Pond-Level Risk Factors for White Spot Disease Outbreaks

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ABSTRACT

White spot disease (WSD) is a pandemic disease of crustaceans caused by White Spot Virus (WSSV). In the earlier stages of the pandemic, the scientific community hypothesised a number of potential risk factors for outbreaks based on information from other diseases and circumstantial evidence. As a result control strategies were recommended to farmers. Highly sensitive diagnostic methods were developed and allowed the detection of WSSV in shrimp broodstock, seed and wild animals leading to further interventions to reduce the risk of introducing the virus into ponds. Aspects of some of the hypothetical risk factors for WSD were tested through experimental trials. Field investigations were also carried out and provided further information on risk factors for outbreaks and the information generated was used to investigate WSSV transmission using mathematical modelling. In this review we summarise and discuss current knowledge of the pond level risk factors for WSD. The importance of different routes of WSSV entry to the pond has been examined. Pond preparation practices proved useful at eliminating the virus from the pond therefore reducing the risk of outbreaks. The importance of stocking WSSV positive post-larvae (PL) was also evaluated and it would appear that the prevalence rather than the mere presence or absence is more important as a predictor of WSD. WSSV presence in crabs and plankton was not associated with an increased risk of WSD in field studies. An association existed between water exchange and the occurrence of WSD cases in some farming systems. Commercial feed as a source of WSSV was also investigated. In one study a large proportion of feed samples tested were WSSV positive. Although no significant association between WSSV in feed and WSD was detected, the inclusion of WSSV-positive tissues in feed pellets should be avoided. The need for pond-level biosecurity is also discussed. Factors responsible for precipitating WSSV infection into a WSD outbreak were examined and poor shrimp health was associated with an increased risk of WSD. Similarly,

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stress was identified as a triggering factor for outbreaks and this was consistent with field observations that WSD cases may be preceded by higher pH and un-ionised [cb1]ammonia. Lower water temperature was associated with an increased risk of outbreaks through its effect on WSSV-induced apoptosis. Application of immunostimulants did not affect the risk for WSD whereas the use of vitamins was associated with a reduced risk of WSD in two separate epidemiological investigations. Factors affecting the epidemic within the pond are also discussed including the role of stocking density and removal of infectious shrimp. The need for further epidemiological studies for the identification of risk factors in different farming systems is discussed.

INTRODUCTION

WSD is a pandemic disease of crustaceans, which made its first appearance in China and Japan in 1993 (Nakano *et al*., 1994; Zhan *et al*., 1998). The disease then spread quickly to other countries in Asia and to the Americas (Flegel and Alday-Sanz, 1998; Lo and Kou, 1998; Mohan *et al*., 1998; Park *et al*., 1998; APHIS, 1999). Its devastating impact on the shrimp farming industry stimulated a quick response from the international scientific community. Control strategies were developed based on basic health control principles and on the knowledge gathered from other viral shrimp diseases (e.g., yellow head virus). These consisted of improving farm level biosecurity, excluding predators and competitors from the pond and reducing water exchange (Chanratchakool *et al*., 1995).

A virus was identified as the etiological agent of the disease (Inouye *et al*., 1994; Wongteerasupaya *et al*., 1995). Various names were used by different research groups. These were grouped under the name of White Spot baculovirus-complex (Lightner, 1996) and, following recommendations from the Office International des Epizooties (OIE), will be referred to as white spot syndrome virus (WSSV) in this review. Research groups worldwide developed a number of diagnostic tests for WSSV. Of these, polymerase chain reaction (PCR), a technique to identify the presence of WSSV DNA, was the most widely adopted because of its high sensitivity, especially the 2-step nested protocol. The 2-step PCR is capable of detecting the presence of WSSV in asymptomatic carriers (Lo *et al*., 1996). Nevertheless, the occurrence of actual disease as opposed to the presence of the virus in the shrimp can be detected only by histopathological identification of the characteristic inclusion bodies indicative of WSSV replication (Wongteerasupaya *et al*., 1995; Wang *et al*., 1999b).

Owing to its increased sensitivity compared with histopathology, PCR was widely used for the identification of the virus from a number of sources such as wild shrimp, crabs and other arthropods (Lo *et al*., 1996). This led to further recommendations for keeping the virus out of ponds.

Experimental studies and mathematical modelling were also used for testing or quantifying the effects of some of the proposed risk factors. However, to date only a limited number of field studies have been conducted and, of these, only a few used an epidemiological approach. Among these, the most extensive were two investigations conducted by the authors in Vietnam (Corsin *et al*., 2002b) and India and another study carried out on the east coast of India, the results of which have been recently published as a “Shrimp Health Management Extension Manual” (MPEDA/NACA, 2003). In these studies, the pond was used as the study unit.

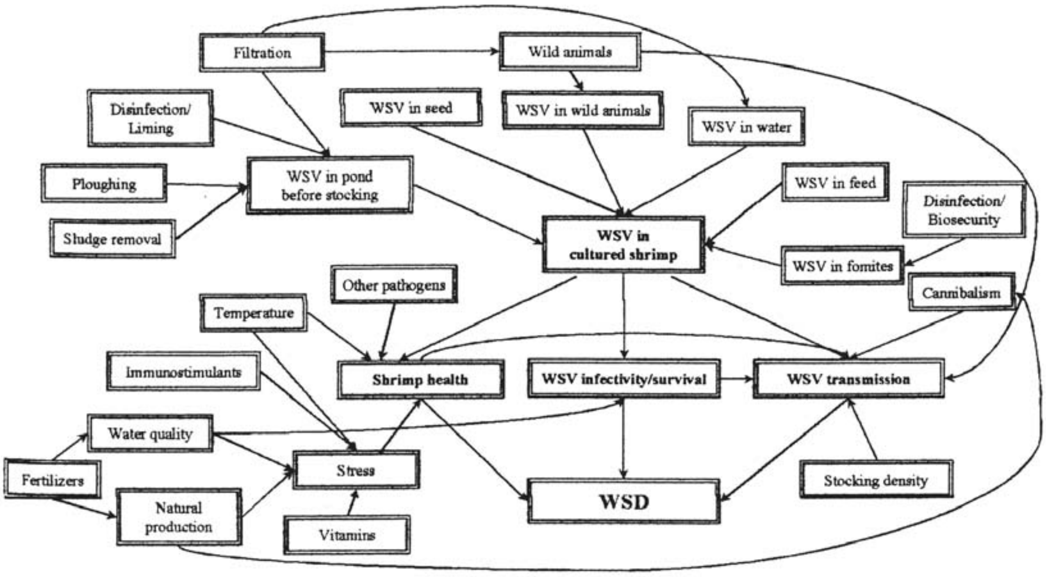

Figure 1. Causal web of WSD outbreaks in shrimp ponds.

This manuscript reviews the current knowledge of factors associated with WSSV infection or the occurrence of WSD, derived from experimental and field studies on penaeids. A causal web summarizing the effect of different pond-level risk factors for WSD outbreaks has been produced (Fig. 1). In this paper, we will refer to risk factors as variables that increase or decrease the risk of a WSD outbreak in a pond. Our objective is to clarify some of the issues that have been raised in previous studies with the aim of identifying control strategies for WSD outbreaks.

DEFINITION OF WSD CASES

To date, most authors involved in field investigations of WSD have used production, mortality, survival or length of production cycle, often in association with the presence of WSSV, as proxy measures for outbreaks (Lo *et al*., 1998; Withyachumnarnkul, 1999; Peng *et al*., 2001).

The OIE Diagnostic Manual for Aquatic Animal Diseases defines a WSD outbreak as the occurrence of "... high and rapid mortality accompanied by gross signs in moribund shrimp of white, initially circular, inclusions or spots in the cuticle ..." (OIE, 2000). The manual further stated that the detection of white spots is unreliable and preliminary diagnosis must include the detection of the characteristic WSD pathology consisting of intra-nuclear inclusion bodies in infected cells located in tissues of ectodermal and mesodermal origin (Momoyama *et al*., 1994; Wongteerasupaya *et al*., 1995; Lightner, 1996). Owing to the large proportion of farmers who engage in emergency harvest after the first detection of dead shrimp and the subsequent difficulties in observing high and rapid mortalities, the OIE definition of WSD is of limited value. This problem is exacerbated by the fact that, although the detection of inclusion bodies is a sign of disease at the animal level, the detection of infected individuals does not necessarily indicate an outbreak at a pond level.

In our studies, we developed a pond-level definition of WSD cases based on presence of both shrimp mortality and WSSV in harvested shrimp (Corsin *et al*., submitted). This definition, however, is most likely specific to the farming system investigated. For this reason, where possible, we include the definition used in the papers reviewed. This, however, limits the comparability of the results and we believe that a pond-level definition of WSD cases which generalizes a range of farming systems is urgently required.

SOURCES OF WSSV AND THEIR CONTROL

Since WSSV is a necessary cause for WSD, the most obvious way to prevent outbreaks is to keep the virus out of the farming system. Virus exclusion has been suggested by some authors (Lotz, 1997; Clifford, 1999). In order to evaluate the value of such a control strategy it is necessary to first examine the possible routes by which the virus can enter the faming system.

Pond

It has been proposed that the virus may be already present in the pond at the time of stocking. This has been tested in only one field study, conducted by the authors in India (unpublished data), in which wild crustaceans were collected before stocking and tested for the presence of WSSV. Only a small proportion of samples tested PCR positive and no association was found between WSSV detection and WSD outbreaks. There was, however, some indication that ponds with a history of disease outbreaks were more likely to experience disease (MPEDA/NACA 2003; unpublished data). Although this does not necessarily support the hypothesis that WSSV survives between crops, the possibility of such survival should not be ignored and steps should be taken to prevent spread between production cycles.

The effect of management practices and environmental conditions before stocking have also been investigated in epidemiological studies. Drying, soil removal and treating the pond with various substances before stocking have been recommended to farmers as a means of prevention by several research groups and extension workers. The purpose of such practices is to eliminate not only possible pathogens and their carriers, but also predators and competitors of the cultured shrimp. Laboratory studies have shown that WSSV can be rendered non-infective by drying or treating with chemicals such as sodium hypochlorite and povidone iodine (Chang *et al*., 1998, Maeda *et al*., 1998b).

A study conducted in India found that removal of bottom sludge, ploughing of soil when wet and liming were indeed associated with a reduced disease risk (MPEDA/NACA, 2003). Water filtration and disinfection before stocking were also found to reduce the risk of outbreaks (MPEDA/NACA, 2003). These findings are similar to the results from our Indian study, where applying at least one kind of pesticide (e.g., teaseed cake, neam cake, commercial treatments, etc.) before stocking was associated with a reduced risk of outbreaks (unpublished data).

Shrimp post-larvae

The presence of WSSV has been reported in post-larvae (PL) from different penaeid species and geographical regions (Kim *et al*., 1998; APHIS, 1999; Mushiake *et al*., 1999, Withyachumnarnkul, 1999; Thakur *et al*., 2002) and a number of possible routes of contamination or infection were hypothesised. The vertical transmission of the virus has been proposed (Mohan *et al*., 1997) and a thorough investigation of WSSV infection in reproductive organs of Penaeus monodon broodstock showed the presence of inclusion bodies in reproductive organs and eggs (Lo *et al*., 1997). Although infected eggs did not seem to develop, trans-ovarian infection was suggested (Lo *et al*., 1997, Lo and Kou 1998) and was supported by the observation that WSSV infection in broodstock was associated with infection in the offspring (Hsu *et al*., 1999, Peng *et al*., 2001). WSSV was also detected in Artemia used as feed for PL (Otta *et al*., 1999) and this was also proposed as a possible route of contamination, although it was later shown that feeding Artemia exposed to WSSV or commercial cysts testing WSSV positive did not lead to WSSV infection in P. monodon nauplii and P. indicus juveniles (Chang *et al*., 2002; Sahul Hameed *et al*., 2002).

The screening of PL for the presence of the virus and avoidance of positive PL was suggested as an intervention strategy on the basis of an association between stocking WSSV positive seed and WSD outbreaks (Nakano *et al*., 1994, Chanratchakool and Limsuwan, 1998, Lo *et al*., 1998, Satoh *et al*., 1999, Withyachumnarnkul, 1999; Liu *et al*., 2001; Peng *et al*., 2001). Although, none of this evidence came from an epidemiological study designed to investigate the effect of WSSV in shrimp seed on the occurrence of WSD while adjusting for the effect of other variables, some of these studies indicated that, at least in intensive systems, "heavy" WSSV infection (i.e., 1-step PCR or high prevalence of WSSV positive shrimp) in the seed might be a risk factor for WSD outbreaks (Withyachumnarnkul, 1999; Peng *et al*., 2001).

In our Vietnamese study, none of the PL samples stocked was WSSV positive (Corsin *et al*., 2001). Nevertheless, a major outbreak of WSD was experienced by 2/3 of the study ponds. We suggested that the PL might still have been WSSV-positive but this was not detected because of the allegedly poor sensitivity of the diagnostic method used (i.e., 1-step PCR of Kim *et al*., 1998). However, our study also indicated that major losses caused by WSD need not necessarily be associated with high levels of WSSV presence in PL. In our study in India, we found that 36 of the 73 PL batches stocked in 70 ponds, tested WSSV positive using a nested PCR protocol (Lo *et al*., 1996) and 3 of these were positive after the 1st step of amplification (Thakur *et al*., 2002). However, we found no association between WSSV in PL by either 1- or 2-step PCR and WSD outbreaks (Turnbull *et al*., 2002). In this study, however, we did not estimate the prevalence of WSSV in the PL batches stocked and the MPEDA/NACA project showed that stocking batches with a higher prevalence of WSSV in PL was associated with disease (MPEDA/NACA, 2003). The MPEDA/NACA results suggest that the prevalence of WSSV among PL might be more important than the mere detection of the virus in the batch. It is also possible that the association between WSSV in PL and WSD outbreaks is not consistent between farming systems. Stocking density, for example, may play a critical role in the effect of WSSV in PL. The system we studied in India was open with regular water exchange with little if any control over virus or carriers entering the pond. Such a system could either provide an alternative source of virus for the PL or the effect of virus coming in during water exchange may have masked any effect from WSSV positive PL.

Wild animals and water during production

The employment of nested PCR led to the detection of the virus in a large number of wild animals. Flegel and Alday-Sanz (1998) summarised the species susceptible to infection either by observation or experimental infection. Among these, there were not only shrimp, prawn and crab species but also planktonic organisms and insect larvae. Since then, many more species have been reported as potential carriers for WSSV and the occurrence of WSD in wild crustaceans was also demonstrated by histopathological examination (Wang *et al*., 1997; Hao *et al*., 1999). A transmission trial demonstrating the water-borne infection of shrimp from experimentally infected crabs was also conducted (Kanchanaphum *et al*., 1998). Although this study showed that infection was possible, the experiment was conducted with a high stocking density (i.e., approximately 15 crabs cohabiting with 50 shrimp per m^{-2}) and the degree of infection induced in the crabs was arguably higher than would normally occur under natural conditions. Based on these findings, interventions (e.g., fencing of the pond) were proposed to eliminate or reduce the exposure of the farmed shrimp to potential carriers.

In Vietnam, we found that ponds closer to the sea were at higher risk of being WSSV positive at harvest (Corsin *et al*., 2001). It was hypothesised that the virus came from the sea, possibly via wild shrimp, crabs or planktonic organisms. However, most of the wild crustacean samples tested WSSV negative by 1-step PCR. Furthermore, no significant association between the presence of wild crustaceans and an increase in the odds of detecting WSSV at harvest was identified (Corsin *et al*., 2001). On the contrary, the presence of crabs (*Hemigrapsus* spp.) during the first month of production and the size of the mud crabs collected at harvest were associated with a reduced risk of WSSV presence at harvest (Corsin *et al*., 2001). In the Indian study, the majority of crabs entered the pond after stocking. However, crab samples were collected from the dike outside the pond and it was assumed that the population of crabs entering the pond was homogeneous with those outside. Almost 10% of the crab samples were WSSV positive by nested PCR, however, this was not associated with either the presence of WSSV at harvest or WSD (unpublished data). In addition, the presence of crabs [cb2]during the production cycle and at harvest had a significant effect on reducing the risk of WSD outbreaks (unpublished data), supporting our findings in Vietnam.

On the basis of these findings it would be inappropriate to suggest that stocking crabs could reduce the risk of WSD. It is possible that the presence and size of crabs and the absence of WSSV or WSD are both associated with better environmental conditions in the pond. At least in the systems studied, PCR testing of crabs collected from ponds would appear to be of little help for predicting outbreaks.

The relation between the presence of non-crustacean animals and WSD was also investigated in our studies. If polychaetes were present [cb3]during the production cycle or if jelly fish were present at harvest, there was a significant increase in the risk of WSD (unpublished data). Whether this was due to the presence of infectious WSSV in those organisms or to an association with environmental variables has not been clarified and deserves further investigation.

Plankton has also been suggested as a potential cause of WSD outbreaks. WSSV detection in plankton has been reported by a number of authors (Lo *et al*., 1996; Ruangsri and

Supamattaya, 1999). It was also reported that filtering of the inflowing water reduced the risk of WSSV contamination in some systems (Lawrence *et al*., 2001). In India, we detected the presence of WSSV by PCR in the plankton samples from almost 10% of the ponds but no association with WSD occurrence was detected. These results might, however, be system specific and further studies should be carried out in more intensive systems.

In addition to zooplankton, water could also be a vehicle for free viral particles. Perhaps as a result of the difficulties in harvesting viruses from water, so far there have been no studies of the potential risk from WSSV in the incoming water. Free WSSV has been shown to be infectious for at least 5 days in seawater at 28°C (Maeda *et al*., 1998b) and up to 30 days at 30°C (Momoyama *et al*., 1998). Therefore, a number of investigations were conducted to develop effective disinfection protocols (Chang *et al*., 1998; Maeda *et al*., 1998b, Nakano *et al*., 1998).

In Vietnam we assessed the relation between water exchange and WSD by examining the temporal relationship between WSD cases and the total volume of water intake in the study ponds. Cases seemed to follow or coincide with periods of high water intake. However, this occurred only when a neap tide coincided with a full moon, possibly indicating an effect of the lunar cycle, and moulting, on the development of outbreaks (Corsin et al., submitted). When data were analysed on a pond by pond basis no significant association was detected (Corsin, 2001) possibly because of the regular and frequent water exchanges carried out in most of the ponds and of the difficulties of investigating the complex hydrography of the study area. There is a great deal of circumstantial evidence that water plays an important role in WSSV spread between ponds. However, it is reasonable to assume that the risk posed by water exchange is system-specific.

Feed

Experimental infection through the feeding of tissue from WSSV infected shrimp has been demonstrated by several research groups (Chou *et al*., 1998; Sahul Hameed *et al*., 1998; Momoyama *et al*., 1999). Nevertheless, in some artisanal farming systems, wild shrimp and crabs of unknown WSSV status are still used as feed (Corsin *et al*., 2001). There are few studies that have assessed the effect of feeding practices on WSD occurrence. In the MPEDA/NACA project there was no effect of feeding management on the risk of disease. However, in Vietnam we found that higher amounts of feed were associated with the occurrence of WSD (Corsin, 2001). Although this may have been associated with a higher probability of introducing WSSV into the pond, the effect of high feeding rates on water quality or as a proxy measure of stocking density cannot be ruled out (see below).

Commercial feed can also be PCR-positive for WSSV (Maeda *et al*., 1998a). In Vietnam we identified an association approaching significance between feeding a specific commercial brand of feed and the presence of WSSV in harvested shrimp (Corsin *et al*., 2001). In our Indian study, we found that 43% of the ponds were fed WSSV-positive commercial feed (Corsin *et al*., 2002a). Some brands of feed had a significantly higher proportion of WSSV-positive samples indicating that they either used WSSV-infected constituents more frequently or carried out less aggressive feed processing practices that increased the chances of viral DNA surviving in the feed. However, no significant association was detected between the WSSV status of feed and WSD occurrence. Furthermore, an experimental infection trial conducted by another research group showed that feeding of WSSV-positive shrimp headmeal did not lead to the detection of WSSV in the shrimp haemolymph (Pongmaneerat *et al*., 2001). In view of the fact that the oral route is the most effective method of WSSV transmission (Soto and Lotz, 2001), it is logical that the presence of WSSV DNA in commercial feed should be avoided and production of WSSV-free feed should be encouraged.

Other sources of infection during production: fomites and role of sanitation

The introduction of pathogens through fomites (i.e., non-infected vehicles) is a well documented risk and improvements in the biosecurity of farms have been suggested as a means of mitigation (Lotz, 1997; Mohan *et al*., 2002a).

In our studies, although we did not find a significant association between outbreaks and sharing equipment between ponds, sharing personnel was found to be associated with WSD to a level approaching significance (unpublished data). In view of these results, we believe that sharing equipment and personnel between ponds should be avoided.

How does WSSV presence develop into a WSD outbreak?

There are various routes by which WSSV can enter a pond and, at least in some farming systems, the majority of ponds harvest WSSV-positive shrimp. Since some of these positive ponds do not experience a WSD outbreak, there must be factors responsible for precipitating WSD outbreaks. Such precipitating factors may affect various combinations of the virus, shrimp and transmission rate.

Factors affecting the virus

A number of treatments have been recommended to farmers for their supposed "antiviral properties". This approach was criticized by one of the co-authors of this review (Mohan and Shankar, 1997) and, to date, there is no conclusive evidence that any substance is capable of controlling WSSV infectivity or survival without negatively affecting the cultured shrimp. It has also been suggested that water quality parameters may reduce WSSV infectivity but this is only likely to occur at levels beyond those encountered in production ponds (Chang *et al*., 1998; Maeda *et al*., 1998b).

Factors affecting the cultured shrimp

Water quality parameters are more likely to have an effect through interaction with the shrimp. An association between general shrimp health and WSD has been suggested by a number of authors. For this reason, methods for checking PL health have been used in many shrimp farming systems in the belief that PL health is a predictor for success of the crop. It was reported that stocking stronger PL was associated with improved yields in a WSSV endemic area (Samocha *et al*., 2001). In India the MPEDA/NACA project found an increased disease risk when poor quality seed was stocked. However, we failed to find an association between PL health or quality and crop success in our Vietnamese study (Corsin *et al*., 2001). In India we did not find any significant association between PL quality and WSSV in PL (Corsin *et al*., 2003) and neither quality nor WSSV were associated with WSD (Turnbull *et al*., 2002). It is also possible that, in some systems, PL quality either does not play a major role in the occurrence of WSD or the effect of WSSV in the PL is overwhelmed by other influences on disease outbreaks.

General shrimp health during production might also have an effect on WSD occurrence. In Vietnam we observed that ponds with relatively small shrimp at one month after stocking were more likely to be WSSV positive at harvest (Corsin *et al*., 2001). Although this may either be a cause or an effect, it is possible for the size and health of the shrimp to be predisposing factors to WSD.

Co-infection with other pathogens might also have an effect on WSD occurrence. Dual viral infection with WSSV and yellow head virus has been reported since the earlier stages of the pandemic (Wongteerasupaya *et al*., 1995) and more recently, multiple infection with other viruses was also reported (Madhavi *et al*., 2002; Manivannan *et al*., 2002). However, to our knowledge, no evidence of an association between multiple viral infection and WSD had ever been demonstrated. Some authors reported that vibriosis often preceded WSD (Hettiarachchi *et al*., 1999). However, our results indicated a reduced risk of WSD or crop failure when signs of bacterial infection are detected at harvest (Corsin *et al*., 2001; Mohan *et al*., 2002b).

It has also been suggested that exposure of shrimp to stressors increases the risk of WSD, since stressors can compromise the shrimp defence system (Takahashi *et al*., 1995). In the early stages of the pandemic, this association was based on circumstantial evidence (Chou *et al*., 1995, Flegel and Alday-Sanz, 1998). However, in later years the effect of stress was elucidated through experimental studies and stress caused by pereiopod excision or spawning was reported to be associated with WSD at the shrimp-level (Peng *et al*., 1998; Hsu *et al*., 1999). In Vietnam, although no significant association was detected with salinity, alkalinity and other water quality variables that might have acted as stressors, we found that WSD outbreaks were preceded or coincided with higher pH and un-ionised [cb4] ammonia (Corsin, 2001). High pH was also a significant variable in the MPEDA/NACA project, as was high salinity. Although stress seems to be the most likely explanation for these associations, it is also possible that these variables exert an effect through other mechanisms.

According to some authors, an increased resistance to stress and bacterial infections in shrimp could be achieved by feeding vitamins which in some cases act as immunostimulants (Merchie *et al*., 1997; Merchie *et al*., 1998; Lopez *et al*., 2003). In Vietnam we found that all the ponds in which vitamins were fed were WSSV-negative by 1-step PCR (Corsin *et al*., 2001). Analysis of the Indian data-set found a similar association with ponds feeding vitamins being less likely to experience a WSD outbreak (unpublished data).

Some authors also reported the beneficial effect of applying other immunostimulant products. The use of substances such as glucans, peptidoglycan, fucoidan and lipopolysaccharide has been reported to be successful in protecting shrimp from WSD under experimental conditions (Song *et al*., 1997; Itami *et al*., 1998; Takahashi *et al*., 1998; Chang *et al*., 1999; Takahashi *et al*., 2000). However, under field conditions in India, we found that the use of these substances had no significant effect on the occurrence of WSD (unpublished data).

In order to investigate the relationship between factors affecting cultured shrimp and WSD outbreaks, we need also to understand the relationship between WSSV and shrimp. A recent study suggested that WSSV leads to shrimp death by acting on mechanisms that regulate apoptosis (Granja *et al*., 2003). This study also showed that WSSV-induced apoptosis occurs at higher levels when shrimp are held at lower temperatures. This was a development from an earlier trial in which an association between lower temperature and WSD was demonstrated (Vidal *et al*., 2001). This observation was also made under field conditions. Circumstantial evidence gathered from several farming systems indicated that crops grown in warmer seasons were less likely to experience WSD outbreaks, although there are many potential reasons for this association other than temperature on its own. In Vietnam we also observed that WSD outbreaks coincided with or were preceded by lower water temperatures or drops in air temperature (Corsin *et al*., submitted). In Ecuador, farmers are already using water level as a way to control water temperature and WSD outbreaks (Lachlan, H., pers. comm.) which supports the validity of this association.

Factors affecting the epidemic within the pond

In addition to factors progressing WSSV infection through to a WSD outbreak, there are also variables that affect the development and maintenance of the epidemic within the pond. WSD in an individual animal does not necessarily indicate or predict an outbreak at a pond level (our observation).

In epidemiology, the basic reproductive ratio (R_0) is the number of individuals that can be infected by an infectious individual during its infectious period (Thrusfield, 1995) in a totally susceptible population. An epidemic can only develop when R_0 is higher than 1 (i.e., when, on average, each infectious animal infects more than one other animal) otherwise, the epidemic will die out.

Horizontal transmission of WSSV infection has been well documented and can occur through a variety of means. Water-borne infection has been reported by several research groups (Chou *et al*., 1995; Chou *et al*., 1998; Venegas *et al*., 1999; Wu *et al*., 2001) although cohabitation trials aimed at measuring the transmission coefficient were performed only recently (Soto and Lotz, 2001). Oral infection has also been demonstrated by several authors (Chou *et al*., 1998; Sahul Hameed *et al*., 1998; Momoyama *et al*., 1999; Wang *et al*., 1999a) and, cannibalism has also been suggested as a major route of infection, based on shrimp behaviour and results from mathematical modelling (Soto and Lotz , 2001).

Some of the variables associated with WSD may act through an effect on R_0. For example, water quality parameters, rather than acting as stressors, may influence WSSV infectivity and affect shrimp behaviour leading to a reduced rate of transmission. Similarly, the protective effect of crabs could be due to removal of moribund shrimp, thereby reducing spread of infection.

It is generally accepted that the R_0 of several diseases e.g. of terrestrial animals can also be reduced by decreasing stocking density. Although WSD outbreaks have occurred in all farming systems from intensive to extensive, an experimental study conducted on P. japonicus demonstrated that shrimp density may be a risk factor for WSD (Wu *et al*., 2001). This study, however, investigated stocking densities ranging between 73 and 260 shrimp m^{-2}, therefore did not reflect most farming systems where stocking densities would be lower. The effect of stocking density was also supported by mathematical models (Lotz and Soto, 2002) and by our field-based observations. In Vietnam, although PL density did not have an effect on the risk of WSSV at harvest (Corsin *et al*., 2001), we found that the first two ponds experiencing WSD were also the ones with the highest stocking density (Corsin *et al*., submitted) and this association was highly significant. Higher shrimp density during production also increased the risk of WSD outbreaks (Corsin 2001) and, in our Indian study, higher PL density was a risk factor for WSD (unpublished data).

The use of fertilisers was associated with decreased risk of WSD but we have no evidence for the mechanism involved. In India we detected a strong association between applying fertilisers either before stocking or during production and a reduced risk of WSD (unpublished data). In Vietnam we also found a decreased risk in ponds that had high amounts of chlorophyll b (Corsin, 2001). Similar results were obtained in the MPEDA/NACA project where applying fertilizers before stocking reduced the risk of disease. It is possible that using fertilizers decreases the chances of cannibalism by increasing natural production and reducing WSSV transmission (R_0). However, fertilizers are also used to encourage a plankton bloom, which shades the pond and incidentally reduces temperature fluctuations and absorbs potential toxic nutrients thereby reducing the effect of several potential stressors. Owing to the consistency of these results across studies, we believe that pond fertilization should be encouraged to limit WSD occurrence.

CONCLUSION

In this review we examined several variables for their importance as pond-level risk factors for WSD. Some of the many routes of WSSV entry, such as the presence of WSSV in PL, appeared to play an important role in the development of WSD outbreaks. However, this association is most likely system-specific. WSSV is indeed a necessary cause for WSD, but its presence, alone, is not sufficient for causing outbreaks, which are often the result of a complex interaction of factors. The complexity of WSD causation shown in Fig. 1 demonstrates the need for a holistic approach to disease control. WSSV entry into the pond should be prevented where possible, but care should also be taken when interpreting WSSV diagnostic techniques, which identify the presence of the pathogen rather than the disease. There are several management practices that can also affect the risk of outbreaks. Stocking density should be reduced where possible, as, in addition to being a stressor itself, it may also be important in exposing shrimp to additional stressors.

Interestingly, many of the putative risk factors hypothesized in the earlier stages of the pandemic have subsequently been shown to be associated with an increased risk for WSD outbreaks in ponds. This indicates that our understanding of the disease and of shrimp health in general has greatly improved in the last decades. Although WSD continues to be a major problem to shrimp farmers, its impact has been greatly reduced and this is at least partly due to the concerted efforts of the scientific community.

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