Shrimp Disease Control: Past, Present and Future

TIMOTHY W. FLEGEL[1], DONALD V. LIGHTNER[2], CHU FANG LO[3] and LEIGH OWENS[4]

[1]Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand
[2]Department of Veterinary Science & Microbiology, University of Arizona, Tucson, AZ 85721, USA.
[3]National Taiwan University, Rm 1216, Life Science Building, Taipei, Taiwan
[4]Department of Biomedical and Tropical Veterinary Sciences, James Cook University of North Queensland, Townsville, Queensland 4811, Australia

ABSTRACT

In the period from 1970-1990, the worldwide shrimp industry was dependent on post larvae (PL) from wild sources. Grow-out systems were relatively simple, water exchange rates were high and few disease agents were known. Diagnostic capability was low and antibiotics and chemical use was common. Explosive increase in global shrimp cultivation was accompanied by viral pandemics starting around 1987 and these quickly revealed the need for changes. The need transformed the shrimp farming industry. Beginning in the early 1990's, dependence on wild PL rapidly declined and use of domesticated stocks rapidly dominated. Many significant diseases were described and diagnostic tests developed. Biosecurity measures practiced in intensive, controlled farming systems became more common, and antibiotic use decreased and became more responsible. Molecular biology is leading to a better understanding of shrimp and pathogen biology and the interaction between the two. The combined result of all of these developments has been a continuing increase in production of cultivated shrimp. In the future, it is expected that the world shrimp industry will have ready access to a variety of domesticated, genetically improved shrimp stocks free of all significant pathogens. Both laboratory and pond-side diagnostic methods/kits will be available for the most significant shrimp diseases and test standardization will be improved. Biosecurity methods will also improve

Flegel, T.W., Lightner, D.V., Lo, C.F. and Owens, L. 2008. Shrimp disease control: past, present and future, pp. 355-378. *In* Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M. and Subasinghe, R.P. (eds.). Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines. 505 pp.

Corresponding author: sctwf@mahidol.ac.th

and will be applied in all systems. Intensive and super-intensive culture systems will become more competitive with more extensive, traditional methods. Overall production efficiency will be facilitated by a better understanding of shrimp, shrimp pathogens and microbial ecology, and by the use of novel and environmentally friendly antiviral and antibacterial agents.

INTRODUCTION

According to FAO statistics (Figure 1), world production of cultivated shrimp has increased steadily since the early 1980's. It is also known that the world shrimp fishery is not growing, while the demand for shrimp is increasing steadily. Only aquaculture can meet this increasing demand. Thailand presents a good example (Figure 2). Since a peak in 1982, captured shrimp production has declined slowly while aquaculture production has steadily grown.

Despite the explosive growth in world production of cultivated shrimp, there have also been staggering, periodic losses due to disease. A global shrimp survey by the Global Aquaculture Alliance (GAA) in 2001 revealed a rough overall loss to disease of approximately 22% in a single year. Given a total production of 700,000 metric tons in 2001 valued at roughly US$8 per kg, this translated into an estimate of about US$1 billion loss in a single year. This was probably a conservative estimate, since farms with very bad results may not have responded to the survey. Thus, a conservative estimate for the total loss to disease over the past 15 years may be in the order of US$15 billion. This illustrates the importance of disease control to the industry.

With respect to disease agents, the GAA survey revealed that 60% of losses were attributed to viruses and about 20% to bacteria (Figure 3). Thus, the majority of our effort on disease control (80%) should clearly be focused on viral and bacterial pathogens. Indeed, that has been the case as the following review of disease control work will exemplify. The control effort has emphasized prevention, and this has required the development of good diagnostic tools, trained personnel and a better understanding of the hosts and their pathogens.

THE PAST (~1970-1990)

The good early years (~1970-1986)

The first shrimp cultivation systems were extensive and used post-larvae (PL) from tidal flow or hand collection, usually from nearby geographical areas. Stocking densities were low, disease problems relatively few and production relatively low. In the early 1980's, explosive, large-scale shrimp production was made possible by development of the eyestalk ablation technique to stimulate maturation of captured female broodstock. At that point, the industry shifted to dependence on hatchery produced PL, and production volume expanded rapidly. At the same time, stocking density gradually increased, especially in Asia.

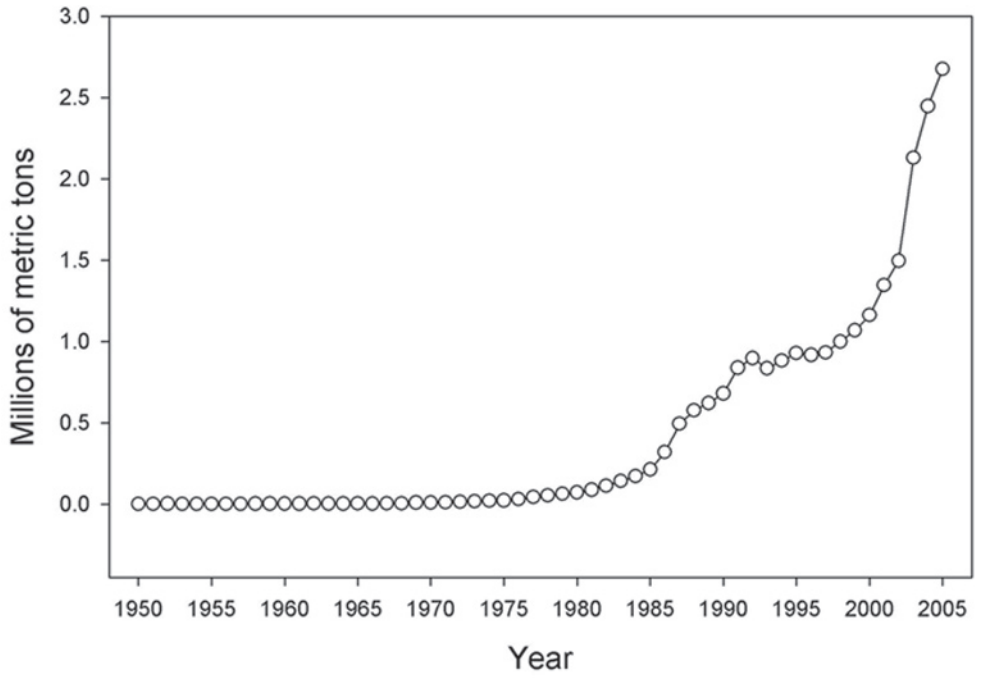

Figure 1. Global production of shrimp from aquaculture 1950 to 2005. Source FAO.

Figure 2. Comparison of captured and cultivated shrimp production in Thailand from 1985 to 2005. Source: http://www.biotec.or.th/shrinfo/.

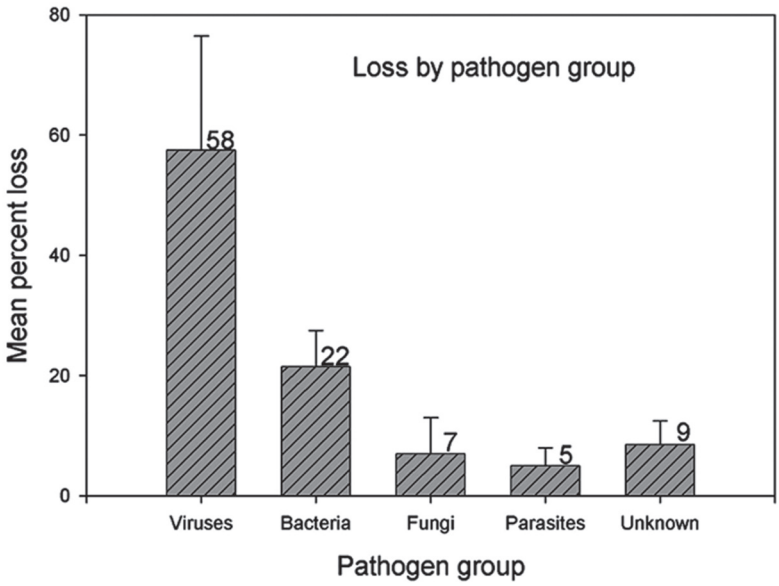

Figure 3. Relative economic loss to disease caused by various pathogen groups in the 2001 world shrimp industry survey by the Global Aquaculture Alliance.

In these early days, there were few disease control measures. Shrimp farmers used high rates of unfiltered water exchange and a wide range of chemicals and antibiotics, especially in the hatchery phase of production. There were few disease specialists available to help shrimp farmers, and diagnostic capabilities in most regions were limited. This was a vulnerable position as the industry grew exponentially with trends towards increasing farm densities in suitable farming areas and increasing rearing (stocking) intensity in individual ponds. Little was known of shrimp defense mechanisms, especially for viral pathogens. This eventually led to severe disease epidemics (epizootics) for which the industry was more or less unprepared.

The first serious disease outbreaks (~1987-1992)

The first widely reported shrimp disease epidemic was for monodon baculovirus (MBV) in Taiwan in the mid-1980's (Lin, 1989; Liao *et al.*, 1992). This was followed by epidemics caused by infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the Americas (Lightner *et al.*, 1983; Lightner, 1996b), yellow head virus (YHV) in Thailand (Chantanachookin *et al.*, 1993; Flegel, 1997) and Taura syndrome virus (TSV) in the Americas (Hasson *et al.*, 1995; Brock *et al.*, 1995; Brock *et al.*, 1997).

THE WHITE SPOT VIRUS ERA (~1993-2002)

While the shrimp industry was still struggling with MBV, IHHNV, YHV and TSV outbreaks, it was hit by an even bigger disaster with the arrival of white spot syndrome virus (WSSV). After its first appearance in China in 1992, it spread rapidly around Asia (Nakano *et al.*, 1994; Flegel, 1997; Flegel and Alday-Sanz, 1998) and eventually to the Americas, first in 1996, but later with devastating losses from 1999 onwards. Total losses for this virus alone have been estimated to be in the range of US$1 billion per year since the middle of the 1990's.

LESSONS LEARNED IN THE PAST

Serious viral disease outbreaks revealed that the shrimp industry had to be better prepared with more knowledge about shrimp and their pathogens so that disease prevention methods could be improved. This need shifted attention to biosecurity, that is, possible methods of cultivating shrimp in restricted systems designed to prevent the entry of potential pathogens. The industry also realized that a good number of disease outbreaks originated from careless transboundary movement of contaminated but grossly normal aquaculture stocks.

More than any other problem, the WSSV pandemic served as a "wake up" call that shocked the industry into concerted actions. The catastrophic losses had serious impacts on whole national economies in Asia and the Americas. They resulted in increased support for research on shrimp diseases (including epidemiology) and in increased farmer awareness of the need for biosecurity.

Research on shrimp defenses and shrimp pathogens increased sharply. Many diagnostic techniques were developed, particularly PCR and RT-PCR. Training programs were carried out (e.g. SEAFDEC and the University of Arizona) and shrimp domestication and breeding programs were started with *P. vannamei* and *P. stylirostris*. In addition, hatchery and farm biosecurity measures were improved.

HIGHLIGHTS FROM THE PRESENT (~2003-2005)

Current industry overview

The shrimp industry has been transformed since 2003 by the widespread use of specific pathogen free (SPF) stocks of domesticated and genetically improved *Penaeus vannamei*, also called the American whiteleg shrimp or *Litopenaeus vannamei*. It has now replaced *P. monodon* as the main cultivated species worldwide. World production of cultivated shrimp reached an all-time high of approximately 1 million metric tons in 2004, and it continues to rise. At the time of writing, the main problem for farmers did not appear to be loss due to disease and resulting low production, but to competition in the global market and resulting low farm-gate prices. This trend is driving competition based on production efficiency, so limiting loss to disease remains an important issue.

Reaping major benefits from the 1990's

As a result of research and training in the 1990's, there are many benefits for shrimp farmers in the new millennium. For example, industry dependence on wild stocks of PL and brooders is rapidly declining. Domesticated, genetically selected stocks are now available for *P. vannamei* and *P. stylirostris*. They are also being developed for *P. chinensis* (Hennig *et al.*, 2005; Pantoja *et al.*, 2005) and *P. monodon*. In addition, many important diseases have been described and commercial diagnostic tests are available for most. A good example is the shrimp viruses (Figure 4), several of which have been listed by OIE. Increased availability of tests, test facilities and trained personnel means that the capacity for diagnosis has greatly improved. Even more important, the response time for characterization and for development of detection methods for new pathogens is more rapid and the time to development and distribution commercial test kits has been greatly reduced. Finally, biosecurity measures are widely applied and shrimp farmers are highly aware of their importance. A major benefit has been that antibiotic and chemical use has decreased and become more responsible.

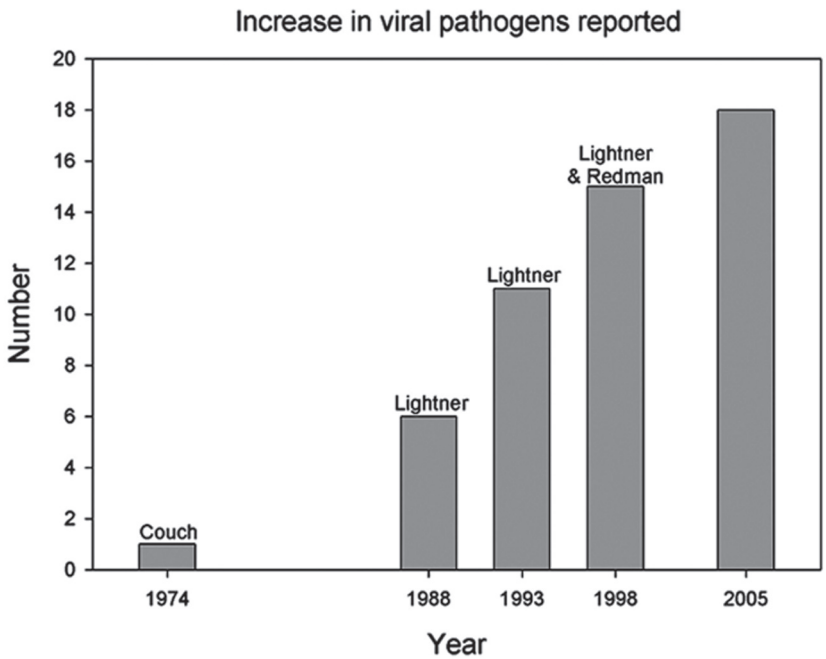

Figure 4. Increase in the number of shrimp viruses described over the period 1974 to 2005.

A number of diagnostic guides and manuals have played a fundamental role in improving diagnostic capability and are now used as standard laboratory references. These include, for example, the following: (i) Manual of diagnostic tests for aquatic animals (OIE, 2003, 2006), (ii) Disease control in fish and shrimp aquaculture in Southeast Asia (Inui and Cruz-Lacierda, 2002), (iii) Asia diagnostic guide to aquatic animal diseases (Bondad-Reantaso

et al., 2001), (iv) Diagnosis of shrimp diseases with emphasis on the black tiger prawn *Penaeus monodon (*Alday de Graindorge and Flegel, 1999), (v) A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp (Lightner, 1996a), (vi) A guide to common problems and diseases of cultured *Penaeus vannamei* (Brock and Main, 1994), (vii) A handbook of normal shrimp histology (Bell and Lightner, 1988).

Several useful resources are also available on epidemiology and for guidance on safe movement of aquaculture stocks such as the following: (i) Asia regional technical guidelines on health management for the responsible movement of live aquatic animals (FAO/NACA, 2000), (ii) Manual of procedures for the implementation of the Asia regional technical guidelines of health management for the responsible movement of live aquatic animals (FAO/NACA, 2001), (iii) Survey toolbox for aquatic animal diseases (Cameron, 2002), and (iv) Diseases in Asian Aquaculture (DAA) series (Volumes 1 to 5). In addition to these books, a substantial amount of related information is available on the internet via gateways such as <www.fao.org>, <www.enaca.org> and <www.was.org>. The trend towards e-learning exemplified by the Southeast Asian Fisheries Development Center (SEAFDEC) program is an especially promising training model that can lead to even wider availability of trained personnel.

Despite current progress in the shrimp industry, a number of outstanding needs still remain to be satisfied. These include the need for more widespread use and standardization of diagnostic tests; wider application and improvement in biosecurity; better control over transboundary movement of live crustaceans for culture; investigation of the efficacy of probiotics in full-scale field trials; full understanding of the host-pathogen interaction in shrimp; more work on epidemiology and on molecular ecological studies of the microbial dynamics in shrimp ponds and tanks.

SOME PROMISING NEW DIRECTIONS

Exciting new directions are opening up in shrimp research. These should lead to new, innovative products and methodologies that will help control shrimp diseases and make shrimp aquaculture more stable and more efficient.

New diagnostic methods

An important aspect of any disease control program is the easy and convenient availability of rapid and reliable pathogen detection methods together with the ability to interpret results and apply them in a proper manner in health management programs. PCR and RT-PCR methods have been very important in helping to control the spread of major shrimp disease agents, but they have the disadvantage of requiring sophisticated equipment and highly trained personnel. Recently, lateral flow chromatographic immunodiagnostic strips similar to common drug-store pregnancy tests have begun to appear for some shrimp diseases. Using these, unskilled farm personnel can easily diagnose shrimp disease outbreaks at the pond side. The strips are relatively cheap and give an answer within 10 minutes (www.

enbiotec.co.jp; www.shrimpbiotec.com) . Other methods comparable to PCR and RT-PCR are now available or being developed for single and dual to multiple viral detection, but they too currently require advanced equipment and personnel.

Probiotics

Many shrimp farmers add preparations of living bacterial cells called "probiotics" to their cultivation ponds prompted by advertising and sales personnel with assurances that these preparations will improve water quality or prevent diseases. Sometimes the sales campaign is supported by positive results from properly controlled laboratory tests, but very few, if any, by properly controlled field tests on commercial farms. The only properly controlled field tests on probiotics revealed no significant effect on measured water quality parameters (Boyd and Gross 1998), but few proper studies on the disease control have been done (Rengpipat *et al.*, 1998; Rengpipat *et al*., 2003). If large-scale trials on commercial farms give positive evidence of efficacy with cost benefit analysis, there would be a good reason to include probiotics as part of integrated health management schemes. If not, discontinued use would reduce production costs and thus improve competitiveness.

Immunostimulant

This topic is often confused with probiotics. In fact, it is a separate issue since immunostimulant efficacy is not dependent upon the presence of living cells. They may be crude preparations such as whole, dead microbial cells (e.g. yeasts or bacteria), semi-purified products from plants and microbes or pure chemicals (Raa, 1996). A large number of these products are on the market and some have been tested and shown to be effective in proper laboratory trials but few have been tested for efficacy in full-scale field trials where laboratory successes may not be realized (Sritunyalucksana *et al.*, 1999). As with probiotics, large-scale trials on commercial farms are needed to provide positive evidence of efficacy with cost benefit analysis, if they are to be rationally included in integrated health management schemes.

Quorum sensing control of bacterial virulence

One of the problems for those who argue in favor of probiotics, especially for water quality improvement, is the small number (in the order of a few hundred or thousand per ml) of probiotic cells usually added to a shrimp pond when compared to the total number of resident bacteria (in the order of 1 to 10's of millions per milliliter). How could cells accounting for only 0.01% of the whole bacterial population have a controlling effect on water quality? For the issue of disease, the situation may not be a simple matter of percentage of population. It has been known for many years that cross-talk takes place amongst microbes via minute quantities of natural chemical messengers. These messengers can sometimes prevent a bacterial pathogen from causing disease without actually killing it. The process is called quorum sensing (Hardman *et al.*, 1998) and it is an intense area of research in many fields including medicine and environmental science but little has yet been done in aquaculture (Flegel, 2002; Defoirdt *et al.*, 2004). Controlling disease by quorum sensing would be advantageous because the chemical messengers are mostly

common and innocuous natural substances that do not kill target cells. Thus, there is no pressure for selection of non-sensitive cells, as occurs with the use of antibiotics. There have been some promising recent results for control of virulence in *Vibrio* (Misciattelli *et al.*, 1998, Manefield *et al.*, 2000; Dunlap, 2002). Perhaps some of the current probiotics actually are efficacious because they work by quorum sensing. However, more research is needed to investigate this possibility, to isolate the most effective microbes and to determine whether the can control bacterial pathogens in a cost effective manner in commercial farm settings.

Phage therapy

Another exciting area of research focuses on the use of natural bacterial viruses (called bacteriophages) to control bacterial populations. This is not a new science. The practice has reached a high level of sophistication in Russia where a common therapy for human gastrointestinal diseases such as salmanellosis is to simply drink a solution containing a mixture of appropriate bacteriophages (e.g.,www.phageinternational.com) . The technology is only just being adopted in Western countries (Sulakvilidze *et al.*, 2001) and has not yet been applied widely in aquaculture (Karunasagar *et al.*, 2005). It may be particularly useful for application in shrimp hatcheries where larval death from bacterial infections can sometimes be high (Lavilla-Pitogo *et al.*, 1990).

Shrimp-virus interactions

Although we have some knowledge of how shrimp interact with bacterial and fungal pathogens (Soderhall *et al*., 1994; Soderhall and Thornqvist, 1997; Thornqvist and Soderhall, 1997; Soderhall, 1999; Bachere *et al*., 2000b; Sritunyalucksana and Soderhall, 2000; Young Lee and Soderhall, 2002; Stet and Arts, 2005), we still know very little about how they interact with viral pathogens (Flegel, 2001). Work in this area is just beginning, but it is developing rapidly and many interesting discoveries have been made that may lead to the development of new disease control mechanisms.

There is much recent work on shrimp-viral interaction at the molecular and genetic level. Many new shrimp genes have been discovered and we hope that some of these will lead to new products for disease control. One group in Thailand has a particular interest in the process called programmed cell death (PCD) that is also called apoptosis (Kerr *et al.*, 1972). This is a common program for cell suicide in all multicellular organisms and we have some evidence that viruses may sometimes trigger this program to cause shrimp death (Sahtout *et al.*, 2001; Khanobdee *et al.*, 2002; Wongprasert *et al*., 2003). If we can find the key to the trigger, perhaps we can find novel ways of blocking it to prevent shrimp death until harvest. A few genes in the complex pathway have been discovered in shrimp (Phongdara *et al.*, 2006; Bangrak *et al*., 2002; Bangrak *et al*., 2004; Tonganunt *et al.*, 2005). The viral pathogens can also carry genes involved in controlling this host-cell process (Wang *et al.*, 2004).

We know that low temperature triggers white spot virus disease (WSD) and that hyperthermia or hypothermia can prevent it in several WSSV-infected shrimp species (Vidal *et al.*, 2001; Guan *et al.*, 2003; Jiravanichpaisal *et al.*, 2004). Although the mechanism for hyperthermic

protection has been proposed to be an increased level of apoptosis (Granja *et al*., 2003), the proposal is controversial (Wu and Muroga, 2004). It has also been suggested that osmotic stress can trigger WSSV disease outbreaks (Liu *et al.*, 2006; Yu *et al.*, 2003) and this may be true for other viruses as well. For example, it has been found that survival of viral-infected shrimp improved 13.53% ($P<0.005$) upon feeding the osmolyte betaine (Owens *et al*., unpublished). The relative risk of not responding to osmotic shock was 0.23 for shrimp fed betaine and since this was less than 1, it indicated that feeding betaine was beneficial. It is known that cultured prawns have 43% lower levels of betaine than wild prawns. Does this mean that cultured prawns are often betaine deficient? Obviously, the role for osmolytes in the diet should be further explored. Together, these examples clearly illustrate how environmental changes may trigger viral disease outbreaks. Again, understanding the process may lead to novel control measures

Another intriguing phenomenon recently reported was protection against WSD by persistent IHHNV infections in *P. stylirostris* (Tang *et al.*, 2003a). No such protection was obtained with acutely infected *P. vannamei*. It is important to realize that infection was not prevented but that disease severity was reduced. A similar phenomenon has been reported for insect cells (Burivong, 2003), suggesting that it may be a general mechanism in arthropods. This raises the question as to whether such protection is a general benefit of persistent infections, and is the reason why dual to multiple viral infections are common in shrimp (Manivannan *et al.*, 2002; Chayaburakul *et al.*, 2004; Flegel *et al*., 2004) and other arthropods (Chen *et al*., 2004).

Results from the work on insect cells (Burivong *et al.*, 2004) and whole insects (Roekring, 2004; Roekring *et al.*, 2006) revealed that defective interfering particles (DIP) of viruses may be an important product of persistent viral infections and may play some role in the lack of disease expression. Thus, it was suggested that persistent infections may serve as a kind of "memory" to somehow reduce the severity of disease (*i.e*. result in disease tolerance) possibly by preventing viral triggered apoptosis, as earlier hypothesized (Flegel and Pasharawipas, 1998). In any case, the work has shown that research on insect models can complement that done on shrimp.

Finally, there are some curious results on viral protein expression in shrimp that survive viral challenges. Palaemonid shrimp tolerant to YHV produce the viral capsid protein (p20) and one envelope protein (gp64) but not the other (gp116) (Longyant *et al.*, 2005). Similar results are sometimes seen with *P. monodon* that survive YHV-challenge (T.W. Flegel, unpublished). This is particularly intriguing because gp116 and gp64 are products of a single messenger RNA that produces a polyprotein that is later cleaved into gp116 and gp64 before viral envelope assembly (Jitrapakdee *et al.*, 2003). Since gp116 precedes gp64 in the mRNA and in the polyprotein, it is curious that gp64 can be produced in the absence of gp116.

In summary, this and other research to understand the molecular mechanisms behind shrimp tolerance to viral infections (Luo *et al.*, 2003; Xu *et al*., 2003) is showing promise but is still in early stages. It is hoped that results from continued work with shrimp and from related studies in insects will lead to a better understanding of host-viral interaction and to the development of novel methods of disease control.

Shrimp vaccines

In the year 2000, Dr. Muroga's research group in Japan reported that Kuruma shrimp survivors (*Penaeus japonicus*) in a pond two months after a WSSV outbreak could not be killed by injection of WSSV. They found a factor(s) in shrimp hemolymph that could prevent naïve shrimp from dying upon injection of WSSV (Venegas *et al.*, 2000). They called this a quasi-immune response. It is important to understand that the WSSV outbreak survivors and the protected naïve shrimp were mostly infected with WSSV after challenge, so they were not protected from infection, but protected from disease. We still do not understand the basis of this "quasi-immune" response, but the report about it led many scientists to examine the possibility of protecting shrimp from viral pathogens by using so called "vaccines". Some examples follow on the testing of new reagents with shrimp. Perhaps the most valuable thing that will come out of these tests will be a better understanding of shrimp-viral interaction. That knowledge may lead to even better methods of viral disease control.

It is unfortunate that some scientists and commercial companies refer to shrimp viral protective reagents as "vaccines". This gives listeners the false impression that the reagents can stop shrimp from getting infected by a process resembling the one that occurs in vaccinated people and other vertebrate animals. The vertebrate process involves antibodies, and we know that antibodies do not occur in shrimp. In addition, "vaccinated" shrimp generally do get infected, they just don't get sick as a result. To distinguish the difference, it has been recommended that such shrimp reagents be called "tolerines" (Flegel and Pasharawipas, 1998) rather than vaccines. The term tolerine clearly indicates that tolerance to infection rather than prevention of infection will be the result of its use.

Two general types of tolerines have been studied in shrimp. The first type was developed in Thailand in the mid-1990s and is still marketed by Charoen Pokphand Co. Ltd. (CP) under the brand name SEMBVAC. However, their developmental work for the reagent was not published. SEMBVAC and other similar products consist of inactivated whole particles of WSSV (Bright Singh *et al.*, 2005). After ingesting these products, the shrimp acquire some tolerance to WSSV, they suffer less from disease after being infected and this tolerance is very long lasting (Bright Singh *et al.*, 2005). However, field practice has proven that the protection is not absolute and can be overridden by environmental effects (unpublished field results from the use of SEMBVAC in Thailand).

The other types of tolerine consist of individual or mixed protein subunits of viral particles that are administered either by injection or by mixing with shrimp feed (Namikoshi *et al.*, 2004; Witteveldt *et al*., 2004a; Witteveldt *et al*., 2004b; Li *et al.*, 2005). In contrast to feeding inactivated whole viral particles, the shrimp apparently do not become infected when challenged with the source virus and they must be boosted at 10 to 15 day intervals to remain protected. As far as we know, no commercial product is yet available. The main factors in determining marketability will probably be cost and safety issues. With respect to novel methods of reducing production cost, some Chinese scientists (Xu *et al*., unpublished) used baculovirus-infected silkworms to express WSSV proteins and then protected shrimp by mixing the ground-up silkworms with shrimp feed.

RNA interference

RNA interference is another advanced technology that has recently been used in the laboratory to protect shrimp from viral diseases (Robalino *et al.*, 2004; Robalino *et al.*, 2005; Tirasophon *et al*., 2005). The process consists of making small fragments of double-stranded RNA with sequences that match those of viral genes. When these are injected into shrimp or exposed to shrimp cells in culture, disease protection results. It remains to be seen whether the protection is long-lived and whether the shrimp remain infected after viral challenge. Although this concept is very interesting, issues of cost, safety and public acceptance of a genetic engineering technique remain unanswered.

Antiviral and antbacterial substances in shrimp

Since the publication of ground-breaking work on the presence of antimicrobial peptides (penaeidins) in shrimp (Destoumieux *et al.*, 1997, 1999), there has been a growing interest in the field (Bachère *et al.*, 2000a, 2003, 2004; Destoumieux *et al*., 2000a, 2000b; Destoumieux-Garzon *et al.*, 2001; Munoz *et al.*, 2003; Chen *et al.*, 2005; Gueguen *et al.*, 2006; Gross *et al.*, 2001; Kang *et al.*, 2004; Supungul *et al.*, 2004). Apart from potential production for general use as anti-bacterial, -fungal and –viral agents in human and veterinary medicine, production of these substances is induced in shrimp after they become infected with microbes. Understanding the nature of these molecules, their specific antimicrobial activities and specific ways of induction could lead to new methods of disease control. Since these substances are natural shrimp compounds with homologues from invertebrates to humans they would be much better therapeutics for shrimp aquaculture than antibiotics. This is because they are already present in shrimp and their use would not lead to the development of bacterial resistance to antibiotics that are important in human medicine.

In addition to antimicrobial substances, many other kinds of proteins are also produced in response to viral pathogens. For example, over 60 proteins are up-regulated in the hepatopancreas and hemocytes of WSSV-resistant shrimp when compared to normal shrimp (He *et al.*, 2004; Pan *et al.*, 2000, 2005). These include things such as C-type lectin and an interferon-like protein (He *et al*., 2004). These results raise questions as to whether and how these substances are involved in crustacean anti-viral activity. If so, is their production a common, generic response to all viruses or does it vary specifically with each virus? Answers to such questions may lead to development of novel disease prevention methods.

Molecular epidemiology

Recent molecular work on several shrimp viruses has shown that a variety of genetic types often exist for each, either within their endemic region or elsewhere. The most extensive work in this area has been done for WSSV, YHV, TSV and IHHNV. Less has been done with other viruses such as BP and HPV and little or none with the other 10 or so remaining viruses currently known. The work is important because the types sometimes differ in pathogenicity. A good example is yellow head virus (YHV). We now know that there are 6 or more types of YHV in Asia (Walker *et al*., 2001; Peter Walker, pers. comm.) but that

only one type (Type 1) is really dangerous. In Thailand, 3 native YHV types and possibly 1 type imported from Australia are known (Soowannayan *et al*., 2003; T. Flegel, pers. comm.). Fortunately, there is a good RT-PCR diagnostic kit (Farming Intelligene, Taipei) that is useful for distinguishing amongst these 3 types. People screening broodstock and larvae can identify stocks infected with the dangerous type. Similarly, TSV is known to exist in a variety of types (Nielsen *et al*., 2005; Tang and Lightner, 2005), some of which are more virulent than others (Erickson *et al*., 2002; Erickson *et al.*, 2005; Tang and Lightner, 2005).

Although work with WSSV has shown that portions of its genome targeted by common detection systems vary insufficiently to interfere with detection (Lo *et al*., 1999; Kiatpathomchai *et al*. 2005), it has also been found that other portions contain highly variable repeat regions that can be used for molecular epidemiological work (van Hulten *et al.*, 2000, van Hulten *et al.* 2001, Wongteerasupaya *et al*., 2003). When comparisons were made in Vietnam (Hoa *et al.*, 2005) there was some indication that a particular repeat-type predominated in outbreak ponds. Similarly, variants of IHHNV in *Penaeus monodon* from various geographical regions appear to differ in virulence for *Penaeus vannamei* (Tang *et al.*, 2003b). It is not yet known whether variation found in other viruses such as BP and HPV is associated with differences in virulence.

More work is needed in this field so that shrimp farmers can be provided with tools to assess whether the viral and other pathogen types they may find in their ponds are dangerous or not. In addition, development of generic analytical tools would also allow shrimp breeders to verify that their specific pathogen free stocks were free of a wide range of potential variants.

Shrimp breeding and selection

Work with both shrimp (Moss *et al*., 2005) and their insect relatives (Roekring *et al.*, 2006; Roekring, 2004) has shown that it is possible to select for populations that do not suffer disease when infected with dangerous viral pathogens. This has been done successfully for Taura syndrome virus (TSV) in *Penaeus vannamei* and for infectious hypodermal and hematopoietic necrosis virus in *Penaeus stylirostris*. It may not be that these shrimp generally resist infection but that they simply do not become diseased after infection. Thus, it is best to say that they are tolerant to the viruses rather than resistant. Unfortunately, selection of tolerance for one virus does not seem to result in tolerance for another (Moss *et al.*, 2005). Indeed, tolerance may be specific to the strain of virus used in the selection process. For example, a new type of TSV was found to be highly virulent for a family of shrimp selected for specific tolerance to a different type of TSV (Erickson *et al.*, 2005). If we can understand the basic mechanisms of viral tolerance, we would be able to design more effective selection programs to develop disease tolerant shrimp.

There is wide interest in the shrimp research community to set up an international shrimp genome project. If this could be achieved, it would be the best way to efficiently gather shrimp genome sequences into a central, public database and assemble genetic maps. As with other economically important species, availability of complete genome sequences and genetic maps will help breeders to accelerate the process of identifying and selecting for economically desirable traits.

THE FUTURE (BEYOND 2005)

Within the next 10 years, we believe that the world shrimp industry will be overwhelmingly dominated by cultivation of domesticated lines of shrimp that are free of most, if not all, of the significant shrimp diseases. Most of the stocks used will also be improved by genetic selection for growth rate and other desirable traits like disease tolerance. We already know from experience with *P. vannamei* that such stocks are highly successful when reared with good biosecurity and good management of feed and the pond environment. The latter can be achieved by following Good Aquaculture Practices recommended by the Global Aquaculture Alliance. This combination of SPF stocks and proper management greatly reduces the risk of disease outbreaks and essentially eliminates the need for chemotherapy.

We expect that an international shrimp genome project will become a reality and that it will succeed in producing one or more genetic maps and complete shrimp genome sequences. These will constitute a valuable resource for research on all aspects of shrimp biology, including health.

Both in the laboratory and on the farm, diagnostic methods/kits will be available for the most significant shrimp diseases and many of these will be multiplex tests that can be used at the pond-side by farmers themselves. This will be complemented by easier access to information via e-learning, e-monitoring and e-assistance. Many of the tolerines, probiotics, immunostimulants, quorum sensing modulators and other disease prevention methods or tools now under development may be introduced into the industry, and biosecurity methods will be widely applied. Thus, it is expected that biosecure, controlled-environment, intensive and super-intensive culture systems will become more common and will compete well with traditional pond methods.

Increasing sophistication in the industry will essentially mimic that which has occurred with other domesticated animals such as chickens, pigs, salmon and trout. The net result will probably be a consolidation of the industry and a fall in farm-gate profit margins. Overall, production efficiency will become increasingly important, and disease control will continue to be a major factor in maintaining high efficiency. Our vision for DAA-VII in 2008 is the widespread cultivation of healthy and healthful, domesticated shrimp in biosecure ponds with no significant negative impact on the environment. We expect that new pathogens will continue to be discovered, especially if living shrimp and other crustaceans are translocated without sufficient precautions. On the other hand, the impact of newly-emerging pathogens will be counteracted by the rapid response time for their characterization and for development of diagnostic tools and by the use of domesticated SPF stocks in biosecure rearing ponds.

REFERENCES

Alday de Graindorge, V. and Flegel, T.W. 1999. Diagnosis of shrimp diseases with emphasis on the black tiger prawn *Penaeus monodon*. Interactive CD. Multimedia Asia, Bangkok.

Bachère, E. 2003. Anti-infectious immune effectors in marine invertebrates: Potential tools for disease control in larviculture. *Aquaculture* 227:427-438.

Bachère, E., Destoumieux, D. and Bulet, P. 2000a. Penaeidins, antimicrobial peptides of shrimp: a comparison with other effectors of innate immunity. *Aquaculture* 191:71-88.

Bachère, E., Fuchs, R. and Soderhall, K. 2000b. Special issue on shrimp response to pathogens. *Aquaculture* 191: 1-270.

Bachère, E., Gueguen, Y., Gonzalez, M., De Lorgeril, J., Garnier, J., and Romestand, B. 2004. Insights into the anti-microbial defense of marine invertebrates: The penaeid shrimps and the oyster *Crassostrea gigas*. I*mmunological Reviews* 198:149-168.

Bangrak, P., Graidist, P., Chotigeat, W., Phongdara, A. 2004. Molecular cloning and expression of a mammalian homologue of a translationally controlled tumor protein (TCTP) gene from *Penaeus monodon* shrimp. *J. Biotechnol*. 108:219-226.

Bangrak, P., Graidist, P., Chotigeat, W., Supamattaya, K. and Phongdara, A. 2002. A syntenin-like protein with postsynaptic density protein (PDZ) domains produced by black tiger shrimp *Penaeus monodon* in response to white spot syndrome virus infection. *Dis. Aquat. Org.* 49:19-25.

Bell, T. and Lightner, D.V. 1988. Bell, T.A. and Lightner, D.V., 1988. A handbook of normal shrimp histology. World Aquaculture Society, Baton Rouge, LA.

Bondad-Reantaso, M.G., McGladdery, S., East, I. and Subasinghe, R.P. 2001. Asia diagnostic guide to aquatic animal diseases. *FAO Fisheries Technical Paper No*. 402, Supplement 2. Rome. FAO. 240 p.

Boyd, C and Gross, A. 1998. Use of probiotics for improving soil and water quality in aquaculture ponds, pp. 101-106. *In* Flegel T. (ed.). Advances in shrimp biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand.

Bright Singh, I.S., Manjusha, M., Somnath Pai, S. and Philip, R. 2005. *Fenneropenaeus indicus* is protected from white spot disease by oral administration of inactivated white spot syndrome virus. *Dis. Aquat. Org*. 66:265-270.

Brock, J.A., Gose, R., Lightner, D.V. and Hasson, K.W. 1995. An overview on Taura syndrome, an important disease of farmed *Penaeus vannamei*, pp. 84-94. *In* Browdy, C.L. and Hopkins, J.S. (eds.). Swimming through troubled water. Proceedings of the special session on shrimp farming. World Aquaculture Society, Baton Rouge, LA.

Brock, J.A., Gose, R.B., Lightner, D.V. and Hasson, K.W. 1997. Recent developments and an overview of Taura Syndrome of farmed shrimp in the Americas, pp. 267-283. *In* Flegel T.W. and MacRae, I.H. (eds.). Diseases in Asian Aquaculture III. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Brock, J.A. and Main. 1994. Brock, J.A. and Main, K., 1994. A guide to the common problems and diseases of cultured *Penaeus vannamei*. World Aquaculture Society, Baton Rouge, LA.

Burivong, P.2003. Densovirus: its kinetics of growth and interaction with infectivity of Dengue virus in mosquito cells. MSc. Thesis. Mahidol University.

Burivong, P., Pattanakitsakul, S.-N., Thongrungkiat, S., Malasit, P. and Flegel, T.W. 2004. Markedly reduced severity of Dengue virus infection in mosquito cell cultures persistently infected with *Aedes albopictus* densovirus (AalDNV). *Virology* 329:261-269.

Cameron, A., 2002. Survey toolbox for aquatic animal diseases; a practical manual and software package. Australian Centre for International Agricultural Research, Canberra. 375 p.

Chantanachookin, C., Boonyaratpalin, S., Kasornchandra, J., Direkbusarakom, S., Ekpanithanpong, U., Supamataya, K., Siurairatana, S. and Flegel, T.W. 1993. Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by "yellow-head" disease. *Dis. Aquat. Org.* 17:145-157.

Chayaburakul, K., Nash, G., Pratanpipat, P., Sriurairatana, S., Withyachumnarnkul, B. 2004. Multiple pathogens found in growth-retarded black tiger shrimp *Penaeus monodon* cultivated in Thailand. *Dis. Aquat. Org*. 60:89-96.

Chen, J.-Y., Chuang, H., Pan, C.-Y., Kuo, C.-M. 2005. cDNA sequence encoding an antimicrobial peptide of chelonianin from the tiger shrimp *Penaeus monodon*. *Fish Shellfish Immunol.* 18:179-183.

Chen, Y., Zhao, Y., Hammond, J., Hsu H.-T., Evans, J. and Feldlaufer, M. 2004. Multiple virus infections in the honey bee and genome divergence of honey bee viruses. *J. Invertebr. Pathol.* 87:84-93.

Defoirdt, T., Boona, N., Bossierb, P. and Verstraete, W. 2004. Disruption of bacterial quorum sensing: an unexplored strategy to fight infections in aquaculture. *Aquaculture* 240:69-88.

Destoumieux, D., Bulet, P., Loew, D., Van Dorsselaer, A., Rodriguez, J. and Bachère, E. 1997. Penaeidins, a new family of antimicrobial peptides isolated from the shrimp *Penaeus vannamei* (Decapoda). *J. Biol. Chem.* 272:28398-28406.

Destoumieux, D., Bulet, P., Strub, J.M., Van Dorsselaer, A. and Bachère, E. 1999. Recombinant expression and range of activity of penaeidins, antimicrobial peptides from penaeid shrimp. *Eur. J. Biochem./FEBS* 266:335-346.

Destoumieux, D., Munoz, M., Bulet, P. and Bachère, E. 2000a. Penaeidins, a family of antimicrobial peptides from penaeid shrimp (Crustacea, Decapoda). *Cell. Mol. Life Sci.* 57:1260-1271.

Destoumieux, D., Munoz, M., Cosseau, C., Rodriguez, J., Bulet, P., Comps, M. and Bachere, E. 2000b. Penaeidins, antimicrobial peptides with chitin-binding activity, are produced and stored in shrimp granulocytes and released after microbial challenge. *J. Cell Sci*. 113:461-469.

Destoumieux-Garzon, D., Saulnier, D., Garnier, J., Jouffrey, C., Bulet, P. and Bachere, E. 2001. Crustacean immunity. Antifungal peptides are generated from the C terminus of shrimp hemocyanin in response to microbial challenge. *J. Biol. Chem*. 276:47070-47077.

Dunlap, P.V. 2002. Quorum sensing and its potential application to vibriosis, pp. 59-71. *In* Lavilla-Pitogo, C.R, and Cruz-Lacierda, E.R. (eds.). Diseases in Asian aquaculture IV. Fish Health Section, Asian Fisheries Society, Manila.

Erickson, H.S., Poulos, B.T., Tang, K.F.J., Bradley-Dunlop, D. and Lightner, D.V. 2005. Taura syndrome virus from Belize represents a unique variant. *Dis. Aquat. Org*. 64:91-98.

Erickson, H.S., Zarain-Herzberg, M. and Lightner, D.V. 2002. Detection of Taura syndrome virus (TSV) strain differences using selected diagnostic methods: diagnostic implications in penaeid shrimp. *Dis. Aquat. Org*. 52:1-10.

FAO/NACA. 2000. Asia regional technical guidelines on health management for the responsible movement of live aquatic animals and the Beijing consensus and implementation strategy. FAO Fisheries Technical Paper No. 402. Rome, FAO. 53 p.

FAO/NACA. 2001. Manual of procedures for the implementation of the Asia regional technical guidelines of health management for the responsible movement of live aquatic animals. FAO Fisheries Technical Paper No. 402, Supplement 1. Rome, FAO. 106 p.

Flegel, T. and Alday-Sanz, V. 1998. The crisis in Asian shrimp aquaculture: current status and future needs. *J. Appl. Icthyol.* 14:269-273.

Flegel,T.W. 1997. Special topic review: Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. *World J. Microbiol. Biotechnol.* 13:433-442.

Flegel, T.W. 2001. The shrimp response to viral pathogens, pp. 190-214. *In* Browdy, C.L. and Jory, D.E. (eds.). The new wave. Proceedings of the special session on sustainable shrimp aquaculture, World Aquaculture 2001 , Orlando. World Aquaculture Society, Boca Raton.

Flegel, T.W. 2002. Emerging shrimp diseases and innovations to prevent their spread, pp. 137-149. *In* Lavilla-Pitogo, C.R. and Cruz-Lacierda, E.R. (eds.). Diseases in Asian Aquaculture 1V. Fish Health Section, Asian Fisheries Society, Manila.

Flegel, T.W., Nielsen, L., Thamavit, V., Kongtim, S. and Pasharawipas, T. 2004. Presence of multiple viruses in non-diseased, cultivated shrimp at harvest. *Aquaculture* 240:55-68.

Flegel, T.W. and Pasharawipas, T. 1998. Active viral accommodation: a new concept for crustacean response to viral pathogens, pp. 245-250. *In* Flegel, T.W. (ed). Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok.

Granja, C.B., Aranguren, L.F., Vidal, O.M., Aragon, L. and Salazar, M. 2003. Does hyperthermia increase apoptosis in white spot syndrome virus (WSSV)-infected *Litopenaeus vannamei*? *Dis. Aquat. Org*. 17:73-78.

Gross, P.S., Bartlett, T.C., Browdy, C.L., Chapman, R.W. and Warr, G.W. 2001. Immune gene discovery by expressed sequence tag analysis of hemocytes and hepatopancreas in the Pacific White Shrimp, *Litopenaeus vannamei*, and the Atlantic White Shrimp, *L. setiferus*. *Dev. Comp. Immunol.* 25:565-577.

Guan, Y.Q., Yu, Z.M. and Li, C.W. 2003. The effects of temperature on white spot syndrome infections in *Marsupenaeus japonicus*. *J. Invertebr. Pathol*. 83:257-260.

Gueguen, Y., Garnier, J., Robert, L., De Lorgeril, J., Janech, M., Bachère, E., Lefranc, M.-P., Mougenot, I., Gross, P.S., Warr, G.W., Cuthbertson, B., Barracco, M.A., Bulet, P., Aumelas, A., Yang, Y., Bo, D., Xiang, J., Tassanakajon, A. and Piquemal, D. 2006. PenBase, the shrimp antimicrobial peptide penaeidin database: Sequence-based classification and recommended nomenclature. *Dev. Comp. Immunol*. 30:283-288.

Hardman, A.M., Stewart, G.S. and Williams, P. 1998. Quorum sensing and the cell-cell communication dependent regulation of gene expression in pathogenic and non-pathogenic bacteria. *Antonie Van Leeu* 74:199-210.

Hasson, K.W., Lightner, D.V., Poulos, B.T., Redman, R.M., White, B.L., Brock, J.A., Bonami, J.R. 1995. Taura Syndrome in *Penaeus vannamei*: demonstration of a viral etiology. *Dis. Aquat. Org.* 23:115-126.

He, N., Liu, H. and Xu, X. 2004. Identification of genes involved in the response of haemocytes of *Penaeus japonicus* by suppression subtractive hybridization (SSH) following microbial challenge. *Fish Shellfish Immunol.* 17:121-128.

Hennig, O.L., Arce, S.M., Moss, S.M., Pantoja, C.R. and Lightner, D.V. 2005. Development of a specific pathogen free population of the Chinese fleshy prawn, *Fenneropenaeus chinensis*: Part II. Secondary quarantine. *Aquaculture* 250:579-585.

Hoa, T.T.T., Hodgson, R.A.J., Oanh, D.T.H., Phuong, N.T., Preston, N.J. and Walker, P.J. 2005. Genotypic variations in tandem repeat DNA segments between ribonucleotide reductase subunit genes of white spot syndrome virus (WSSV) isolates from Vietnam, pp. 339-351. *In* Walker, P., Lester, R. and Bondad-Reantaso, M.G. (eds.). Diseases in Asian Aquaculture V. Fish Health Section, Asian Fisheries Society, Manila.

Inui and Cruz-Lacierda (eds.). 2002. Disease control in fish and shrimp aquaculture in Southeast Asia. SEAFDEC, Ilo-Ilo, Philippines.

Jiravanichpaisal, P., Soderhall, K. and Soderhall, I. 2004. Effect of water temperature on the immune response and infectivity pattern of white spot syndrome virus (WSSV) in freshwater crayfish. *Fish Shellfish Immunol.* 17:265-275.

Jitrapakdee, S., Unajak, S., Sittidilokratna, N., Hodgson, R.A.J., Cowley, J.A., Walker, P.J., Panyin, S. and Boonsaeng, V. 2003. Identification and analysis of gp116 and gp64 structural glycoproteins of yellow head nidovirus of *Penaeus monodon* shrimp. *J. Gen. Virol.* 84:863-873.

Kang, C.-J., Wang, J.-X., Zhao, X.-F., Yang, X.-M., Shao, H.-L. and Xiang, J.-H. 2004. Molecular cloning and expression analysis of Ch-penaeidin, an antimicrobial peptide from Chinese shrimp, *Fenneropenaeus chinensis*. *Fish Shellfish Immunol.* 16:513-525.

Karunasagar, I., Vinod, M.G., Kennedy, B., Vijay, A., Deepanjali, K.R., Umesha, K.R. and Karunasagar, I. 2005. Biocontrol of bacterial pathogens in aquaculture with emphasis on phage therapy, pp. 535-542. *In* Walker, P.J., Lester, R.G. and Bondad-Reantaso, M.G. (eds.). Diseases in Asian Aquaculture V. Fish Health Section, Asian Fisheries Society, Manila.

Kerr, J.F.R., Wyllie, A.H. and Currie, A.R. 1972. Apoptosis: A basic biological phenominal with wide ranging implication in tissue kinetics. *Br. J. Cancer* 26:239-257.

Khanobdee, K., Soowannayan, C., Flegel, T.W., Ubol, S. and Withyachumnarnkul, B. 2002. Evidence for apoptosis correlated with mortality in the giant black tiger shrimp *Penaeus monodon* infected with yellow head virus. *Dis. Aquat. Org*. 48:79-90.

Kiatpathomchai, W., Taweetungtragoon, A., Jittivadhana, K., Wongteerasupaya, C., Boonsaeng, V., Flegel, T.W. and Meth, J.V. 2005. Target for standard Thai PCR assay identical in 12 white spot syndrome virus (WSSV) types that differ in DNA multiple repeat length. *J. Virol. Meth.* 130:79-82.

Lavilla-Pitogo, C.R., Baticados, M.C.L., Cruz-Lacierda, E.R., de la Pena, R. 1990. Occurence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture* 91:1-13.

Li, H.-X., Meng, X.-L., Xu, J.-P., Lu, W. and Wang, J. 2005. Protection of crayfish, *Cambarus clarkii*, from white spot syndrome virus by polyclonal antibodies against a viral envelope fusion protein. *J. Fish Dis*. 28:285-291.

Liao, I.C., Su, M.S., Chang, C.F. 1992. Diseases of *Penaeus monodon* in Taiwan: a review from 1977 to 1991, pp. 113-137. *In* Fulks, W. and Main, K.L. (eds.). Diseases of Cultured Penaeid Shrimp in Asia and the United States. Oceanic Institute, Honolulu, HI.

Lightner, D.V. 1996a. Lightner, D.V., 1996. A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquaculture Society, Baton Rouge, LA.

Lightner, D.V. 1996b. Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas. *Rev. Sci. Tech*. 15:579-601.

Lightner, D.V., Redman, R.M. and Bell, T.A. 1983. Infectious hypodermal and hematopoietic necrosis, a newly recognized virus disease of penaeid shrimp. *J. Invertebr. Pathol*. 42:62-70.

Lin, C.K. 1989. Prawn culture in Taiwan, What went wrong? *World Aquaculture* 20:19-20.

Liu, B., Yu, Z., Song, X., Guan, Y., Jian, X. and He, J. 2006. The effect of acute salinity change on white spot syndrome (WSS) outbreaks in *Fenneropenaeus chinensis*. *Aquaculture* 253:163-170.

Lo, C.F., Hsu, H.C., Tsai, M.F., Ho, C.H., Peng, S.E., Kou, G.H. and Lightner, D.V. 1999. Specific genomic DNA fragment analysis of different geographical clinical samples of shrimp white spot syndrome virus. *Dis. Aquat. Org.* 35:175-185.

Longyant, S., Sithigorngul, P., Chaivisuthangkura, P., Rukpratanporn, S., Sithigorngul, W. and Menasveta, P. 2005. Differences in susceptibility of palaemonid shrimp species to yellow head virus (YHV) infection. *Dis. Aquat. Org*. 64:5-12.

Luo, T., Zhang, X., Shao, Z. and Xu, X. 2003. PmAV, a novel gene involved in virus resistance of shrimp *Penaeus monodon*. *FEBS Letters* 551:53-57.

Manefield, M., Harris, L., Rice, S.A., de Nys, R. and Kjelleberg, S. 2000. Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. *Appl. Environ. Microbiol.* 66:2079-2084.

Manivannan, S., Otta, S.K., Karunasagar, I. and Karunasagar, I. 2002. Multiple viral infection in *Penaeus monodon* shrimp postlarvae in an Indian hatchery. *Dis. Aquat. Org.* 48:233-236.

Misciattelli, N., Jones, D., Simoes, N., Latchford, J. and Bridson, P. 1998. Manipulation of bacterial populations in shrimp larval cultures fed artificial diets, pp. 101-105. *In* Flegel, T.W. (ed.). Advances in shrimp biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok.

Moss, S.M., Doyle, R.W. and Lightner, D.V. 2005. Breeding shrimp for disease resistance: challenges and opportunities for improvement, pp. 379-393. *In* Walker, P.J., Lester, R.G. and Bondad-Reantaso, M.G. (eds.). Diseases in Asian Aquaculture V. Proceedings of the 5th Symposium on Diseases in Asian Aquaculture. Asian Fisheries Society, Manila.

Munoz, M., Vandenbulcke, F., Gueguen, Y. and Bachere, E. 2003. Expression of penaeidin antimicrobial peptides in early larval stages of the shrimp *Penaeus vannamei*. *Dev. Comp. Immunol.* 27:283-289.

Nakano, H., Koube, H., Umezawa, S., Momoyama, K., Hiraoka, M., Inouye, K. and Oseko, N. 1994. Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus*, in Japan in 1993: Epizootiological survey and infection trials. *Fish Pathol*. 29:135-139.

Namikoshi, A., Wu, J.L., Yamashita, T., Nishizawa, T., Nishioka, T., Arimoto, M. and Muroga, K. 2004. Vaccination trials with *Penaeus japonicus* to induce resistance to white spot syndrome virus. *Aquaculture* 229:25-36.

Nielsen, L., Sang-oum, W., Cheevadhanarak, S. and Flegel, T.W. 2005. Taura syndrome virus (TSV) in Thailand and its relationship to TSV in China and the Americas. *Dis. Aquat. Org.* 63:101-106

OIE. 2003. Manual of diagnostic tests for aquatic animals. 2nd edtition. World Organization for Animal Health, Paris.

OIE. 2006. Manual of diagnostic tests for aquatic animals. 3rd edition.World Organization for Animal Health, Paris.

Pan, D., He, N., Yang, Z., Liu, H. and Xu, X. 2005. Differential gene expression profile in hepatopancreas of WSSV-resistant shrimp (*Penaeus japonicus*) by suppression subtractive hybridization. *Dev. Comp. Immunol.* 29:103-112.

Pan, J., Kurosky, A., Xu, B., Chopra, A.K., Coppenhaver, D.H., Singh, I.P. and Baron, S. 2000. Broad antiviral activity in tissues of crustaceans. *Antiviral Res*. 48:39-47.

Pantoja, C.R., Noble, B., Lightner, D.V., Song, X., Xia, L., Gong, H. and Wilkenfeld, J. 2005. Development of a specific pathogen-free (SPF) population of the Chinese fleshy prawn *Fenneropenaeus chinensis* - Part 1: Disease pre-screening and primary quarantine. *Aquaculture* 250:573-578.

Phongdara, A., Wanna, W. and Chotigeat, W. 2006. Molecular cloning and expression of caspase from white shrimp *Penaeus merguiensis*. *Aquaculture* 252:114-120.

Raa, J. 1996. The use of immunostimulatory substances in fish and shellfish farming. *Rev. Fish. Sci.* 4:229-288.

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S. and Menasveta, P. 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture* 167:301-313.

Rengpipat, S., Tunyanun, A., Fast, A.W., Piyatiratitivorakul, S. and Menasveta, P. 2003. Enhanced growth and resistance to *Vibrio* challenge in pond-reared black tiger shrimp *Penaeus monodon* fed a *Bacillus* probiotic. *Dis. Aquat. Org*. 55:169-173.

Robalino, J., Bartlett, T., Shepard, E., Prior, S., Jaramillo, G., Scura, E., Chapman, R.W., Gross, P.S., Browdy, C.L. and Warr, G.W. 2005. Double-stranded RNA induces sequence-specific antiviral silencing in addition to nonspecific immunity in a marine shrimp: convergence of RNA interference and innate immunity in the invertebrate antiviral response? *J. Virol.* 79:13561-11371.

Robalino, J., Browdy, C.L., Proir, S., Metz, A., Parnell, P., Gross, P. and Warr, G. 2004 Induction of antiviral immunity by double-stranded RNA in a marine invertebrate. *J. Virol.* 78:10442-10448.

Roekring, S. 2004. Study on invertebrate host-viral interaction using a mosquito-parvovirus model. Ph.D. Thesis. Mahidol University.

Roekring, S., Flegel, T.W., Kittayapong, P. and Malasit, P. 2006. Challenging successive mosquito generations with a densonucleosis virus yields progressive survival improvement but persistent, innocuous infections. *Dev. Comp. Immunol*. 30: 878-892.

Sahtout, A.H., Hassan, M.D. and Shariff, M. 2001. DNA fragmentation, an indicator of apoptosis, in cultured black tiger shrimp *Penaeus monodon* infected with white spot syndrome virus (WSSV). *Dis. Aquat. Org.* 44:155-159.

Soderhall, K. 1999. Invertebrate immunity. *Dev. Comp. Immunol.* 23:263-442.

Soderhall, K., Cerenius, L. and Johansson, M.W. 1994. The prophenoloxidase activating system and its role in invertebrate defence. *Ann. N. Y. Acad. Sci*. 712:155-161.

Soderhall, K. and Thornqvist, P.O. 1997. Crustacean immunity--a short review. *Dev. Biol. Stand.* 90:45-51.

Soowannayan, C., Flegel, T.W., Sithigorngul, P., Slater, J., Hyatt, A., Cramerri, S., Wise, T., Crane, M.S.J., Cowley, J.A. and Walker, P.J. 2003. Detection and differentiation of yellow head complex viruses using monoclonal antibodies. *Dis. Aquat. Org.* 57:193-200.

Sritunyalucksana, K., Sithisarn, P., Withayachumnarnkul, B. and Flegel, T.W. 1999. Activation of prophenoloxidase, agglutinin and antibacterial activity in haemolymph of the black tiger prawn, *Penaeus monodon*, by immunostimulants. *Fish Shellfish Immunol.* 9:21-30.

Sritunyalucksana, K. and Soderhall, K. 2000. The proPO and clotting system in crustaceans. *Aquaculture* 191:53-69.

Stet, R.J. and Arts, J.A. 2005. Immune functions in crustaceans: lessons from flies. *Devel. Biologicals* 121:33-43.

Sulakvelidze, A., Alavidze, Z. and Morris, J.G. Jr. 2001. Bacteriophage therapy. *Antimicrob. Agents Chemother*. 45:649-659.

Supungul, P., Klinbunga, S., Pichyangkura, R., Hirono, I., Aoki, T. and Tassanakajon, A. 2004 Antimicrobial peptides discovered in the black tiger shrimp *Penaeus monodon* using the EST approach. *Dis. Aquat. Org.* 61:123-135

Tang, K.F.J., Durand, S.V., White, B.L., Redman, R.M., Mohney, L.L. and Lightner, D.V. 2003a. Induced resistance to white spot syndrome virus infection in *Penaeus stylirostris* through pre-infection with infectious hypodermal and hematopoietic necrosis virus - a preliminary study. *Aquaculture* 216:19-29.

Tang, K.F.J. and Lightner, D.V. 2005. Phylogenetic analysis of Taura syndrome virus isolates collected between 1993 and 2004 and virulence comparison between two isolates representing different genetic variants. *Virus Res*. 112:69-76.

Tang, K.F.J., Poulos, B.T., Wang, J., Redman, R.M., Shih, H.H. and Lightner, D.V. 2003b. Geographic variations among infectious hypodermal and hematopoietic necrosis virus (IHHNV) isolates and characteristics of their infection. *Dis. Aquat. Org*. 53:91-99.

Thornqvist, P.O. and Soderhall, K. 1997. Crustacean immune reactions, a short review, pp. 203-218. *In* Flegel, T.W. and MacRae, I.H. (eds.). Diseases in Asian Aquaculture III. Fish Health Section, Asian Fisheries Society, Manila.

Tirasophon, W., Roshorm, Y. and Panyim, S. 2005. Silencing of yellow head virus replication in penaeid shrimp cells by dsRNA. *Biochem. Biophys. Res. Comm.* 334:102–107.

Tonganunt, M., Phongdara, A., Chotigeat, W. and Fujise, K. 2005. Identification and characterization of syntenin binding protein in the black tiger shrimp *Penaeus monodon*. *J. Biotechnol.* 120:135-145.

van Hulten, M.C., Tsai, M.F., Schipper, C.A., Lo, C.F., Kou, G.H. and Vlak, J.M. 2000. Analysis of a genomic segment of white spot syndrome virus of shrimp containing ribonucleotide reductase genes and repeat regions. *J. Gen. Virol.* 81 Pt 2:307-316.

van Hulten, M.C., Witteveldt, J., Peters, S., Kloosterboer, N., Tarchini, R., Fiers, M., Sandbrink, H., Lankhorst, R.K. and Vlak, J.M. 2001. The white spot syndrome virus DNA genome sequence. *Virology* 286:7-22.

Venegas, C.A., Nonaka, L., Mushiake, K., Nishizawa, T. and Muroga, K. 2000. Quasi-immune response of *Penaeus japonicus* to penaeid rod-shaped DNA virus (PRDV). *Dis. Aquat. Org.* 42:83-89.

Vidal, O.M., Granja, C.B., Aranguren, F., Brock, J.A. and Salazar, M. 2001. A profound effect of hyperthermia on survival of *Litopenaeus vannamei* juveniles infected with white spot syndrome virus. *J. World Aquacul. Soc.* 32:364-372.

Walker, P.J., Cowley, J.A., Spann, K.M., Hodgson, R.A.J., Hall, M.R. and Withyachumanarnkul, B. 2001. Yellow head complex viruses: transmission cycles and topographical distribution in the Asia-Pacific region, pp. 292-302.. *In* Browdy, C.L. and Jory, D.E. (eds.). The New Wave, Proceedings of the special session on sustainable shrimp culture, Aquaculture 2001. World Aquaculture Society, Baton Rouge, LA.

Wang, Z., Hu, L., Yi, G., Xu, H., Qi, Y. and Yao, L. 2004. ORF390 of white spot syndrome virus genome is identified as a novel anti-apoptosis gene. *Biochem. Biophys. Res. Comm*. 325:899-907.

Witteveldt, J., Cifuentes, C.C., Vlak, J.M. and Van Hulten, M.C.W. 2004a. Protection of *Penaeus monodon* against white spot syndrome virus by oral vaccination. *J. Virol.* 78:2057-2061.

Witteveldt, J., Vlak, J.M., van Hulten, M.C.W. 2004b. Protection of *Penaeus monodon* against white spot syndrome virus using a WSSV subunit vaccine. *Fish Shellfish Immunol*. 16:571-579.

Wongprasert, K., Khanobdee, K., Glunukarn, S., Meeratana, P. and Withyachumnarnkul, B. 2003. Time-course and levels of apoptosis in various tissues of black tiger shrimp *Penaeus monodon* infected with white spot syndrome virus. *Dis. Aquat. Org*. 55:3-10.

Wongteerasupaya, C., Pungchai, P., Withyachumnarnkul, B., Boonsaeng, V., Panyim, S., Flegel, T.W. and Walker, P.J. 2003. High variation in repetitive DNA fragment length for white spot syndrome virus (WSSV) isolates in Thailand. *Dis. Aquat. Org.* 54:253-257.

Wu, J.L. and Muroga, K., 2004. Apoptosis does not play an important role in the resistance of "immune" *Penaeus japonicus* against white spot syndrome virus. *J. Fish Dis*. 27, 15-21.

Xu, Z.K., Wyrzykowski, J., Alcivar-Warren, A., Argue, B.J., Moss, S.M., Arce, S.M., Traub, M., Calderon, F.R.O., Lotz, J. and Breland, V. 2003. Genetic analyses for TSV-susceptible and TSV-resistant Pacific white shrimp *Litopenaeus vannamei* using M1 microsatellite. *J. World Aquacul. Soc.* 34:332-343.

Young, Lee S. and Soderhall, K. 2002. Early events in crustacean innate immunity. *Fish Shellfish Immunol* 12:421-437.

Yu, Z.M., Li, C.W. and Guan, Y.Q. 2003. Effect of salinity on the immune responses and outbreak of white spot syndrome in the shrimp *Marsupenaeus japonicus*. *Ophelia* 57:99-106.