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Facts, truths and myths about SPF shrimp in Aquaculture

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Abstract

Shrimp domestication and genetic improvement programmes began in late 1980s, in the United States of America, under the United States Marine Shrimp Farming Program (USMSFP), using the Pacific whiteleg shrimp *Penaeus vannamei*. The USMSFP was based on proven concepts from the livestock and poultry industries and began with establishing a specific pathogen-free (SPF) shrimp stock. The original shrimp stock was obtained using rigorous screening of captured wild shrimp for selection of individuals naturally free of major shrimp pathogens. Although the concept of SPF animals was well defined for terrestrial animals, it was relatively new for aquaculture, and it took some time to be adopted by the aquaculture community. In the early 1990s, parallel to USMSFP, several other programmes on genetic improvement of shrimp were also initiated in Latin America. Subsequently, several new terminologies and products, such as specific pathogen resistant (SPR) shrimp, specific pathogen tolerant (SPT) shrimp and even 'all pathogen exposed' (APE) shrimp, entered the shrimp industry vocabulary and became commercial. This led to confusion in the shrimp industry about the meaning, relationship and significance of these new terms with respect to SPF. This position paper attempts to clarify these concepts, provide science-based definitions, reconfirms the importance of developing, maintaining and using domesticated, specific pathogen-free (SPF) shrimp stocks (which may also achieve SPR and/or SPT status) to reduce the risk of disease outbreaks and increase production and profit. The same principles would apply to development of domesticated SPF stocks for other species used in aquaculture. The paper also discusses the difficulties of confirming and certifying SPF status due to the presence of endogenous viral elements (EVEs) and calls for internationally agreed science and evidence-based technical guidelines for producing healthy shrimp.

Key words: aquaculture, diseases, shrimp, SPF, SPR, SPT.

Introduction

The concept of specific pathogen-free (SPF) animal stocks and the technology to create and manage them evolved primarily in the Western hemisphere (United States and Europe). It originated in the early 1940s and lies within the scope of laboratory animal medicine. Specifically, SPF chicken eggs were developed for the culture and propagation of live organisms for vaccine production (Luginbuhl 2000). Thereafter, over the subsequent 30–40 years, SPF technology was adopted, developed and applied to commercial poultry, and in the 1960s, extended to swine and other domestic animal production systems. It was also used in veterinary applications for the production and maintenance of standardised and genetically inbred animal stocks to serve as 'white mice' for medical and veterinary research.

The United States Marine Shrimp Farming Program (USMSFP) of the United States Department of Agriculture (USDA) was formed in 1984, made up of several institutions in different States in the U.S. with the objective of increasing local production of marine shrimp while decreasing the reliance on importation. The response of USMSFP, after having its breeding programme hit by a disease outbreak, was a paradigm shift towards designing, developing and implementing an integrated SPF herd-health, infectious disease management programme that would thereafter be applied to all the USMSFP participant institutions and eventually be commercialised in the US shrimp industry. Consequently, the first commercial programme for domestication and genetic improvement of penaeid shrimp was initiated under the USMSFP using Pacific whiteleg shrimp (*Penaeus vannamei*) in 1989 (Lotz 1992; Wyban *et al.* 1992; Lotz *et al.* 1995; Moss 2002; Moss *et al.* 2012; #8697). The primary objective of the programme was to produce broodstock, free of specific pathogens, that could be bred and produce postlarvae that could be raised in biosecure production facilities and systems, to reduce mortality and increase production (Moss 2002; Lightner 2011).

Basically, the USMSFP programme adopted the breeding and selection concepts from the livestock and poultry industries to establish specific pathogen-free (SPF) stocks[1] of shrimp that would provide high health and genetically improved postlarvae (Gjedrem & Fimland 1995). The stocks were obtained by rigorous screening of captured, wild shrimp for selection of individuals naturally free from a list of known and easily detectable shrimp pathogens that it would be possible to permanently exclude from the stock under strict quarantine conditions in a nucleus breeding center (NBC) housing many founder families. These stocks could then be subjected to a domestication and genetic improvement programme, where better performing families from each generation could be used to produce postlarvae destined to become SPF broodstock in an adequately biosecure broodstock multiplication center (BMC). The broodstock would be supplied to commercial hatcheries where postlarvae would be produced for farmers to stock in ponds.

In parallel, several breeding and selection programmes were carried out with *P. vannamei* in Latin America. In Venezuela a mass selection programme began in 1990 to produce shrimp well-adapted to the local rearing conditions (De Donato *et al.* 2005). Similarly, in Colombia commercial producers mass selected TSV resistant shrimp in the early 1990s. These early efforts later developed into fully fledged family selection breeding programmes that resulted in some improved populations for the local industry (Cock *et al.* 2009). The programmes in Latin America were based on the concept that the populations should be well adapted to local conditions and should be resistant or tolerant to the major disease problems endemic in the region. Thus, a major dichotomy in breeding strategies emerged in the 1990s, with selection, maintenance and multiplication of populations in essentially disease-free conditions under the SPF protocols of the USMSFP while other programmes used populations selected in the presence of multiple disease pressures that are common in commercial production.

Although the concept of SPF animals was well defined for terrestrial animals that could be grown out in isolated installations, it was relatively new for aquaculture where it is difficult to isolate the animals in the aquatic environment. A major impetus for eventual wide adoption of the SPF shrimp concept was the emergence and spread of white spot disease (WSD) of shrimp caused by white spot syndrome virus (WSSV) in the mid-1990's (Flegel & Alday-Sanz 1998). At that time, *Penaeus monodon* was the main cultivated shrimp species in Asia, and it was soon realised that the major source of WSSV in shrimp grow-out ponds was infected postlarvae derived from captured WSSV-carrying broodstock (Withyachumnarnkul 1999) and that PCR monitoring was not sufficiently effective to minimise the level of WSSV in PL to acceptable levels for sustainable shrimp production (Withyachumnarnkul *et al.* 2003). As pointed out by (Briggs 2005), the main reason behind the importation of *P vannamei* to Asia was the perceived poor performance, slow growth rate and disease susceptibility of the major indigenous cultured shrimp species, *P. chinensis* in China and *P. monodon* virtually everywhere else. These were the consequences of using infected broodstock that would transmit pathogens to their offspring. The availability of SPF stocks of *P. vannamei* together with pathogen exclusion biosecurity strategy was very effective and rapidly led to it becoming the dominant cultivated shrimp species in Asia (Wyban 2007).

Because of the benefits of using domesticated and genetically improved SPF stocks of *P. vannamei* to produce healthy PLs for farmers to use in stocking their ponds, the term SPF in Asia began to be related to stocks with higher disease resistance or tolerance. The opposite situation occurred in Latin America where SPF shrimp were stocked in ponds with no pathogen exclusion biosecurity leading to mass mortalities and leading to farmer perception that SPF status implied higher disease susceptibility. This perception was incorrect. SPF only indicates the sanitary status of a stock and gives no indication of its susceptibility, resistance or tolerance to infection and disease.

This dichotomous approach resulted in the creation of new terms such as specific pathogen resistant (SPR) stocks and specific pathogen tolerant (SPT) stocks that led to

[1]The terminology, specific pathogen free (SPF) is later defined in the document.

confusion in the shrimp industry regarding the meaning, relationship and significance of these new terms with respect to SPF. While mistaken perceptions of SPF and SPR have long been recognised (Briggs *et al.* 2004), the paramount need for SPF domesticated shrimp stocks and SPF as a novel and emerging technology that will support sustainable shrimp aquaculture were emphasised during the Global Conference on Aquaculture 2010 (Browdy *et al.* 2012; Hine *et al.* 2012).

The objective of this position paper is to clarify these concepts and terminologies and to reconfirm the importance and the benefits of developing and maintaining domesticated, healthy shrimp stocks that are effectively free from major pathogens and make shrimp farming more profitable and sustainable. An expert meeting was convened by the Food and Agriculture Organization of the United Nations (FAO) from 26 to 28 May 2016 in Bangkok, Thailand. This paper reflects the outcomes of that meeting, the further debate that followed and the consensus reached.

Major definitions

With respect to pathogen status, the only technical terminology used for terrestrial animals is specific pathogen-free (SPF) stocks, as defined below. That definition can be applied to all animals, terrestrial or otherwise. However, in the shrimp industry during the past 2 decades, several other terms have been proposed without 'agreed definitions'. Therefore, this paper proposes scientifically accurate definitions for these newly proposed terms for use in the shrimp farming industry and for application to other aquaculture species.

As a background for this section, it is important to understand that shrimp interactions with viral pathogens are not fully understood. Unlike vertebrates, they do not produce antibodies (Cerenius *et al.* 2010; Wang *et al.* 2014; Tassanakajon *et al.* 2018) and it is well known that survivors from disease outbreaks with a normally lethal virus may remain infected with that lethal virus at a low level for up to a lifetime, without showing any gross signs of disease. In this state, they maintain the potential to transmit the pathogen to their offspring and to naïve shrimp that may become diseased. They also have the potential to develop disease due to environmental or other stressors. The phenomenon of tolerating infectious viral pathogens for long periods of time without signs of disease has been called viral accommodation (Flegel 2007, 2009; Utari *et al.* 2017), but the mechanisms underlying it are still unclear. Shrimp stocks tolerant to TSV but uninfected with TSV have been developed using genetic selection (Moss *et al.* 2005; Cock *et al.* 2017). When these stocks are challenged with lethal isolates of TSV they become infected but show no gross signs of disease. However, they carry the lethal virus and are capable of transmitting it to naïve, susceptible shrimp. Thus, shrimp stocks that tolerate and carry viral pathogens may lack gross signs of disease (including histological lesions) and may give negative results with molecular detection methods of low sensitivity. This constitutes a special danger that must be guarded against in the transboundary movement of shrimp stocks for aquaculture (Flegel 2006), and it has consequences for stocks labelled with the terms defined below. It is important that these terms be clearly defined and understood to avoid confusion that unscrupulous individuals might use to take advantage of shrimp farmers.

Pathogen-free (PF) stocks (New term)

These are stocks that are free from any known or unknown pathogen. Since the definition includes 'unknown pathogens', it is obvious that PF cannot be used to refer to any actual animal stock, and that it must be reserved only for theoretical discussions. This is especially true for shrimp due to their ability to carry viruses, including those still unknown, for long periods of time without showing any signs of disease (see above).

Specific pathogen free (SPF) stocks (Existing, defined term)

SPF animal stocks must come from a population that has tested negative for specific pathogens for a period of at least 2 consecutive years, has been raised in highly biosecure facilities[2] following stringent biosecurity management measures and has been fed with biosecure feeds. To be able to maintain and claim SPF status, a suitable surveillance program for the specific pathogens, including both molecular and histopathological tools, must be in place.

As mentioned above, SPF stocks are not necessarily free of all pathogens. Thus, a list of pathogens from which the animals are claimed to be free should always accompany them. SPF status may refer, not only to relevant OIE listed pathogens, but also to any other pathogen/s deemed necessary by the SPF stock producer. Any shrimp stock claimed to be SPF should, at least, be free from the following pathogens listed in the OIE Code and Manual (Anonymous, 2017, 2018): *Vibrio* isolates that cause acute hepatopancreatic necrosis disease (AHPND), infection with *Hepatobacter penaei* that causes necrotising hepatopancreatitis (NHP), infectious hypodermal and haematopoietic necrosis virus (IHHNV), infectious myonecrosis virus (IMNV), Taura

[2]To qualify as highly biosecure facilities, the risk of introduction of pathogens needs to be negligible. This may refer to indoor/greenhouse facilities and with effective water disinfection. Areas endemic with insect transmitted pathogens, i.e. YHV would require double doors and insect control.

syndrome virus (TSV), white spot syndrome virus (WSSV) and yellow head virus (YHV - genotype 1).

As of today, the other known pathogens that might be considered for exclusion in a shrimp SPF program would be: *Penaeus monodon*-type baculovirus (MBV), *Baculovirus penaei* (BP), hepatopancreatic parvovirus (HPV), the microsporidian *Enterocytozoon hepatopenaei* (EHP), *Streptococus* sp., *Spiroplasma* sp., *Penaeus vannamei* nodavirus (PvNv), Laem Singh virus (LSNV) a component cause of monodon slow growth syndrome (MSGS), muscle microsporidia and gregarines.

The SPF list of pathogens should be dynamic and be regularly updated with recently discovered pathogens. It should be noted that SPF animals (including shrimp) are not necessarily more susceptible to infection or disease than non-SPF animals. Nor are they more resistant or tolerant to any pathogen. SPF refers exclusively to the health (infection) status of a shrimp stock.

We currently accept that there are two ways to generate SPF shrimp stocks. One way (used by USMSFP, for example) is to find a geographical area where major shrimp pathogens are known to be absent or at low prevalence, to capture and screen wild shrimp from that area and to select individuals that are shown to be naturally free of a specified list of pathogens for a period of at least 2 consecutive years. A stock generated in this fashion could be called a 'natural SPF stock'. Another way to generate an SPF stock is to choose a shrimp farming area where major shrimp pathogens such as WSSV, TSV and IHHNV are present and to use a process of continuous screening to select individuals that are shown to be free of a specified list of pathogens for a period of at least 2 consecutive years. A stock generated in this fashion could be called a 'cleansed SPF stock'. Cleansed SPF stocks of *P. vannamei* have been produced successfully in (Alday-Sanz, 2018). Obviously, it should be easiest to develop a 'natural SPF stock', but it may be that its lack of previous exposure to major pathogens would reduce the probability of its potential for subsequent selection of genetic factors for resistance and/or tolerance to those major pathogens. By contrast, it should be hardest to develop a 'cleansed SPF stock' but the effort might be compensated by a higher probability for subsequent selection of genetic factors for resistance and/or tolerance that may have developed in response to prior pathogen exposure.

Resistance and tolerance concepts have a different meaning when studied from the genetic or the sanitary point of view. For geneticists, resistance is defined as the ability to limit the burden of a pathogen in an infected animal while tolerance is defined as the ability to limit the severity of disease induced by a given pathogen burden (Råberg *et al.* 2007). Both traits are quantitative. However, the clarity of these definitions is blurred from the sanitary point as the outcome of disease is not related exclusively to the genetic trait of the animal. It has long been understood (Snieszko 1974) that the disease state arises from an interaction of host (genetics), pathogen and environmental factors. So, from the sanitary point of view, resistance is the ability to be refractory to infection (qualitative trait), while tolerance is the ability to reduce the expression of disease (quantitative trait).

Specific pathogen resistant (SPR) stocks (New term)

These are animal stocks that remain refractory to infection without showing gross signs of infection and/or disease, even after challenge with a lethal dose of one or more specific pathogens. Resistance may be specific to those pathogens or strains of them. However, some stocks may manifest resistance to more than one pathogen, while being susceptible to others. Unlike SPF, SPR does not refer to the health status of a shrimp stock but to its genetic characteristics (i.e. its genetic status). Indeed, it is possible that a non-SPF stock advertised as SPR for one pathogen may be infected with one or more other pathogens. Nowadays, a population of *P. monodon* WSSV SPR has been developed starting from naturally resistant mutants collected from the wild (G. Lo, personal communication).

Specific pathogen tolerant (SPT) stocks (New term)

These are stocks that are susceptible to infection by a specific pathogen but do not normally develop clear signs of disease as a result of such infection, i.e. they are tolerant to disease expression in a quantitative manner dependent on their genetics, on the pathogen strain and on environmental conditions that influence the disease. Tolerance may be specific to a pathogen, to a strain of a pathogen or to a group of pathogens. However, non-SPF stocks that have SPT status could be susceptible, get infected and manifest clinical disease with other pathogens. In addition, if they are not SPF for the pathogens they tolerate, they might be grossly normal carriers for those pathogens and be capable of transmitting them to naïve shrimp. As with SPR above, SPT does not refer to the health status of an animal stock but to its genetic characteristics.

Combined SPF and SPR or SPT stocks (New terms)

While SPF refers to animal health status (backed up by a 2-year stock history at a certified rearing facility for absence of specific pathogens), it is possible and logical to combine SPF health status with genetic status as in SPF+SPR, SPF+SPT or SPF+SPT+SPR stocks. In other words, a stock characterised as SPF based on health status can be subjected to a subsequent genetic selection programme designed to identify, characterise and select for genetic attributes in the

stock population that could lead to specific disease resistance and/or tolerance for one or more pathogens. However, to achieve such combined status and to claim it would have to be based on the biosecurity strategy and stock history for each facility as described above individually for SPR and SPT status.

Uncharacterised, selected survivor (USS) stocks (New term)

These are animal stocks that have been produced by selecting survivors (based on size and gross health appearance) from several successive generations under non-biosecure farming conditions in a region where several known and unknown pathogens occur. Such stocks have previously been referred to as 'all pathogen exposed' (APE) stocks. However, 'all' pathogens do not occur in every geographical region and those known and unknown in any particular region are not always present in every pond. Thus, development of a stock exposed to 'all' known and unknown pathogens is impossible, and the designation 'APE stock' is technically and scientifically unsupportable. For these reasons, we propose that the term 'APE stock' be regarded as unacceptable by the shrimp industry and that it be replaced by 'USS stock' as defined herein. It is important to understand that although USS stocks may appear grossly normal, they may be infected with pathogens that could be transmitted horizontally or vertically to naïve animals, unless they have subsequently been certified free of specific pathogens using negative testing for a period of at least 2 consecutive years (i.e. been converted to 'cleansed SPF status' as described above). From these definitions, it is obvious that USS shrimp stocks are riskier than SPF shrimp stocks for transboundary movement.

High health (HH) stocks (New term)

This is a commercial term frequently used but not clearly defined. It often refers to descendants of an SPF stock. Since it does not specify the pathogens, genetic, epigenetic or rearing conditions or status, use of the term 'HH stock' should be avoided and instead, one of the above terms describing health status and pathogen response should be used to characterise a stock.

These definitions apply to the health status and genetic characteristics of stocks with respect to pathogens. However, it is important to understand that they do not give any indication of other stock characteristics such as growth rate or of how the stocks will respond to genetic changes in pathogens and to variations in environmental conditions outside the ranges under which the stocks were developed and tested.

Importance of pathogen detection and monitoring

Sensitive and specific diagnostic tests are an essential component to infectious disease prevention, management and control. Prior to and through the 1980's, shrimp pathogen detection relied upon physical changes detected using gross appearance, wet-mount microscopy, histopathology and culture/identification of microbial agents that had the capacity to grow independently on formulated culture media. Except for culture and isolation on artificial media, which worked well for bacterial and fungal pathogens of shrimp, there were no cell culture systems available at the time (as well as now) applicable for shrimp virus diagnostics. Alongside the progress in development of domesticated SPF shrimp (*P. vannamei*) in the US under the USMSFP programme, there was a rapid evolution and advancement of diagnostic testing methodologies and specifically, rapid diagnostic tests that relied on detection of nucleic acid sequences specific for each pathogen. For the first time, in 1995, OIE published a list of penaeid shrimp pathogens, and recommended methods and procedures for testing and detecting them, in the OIE Aquatic Animal Code and Manual (Anonymous, 2001a,b) and they have since been continually updated (Anonymous, 2017, 2018).

Validation and maintenance of SPF status

Validation and maintenance of the SPF status for shrimp is a time consuming and expensive process. It requires that the facility, the biosecurity standard operating procedures (SOPs) and the shrimp within the facility conform to standards suitable for the location of the facility to ensure that the shrimp housed in the facility can be maintained as SPF (Lotz 1994). Stated differently, the validation is for the facility as well as for a specific lot or group of shrimp. For an SPF facility sited in a coastal zone, the difficulty and risk is high for pathogen contamination from operations and/or effluents from neighbouring shrimp maturation facilities, hatcheries and/or farms engaged in non-SPF shrimp production. In contrast, the risk of exogenous pathogen contamination is reduced considerably for an SPF facility located in the same region but inland, away from the coastal zone and utilising recirculation technology with appropriate biosecurity practices.

Periodic shrimp tissue sample collection, submission and testing using the appropriate, diagnostic methods must be used (Lightner 2011; Anonymous, 2018) (where these apply) and must be based on scientifically sound sample collection sizes. Knowledge of new shrimp stock entries into the facility must be taken into consideration as well as the primary and secondary quarantine steps, processes and protocols applied prior to entry into the SPF facility. Professional, certified and licensed staff and/or recognised

government or third party diagnostic laboratories should be engaged to carry out the sampling, chain of custody, laboratory assays and reporting of results. Currently, the estimated standard duration for SPF facility testing is 2 years (Lightner 2011 and OIE, 2018), unless the SPF facility was stocked with SPF founder shrimp that were an offspring generation derived from an existing population of SPF shrimp that had previously passed through the quarantine and generational testing processes. Under these circumstances, 6 months to 1 year may be an acceptable standard.

Validation of SPF quality for the OIE-listed pathogens is well established. However, emerging pathogens should also be included in the screening process as soon as these are identified. A discriminating client of SPF shrimp should exercise caution, request further testing not only for OIE-listed pathogens and consider employing a quarantine phase upon arrival in the importing country where additional PCR testing, histopathology examination, microbial culture and, perhaps, bioassay challenge protocols can be conducted as precautionary steps to reduce risk and to protect themselves. This additional layer of risk mitigation is optional but in some circumstances appropriate for facilities that house high-value shrimp stocks, domesticated over many generations and genetically improved such that losing the SPF status of their herd would have devastating economic consequences for their company.

Problems confirming SPF status

Although internationally approved procedures are in place to detect most of the important pathogens of shrimp that should be included in an SPF program, the issue of endogenous viral elements (EVEs)[3] has become a scientific challenge for confirming and certifying the SPF status of a shrimp stock. Although many EVE originate from retroviuses, non-retroviral EVE was first reported in shrimp (Tang & Lightner 2006) for two EVEs of IHHNV. However, at the time, the term EVE did not exist and was not coined until the discovery of previously unknown and unexpected, non-retroviral EVE in vertebrates (Katzourakis & Gifford 2010). The vertebrate discovery greatly increased interest in EVE in the general scientific community. Subsequently, many more EVE for IHHNV were reported for *P. monodon* and *P. vannamei* and many of them gave false-positive PCR test results for IHHNV using the OIE recommended detection method, even though the shrimp were not infected with IHHNV (Saksmerprome *et al.* 2011; Brock *et al.* 2013). Such false-positive test results for an infectious virus could have serious international trade implications for shrimp breeders. In addition, a population or family of shrimp that has tested negative for infectious with IHHNV using the recommended OIE method may generate occasional offspring that suddenly appear as 'pop-up' false positives (PUPs) via genetic recombination between EVE that carry incomplete but complementary fragments of the target sequence for the OIE method. As with IHHNV, EVEs for WSSV were also found in *P. monodon* where they also occurred in a random manner with respect to type and number in each individual shrimp tested (Utari *et al.* 2017).

The relatively common occurrence of EVE in shrimp (Flegel 2009) and other crustaceans (Thézé *et al.* 2014) cannot be disputed, and this can lead to problems in obtaining false-positive test results for infectious viruses. This could lead to restriction in the international trade of SPF shrimp stocks, limiting their supply to shrimp farmers. It is possible to eliminate the problem of false-positive test results that arise from EVE by developing an international, mutual agreement on a fixed, standard target region for each shrimp viral pathogen. The agreement process could be arranged and managed by FAO/OIE at coordinated meetings with shrimp breeding companies and shrimp farmer associations. This would allow SPF stock developers to eliminate EVE for the agreed target region from their breeding stocks. After such an agreement, any subsequent positive test result for that target region from a breeding stock would indicate the presence of the infectious virus while continual negative test results for that target region would indicate absence of the infectious virus in a stock, even if that stock gave a positive PCR test result for a different target gene sequence from the same virus.

Importance and benefits of SPF shrimp

Naturally derived SPF *P. vannamei* from Hawaii were first introduced (imported) to Thailand in 2002 (Wyban 2007; Lightner 2011). Following this introduction, shrimp production was revolutionised in Asia (mainly in Southeast Asia), with *P. vannamei* almost completely replacing *P. monodon* in regional shrimp production within a decade. In 2003, Charoen Pokphand Foods (CPF) in Thailand started their SPF breeding programme with high biosecurity protocols. This programme contributed significantly to exponential growth of the industry in Southeast Asia for nearly a decade, until a new disease, acute hepatopancreatic necrosis disease (AHPND) emerged in 2009. This new

[3]For this manuscript, EVEs are defined as sequences of whole genomes or genome fragments from RNA or DNA viruses that have become integrated into host DNA. If inserted into germ cells, they are heritable and may become fixed in a population Cui & Holmes 2012; Endogenous RNA viruses of plants in insect genomes. Virology. 427, 77–79, ibid., Feschotte 2010; Virology: Bornavirus enters the genome. Nature. 463, 39–40, Feschotte & Gilbert 2012; Endogenous viruses: Insights into viral evolution and impact on host biology. Nature Reviews Genetics. 13, 283–296, Flegel 2009; Hypothesis for heritable, anti-viral immunity in crustaceans and insects. Biology Direct. 4, 32, ibid., Katzourakis & Gifford 2010; Endogenous viral elements in animal genomes. PLoS Genet. 6, e1001191.

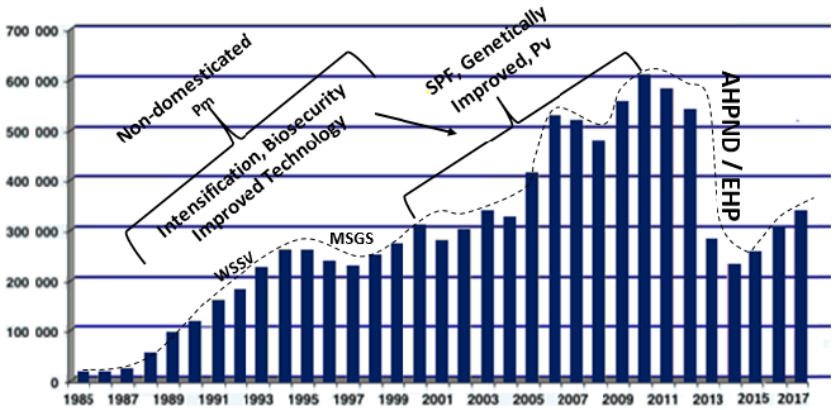

Figure 1 Thai shrimp production, the impact of diseases and the influence of non-domesticated and SPF stocks.

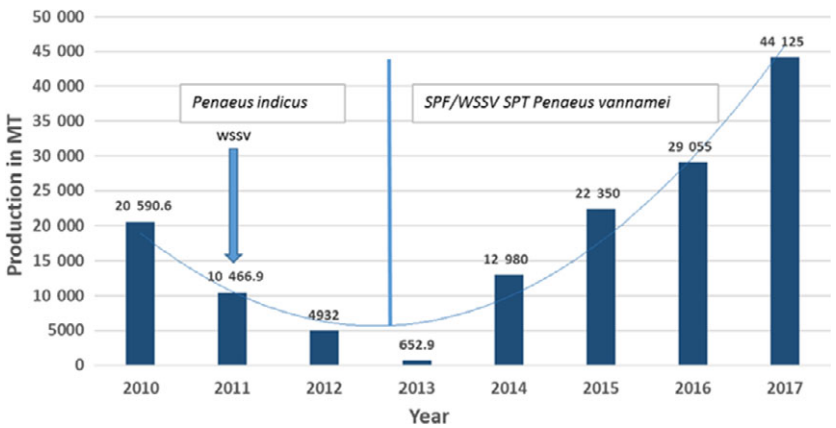

Figure 2 Recovery of the shrimp production in the Kingdom of Saudi Arabia after *Penaeus indicus* was wiped out by WSSV and SPF+WSSV/SPT *Penaeus vannamei* was introduced. Source: Saudi Aquaculture Society.

pathogen escaped the biosecurity measures previously implemented for viral pathogens (Fig. 1). Similarly, dramatic recovery from a national epidemic of white spot disease (WSD) was achieved in Saudi Arabia (Fig. 2), including a rapid increase in production, after the introduction of a cleansed SPF stock that was also SPT/SPR for WSD (Alday-Sanz 2018). In this case, certain biosecurity measures adapted to the local farming conditions (mostly 10 ha ponds) were implemented such as viral exclusion up to postlarvae stocking and water filtration in the ponds which ranged from 250 to 1000 microns.

The relevance that SPF stocks have had for shrimp farming varies greatly with region and farming practices, but it has clearly moved the industry forward in several ways. These include the following. They have reduced the introduction of pathogens and disease expression in farms leading to an immediate and exponential increase in culture performance as seen in the Figure 2. They have provided a means for safe introduction of *P. vannamei* shrimp around the world until it became the species of choice and the dominant one farmed. They have provided an important platform for the application of selective genetics by removing the variability of pathogen infection from individual to individual and generation to generation. Growth is a good example where the SPF approach has resulted in 15% improvement per generation, while programs that have used an SSU approach have had difficulty in obtaining growth improvements of 3–5% per generation (R. McIntosh, personal communication). Finally, the availability of SPF shrimp has been a very important asset for use as test animals in disease challenge, nutritional, physiological and biochemical studies performed in the laboratory or at the pilot scale, where minimisation of non-controlled variables is an important aspect to the study design.

Conclusions

SPF refers to the health status of a shrimp stock while SPR and SPT statuses refer to defined genetic characteristics of stocks in response to pathogens and disease. SPF lists for

particular shrimp species may include pathogens that do not affect it, or affect it little but may be carried by it and be transmissible to other disease-susceptible shrimp species. The objective is to avoid negative impacts on production, transmission among species and trade barriers that might arise from pathogen detection in shrimp and shrimp products. The designation 'USS stock' alone gives no indication of either the specific health status or specific genetic characteristics of a shrimp stock with respect to pathogen and disease response. However, USS stocks may be suitable for conversion to SPF stocks using screening to select individual animals free from a specific list of pathogens for two consecutive years. It is also possible to combine strategies such as SPF+SPR, SPF+SPT or SPF+SPR+SPT in order to help shrimp farmers prevent disease outbreaks in grow-out ponds. The success of these approaches may depend on the biosecurity strategy defined for each facility. Farmers must also consider other aspects of stock performance such as growth, survival, etc. that may be related to stock health status and genetic status. We recommend that farmers cooperate with one another in critical evaluation of stocks provided by commercial suppliers. This can be done by simple epidemiological techniques to determine the relationship between their stock sources and stock performance including such things as disease response, growth, survival, etc. Over time, this analytical process should reveal the identity of the most reliable stock suppliers with respect to overall stock performance.

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