNBV, called in mainland China). Local researchers contributed greatly to the knowledge on the early stages of the WSSV epidemic in China, and we now know something about its origins (Huang *et al*., 1995a,b; Cai *et al.,* 1995; Wang 1995). Outbreaks of WSSV were first found in *P. japonicus* in China's Fujian Province. The disease then spread very rapidly southward to Shantou (and possibly to Taiwan at the same time) and northward to Wenzhou before the end of 1992. By then, the disease had spread to all the coastal shrimp farms from Zhanjiang to Bohai and to inland ponds north of the Yangtze River. These outbreaks started when ponds were stocked with kuruma shrimp postlarvae from the south (Fujian Province). Subsequently, the disease was transmitted to *P. chinensis* (the major cultured penaeid in China) and other penaeids. In 1994, the disease was widely reported from almost shrimp culture sites. It was also in 1993 that the disease was transmitted to Japan via kuruma shrimp postlarvae imported from Fujian Province. (Inouye *et al.*, 1994).

In Taiwan, WSS was first observed in *P. japonicus* in 1992 in the farms of northern I-Lan County, possibly through the import of living broodstock or postlarvae from Fujian Province directly to culture facilities. The disease soon spread southward, and in 1993 *P. monodon* and other shrimp species in farms in the south of Taiwan were affected (Chou *et al.*, 1995). The Taiwan, kuruma shrimp farming industry was seriously affected by the outbreak of WSS in 1992 (although at that time the causative agent had not yet been identified by local researchers), with the yield falling over 90% from 1991 to 1993. Yields continue to be affected even today. The effects of WSSV were not so obvious on the *P. monodon* industry, which had already been seriously reduced in 1987/88 (Fig. 1), but its dramatic effects on cultured *P. japonicus* can be seen in Figure 2.

VIRAL INFECTION STATUS IN CULTURE SHRIMPS AND WILD-CAUGHT BLACK TIGER SHRIMP IN TAIWAN

In Taiwan, cultured shrimp have been found to be infected with MBV (Lightner *et al.,* 1987; Hsu *et al.,* 2000), infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Lightner *et al.,* 1983; 1987), WSSV (Chou *et al.,* 1995), yellow head virus (YHV) (Boonyaratpalin *et al.,* 1993; Wongteerasupaya *et al.,* 1995a; Wang *et al.,* 1996; Spann and Lester 1997; Spann *et al.,* 1997; Cowley *et al.,* 2000; Wang and Chang 2000), and Taura syndrome virus (TSV) (Hasson *et al.,* 1995; Tu *et al.,* 1999; Yu and Song, 2000). However, of the twenty known shrimp viruses, only four are classified as category 1 (C1; see the review by Lotz, 1997), that is, viruses that cause mass mortalities and may threaten the shrimp farming industry of a whole geographic region. To determine the present shrimp infection status for these four C1 pathogens in Taiwan, we used four single-tube nested detection kits

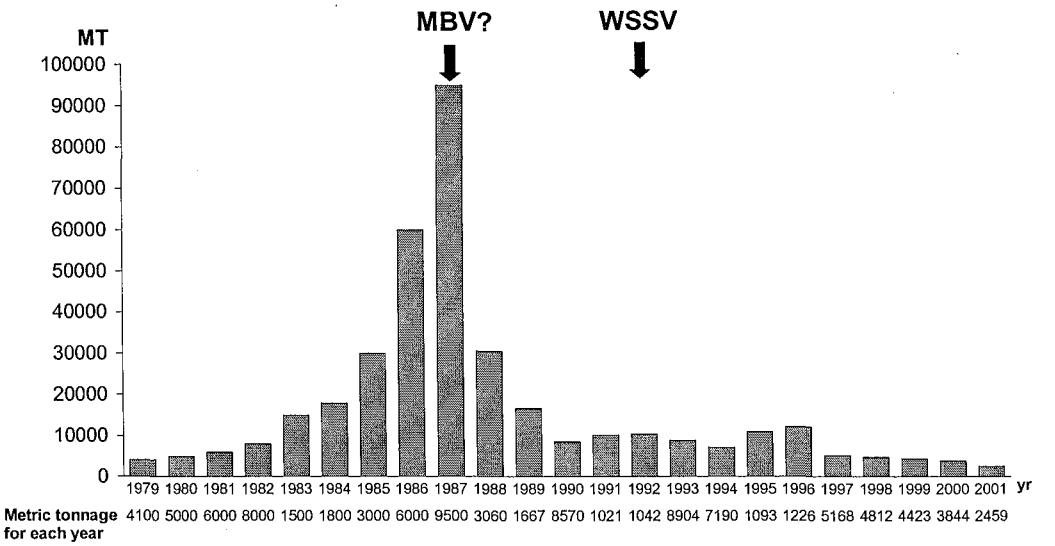

Fig. 1. Annual productions of cultured *Penaeus monodon* in Taiwan. (Data from: Fisheries statistical yearbook, Taiwan area, 1980-2002 and Liao *et al.*, 1992).

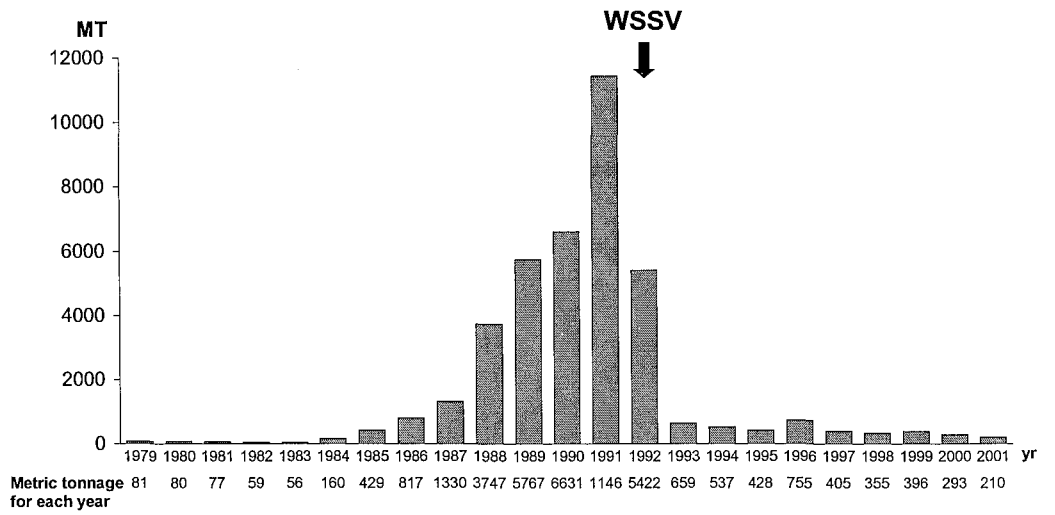

Fig. 2. Annual productions of cultured *Penaeus japonicus* in Taiwan. (Data from: Fisheries statistical yearbook, Taiwan area, 1980-2002).

(Farming IntelliGene Tech. Co., Taipei, Taiwan) for detecting IHHNV, WSSV, TSV and YHV/GAV (gill associated virus) in *P. monodon, P. vannamei, Meta- penaeus ensis* in 2000. In this trial, we used the kit that was not able to distinguish between the YHV and the GAV, a non-virulent YHV-related virus (Cowley *et al.*, 1999). Our data showed that all four of these viruses were found in each of the shrimp species tested. This is the first time that a TSV infection has been reported for either *P. monodon* or *M. ensis*. Also, as recently as 1997, TSV was still

considered a western hemisphere virus that primarily affected the Americas (Lotz, 1997), and until now, TSV infections in Taiwan had only been reported in the imported *P. vannamei* (Tu *et al.*, 1999; Yu and Song, 2000). The detection of TSV in wild- caught *P. monodon* brooders was also particularly notable because *P. monodon* was thought resistant to TSV infection.

The infection status of *P. monodon* broodstock collected from the Taiwan coastal waters in 2000 was also studied (Kou *et al.*, 2001). There was a high proportion of double- and multiple- infections, and the implications of this for cultured shrimp will need to be investigated. One curious and unexpected preliminary observation is that a co-infection of WSSV and IHHNV was actually associated with a lower mortality rate in *P. monodon* brooders after spawning. It is notable too that IHHNV was detected in around 40% of these brooders. Like TSV, IHHNV is a virus that was originally associated with *P. vannamei* and *P. stylirostris,* and its high prevalence now in Taiwanese *P. monodon* broodstock has very likely resulted from infected *P. vannamei* and *P. stylirostris* broodstock and/or postlarvae being imported to Taiwan for culturing in recent years. International transport of live shrimp for aquaculture is attractive economically, but it is also a very rapid and effective means of spreading viruses. The movement of live shrimp (and other aquatic organisms) should be strictly regulated.

A BRIEF REVIEW OF THE STUDIES ON WHITE SPOT SYNDROME VIRUS

Outbreaks of WSS were reported from farmed shrimp in Japan (Inouye *et al.*, 1994, 1996; Nakano *et al.*, 1994; Momoyama *et al.*, 1994, 1995; Takahashi *et al.*, 1994; Kimura *et al.*, 1995), China (Huang *et al.*, 1995a,b), Taiwan (Chou *et al.*, 1995; Wang *et al.*, 1995, 1997a; Lo *et al.*, 1996a, 1997), Thailand (Wongteerasupaya *et al.*, 1995b, 1996), Korea (Kim *et al.*, 1998; Park *et al.*, 1998), India (Karunasagar *et al.*, 1997, 1998; Mohan *et al.*, 1998), the USA (Lu *et al.*, 1997a, Loh *et al.*, 1998; Lightner, 1996), and in the main shrimp farming countries of Central and South America (GAA 1999a,b). The disease can cause up to 100% mortality, with a correspondingly devastating economic impact. So far no significant resistance to this disease has been reported for any species of shrimp.

WSSV has an extremely wide range of potential hosts. It infects not only the penaeid and non- penaeid shrimp but also a wide range of other decapods, including crab, crayfish, and lobster and perhaps artemia, copepod and insect larvae, which have all detected positive for PCR and some have not been observed to be actually infected (Flegel 1997; Lo *et al.*, 1996a; Kanchanaphum *et al.*, 1998; Supamattaya *et al.*, 1998). The virus is transmitted both horizontally and vertically (Chou *et al.*, 1995, 1998; Lo *et al.*, 1997).

Another notable feature of WSSV is that its replication is easily triggered by stressful conditions. We classify WSSV infection into three stages: the asymptomatic carrier, transition, and acutely affected stages (Lo and Kou, 1998). The thing to note here is that the carrier stage may persist for months, but as soon as certain triggering conditions are reached, the disease progresses to the transition and patent stages within a few hours. Once the infection becomes patent, mortality inevitably occurs within a few days. In the growout ponds too, if stocking with WSSV carriers is done, one should minimize the environmental stresses. If stresses are mostly eliminated, even a pond stocked with WSSV carrier shrimp still has a good chance for a successful harvest. Conversely, for intensive culture ponds, the use of WSSV-free postlarvae for stocking plays a key role in successful culturing (Lo *et al.*, 1998; 2001; Withyachumnarnkul, 1999; Lo *et al.*, 2001; Peng *et al.*, 2001).

Virions are rod-shaped to elliptical with an envelope, and they are large

(80-120 × 250-380 nm) (Inouye *et al.*, 1994, 1996; Takahashi *et al.*, 1994; Wang *et al.*, 1995; Wongteerasupaya *et al.*, 1995b; Durand *et al.*, 1997). Negatively stained virions show unique, tail-like appendages (Wongteerasupaya *et al.*, 1995b; Wang *et al.*, 1995). WSSV was initially described as a non-occluded baculovirus, but even while the molecular data were still limited (the preliminary WSSV-DNA sequence analysis), the morphological characteristics and the general biological properties of the virus had already highlighted its uniqueness (Wongteerasupaya *et al.*, 1995b; Lo *et al.*, 1996a; Lo *et al.*, 1997). Recent data, including the genome sequence and phylogenies based on DNA polymerase and protein kinase, suggest that WSSV is a member of a new virus family tentatively named as Nimaviridae (Yang *et al.*, 2001; van Hulten *et al.*, 2001a; Liu *et al.*, 2001; Chen *et al.*, 2002a; Vlak *et al.*, 2001).

The size of the WSSV genome has been differently reported for different isolates: 305107 bp (GenBank Accession No. AF332093; Yang *et al.*, 2001), 292967 bp (GenBank Accession No. AF369029; van Hulten *et al.*, 2001a), and 307287 bp (GenBank Accession No. AF440570) for viruses isolated from China, Thailand and Taiwan, respectively. Only a few WSSV genes have been studied beyond this sequence analysis (Tsai *et al.*, 2000a,b; van Hulten *et al.*, 2000, 2001a,b, 2002; Liu *et al.*, 2001; Zhang *et al.*, 2001; Chen *et al.*, 2002a,b; Lin *et al.*, 2002; Huang *et al.*, 2002a,b; Tzeng *et al.*, 2002; Zang *et al.*, 2002).

It is good to report that scientists reacted very quickly to this disease. For example, within a very short time after the WSS outbreak, the causative agent was identified. Many effective diagnostic tools/ strategies were developed by several groups working in Taiwan, Japan, Thailand, Korea, and the USA (Momoyama *et al.*, 1994, 1995; Takahashi *et al.*, 1994, 1996; Wang *et al.*, 1995, 1997b; Wongteerasupaya *et al.*, 1995b, 1996; Durand *et al.*, 1996, 1997; Kimura, 1996; Lightner, 1996; Lo *et al.*, 1996 a,b, 1999; Flegel *et al.*, 1997; Lu *et al.*, 1997a,b; Nadala *et al.*, 1997; Nunan and Lightner, 1997; Tapay *et al.*, 1997; Kasornchandra and Boonyaratpalin, 1998; Kasornchandra *et al.*, 1998; Kim *et al.*, 1998; Kou *et al.*, 1998; Loh, *et al.*, 1997, 1998, Hsu *et al.*, 2000). In addition, molecular tools, including WSSV diagnostic polymerase chain reaction (WSSV PCR), soon proved very useful for screening carriers in shrimp larvae, bloodstocks, and invertebrate populations which shared the same habitat as well as in helping to ascertain the transmission and infection cycle of WSSV. All of these helped us better understand the virus and its transmission modes, and this brought about changes in hatchery and farming practices, including the use of PCR technology to screen broodstock and larvae, and widespread adoption of closed or semiclosed cultivation (Flegel and Alday-Sanz, 1998).

The efforts of the members of an international WSSV study group initiated by Prof. Just Vlak, Wageningen University, to establish a new virus family (proposed name Nimaviridae) that would accommodate WSSV and other closely related viruses is also worthy of reporting here. Through international collaboration, the taxonomic question was thus efficiently solved (Vlak *et al.*, 2001).

CONCLUSIONS AND PROSPECTIVES

WSSV can be transmitted orally as well as via water across shrimp species and possibly arthropods (Chang *et al.*, 1996; Chou *et al.*, 1998). Due to its rapid spread and broad host range, we anticipate WSSV will continue to inflict serious damage on the shrimp aquaculture industry worldwide. We now have a WSSV diagnostic PCR, which can be applied immediately to screen for carriers in the shrimp larvae used for stocking as well as captured broodstock, and this allows for better control of WSSV

in a cultivation system. However, if the disease is to be successfully combated, perhaps a WSSV-resistant strain of shrimp will eventually need to be developed.

In brooders, spawning usually increases the severity of a WSSV infection. Yet we found that some brooders (less than 25%) were able to contain the virus and thus prevent them from rapid replication during spawning. It would be very interesting to compare the gene expression profiles of shrimps with different performances in response to WSSV infection. This may facilitate the investigation into the molecular mechanisms that confer on certain shrimp the ability to contain the virus under stressful conditions. Expressed sequence tag (EST) analysis (Rojtinnakorn *et al.*, 2002; Lehnert *et al.*, 1999; Gross *et al.*, 2001; Supungul *et al.*, 2002) and DNA microarray technology (Kurella *et al.*, 2001) may be powerful tools in this study.

Many studies demonstrate that the WSSV infection status of shrimp broodstock and postlarvae for stocking plays a key role in successful culturing (Lo *et al.*, 1998; Hsu *et al.*, 1999; Withyachumnarnkul, 1999; Peng *et al.*, 2001), yet the domestication technology of *P. monodon* has not matured, and the source of the healthy *P. monodon* broodstock has become a key bottleneck in the struggle to create a sustainable shrimp culture industry. The functional genomics of the shrimp *P. monodon* have become increasingly important. We need a genome-wide analysis of the black tiger shrimp to gain a better understanding of the molecular biology of the shrimp especially in the fields of reproduction, growth, and disease defense. Functional genomic studies will generate useful information by which many biologically and economically significant genes will be identified. Furthermore, a genetic analysis of the black tiger shrimp, including development of molecular markers for the use in gene mapping, marker-assisted breeding, stock improvement, and gene function analysis should be performed. All of these will help to develop genetic improvement programs for *P. monodon* to enhance disease resistance, increase productivity, and reduce dependency on wild stocks and thus provide lasting benefits to the shrimp aquaculture industry.

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台灣草蝦主要病毒症之研究回顧與前瞻

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臺灣的蝦類養殖大多以集約及半集約方式為主，雖然單種養殖的方式可以顯著提高單位產量，但在這種非自然的生態環境中，養殖蝦類易產生生理緊迫，因此一旦有病原出現，則池蝦常發病迅速、死亡率也高。近十年來傳染性疾病的突發，不僅使養蝦產業損失慘重，更阻礙了本產業的發展。在所有目前已知的蝦類病原當中，以病毒對蝦類養殖產業所造成衝擊最大，因此本文將報導臺灣養殖蝦目前感染病毒的現況，也將討論病毒於蝦類本身—種蝦和子代—所造成的影響。我們回顧了各研究團隊在究明白點症病毒本身及建構對抗此病毒所需之工具及知識等相關議題上的貢獻，同時也提出未來研究方向及展望，以利養蝦產業的永續經營。

關鍵詞：草蝦(*Penaeus monodon*)，病毒症，台灣

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