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## Current Status of Viral Diseases in Asian Shrimp Aquaculture

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Key words: shrimp viruses, white spot syndrome virus (WSSV), yellow head virus (YHV), hepatopancreatic parvovirus (HPV), monodon baculovirus (MBV), taura syndrome virus (TSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), Laem-Singh virus (LSNV), infectious myonecrosis virus (IMNV), abdominal segment deformity disease (ASDD)

### Abstract

The giant or black tiger shrimp, *Penaeus monodon*, was formerly the dominant cultured shrimp species in Asia. Since approximately 2002, it has been essentially replaced by the domesticated American whiteleg shrimp *P. vannamei*. The change in dominant species has affected disease concerns. For both species, white spot syndrome virus (WSSV) and yellow head virus (YHV) are the most lethal. For *P. monodon*, the next most important diseases are hepatopancreatic parvovirus (HPV) and monodon baculovirus (MBV). For *P. vannamei*, they are taura syndrome virus (TSV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV). TSV was introduced to Asia in 1998 by careless importation of shrimp stocks for aquaculture but has not been reported to cause problems with local crustacean species. IHHNV, which is endemic in Asia, is harmless to *P. monodon* but poses a constant threat to IHHNV-free stocks of *P. vannamei* if they are hatched and reared in Asia under non-biosecure conditions. An emerging disease for *P. monodon* is monodon slow growth syndrome (MSGs), a component of which seems to be Laem-Singh virus (LSNV). Infectious myonecrosis virus (IMNV) is a *P. vannamei* disease, first reported from Brazil but now reported in Indonesia where it was probably introduced by careless importation of shrimp aquaculture stocks. So far, IMNV has not been reported in other Asian countries. *Penaeus vannamei* nodavirus (PvNV) is a new pathogen first reported from Belize in 2004 with gross and histological signs that are indistinguishable from those of IMNV. The disease has not yet affected Asian culture. A more recent disease of *P. vannamei* in Asia is abdominal segment deformity disease (ASDD), possibly caused by a yet unknown local virus.

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### Introduction

This review presents an overview of current viruses in cultivated shrimp in Asia. To understand the situation, it is important to realize that the three largest shrimp producing countries in Asia (China, Thailand, Indonesia) shifted from cultivating the native giant tiger shrimp, *Penaeus monodon*, to the exotic American whiteleg shrimp, *Penaeus (Litopenaeus) vannamei*, in approximately 2002 (Wyban, 2007a,b). The shift resulted in dominance of *P. vannamei* in the global cultivated shrimp market. The main reason for this shift was linked to the supply of shrimp seed (postlarvae) for farms. Traditionally dominant *P. monodon* farming was based on postlarvae reared from captured broodstock that often appeared normal but were infected with white spot syndrome virus (WSSV). The broodstock transmitted the WSSV (along with other viruses) to their offspring. Thus, postlarvae used to stock cultivation ponds were often the source of continuing WSSV disease (WSD) outbreaks.

This problem was solved by using domesticated and genetically improved stocks of *P. vannamei* that were specific pathogen free (SPF) of major shrimp pathogens including WSSV. These stocks originated at the Oceanic Institute in Hawaii as a result of cooperative efforts in the US Marine Shrimp Research Program (Moss et al., 2005). The exotic shrimp stocks grew well in the intensive culture systems used in Asia and resulted in an increase in world production of cultivated shrimp, previously dominated by *P. monodon* (Fig. 1). This increase is rapidly bringing total shrimp aquaculture production near that of the shrimp capture fishery.

As an example of the general trend in Asia, *P. monodon* production in Thailand grew explosively from 1987 until the early 1990s when it was hit by yellow head virus (YHV) and, soon

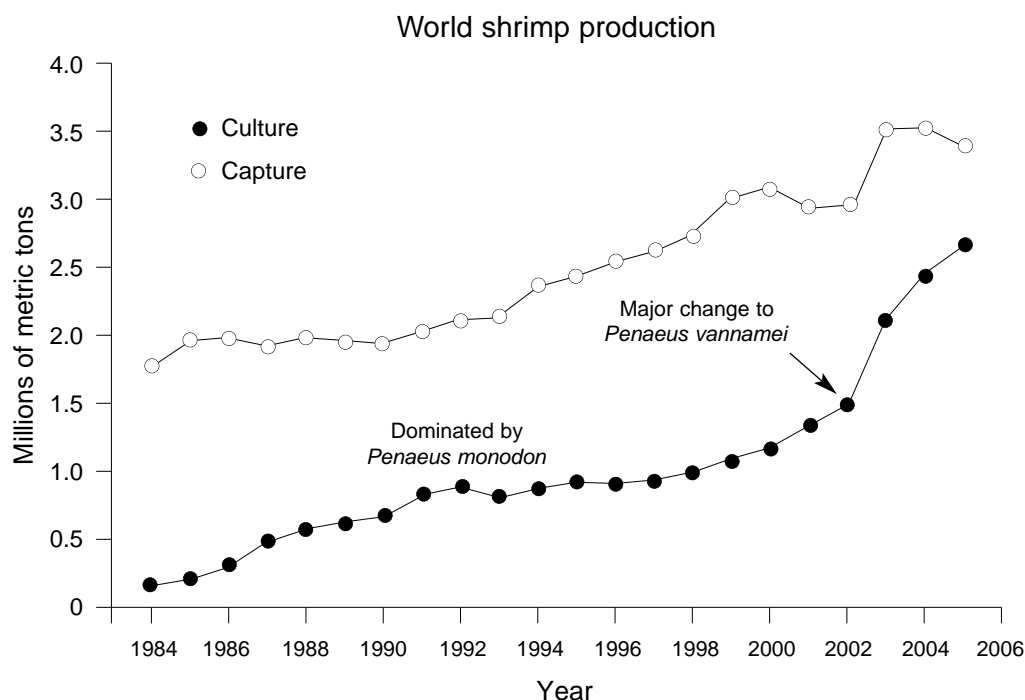


Fig. 1. World production of shrimp from capture fisheries and aquaculture. Production was dominated by *Penaeus monodon* until 2002 and by *Penaeus vannamei* thereafter.

thereafter, WSSV (Fig. 2). There followed a period of recovery up to the early 2000s when the industry was again hit by disease, this time monodon slow growth syndrome (MSGs), one of the major reasons for the shift to *P. vannamei*. After that, production increased rapidly, exceeding 400,000 tons in 2006. Meanwhile, the shrimp capture fishery peaked in 1982, then declined rapidly to 1985, before the boom in shrimp cultivation began. From 1985 onwards, it declined steadily, but not as sharply as the culture industry during the WSSV outbreaks in 1994-1997. The decline in the capture fishery from 1998 to 2000 may have been the result of the high demand for captured shrimp broodstock needed to supply postlarvae for continuously expanding shrimp farms.

Although China, Thailand, and Indonesia have made major shifts towards the cultivation of *P. vannamei*, this species is banned in India and restricted in Vietnam and the Philippines where *P. monodon* is still the dominant cultivated species. Due to the dramatic change in cultivated species in Asia, concerns regarding shrimp diseases have changed to include pathogens of *P. vannamei* that did not previously concern Asian farmers. The major diseases of *P. monodon* in Asia, including WSSV and YHV that can also be serious pathogens for *P. vannamei*, have already been reviewed (Lightner and Redman, 1998; Flegel, 2006a). Thus, most of the information in this review relates to new emerging pathogens that threaten the cultivation industry, i.e., taura syndrome virus (TSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), infectious myonecrosis virus (IMNV), and *Penaeus vannamei* nodavirus (PvNV). Also included is a new disease of currently unknown etiology called abdominal segment deformity disease (ASDD).

#### Taura Syndrome Virus (TSV)

A disease caused by TSV was first described from Ecuador in the early 1990s (Jimenez, 1992) but the etiological agent was not discovered for several years (Hasson et al., 1995). Thereafter, laboratory tests indicated that it had low virulence for the giant tiger shrimp *P. monodon* (Brock

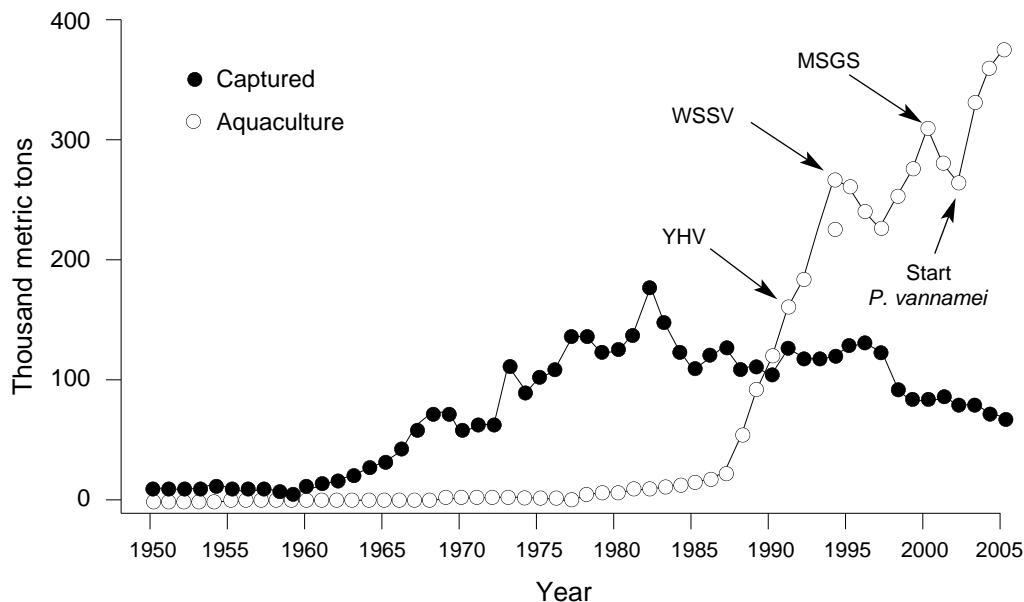


Fig. 2. Production of shrimp from capture fisheries and aquaculture in Thailand. Aquaculture production rose in 2002 after *Penaeus vannamei* replaced *Penaeus monodon*.

et al., 1997; Srisuvan et al., 2005). It was not reported in Asia until after introduction of *P. vannamei* for aquaculture in Taiwan in the late 1990s (Tu et al., 1999) and was subsequently reported from all Asian countries to which *P. vannamei* were imported for aquaculture. Genetic comparison of isolates revealed that the virus was almost certainly brought to Asia with contaminated shrimp stocks used for aquaculture (Nielsen et al., 2005; Tang and Lightner, 2005). This is an example of how viral diseases spread via transboundary movement of aquaculture stocks (Flegel, 2006b).

The current overall impact of TSV in Asia is comparatively low because most of the domesticated and cultivated SPF stocks of *P. vannamei* have been selected for resistance to TSV (Moss et al., 2005; Wyban, 2007a,b). In addition, there have been no reports of mortality caused by TSV in other cultivated or wild Asian crustaceans since its introduction over 10 years ago. On the other hand, laboratory challenge tests by injection or feeding show that common Thai species of native crabs (fiddler crab, *Uca vocans*; mangrove crab, *Sesarma mederi*) and palaemonid shrimp (*Palaemon styliferus*; *Macrobrachium lancesteri*) can be infected with TSV (Kiatpathomchai et al., 2008). Although they did not die, they were infectious when fed to *P. vannamei* and are thus potential carriers of TSV. Despite this potential carrier role, limited tests of captured specimens were negative for TSV when tested by RT-PCR.

#### Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)

IHHNV is an endemic virus in the geographical range of *P. monodon*. Since the beginning of intensive culture of *P. monodon* in the mid 1980s, there have been only two reports of losses attributed to IHHNV disease outbreaks in Asian *P. monodon* shrimp farms: one in hybrid Asian penaeid shrimp (Owens et al., 1992) and the other in captive breeding stock of *P. monodon* (Primavera and Quinitio, 2000). Indeed, publications on domesticated *P. monodon* stocks in Thailand indicate a very low impact (if any) of IHHNV on growth and reproduction in *P. monodon* (Chayaburakul et al., 2005; Withyachumnarnkul et al., 2006). This contrasts with the high virulence of the virus for the American blue shrimp *P. stylirostris* and the stunting it causes (i.e., runt deformity syndrome or RDS) in the American whiteleg shrimp *P. vannamei* (Bell and Lightner, 1984; Bonami et al., 1990; Lightner, 1996). RDS is characterized by gross signs of distorted form, particularly short bent rostra, a downshift of mean weight, and a high coefficient of variation for weight when compared to uninfected shrimp. Although RDS is not associated with mortality, it can result in unprofitable crops.

Prior to the introduction of *P. vannamei*, IHHNV was of little concern in Asia. It was of such little concern that it was not generally included in disease screening of broodstock and postlarvae used in pond stocking programs. Nor is IHHNV problematic in farmed *P. vannamei* in the Americas that is based on SPF stocks. However, farming of SPF *P. vannamei* in Asia where IHHNV is endemic requires special precautions to insure that the stocks do not become contaminated by horizontal transmission from natural or farmed carriers. Unfortunately, the lack of impact of IHHNV on *P. monodon* culture meant that no work was done on other possible native hosts. It is certain, however, that wild-captured *P. monodon* broodstock and other native crustaceans should never be held in the same facility as SPF *P. vannamei* broodstock that are to be used for producing postlarvae to stock grow-out ponds. This advice was widely disseminated in Thailand when *P. vannamei* began to be cultivated on a large scale.

#### Infectious Myonecrosis Virus (IMNV)

IMNV first caused disease outbreaks in 2002 on Brazilian shrimp farms where exotic *P. vannamei* were being cultivated; a new virus was found to be the cause (Poulos et al., 2006). Its viral particles are icosahedral in shape, 40 nm in diameter, and contain a genome consisting of a single, double-stranded RNA (dsRNA) molecule of 7560 bp. Phylogenetic analysis based on its RNA-dependent RNA polymerase (RdRp) indicated that it is related to the *Giardia lamblia*

virus in the family *Totiviridae* (Poulos et al., 2006). The virus caused low but steady mortality leading to accumulated losses up to 70%. Moribund shrimp are characterized by whitened, opaque tail muscles. Histologically, the muscle tissue shows coagulative necrosis together with massive accumulation of hemocytes and basophilic inclusions within the muscle fibers. It seems most likely that IMNV originates from local host species in which it causes no noticeable problems.

In Asia, we first received samples of IMNV-positive shrimp from Indonesia in June 2006 (Senapin et al., 2007). Amplification of the whole gene sequence based on primers designed from the IMNV genome at GenBank revealed that the virus involved in the Indonesian outbreak had 99.6% nucleic acid sequence identity (with 29 differences in the 7.5 kb genome) to that of the Brazilian IMNV reported at GenBank. The most likely explanation was that shrimp stocks for aquaculture had been imported from Brazil to Indonesia without proper quarantine.

Since June 2006, we have received many samples of *P. vannamei* with whitened muscles from China, Malaysia, and Thailand (unpublished). However, all these samples were negative for IMNV. Histopathologically, these samples usually showed signs of muscle necrosis without accumulation of hemocytes. This is typical of lesions caused by muscle cramp syndrome (Lightner, 1996) that can occur spontaneously in stressed *P. vannamei* and cause mortality if the cramps are not quickly resolved. Muscle cramp syndrome also causes whitening of abdominal muscle tissue that usually begins in the middle of the muscle as opposed to whitening caused by IMNV that apparently begins in the sixth abdominal segment. It is probable that many suspected IMNV cases in Asia are in fact cases of muscle cramp syndrome.

#### *Penaeus vannamei* Nodavirus (PvNV)

PvNV is a new pathogen first reported from Belize in 2004 as the cause of muscle necrosis leading to as much as 50% reduction in production (Tang et al., 2007). The gross and histological signs were indistinguishable from the signs of IMNV, i.e., whitened abdominal muscles, coagulative muscle necrosis with hemocytic aggregation, and basophilic inclusions. However, none of the IMNV tests were positive and the causative agent was eventually found to be a new nodavirus called *Penaeus vannamei* nodavirus (PvNV). Unfortunately, the only way to currently distinguish between PvNV and IMNV is to use molecular tests such as RT-PCR or *in situ* hybridization (Poulos and Lightner, 2006; Andrade et al., 2007; Tang et al., 2007). After receiving a positive control for the RT-PCR assay for PvNV kindly provided by Dr. Lightner (University of Arizona) in early 2007, we carried out tests on all the samples we had previously received for IMNV analysis and all were negative for PvNV. I believe there is very little chance that PvNV could come to Asia except by the careless translocation of infected stocks for aquaculture, as most likely occurred with IMNV in Indonesia. In any case, diagnosticians should be on the lookout for both these viruses and reported cases should be handled as emergency containment cases to limit their spread.

#### Abdominal Segment Deformity Disease (ASDD)

This is a relatively new disease reported from Thailand and Indonesia since the introduction of *P. vannamei*. It is characterized by twisting and bulging of the abdominal segments of juvenile shrimp in grow-out ponds, sometimes accompanied by limited muscle whitening (Fig. 3). Unlike RDS, mean weight and coefficient of variation for weight are not abnormal, and rostra are not bent or twisted.

Histologically, the areas of distorted muscle tissue show aggregation of hemocytes that occurs in the absence of basophilic cytoplasmic inclusions and coagulative muscle necrosis. The number of shrimp affected in a pond varies from a few to many. It can have an economic impact since distorted shrimp cannot be marketed as a fresh frozen product but must be processed, at least by peeling and cooking. In Thailand, this reduces the farm-gate selling price by approxi-

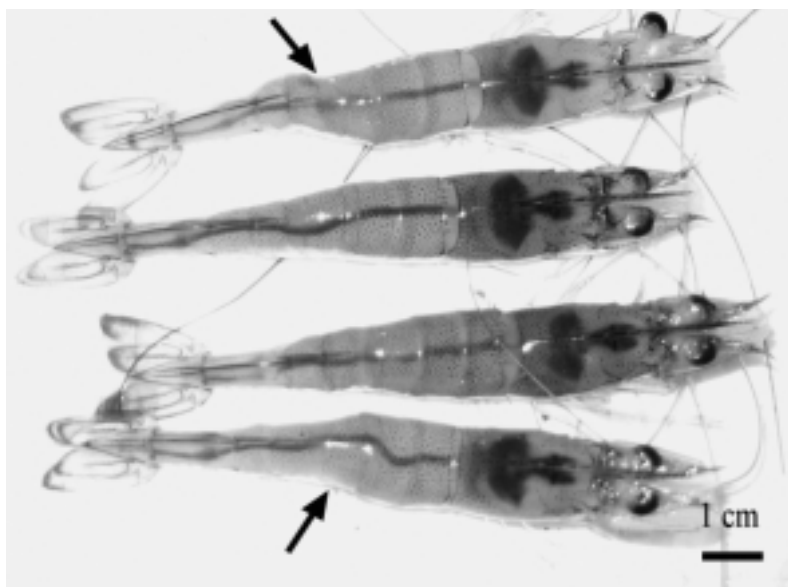


Fig. 3. Twisting and bulging of the abdominal segments of juvenile *Penaeus vannamei* affected by abdominal segment deformity disease (ASDD) in Thailand (photograph courtesy of Waraporn Sakaew).

mately 10%. Thus, the impact depends upon the quantity of shrimp in a pond that are affected.

Injection of membrane-filtered (0.22  $\mu$ m) tissue homogenates can result in ASDD in challenged shrimp (unpublished), suggesting that it might be an infectious disease caused by a viral agent. All our PCR and RT-PCR tests on ASDD specimens for known viruses, including IHNV, hepatopancreatic parvovirus (HPV), TSV, IMNV, PNV, and Laem-Singh virus (LSNV), were negative (unpublished). Examination of tissues from affected shrimp by transmission electron microscopy revealed the presence of viral-like particles in affected muscles and in the ventral nerve cord (unpublished).

#### Diseases of Importance in *P. monodon*

Known viral diseases of importance for *P. monodon* in approximate order of highest to lowest negative economic impact include WSSV, YHV, HPV, and monodon baculovirus (Flegel, 2006a). Only WSSV and YHV are known to cause high mortality. For information on these viruses, the reader is referred to reviews by Lightner and Redman (1998) and Flegel (2006a). Losses from HPV and monodon baculovirus (MBV) are associated with retarded growth, with the effects of HPV being more severe than those of MBV (Flegel et al., 1999, 2004). These viruses are adequately covered in Lightner and Redman (1998) and Flegel (2006a) and will not be considered here. IHNV which, despite its relatively high prevalence in captured broodstock, postlarvae, and cultured shrimp, has a low impact on *P. monodon* and is discussed above.

Some brief discussion is warranted for viruses of *P. vannamei* that are not endemic in Asia. There are no reports of disease outbreaks or other losses in *P. monodon* culture due to TSV since its introduction to Asia in the late 1990s, despite the fact that it has been detected in farmed specimens (Nielsen et al., 2005). Field information is supported by laboratory tests that show *P. monodon* can be infected with TSV by injection (Brock et al., 1997; Srisuvan et al., 2005) but that mortality is low (about 20%) when compared to a highly susceptible variety of *P. vannamei*.

Injection is not a natural route for TSV infection and this may explain the fact that despite the mortality in laboratory challenge tests, no outbreaks have been reported from the field.

Similar to TSV, injection challenge tests with IMNV (Tang et al., 2005) and PNV (Tang et al., 2007) revealed that *P. monodon* can be infected with both viruses but that they were much less virulent (i.e., no mortality) than in *P. vannamei*. If no mortality resulted in injection challenge tests, we may hope by analogy to TSV that IMNV and PNV will not cause difficulties in Asian crustaceans if and when they are introduced by careless importation of aquaculture stocks. On the other hand, TSV, IMNV, and PNV are all RNA viruses with a propensity for high mutation rates and it is possible that more virulent variants could arise post introduction, resulting in significant economic losses. To avoid this possibility, strict quarantine measures should be enforced for all transboundary movement of shrimp stocks for aquaculture because of their ability to carry viral pathogens in long-term, persistent infections without gross signs of disease (Flegel, 2006b; Flegel, 2007).

Having indicated the need for this precaution, it should not be erroneously concluded that similar dangers apply to fresh frozen shrimp products prepared and packaged for retail sale for human consumption (Flegel and Fegan, 2002). Indeed, it is curious that the Aquatic Animal Health Code of the World Organization for Animal Health (OIE) on fish viruses indicates that no special import restrictions need be applied to chilled and fresh frozen eviscerated fish products prepared and packaged for direct retail sale for human consumption, but there is no such waiver regarding similarly treated shrimp products.

Having put aside WSSV, YHV, HPV, MBV, and IHNV, there remains one major problem with *P. monodon*, monodon slow growth syndrome (MSGs). This term is applied to *P. monodon* cultivation ponds where the mean growth rate is less than half the normal, the coefficient of variation for shrimp size is equal to or greater than 35% (as opposed to approximately 20% in normal ponds), and severe infections of HPV and MBV are absent in the affected shrimp.

#### **Monodon Slow Growth Syndrome (MSGs) and Laem-Singh Virus (LSNV)**

The term MSGS is used by Thai shrimp farmers to refer to unusual retarded growth that has occurred in cultivated *P. monodon* since 2002. It has been suggested that an infectious agent may be the cause (Sritunyalucksana et al., 2006). This contention was supported by the rapid spread of the problem and preliminary laboratory challenge tests which showed that microfiltered (i.e., bacteria-free) tissue extracts from MSGS shrimp caused MSGS when injected into *P. monodon* but not when injected into *P. vannamei* (Withyachumnarnkul et al., 2004).

Thai researchers agreed to adopt a case definition to distinguish MSGS ponds from ponds with slow growth caused by other problems (Sritunyalucksana et al., 2006). According to the definition, the coefficient of size variation in an MSGS pond must be at least 35%, the pond must be free of HPV or any other severe hepatopancreas infection, and the shrimp must exhibit any three of the following five characteristics: (a) unusually dark color, (b) average daily weight gain of less than 0.1 g/day at 4 months, (c) unusually bright yellow markings, (d) "bamboo-shaped" abdominal segments, and (e) brittle antennae. These features distinguish MSGS from stunted growth caused by MBV or HPV (Flegel et al., 1999, 2004).

There is no correlation between the presence of several known shrimp pathogens and MSGS (Chayaburakul et al., 2004). Thus, a new pathogen might be the cause of MSGS. Separation of the purported agent from shrimp tissue homogenates has been difficult because of the presence of one or more known viruses and because bioassays require long incubation periods (one month or more) before results can be assessed. Therefore, we adopted a general "shot-gun" strategy to screen MSGS cases for the presence of unknown pathogens (Sritunyalucksana et al., 2006).

Briefly, it consists of obtaining shrimp from MSGS ponds and preparing "viral" extracts for fractionation by gradient centrifugation. The resulting bands are removed and used for total



nucleic acid extraction before shotgun cloning of cDNA prepared using random hexamer primers. Since total nucleic acid is used as the template, both RNA and DNA sequences were amplified. Clones that hybridized with labeled total DNA from normal *P. monodon* are eliminated and negative hybridization clones are sent for sequencing. Clones identified by Blast analysis to contain sequences of shrimp or previously screened pathogens were discarded and the remaining clones were used for *in situ* hybridization assays with shrimp from MSGS ponds and from normal growth ponds.

Using this strategy, a new RNA virus (Laem Singh virus or LSNV) (Sritunyalucksana et al., 2006) and a new variety of YHV (unpublished) were discovered, but their presence in shrimp was not directly correlated with the occurrence of MSGS. A third viral candidate, tentatively called tegumental gland associated virus (TGAV) is still being investigated, but too little information is yet available to make any firm conclusions. Subsequent work with LSNV revealed that, in MSGS ponds, only small shrimp showed retinopathy associated with strong *in situ* hybridization reactions for LSNV. Large shrimp that were positive for LSNV by RT-PCR did not show retinopathy or positive *in situ* hybridization reactions for LSNV. Nor did shrimp in normal growth ponds show retinopathy, even when positive for LSNV by RT-PCR testing (Pratoomthai, 2007).

These results suggest that LSNV is a necessary component of MSGS but that another contributing pathogen(s) or factor(s) is required for the disease to occur. Potential partner pathogens include the new type of YHV and TGAV. In the interim, we recommend that postlarvae be screened for LSNV and that positive batches be eliminated for stocking grow-out ponds. This will potentially remove LSNV as a necessary component of MSGS.

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#### Addendum – August 5, 2009

The SEAFDEC International Workshop on Emerging Fish Diseases in Asia was held in Bangkok in December 2007. Since then, a number of important events have occurred that should be noted here. First, there is a movement to make shrimp virus names conform to those used by the International Committee on Taxonomy of Viruses. Accordingly, the virus referred to as IHHNV in this review is now officially known as *Penaeus stylirostris* densovirus (PstDNV) in the genus *Brevidensovirus* (Tattersall et al., 2005), HPV should be referred to as *Penaeus monodon* densovirus (PmDNV) which will probably be assigned to a new genus *Hepanvirus* (Tijssen, 2008), and monodon baculovirus (MBV) should be referred to as *Penaeus monodon* nucleopolyhedrovirus (PemoNPV; Theilmann et al., 2005).

With respect to diseases of *Penaeus vannamei*, it was recently shown that local Asian crabs and palaemonid shrimp could be infected with TSV and transmit it to *P. vannamei*, but no naturally infected specimens were found in a limited survey (Kiatpathomchai et al., 2008). For white muscle disease in *P. vannamei*, a very recent retrospective study of archived material (unpublished) found that some white muscle specimens from Vietnam and China that had tested negative for IMNV and PvNV tested positive for *Macrobrachium rosenbergii* nodavirus (MrNV) by RT-PCR. This was confirmed by further sequencing of the whole genome (2 RNA fragments). Although no bioassay experiments were carried out, affected ponds experienced high shrimp mortality, accompanied by whitened muscles but in the absence of IMNV and PvNV. Thus, Asian shrimp farmers should note that MrNV may be dangerous for *P. vannamei*, as recently reported for *P. indicus* and *P. monodon* (Ravi et al., 2009).

With respect to viruses of *P. monodon*, recent work on YHV revealed that there are at least five geographical types of the virus and that YHV Type 1 originally reported from Thailand is the only highly virulent type (Wijegoonawardane et al., 2008). It is followed by the less virulent YHV Type 2 (also called gill associated virus or GAV) from Australia. All other types are non-virulent. Curiously, YHV Type 1 has not been reported to be associated with massive mortality anywhere except in Thailand (Flegel, 2009). Since the 2007 workshop, we published our work on ASDD (Sakaew et al., 2008) and retinopathy associated with MSGS (Pratoomthai et al., 2008). In addition, our unusual YHV isolate discovered in MSGS ponds was not associated with MSGS and turned out to be a non-virulent recombinant type between YHV Types 1b and 5 (Gangnonngiw et al., 2009).

Finally, I wrote a review on the safety of exported shrimp products from the point of view of exotic disease transmission (Flegel, 2009) and two articles encouraging (and justifying) the continued use of traditional names for penaeid shrimp species (Flegel, 2007, 2008) rather than the new names proposed in 1997 (Perez Farfante and Kensley, 1997).

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