Status of shrimp diseases and advances in shrimp health management

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ABSTRACT

Disease has had a major impact on shrimp aquaculture since shrimp farming became a significant commercial entity in the 1970s. Diseases due to viruses, rickettsial-like bacteria, true bacteria, protozoa, and fungi have emerged as major diseases of farmed shrimp. Many of the diseases caused by bacteria, fungi and protozoans are now managed using improved culture practices, routine sanitation, and the use of probiotics and chemotherapeutics. However, the virus diseases have been far more problematic to manage and they have been responsible for the most costly epizootics. Because of their socioeconomic significance to shrimp farming, seven of the nine crustacean diseases currently listed (and two of three proposed for listing) by the World Organisation for Animal Health (= Office International des Epizooties or the OIE) are virus diseases of shrimp. The development and export of Specific Pathogen Free (SPF) stocks of *Penaeus vannamei* (the Pacific white shrimp) from the USA to the major shrimp farming countries of Latin America and SE Asia is cited by FAO as being the main contributor to the industry's recovery and subsequent expansion following the viral pandemics of the early 1990's. The development of SPF stocks of *P. vannamei* is the topic of this review.

Key words: shrimp diseases, shrimp health management, aquaculture, OIE, SPF

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INTRODUCTION

As recently as a decade ago, much of the world's production of farmed shrimp was directly or indirectly dependent on wild shrimp stocks for the "seed" stock used to populate its farms. In the Americas the most common practice was the collection and use of "wild seed" (postlarvae or PLs), while in Asia it was more typical to produce PLs from wild-sourced broodstock. While the practice of relying on the sea to provide its seed stock worked well for nearly two decades, the practice provided the industry with little protection from significant losses due to infectious diseases, such as those that were ultimately responsible for the major global shrimp disease pandemics that began around 1991-1992 (Flegel, 2006; Lightner, 2005; Lightner *et al*., 2009).

DOMESTICATED STOCKS - THE FIRST STEP TO SPF STOCKS

By the mid-1970s a number of penaeid shrimp research programs were developing culture systems and methods to close the life cycle of several penaeid shrimp species in captivity (Forester and Beard, 1974; Wickins and Beard, 1978; Salser *et al*., 1978; Aquacop, 1983; Moore and Brand, 1993). Some of these early research groups and institutions were successful in growing, maturing, mating, spawning, and producing progeny from founder shrimp stocks that had been reared for a full generation in captivity. Despite the early successes in developing captive breeding populations of penaeid shrimp at these various facilities, most of the shrimp farming industry remained dependent on the direct or indirect use of wild or captive-wild shrimp stocks for the PLs used to stocks its farms (Argue and Warren, 1999; Lightner, 2005; FAO, 2006). Nonetheless, during this period (~1980 to ~2000), the industry was experiencing much of its initial rapid growth. For example, before WSSV was introduced into Ecuador in 1999, more than 100,000 people were involved in the collection of wild postlarvae from the littoral zone for use in stocking Ecuador's more than 175,000 ha of shrimp ponds (Rosenberry, 2006).

The reasons for the dependence of the shrimp farming industry on wild shrimp stocks for seed were partially technical, but mostly economic. In most large shrimp farming regions of the Americas, the PL requirements were highly seasonal. Hatcheries (called "laboratories" in most of Latin America) were expensive to build, staff and run, and the seasonal requirements for PLs left them operating below capacity for lengthy periods each year. Further, wild PLs ("wild seed") could be obtained in large numbers seasonally (and often when needed most for seasonal stocking plans for farms) and for lower cost than hatchery produced PLs ("lab seed"). Another reason, with both economic and technical implications, was that the prevalence of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in captive-wild *Penaeus vannamei* (the Pacific white shrimp) broodstock typically increased the longer the captive-wild stocks were held in maturation and/or hatchery facilities (Motte *et al*., 2003; Lightner *et al*., 2009). This made persistently IHHNV-infected captive-wild broodstock essentially worthless within 2-3 months of use as broodstock due to their declining performance. The use of *P. vannamei* broodstock with high IHHNV prevalence resulted in poor survival of infected larvae and the production of poor quality PLs (Lightner and Redman, 1998; Motte *et al*., 2003). The surviving PLs ("lab seed" or "maturation seed") had a very high IHHNV prevalence relative to wild PLs. Ponds stocked with such PLs typically had poorer production levels due to the development of IHHNV-caused Runt-Deformity Syndrome (RDS) than did ponds stocked with wild PLs. Long term and increasing problems with IHHNV, and subsequently with Taura Syndrome (caused by TSV), and the arrival of White Spot Disease (caused by WSSV) in 1999 to Central America, Mexico, Ecuador and Peru resulted in rapid changes in shrimp farming strategies in the Americas. The yellow head and white spot disease pandemics in Asia that began about 1992 and the paucity of domesticated SPF stocks of Asian penaeid shrimp species, forced the worlds' shrimp farming industries to change how shrimp are farmed (Lightner, 2005; FAO, 2006; Lightner *et al*., 2009).

FAO (2006) credits the development and export from producers in the USA of SPF (specific pathogen-free) *P. vannamei* and *Penaeus stylirostris* (the Pacific Blue Shrimp) for this paradigm shift in shrimp farming. The FAO report goes on to comment that while the export of SPF shrimp stocks from the USA to Asia and elsewhere in the world may not have been significant in their quantity or total value, their impact has been considerable on both the total quantity of shrimp produced and on global shrimp pricing. The FAO report concludes that without the import and use of USA-produced SPF shrimp stocks it is arguable if Asia's major shrimp producing countries could have recovered from disease outbreaks and the severe shortage of healthy wild-caught broodstock of native penaeids, much less grown to achieve the record levels of production that currently characterize the Asian shrimp farming sector (FAO, 2006).

In the wake of the extraordinary losses that occurred as a result of the viral pandemics of the early 1990's, the industry began to mature into a much more sustainable, technology-based industry. The industry has largely recovered from the major viral pandemics and it has begun a new phase of rapid growth (FAO, 2006). The adoption of new shrimp farming technologies and the abandonment of practices which posed high disease risks have contributed to the industry's recovery and current expansion. Among the most notable changes in culture practices has been the shift of the industry away from using wild stocks for seed production to the use of domesticated stocks. This has been a consequence of the ever increasing incidence in wild shrimp stocks of diseases like WSSV, IHHNV, and other significant diseases that negatively affect broodstock or their progeny (Lightner, 2005; Flegel, 2006). This has made the collection of wild postlarvae (PLs) and wild adult broodstock for the production of PLs for use as seed stock, a risky practice. With the declining dependence of the industry on wild stocks in Asia and in the Americas, the use of domesticated lines of specific pathogen-free (SPF) *P. vannamei* recently surpassed *Penaeus monodon* (the Giant Black Tiger Shrimp) as the dominant farmed shrimp species in Asia. This paradigm switch in the species being farmed occurred within 5 years after the first SPF *P. vannamei* stocks were introduced in quantity to Asia. The use of SPF *P. vannamei* has led to improved production and predictable crops virtually everywhere that was once dominated by the culture of *Penaeus chinensis* (the Chinese White Shrimp) or *P. monodon*. Hence, the most significant single advance in shrimp health management was perhaps the development of domesticated lines of SPF *P. vannamei.*

DEVELOPMENT OF SPF STOCKS

The term "SPF" was in widespread use in large number of terrestrial animal, aquatic animal and plant agriculture industries prior to its being applied to shrimp aquaculture (Wyban, 1992; Wyban *et al*., 1992; Lotz, 1992; Carr *et al*., 1994; Pruder *et al*., 1995; Lotz, 1997a, 1997b; Moss and Moss, 2009). SPF culture practices were commonplace in the poultry, swine and trout producing industries for many years and are still used as a way to avoid disease in otherwise susceptible stocks, when no other means of therapy or prevention (without the increased costs or other problems associated with vaccination) are available (Zavala, 1999). The application of the SPF concept to shrimp farming was a relatively recent event and it occurred well after the technologies had been developed that were necessary to close the life cycle of the penaeid shrimp in the laboratory and begin the process of producing domesticated breeding lines of penaeid shrimp. The development of SPF shrimp stocks became possible with the simultaneous development of the necessary infrastructure, in terms of biosecurity, diagnostic methods and trained personnel, to successfully select founder populations of candidate SPF stocks from wild or cultured stocks and domesticate those stocks, following the ICES Guidelines (ICES, 1995), in the absence of specific disease agents under biosecure conditions (Moss and Pruder, 1999; Bullis and Pruder, 1999; Lightner, 2003a, 2003b, 2005; Lee and O'Bryen, 2003; Scarfe *et al*., 2006; Lightner *et al*., 2009; Moss and Moss, 2009).

The first SPF stocks developed by the U.S. Marine Shrimp Farming Program (USMSFP) were developed in the spirit of the ICES Code (The International Council for the Exploration of the Sea; Code of Practice to Reduce the Risks of Adverse Effects Arising from the Introduction on Non-indigenous Marine Species - Bartley *et al*., 1996) (Table 1) (Wyban *et a*l., 1992; Carr *et al*., 1994; Pruder *et al*., 1995). The determination of which specific pathogens the selected stocks of candidate SPF shrimp were to be free of was based on a working list of pathogenic, diagnosable, and excludable pathogens. The SPF list necessarily changed over time as new diseases, such as those due to WSSV, TSV, IMNV and others, emerged and caused or showed the potential to cause serious pandemics (Lightner *et al*., 2009). The most current working list for the U.S. Marine Shrimp Farming Program includes 10 viruses or virus groups (WSSV, the YHV group, TSV, IHHNV, hepatopancreatic parvovirus (HPV), *Baculovirus penaei* (BP), monodon baculovirus (MBV), baculoviral mid-gut gland necrosis (BMN), and infectious myonecrosis (IMNV) and *Penaeus vannamei* nodavirus (*Pv*NV)), certain classes of parasitic protozoa (microsporidians, haplospordians, and gregarines), and the bacterial agent of necrotizing hepatopancreatitis, or NHP (Table 2). The USMSFP list closely approximates the OIE listed diseases of penaeid shrimp, with all seven of the currently OIE-listed virus diseases of shrimp (and one viral and one bacterial disease being considered for listing) being on the USMSFP list.

Table 1. Recommended steps in the ICES guidelines for risk reduction in aquatic species introductions (modified from Sindermann, 1988, 1990 and Lightner, 2005).

Original ICES Guidelines	Adapted to SPF shrimp development
1. Conduct comprehensive disease study in native habitat.	1. Identify stock of interest (i.e., cultured or wild).
2. Transfer {founder stock} system in recipient area.	2. Evaluate stock's health/disease history.
3. Maintain and study closed system population.	3. Acquire and test samples for specific listed pathogens (SLPs) and pests.
4. Develop broodstock in closed system.	4. Import and quarantine founder (F0) population; monitor F0 stock.
5. Grow isolated F1 individuals; destroy original introductions.	5. Produce F1 generation from F0 stock.
6. Introduce small lots to natural waters - continue disease study.	6. Culture F1 stock through critical stage(s); monitor general health and test for SLPs.
	7. If SLPs, pests, other significant pathologies are not detected, F-1 stock may be defined as SPF and released from quarantine.

To begin the process of developing an SPF stock a candidate wild or farmed stock of interest was identified (Fig. 1). If available, samples of the stock were taken and tested using the most appropriate diagnostic and pathogen detection methods available for the specific pathogens of concern. If none were found, a founder population (F_0) of the "candidate SPF" stock was acquired and reared in primary quarantine. During primary quarantine, the F_0 stock was monitored for signs of disease, sampled, and tested periodically for specific pathogens. If any pathogens of concern were detected, the stock was destroyed. Those stocks that tested negative for pathogens of concern through primary quarantine (which ran from 30 days to as much as one year for some stocks) were moved to a separate secondary quarantine facility for maturation, selection, mating, and production of a second (F_1) generation. The F_1 stocks were maintained in quarantine for further testing for specific pathogens of concern. Those that tested negative were designated as SPF, and used to produce domesticated lines of SPF shrimp (Moss *et al*., 2003; Lightner, 2005; Lightner *et al*., 2009) (Fig. 1). With this practice, the definition for SPF shrimp stocks produced by the USMSFP meant that the stock of interest had at least two years of documented historical freedom of the disease agents listed on its working list of specific pathogens, that the stock has been cultured in biosecure facilities, and that the stock was either cultured under conditions where the listed disease agents would have produced recognizable disease if any were present and/or that the stock has been subjected to routine surveillance and testing for the listed pathogens (Lightner, 2005; Lightner *et al*., 2009) . Those pathogens on the USMSFP SPF list have also met

Table 2. Current U.S. Marine Shrimp Farming Consortium (USMSFC) working list of "specific" and excludable pathogens of American penaeids and Asian penaeids for 2008-2009 (adapted from and Lightner *et al*., 2009).

Pathogen Type	Pathogen/Pathogen Group	Pathogen Category[a]
Viruses	* WSSV - white spot syndrome virus (Nimaviridae, new family)[b]	C-1
	* YHV, GAV, LOV - the Oka viruses (Roniviridae, new family)[b]	C-1
	* TSV - (Dicistroviridae, new family in the picornavirus superfamily) [b]	C-1
	* BP[c] - an occluded enteric baculovirus	C-2
	* MBV[c] - an occluded enteric baculovirus	C-2
	BMN[c] - a nonoccluded enteric baculo-like virus	C-2
	* IHHNV - a systemic parvovirus	C-1
	SMV - an enteric parvovirus	C-2
	** HPV - enteric parvoviruses	C-2
	* IMNV - Infectious myonecrosis virus (putative totivirus)	C-1,2
	PvNV - *Penaeus vannamei* nodavirus	C-2
Procaryotes	** NHP-bacterium - Alpha proteobacteria	C-2
Protozoa	Microsporidians	C-2
	Haplosporidians	C-2
	Gregarines	C-3

* OIE listed pathogen (OIE 2008).

** Listed as "under study" (OIE 2008).

[a] Pathogen category (modified from Lotz *et al*., 1995), with C-1 pathogens defined as excludable pathogens that can potentially cause catastrophic losses in one or more American penaeid species; C-2 pathogens are serious, potentially excludable; and C-3 pathogens have minimal effects, but may be excluded from breeding centers, hatcheries, and some types of farms.

[b] Mayo, 2002a, 2002b.

[c] The 1995 Committee report on virus taxonomy (Murphy *et al*., 1995) removed crustacean baculoviruses from the *Baculoviridae* and assigned them to a position of unknown taxonomic position. Nonetheless, BP, MBV, and BMN are most like members of the *Baculoviridae* (Faquet *et al*., 2005), and for practicality, they are listed here as baculoviruses.

certain criteria including: (a) the pathogen(s) must be excludable; (b) adequate diagnostic and pathogen detection methods are available; and (c) that the pathogen(s) poses significant threat of causing disease and production losses, criteria which are also among those required for disease listing by the OIE.

After the criteria set forth in the ICES Code had been met and a particular stock is declared SPF of specified diseases/pathogens, maintenance of SPF status required that the domesticated SPF stocks be the subject of a routine surveillance program. To be functional,

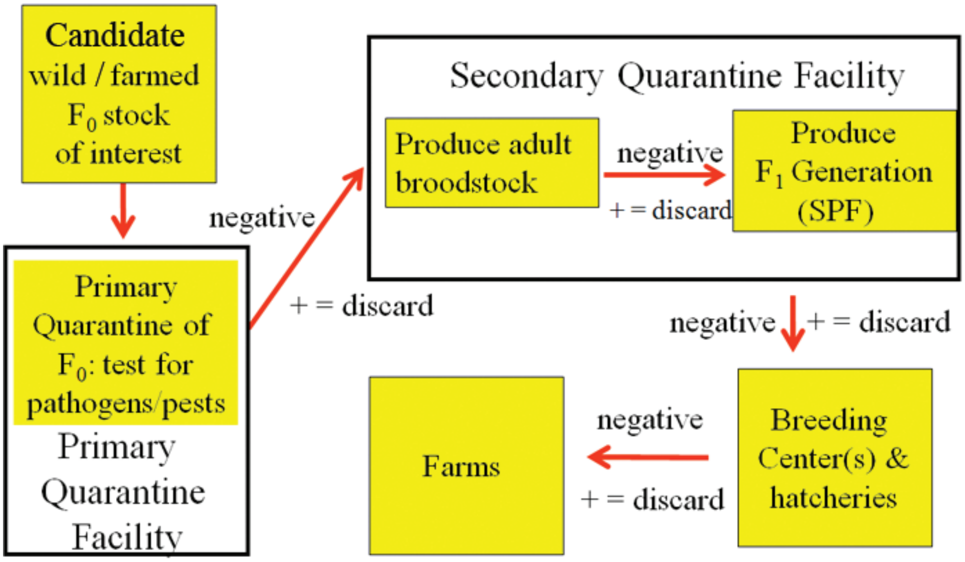

Figure 1. Steps to SPF stock development as developed by the U.S. Marine Shrimp Farming Program

an SPF breeding program must have a surveillance program with both regularly scheduled targeted and general (passive) surveillance components. Molecular diagnostic methods have become as important as classical methods (such as routine histopathology and microbiology) to the shrimp culture industry in recent years, and are especially applicable to routine surveillance programs that are necessary to support claims of disease freedom (such as with SPF stocks) and to monitor shrimp stocks in farms (Subasinghe *et al*., 2004; OIE, 2006; OIE, 2008; Lightner, 2005; Lightner *et al*., 2009).

Because the term SPF is poorly understood and often misused, the term "high health" has also been borrowed from other animal producing industries for use with shrimp to designate shrimp stocks that were developed as SPF, and which may be free of infection by specific disease agents, but which are no longer contained within a designated biosecure SPF facility (Pruder *et al*., 1995). SPF and high health stocks of *P. vannamei* were introduced by the USMSFP and used successfully in U.S. shrimp farms in the mid-1990's and this resulted in nearly doubling the production per crop that had been previously obtained at the same farms in previous years when the farms cultured non-selected lines of *P. vannamei*, which in previous crops, had been persistently affected by RDS due to chronic infection by IHHNV (Wyban, 1992; Pruder *et al*., 1995; Lotz *et al*., 1995; Moss *et al*., 2003).

Beginning in 1999, significant quantities of SPF *P. vannamei* had been introduced into East Asia and found to perform well. By 2006, nearly 3 million metric tonnes of the marine penaeid shrimp were being produced from farms and these shrimp accounted for almost half of the world's total shrimp supply (FAO, 2006). More than half (~57%) of that 3 million pounds was made up of *P. vannamei.* Ironically, more *P. vannamei* were farmed in 2006 in Asia, where the species was introduced, than in the Americas where it is native. FAO in its 2006 publication, "State of world Aquaculture," credited the development and export (from producers in the USA) of SPF *P. vannamei* for this paradigm shift in shrimp farming. The use of SPF *P. vannamei* has led to less disease, improved survival and predictable crops virtually everywhere that was previously dominated by *P. chinensis* and *P. monodon* (FAO, 2006).

SPECIFIC PATHOGEN RESISTANT (SPR) SHRIMP STOCKS

After domesticated SPF stocks were developed some SPF/SPR stocks were successfully developed and applied to management of certain shrimp virus diseases, specifically Taura Syndrome (TS) and Infectious Hypodermal and Hematopoietic Necrosis (IHHN) in some locations (Lightner and Redman, 1998; Fegan and Clifford, 2001; Moss and Moss, 2009; Lightner *et al*., 2009). While unselected stocks of *P. vannamei* have a high degree of resistance to IHHN, the degree of resistance has been improved in some locations by the breeding of selected individuals which show greater resistance to IHHN and RDS (a chronic form of IHHN disease in *P. vannamei*) than unselected stocks (Fegan and Clifford, 2001; Lightner *et al*., 2009). From some of these stocks, IHHNV-free founder stocks have been developed, and these have the advantage of being SPR for IHHN disease, but also being SPF for IHHNV and other diseases.

SPR stocks have been most successfully used in culture regions where TSV, IHHNV or both diseases are enzootic in wild penaeid shrimp stocks or where the viruses are readily transmitted within or between farms. "SPR-43" stocks of *P. stylirostris* were the first SPR stocks developed and they are the primary stocks currently farmed in New Caledonia and French Polynesia. Before WSSV reached Mexico in 1999, SPR stocks of *L. stylirostris* (SPR-43 developed in French Polynesia and Super Shrimp developed in Venezuela) were the dominant shrimp stocks cultured in Mexico and they accounted for nearly 80% of the farmed shrimp produced in Mexico in 1998.

The SPR-43 and Super Shrimp lines of *P. stylirostris* were developed over time in captive stocks of *P. stylirostris* by breeding survivors that were persistently infected with IHHNV. Breeding survivors to survivors eventually resulted in continuous domesticated lines of *P. stylirostris* with a high degree of resistance to IHHN disease, despite being persistently infected with the virus. Some lines of Super Shrimp were found in laboratory challenge studies with IHHNV to be resistant even to infection and to quickly clear the virus after challenge (Tang *et al*., 2010). The Super Shrimp line was developed using the same strategy as was used for SPR-43. Super Shrimp came from breeding survivors of stocks initially imported into Venezuela from Panama. When the stocks were introduced into Mexico in ~1994 1995, the SPR-43 stock was at about its 18th generation in captivity and the Super Shrimp lines were at about their 16th generation (Fegan and Clifford, 2001; Lightner, 2005; Lightner *et al*., 2009).

More recently, SPR stocks of SPF *P. vannamei* were developed by the USMSFP by selectively breeding for TSV resistance. In screening the various lines of SPF, domesticated *P. vannamei* reared by the program for TSV resistance in controlled laboratory challenge trials, certain geographic stocks were found to present better survival than stocks whose founders were derived from other geographic regions. These were selectively bred for multiple generations, and with each generation checked experimentally for TSV resistance. Such SPR stocks of *P. vannamei* (which are also SPF for all OIE and USMSFP listed diseases of penaeid shrimp - Table 2), are currently commercially available from most broodstock suppliers in the USA. Most of the TSV resistant SPF/SPR stocks currently available have documented survival rates of 80 to 100% in laboratory challenge studies with four TSV isolates that represent the main geographic genotypes/biotypes of this virus. The Belize genotype of TSV is the most virulent biotype of the four known TSV genotypes (Moss and Moss, 2009; Lightner *et al*., 2009). Fortunately, selection for resistance to the reference TSV genotype/biotype (obtained from Ecuador and Hawaii in 1994) has been found to work well for all TSV genotypes/biotypes to date (Lightner *et al*., 2009).

FUTURE OF SPF STOCK DEVELOPMENT

Following the model for SPF stock development used by the USMSFP for the development of SPF *P. vannamei*, companies in Asia, Madagascar and the USA have developed some SPF *P. monodon* and *Penaeus chinensis* stocks and others are developing SPF *P. indicus* (Hennig *et al*., 2005; Pantoja *et al*., 2005; Lightner *et al*., 2009). Despite the significant challenges posed by disease, the shrimp farming industry has responded to the challenges posed and it has developed methods to manage disease and mature into a more sustainable industry. Adoption of the SPF concept in the domestication of *P. vannamei* and development of the species for aquaculture was among the milestones that have led to the industry's current explosive growth and apparent sustainability (FAO, 2006). As it has in other meat producing industries, the development of SPF stocks of *P. vannamei, P. stylirostris, P. monodon*, and other penaeid species has become central to the sustainability of modern shrimp farming. Further development through selective breeding of SPF stocks for disease resistance and other desired characteristics is very likely to be a principle characteristic of the industry for the indefinite future.

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