Asian Fisheries Science ***31S*** (2018): 112–120
©Asian Fisheries Society
ISSN 0116-6514
E-ISSN 2071-3720
https://doi.org/10.33997/j.afs.2018.31.S1.008

Specific Pathogen Free (SPF), Specific Pathogen Resistant (SPR) and Specific Pathogen Tolerant (SPT) as Part of the Biosecurity Strategy for Whiteleg Shrimp (*Penaeus vannamei* Boone 1931)

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Abstract

The sanitary and genetic characterization of farmed penaeid shrimp needs to be selected based on the type of culture system and biosecurity strategy applied. This paper attempts to define the concepts specific pathogen free (SPF), specific pathogen resistant (SPR) and specific pathogen tolerant (SPT) and presents the process for development of a combined approach where white-spot syndrome virus (WSSV) SPT/SPR animals were selected to create SPF stocks for use in a low-biosecurity facility. The success of this approach was proven when they were imported and adopted as the only stocks cultured in the Kingdom of Saudi Arabia after the local species were wiped out by WSSV. Record national production has been achieved since their introduction and WSSV has been eradicated even from the wild population. It is suggested that this type of stock (WSSV SPT+SPF) could be an alternative for countries still impacted by WSSV that do not have the technical competence or the investment capacity to transform the industry to small intensive ponds where viral exclusion strategies could be implemented.

Keywords: biosecurity, disease control, *Penaeus vannamei*, specific pathogen free (SPF), specific pathogen resistant (SPT), specific pathogen tolerant (SPT), white-spot syndrome virus (WSSV)

Introduction

Diseases continue to affect the sustainability of shrimp farming, and their prevention requires the definition and implementation of a biosecurity strategy specific for each facility, culture system and sanitary zone. Biosecurity is a broad term that is often poorly understood. FAO (2003) defines biosecurity as follows:

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"Biosecurity is a strategic and integrated approach that encompasses the policy and regulatory frameworks (including instruments and activities) that analyse and manage risks in the sectors of food safety, animal life and health, and plant life and health, including associated environmental risk. Biosecurity covers the introduction of plant pests, animal pests and diseases, and zoonoses, the introduction and release of genetically modified organisms (GMOs) and their products, and the introduction and management of invasive alien species and genotypes."

In animal farming, we can synthesize that biosecurity includes all those activities necessary to prevent, control and manage the risk to animal health and life with the objective of reducing the economic impact of diseases. In other words, biosecurity is a tool for sustainability. The activities that are a fundamental part of biosecurity range from the international framework through intergovernmental agreements, to the national framework through national legislation and enforcement, to the scientific research that generates the knowledge needed to develop sound preventive measures and finally, to the implementation of such measures in animal production.

These production oriented biosecurity measures have three components: those activities related to the environment and the management of the culture conditions, those involving the pathogen and the sanitary status of the animals, and last, the characteristics of the shrimp and its genetics which is the focus of this paper.

Sanitary and genetic characterization of the shrimp

When considering the type of shrimp for culture and the biosecurity strategy to be applied, their sanitary status and genetic characteristics need to be considered.

Regarding their sanitary status, the stocks can be classified as:

- specific pathogen free (SPF), meaning that they are free from specific pathogens, but not necessarily free of all pathogens;
- pathogen free (PE), meaning that they are free of all pathogens (however, this is difficult to prove and assure);
- all pathogen exposed (APE), meaning that they have been exposed to potential pathogens, (these are, for example, the broodstock collected from ponds); or
- high health (HH), this is a commercial term and vague in terms of description of the stocks.

Regarding their genetic characteristics, the stocks can be classified as:

- susceptible, to infection and disease;
- specific pathogen resistant (SPR), meaning resistant to infection by a specific pathogen (this is a qualitative trait – they can either be infected or not); or
- specific pathogen tolerant (SPT), meaning tolerant to a specific disease, (the animal can be infected but may not develop the disease or it may develop the it to a lesser extent).

Specific Pathogen Free Stocks (SPF)

The term "specific pathogen free" has been often misused, and the proper understanding of the concept is not widespread within the shrimp farming industry. An SPF animal can be defined as one coming from a population that has tested negative for specific pathogens for at least two years (a surveillance programme must be in place), that is raised in highly biosecure facilities (i.e. with appropriate water treatment and an enclosed environment) following biosecure management measures and has been fed with biosecure feeds. Therefore, the SPF characterization refers exclusively to the history of the sanitary status of the animal, the facility and the culture conditions under which it has been raised and maintained.

Claiming SPF status requires transparency through regular auditing. An SPF animal is not necessarily more susceptible or more tolerant to pathogens, and they do not necessarily have less genetic diversity or better or worse growth performance. It only refers to its sanitary status, and this condition is not hereditary. As soon as an SPF animal is exposed to potential pathogens (i.e. by movement to a facility having a lower level of biosecurity), the SPF status is lost. Often these animals are referred to as HH.

As mentioned previously, SPF refers only to freedom from certain pathogens, not freedom from all pathogens. There is no consensus of the list of pathogens that SPF animals need to be free of. In some instances, only those pathogens listed by the World Organisation for Animal Health (OIE) are considered. It is recommended that SPF animals should be free from all known pathogens of penaeid shrimp as OIE list is not dynamic enough to include in a timely manner all relevant pathogens to shrimp culture.

The SPF approach is not a new concept, rather, it is normal practice in terrestrial animal husbandry. In fact, the idea of stocking (i.e. investing) in infected animals is difficult to understand from the veterinary point of view. In addition, the use of SPF animals provides an advantageous starting point for proper progress in breeding programmes, as sanitary variables are removed. They are also fundamental for international trade, in order to prevent the transboundary movement of pathogens and research purposes.

Industry approaches to WSSV Epizootics

Over the past several decades, outbreaks of white spot disease (WSD), caused by WSSV have caused major losses in both Asia and Latin America. In each continent, the industry took different approaches to manage the disease. While Asia chose to use SPF stocks and implement high-biosecurity measures during culture, Latin America chose to cope with the virus and to not try to exclude it out of the system.

As seen in Fig. 1, Thailand, as an example of an Asian culture system, recovered very quickly after WSSV through the use of small ponds that allow control of the culture conditions, the introduction of SPF stocks and biosecurity implementation based on viral carrier exclusion.

In fact, the use of SPF stocks changed the industry, permitting it to reach a productivity never seen before, as it allowed the development of very successful breeding programmes.

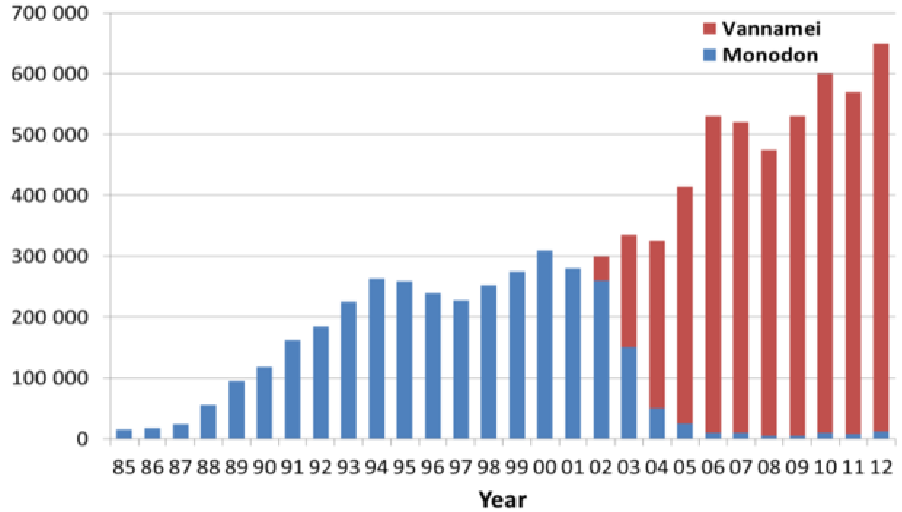

Fig. 1. Thai shrimp production (tonnes), 1985 to 2012.

The opposite approach is represented by Ecuadorian production (Fig. 2). With all the associated economic and social consequences resulting from WSD, the recovery of the industry took a longer period. However, such an approach led to the spontaneous development of shrimp tolerant to WSSV over a period of exposure to the virus, developing what we now refer to as WSSV SPT animals.

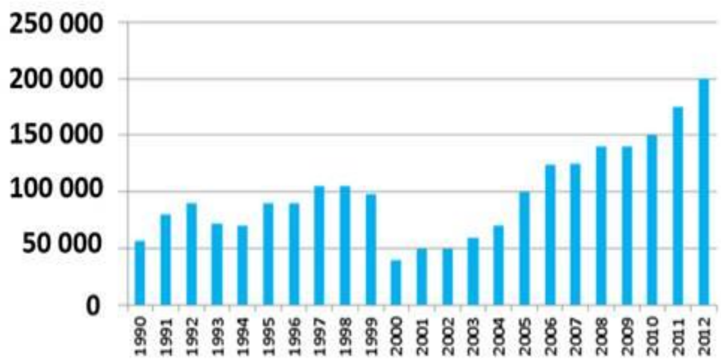

Fig. 2. Ecuadorian shrimp production (thousand tonnes), 1990 to 2012.

Analysing the reaction of both industries in retrospective, the idea of developing an SPF stock from WSSV SPT animals was conceived. From the first outbreaks of WSD in Latin America in 1999 until 2010, the degree of WSSV tolerance achieved in the population was considered acceptable following the increase in survival over the years despite of the presence of WSSV in the ponds.

The WSSV SPT+SPR animals resulting from this programme would be free of infection and therefore, there would be less chance of disease outbreaks and better productivity, while there would be no need to implement strict biosecurity regarding WSD, with the costs associated to it.

The normal practice in Latin America is to collect adult shrimp as broodstock from the ponds. Doing so implies that most of these shrimp are likely to be infected with any pathogen present in the pond, and the impact of any disease is likely to be much more significant when there is vertical transmission rather than horizontal transmission of pathogens in the pond. Considering that acute hepatopancreatic necrosis disease (AHPND) was already affecting Asia and there was the possibility of its spread to new geographical areas, the plan was to develop SPF animals prior to the arrival of a new epizootic. Any selection for increased tolerance to new pathogens should be done on an experimental basis, rather than at an industrial scale, which had happened previously, posing an enormous cost to the farmers.

DEVELOPMENT OF WSSV SPT+SPF STOCKS

Two projects to develop SPF stocks from WSSV SPT stocks were started, one in Ecuador and a second in Nicaragua. The projects started in 2010 and lasted until 2012. The projects targeted the main pathogens of penaeid shrimp as known at that time. These included six OIE-listed pathogens and three others:

- OIE-listed pathogens (2010):
    - White-spot syndrome virus (WSSV)
    - Yellow head virus (YHV)
    - Infectious myonecrosis virus (IMNV)
    - Necrotizing hepatopancreatitis (NHP)
    - Taura syndrome virus (TSV)
    - Infectious hypodermal and haematopoietic necrosis virus (IHHNV)
- Other non-OIE listed pathogens included in the screeningg
    - *Enterocytozoon hepatopenaei* (EHP)
    - *Penaeus vannamei nodavirus* (PVNV)
    - *Streptococcus* spp.

As diagnostic tools, nested polymerase chain reaction (PCR) (IQ2000 kits) and histology were used. The company diagnostic laboratories successfully participated in the Arizona Ring Test. The projects started with a nationwide surveillance for primary pathogens and consultations with research centres and official diagnostic centres to learn about enzootic pathogens. The only primary pathogens found were WSSV, IHHNV and NHP. From then on, these were considered enzootics and selected animals would be tested individually for each of them, while they would be tested in pools of 10 animals for the rest of the pathogens (non-endemic). The first objective was to identify individuals exposed to pathogens from the ponds (APE) that were free of pathogens. The target size was between 23 and 30 g, as it was assumed that if animals could reach this size free of pathogens, then they would have specific characteristics of tolerance (SPT) or resistance (SPR) to infection. Other criteria for selection were that the shrimp should be from ponds identified as having high productivity and that to broaden the genetic diversity, they should have as broad geographic origin as possible.

Vertical transmission of pathogen may have different pathways; it can happen within the egg (intra-ovum), on the egg surface (per-ovum) or through the contaminated faeces of the broodstock (Table 1). From a health-management perspective, we can assume that all systemic viruses have some degree of vertical transmission and therefore, infected animals are not acceptable for an SPF development project. Enteric pathogens allow certain management strategies to prevent the contamination of the nauplia. Of the enzootic pathogens, both WSSV and IHHNV have intra-ovum vertical transmission, which meant that any infected animal had to be discarded, as it could transmit the infection to offspring. As NHP is caused by a bacterium, oral treatment of the selected animals with oxytetracycline upon arrival combined with an egg washing with the same antibiotic resulted in the eradication of NHP in both projects.

Table 1. List of shrimp pathogens, their economic relevance, body distribution, type of vertical transmission and recommended broodstock strategy.

Pathogen[1]	**Economic impact**	**Distribution**	**Vertical transmission**	**Broodstock strategy**
WSSV	High (highly prevalent)	Systemic	Intra-ovum	Eliminate
TSV	High (sporadic)	Systemic	Per-ovum	Eliminate
YHV	High (sporadic)	Systemic	Per-ovum	Eliminate
IHHNV	Medium	Systemic	Intra-ovum	Eliminate
YHV/GAV	High (localized)	Systemic	Per-ovum	Eliminate
IMNV	High (localized)	Systemic	Per-ovum	Eliminate
Streptococcus	High (localized)	Systemic	Per-ovum/ Faeces/opening of mouth	Management
NHP	Medium	Digestive track	Faeces/opening of mouth	Management
BP, MBV, HPV	Low	Digestive track	Faeces/opening of mouth	Management
EHP	High	Digestive track	Faeces/opening of mouth	Management
AHPND	High	Internal & external cuticle & faeces	Surface colonization Faeces/opening of mouth	Management

[1]AHPND = acute hepatopancreatic necrosis disease, BP = *Baculovirus penaei*, EHP = *Enterocytozoon hepatopenaei*, GAV = gill-associated virus, HPV = hepatopancreatic parvo-like virus, IHHNV = infectious hypodermal and haematopoietic necrosis virus, IMNV = infectious myonecrosis virus, MBV = Monodon baculovirus, NHP = necrotising hepatopancreatitis, TSV = Taura syndrome virus, WSSV = white-spot syndrome virus.

To conduct these projects, several additional facilities were needed: quarantine 1 and 2, broodstock multiplication centres and larviculture. Water treatments were implemented, biofloc was established indoors, and all fresh feeds were tested by PCR for known pathogens. Biosecurity protocols were established and training considered crucial for the implementation of biosecurity was provided to staff.

At the time of selection of ponds from which to collect animals, a preliminary screening for enzootic pathogens (WSSV, IHHNV and NHP) of 75 animals was performed. Great variability was found between ponds. Only those ponds with low prevalence and good productivity were used for this purpose.

Animals were brought individually to the facilities where they were cold stressed (22–24 ^{o}C) in individual buckets. After 48 hr, individual testing was done for WSSV and IHHNV (in pleopods) and NHP (in faeces), and then done again for each female after ablation and spawning. In addition, each individual contributed to pool reactions (10) for TSV, IMNV, YGV/GAV, PVNV, BP and EHP. Pools of postlarvae (PL) (n=120) were again tested for WSSV and IHHNV after cold challenge. Finally, histology of the broodstock was performed for unknown pathogens.

Each animal was tested individually 2 times (males) or 3 times (females) and as a pools, at least twice. The process was repeated for three generations with consecutive negative results. Considering that international standards (OIE and European Union, EU) to declare freedom of disease require two years of testing, we could declare the populations to be SPF by 2012.

In order to add confidence to the process and with concern for the possibility of having missed latent infections, other testing procedures were used, such as using alternative tissue to the pleopod (e.g. haemolymph, gills and lymphoid organ) and different types of stressors (e.g. pH, salinity and alkalinity). In none of these trials was a primary pathogen detected from the cleaned population.

Due to its high prevalence which resulted in very high cost, the project in Nicaragua decided not to exclude IHHNV. The project in Ecuador successfully eliminated all primary pathogens, achieving SPF status. The number of PCR tests performed during the two years of development was over 64 000 in Ecuador and over 100 000 in Nicaragua. The percentage of broodstock discarded was 74 % in Ecuador and 47 % in Nicaragua.

The surveillance within the selected population continued for two years with consecutive negative results. At that time, experts from the Department of Infectious Diseases, University of Zaragoza (Spain) who had been appointed by the EU to declare zones/populations disease free, were invited to audit the programme and granted SPF status to the Ecuadorian program. This auditing procedure continues to be conducted on an annual basis.

The use of SPF populations was immediately reflected in better productivity at every step of production:

- Maturation:
    - Mortality in reception room dropped from 24 to 0.5 %.
    - Mortality after ablation dropped from 15 to 0.3 %.
    - Female mortality during production dropped from 5 to 0.1 %.
- Larviculture:
    - Days to PL12 were reduced from 20–21 to 17–18.
    - PL.g^{-1} were reduced from 350 to 200.
    - Survival increased from 45–50 to 70–75 %.
    - Coefficient of variation decreased from >15 to 12 %.
- Grow out:
    - Grow out was 6 weeks shorter to reach 15 g.

In addition, these WSSV SPT+SPF stocks were cultured under intensive conditions at the same time that SPF stocks were brought in from the United States of America that were known to be susceptible to WSSV but were reported to have higher growth rate. In the intensive farm, high biosecurity was implemented; however, two of the ponds were hit by WSSV and harvesting was carried out as soon as possible. The harvested SPF susceptible stocks had a survival rate of approximately 35 %, while the survival rate of the WSSV SPT+SPF stock was approximately 70 %. These results prove the validity of using a combined approach of genetic characterization and sanitary status as a biosecurity strategy.

The proposed name for these type of SPF stocks is "Reverse SPF", as in order to develop them, the reverse process is required when compared to the development of a conventional SPF stock. Conventional SPF are originated from areas with the minimum exposure to pathogens while Reverse SPF are originated from areas where pathogens are highly prevalent and have survived these conditions.

Application of WSSV SPT+SPF Shrimp

The Ecuadorian stocks were exported to the Kingdom of Saudi Arabia, where the industry had been wiped out by WSSV. Culture systems in the Kingdom of Saudi Arabia are similar to those used in Ecuador, with big ponds (10 ha) which do not allow the implementation of viral exclusion biosecurity nor the control of culture conditions. Despite the harsh desert conditions with daily water temperature fluctuations of up to 10 °C and high salinity (often up to 55 ppt), *Penaeus vannamei* Boone 1931 performed very well, leading to national record production (Fig. 3).

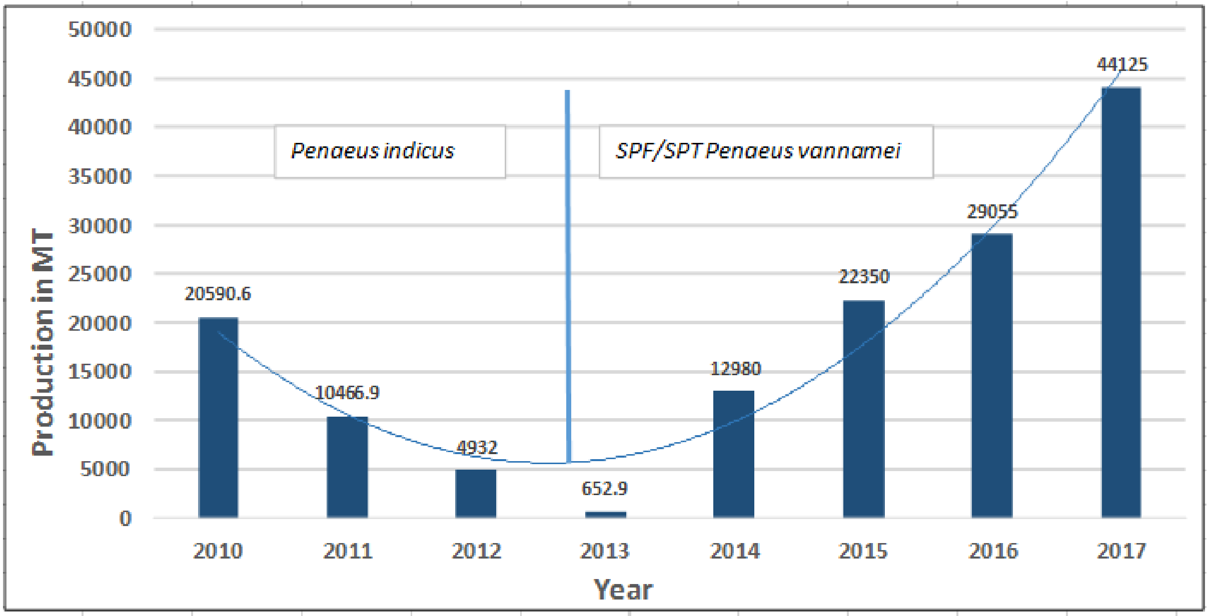

Fig. 3. Shrimp production (tonnes) in the Kingdom of Saudi Arabia, 2010 to 2016.

These are currently the only stocks cultured in the country, and no other source has been approved by the government, as other potential sources are either susceptible SPF, or WSSV SPT but not SPF. The exclusive use of these stocks all over the country is considered to be one of the keys to the success of the biosecurity strategy. In addition to the private-company surveillance programmes, the government has its own monthly surveillance programme. Since 2014, only two detections of WSSV have occurred (in 2015 and 2016), both of them localized in the south. Some mortality was observed and after-harvest survival remained at approximately 70 %. The experience of using WSSV SPT+SPF as demonstrated in the Kingdom of Saudi Arabia could be a solution for countries still impacted by WSSV that do not have the knowledge or the investment capacity to develop small, biosecurity controlled ponds. These stocks could also be used to reduce the cost of biosecurity in intensive systems; however, this would first require the improvement of growth through a breeding programme to be able to match the growth of other SPF stocks currently in the market. From this experience, it can be concluded that the use of SPF stocks is a fundamental strategy to the sustainability of shrimp farming including the extensive and semi-extensive systems with low/none biosecurity. When pathogen exclusion is not possible, SPF status needs to be combined with SPT/SPR characteristics of the stocks for the pathogens endemic in the farming region. SPF claim only defines the health status but there is a common misconception that SPF are more tolerant to diseases (misconception more widespread in Asia) or more susceptible to disease (misconception more widespread in Latin America). As a farmer, we should question stocking, in other words, investing, in animals which are infected by the time we stock.

Reference

FAO. 2003. Biosecurity in food and agriculture. Committee on Agriculture, FAO, Rome.