Certification Process of Imported Pacific White Shrimp *Litopenaeus vannamei* (Boone, 1931) at Aquatic Quarantine Facility, India

M. C. Remany1, DalyCyriac1, D. Kannan1, A. K. Panda1, Jaideep Kumar2 and Y. C. Thampi Samraj2

1. Aquatic Quarantine Facility for *L. vannamei*, Rajiv Gandhi Centre for Aquaculture, TNFDC Hatchery Complex, Neelankarai, Chennai 600041, Tamil Nadu, India
2. Technology Transfer Training & Administrative Complex, Rajiv Gandhi Centre for Aquaculture, Nagapattinam, Sirkali 609109, Tamil Nadu, India

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Abstract: The Aquatic Quarantine Facility (AQF) for *Litopenaeus vannamei* established under the Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture is located on the eastern coastal region of Chennai in Tamil Nadu, India. The facility is an approved quarantine premise for the SPF *vannamei* broodstock imported across India and is probably the only one of its kind to be established in Southeast Asia. The centre is managed and operated by Rajiv Gandhi Centre for Aquaculture (RGCA), a technical extension of the Marine Products Export Development Authority (MPEDA), Ministry of Commerce & Industry, Government of India. The quarantine facility functions on the basis of strict biosecurity protocol and as per the guidelines stipulated in Standard Operating Procedures (SOP) framed by a team of experts. The facility ensures specific pathogen free status of the SPF *L. vannamei* broodstock imported to India, thus playing key role in restricting the entry of diseases to the Country. This article presents the SPF certification process of the imported *L. vannamei* carried out at the facility.

Key words: Aquatic quarantine facility, specific pathogen free, standard operating procedures, *L. vannamei*, broodstock and biosecurity.

1. Introduction

Quarantine is one of the wide ranges of risk management options that can be applied, either alone or in combination, to reduce the risk posed by aquatic animal pathogens [1]. At the international level, the primary purpose of quarantine is to minimize the risk of introducing pathogens into the territory of the importing country and their transmission to susceptible species. Importation of Specific Pathogen Free (SPF) animals to a country provides some assurance that the imported animals will not introduce the listed pathogens to native species and it mitigates the risk associated with the movement of the exotic species. The import of SPF stocks of *Litopenaeus vannamei*, is an initiative by the Government of India to provide shrimp growers and hatcheries with broodstock of known health status with regard to certain pathogens. The initiative was an offshoot of the stagnant shrimp production due to exclusive *Penaeus monodon* culture and its associated disease problems in India. The production, predominantly black tiger prawn (*P. monodon*), has declined from 106,165 tonne in 2007-08 to 75,996 tonne in 2008-09, a fall of 28.41 per cent [2]. To combat the prevailing challenges in farming *P. monodon*, *L. vannamei*, commonly called as the Pacific white shrimp widely cultivated in the US and the Western Hemisphere [3] is being introduced as

Corresponding author: M. C. Remany, assistant project manager, research field: shrimp pathology. E-mail: aqfacility@gmail.com.
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*Litopenaeus vannamei* is considered to be more disease resistant, tolerant to high stocking densities, low salinity and temperature and with high growth rate [4]. The decision to import *L. vannamei* in India was spurred further by the continuous demand of the shrimp growers and traders for the introduction of this shrimp as they believed that there is good export market potential for this species. The importation of this exotic shrimp called for the set up of a quarantine facility which was essential to reduce the risks of adverse effects arising from the introduction of non native species [5]. Subsequently, a dedicated quarantine centre for *L. vannamei* called the “Aquatic Quarantine facility for *L. vannamei*” (AQF), was established in Chennai, Tamil Nadu, as a technical arm of the Rajiv Gandhi Centre for Aquaculture (RGCA) under the Marine Products Export Development Authority (MPEDA), Ministry of Commerce & Industries, Government of India (Fig. 1).

The facility started its operation in July 2009. The Centre is probably the only one of its kind to be in Southeast Asia, set up for the quarantine purpose of imported SPF (Specific Pathogen Free) *L. vannamei*. The AQF serves as an approved quarantine premise for the imported SPF stocks. The centre functions on the basis of strict pre-quarantine and quarantine protocol which was notified under the Act regulated by the Animal Quarantine & Certification Services (AQ & CS) of the Ministry of Agriculture. The quarantine protocol followed for *L. vannamei* falls under the category of “high risk species” which means quarantine of aquatic animals moved either internationally or domestically between regions of different health status that are destined for use in aquaculture [1].

The key role of the facility is to ensure the SPF status of the imported broodstock, thus preventing the entry of any infected broodstock. The centre funded by the National Fisheries Development Board (NFDB), Ministry of Agriculture, operates on Standard Operating Procedures (SOP) framed by a team of technical experts. The member institutions involved in this effort include the Coastal Aquaculture Authority (CAA), the Aquatic Quarantine and Certification Services (AQ & CS, Ministry of Agriculture, Dept. of Animal Husbandry, Dairying & Fisheries), the National Fisheries Development Board (NFDB), the Central Institute of Brackishwater for Aquaculture (CIBA, Indian Council of Agriculture and Research), the Marine Products Export Development Authority (MPEDA) and the Rajiv Gandhi Centre for Aquaculture (RGCA). All activities of the AQF are under the legal provision of Livestock importation Act, 1898. The present paper highlights the quarantine and SPF certification followed at AQF.

### 2. Functioning of AQF

The SOP of AQF aims to confirm the SPF status of the imported shrimp before permitting its transfer to the importing hatcheries. The hatcheries and farms eligible to import and farm *L. vannamei* were identified by the CAA after inspection of the facilities. The yardstick used to identify the hatcheries was the presence of strict biosecurity control [6]. About 24 hatcheries which fulfill the requirements to import *L. vannamei* were approved by the Authority. Similarly, the CAA has also identified eight suppliers of SPF *L. vannamei*, three each from Thailand and USA, and 1 each from Singapore and Hawaii. The broodstock

![Aquatic quarantine facility for *L. vannamei*.](image)
supplier was selected on the basis of genetic base and health status of the shrimps produced by them. Only these suppliers are permitted to provide SPF broodstock to the hatcherries or importers in India. The broodstock imported by the hatcherries were quarantined at the facility for a period of 5 days to ensure that the stock is SPF and of high health. The specific OIE (Office International des Epizooties) listed pathogens diagnosed for SPF *L. vannamei* at the facility are White Spot Syndrome virus (WSSV), Yellow Head/Gill Associated virus (YHV/GAV), Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), Infectious Myonecrosis Virus (IMNV), Taura Syndrome Virus (TSV), Baculovirus *penaei* (BP) and Necrotising Hepatopancreatitis α-Proteobacterium (NHPB).

The operation of the facility, right from the broodstock importation, to the certification of the quarantined broodstock is illustrated in Fig. 2.

### 3. Quarantine Process and SPF certification

The *L. vannamei* broodstock imported from the suppliers were subjected to strict quarantine process as laid down in the guidelines issued by the Ministry of Agriculture (Department of Animal Husbandry, Dairying and Fisheries vide Notification dated 15th October, 2008). The entire process of quarantine and SPF certification of the imported *vannamei* broodstock, in the facility can be categorized under three heads: (1) prequarantine, (2) SPF assurance, (3) quarantine and despatch.

#### 3.1 Prequarantine

The animals on arrival at the airport, are transported to the facility in a sanitized enclosed truck at an ambient temperature maintained at 25 °C. The vehicle is passed through a tyre bath comprising of chlorine before its entry into the facility. The polythene bags containing shrimps are taken inside the primary quarantine unit (prequarantine) for acclimatization (Fig. 3).

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Fig. 2  Standard operating procedures of quarantine broodstock.
Fig. 3  Acclimatization of the broodstock.

Only one representative of the importer/consignee is permitted to observe the shrimps in the receiving area. The general health condition of the lot is then assessed jointly by the scientific staff of AQF and the importer. The salinity, pH, ammonia and temperature of sea water in the spawner bags are recorded on arrival. Within 30 minutes of the broodstock arrival at the facility, pleopod and faecal samples are collected at random for PCR screening of the pathogens. After evaluation and diagnosis of the shrimps for the seven pathogens, the animals are transferred into the quarantine cubicles. The samples if tested positive would be reported to the committee constituted by the technical experts of CIBA and the final decision on destroying the entire consignment would be taken up by them.

3.2 SPF Assurance

The SPF status of the shrimps is ascertained by screening of the seven pathogens which are of industrial concern. PCR technique is employed for the detection of these pathogens. Screening is done as per the OIE manual [7]. WSSV, IHHNV, NHPB, YHV/GAV, IMNV and TSV pathogens are detected by OIE certified kit IQ 2000 Detection and Prevention System (supplied by Farming IntelliGene Tech. Corp, Thailand) and BP by PuRe Taq Ready-To-Go-PCR Beads (supplied by University of Arizona, USA). DNA extracts are prepared from the pleopod samples and faecal strands as suggested by the OIE recognized primer supplier. One half of the extracted DNA is stored for further confirmation test. Screening of RNA viruses is also done in the same manner. Amplifications of WSSV, IHHNV, NHPB, IMNV, TSV, YHV/GAV are performed in a UNIQ programmed thermal cycler (PE Applied Biosystems). The PCR products are then separated in 2% agarose gel, stained in ethidium bromide and the results are then documented using gel documentation system (BIORAD) (Fig. 4).

3.3 Quarantine and Despatch

After evaluation and diagnosis of the shrimps for the seven pathogens, the animals are transferred into the quarantine cubicles, where they are quarantined for a period of 5 days. The facility has four temperature controlled cubicles, each consisting of 158 tanks of 650 liter capacity each (Fig. 5).

The stocking density followed for quarantine is 6-7 per tank. Ambient room temperature is maintained at 25°C-28 °C. Tanks in each cubicle are serially connected to a recirculation system. The recirculation system consists of drain system, sump, physical filtration system and pump. The suspended sediments (left over feed and faeces) are removed from the recirculation sump through a drain pipe. Continuous aeration is provided in all the brood stock quarantine tanks and the water quality parameters like temperature, salinity, pH, ammonia and nitrite are monitored twice daily in each brood stock tanks. Light entry is minimized in all the cubicles so as to reduce the stress caused to the animals.

During the quarantine period, the shrimps are fed *ad libitum* @ of 4%-8% of their biomass with 0.3 mm length and 0.25 mm dia pelletized bio-secured certified feed, in addition to SPF frozen polychaete worms. The feed consumption is monitored in each tank at two hours interval after each feeding. Unfed feed and faeces were siphoned out twice daily from the tanks.
On completion of 5 days quarantine, a test report highlighting the SPF status of the broodstock and the broodstock condition is sent by the Aquatic Quarantine Officer of AQF to the Animal Quarantine Officer of AQ & CS, wherein the latter furnishes a quarantine clearance report to the concerned stakeholder. With the receipt of the quarantine clearance report, the broodstock is released to the stakeholder and is permitted to transport the stock to the hatchery for nauplii production. Till date, 20 stakeholders have utilized the quarantine facility in a total of 62 consignments.

The percentage of SPF vannamei broodstock lots received by the facility from the CAA approved suppliers located in three main regions is depicted in Fig. 6. Table 1 shows the broodstock details imported from these suppliers.

The total number of broodstock imported by the stakeholders was 25253 and the number of broodstock quarantined and despatched was 23317. The overall mean survival rate estimated in terms of live received by the facility was recorded to be 94.01%.
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Controlled introduction of *vannamei* to India in selected farms yielded impressive yields and attractive production costs (unpublished information), giving farmers a free choice on which species to culture. In the year 2010 the production was estimated at 20,000 tons mostly contributed from Andhra Pradesh. The production is expected to reach up to 40,000 tons in 2011. Short listing of more SPF *vannamei* suppliers based on the genetic programme and status of the SPF facility and permit to more number of hatcheries based on strict biosecurity would help more shrimp farmers to switch to *vannamei* culture to augment their income.

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