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Identifying diseases affecting farmed *Litopenaeus vannamei* in different areas of Nellore district in Andhra Pradesh, India

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Abstract
Pacific white shrimp (*Litopenaeus vannamei*) is the most extensively farmed crustacean species in the world. A study was conducted throughout one year from January, 2015 to December, 2015 in shrimp culture ponds of Kavali, Gudur and Nellore divisions of Nellore district in Andhra Pradesh (A.P), India. The study involved 287 ponds in the selected three divisions of 9 Mandals spread in 32 villages and seven diseases are reported from the culture ponds of *L. vannamei*. Disease outbreaks occurred in most of the culture ponds particularly in Gudur and Nellore divisions. With the development of shrimp culture practice from traditional form to modern intensive culture practice, the complexity of diseases has been equally magnified in Andhra Pradesh. The frequent outbreaks of diseases such as White Spot Syndrome Virus (WSSV), Black Gill Disease (BGD), Running Mortality Syndrome (RMS), Loose Shell Syndrome (LSS), White Faecal Syndrome (WFS), White Muscle Disease (WMD) and Infectious Hypodermal and Haematopoietic Necrosis (IHHN) in shrimps causing economic loss to the aquaculture industry in Nellore district. One or more diseases are common in culture ponds of *L. vannamei* due to various reasons. About 50% diseases occurred in the culture ponds of Kavali division, 94% in Gudur and Nellore divisions. Farmers are losing about 60 per cent of their investment due to these diseases in every crop in the season. Shrimp aquaculture industry has experienced severe setbacks due to devastating viral diseases. *L. vannamei* is an exotic species and culturing both in freshwater and saline waters in A.P. The viral outbreaks are minimal in low saline waters compared to the high saline waters with the best management practices. The prevention, control and awareness of disease are very crucial for successful shrimp culture farming. The farmers should aware that, the *L. vannamei* seed stocked is only specific pathogen free (SPF) but not free from all pathogens.

Keywords: *Litopenaeus vannamei*, Shrimp farming, Nellore District, Andhra Pradesh

1. Introduction
Shrimp farming has grown from a traditional, small scale business in South East Asia into a global industry than other culture systems [1]. The rapid growth of shrimp farming led to an economic boom but, unfortunately, the outbreak of viral diseases has increased the economic risks and slowed the industry development [2]. The culture of tiger shrimp *P.monodon* was in a steady progress globally until early 90's, when it was struck by white spot syndrome disease [3]. The shrimp aquaculture in India too suffered significantly due to WSSV infection [4]. Difficulties in captive breeding of tiger shrimp could not make it possible for the development of Specific Pathogen Free (SPF) and genetically improved stains with disease resistance [5]. In this context SPF stain of pacific white leg shrimp (*Litopenaeus vannamei*) was introduced in India in 2009 which revived the shrimp culture in India. Unfortunately, the SPF shrimp was introduced in those areas where infections already existing.
The SPF status does not ensure that it is free from all the diseases. Therefore, the threat of shrimp diseases continued to remain. The practice of bio-security measures like bird fencing, crab fencing, filtration and disinfection of water before pumping into the main pond helped to contain the spread of the disease particularly the carriers of the pathogens. The implementation of Better Management Practices (BMP) at every stage also helped to contain the spread of the disease.
However, of late some unregistered hatcheries supplied the post larvae developed from domesticated SPF brood stock led to deterioration of quality in the seed creating the stress in this post larva, the stress is further aggravated by abiotic factors leading to the bacterial and

viral infections. Further, the diseases of *P.monodon* have also been found in *L. vannamei* indicating the transfer of pathogens from *P.monodon* to *L. vannamei*. However, data on the diseases outbreaks of the *L. vannamei* have not been surveyed extensively in Nellore district. Therefore, the present work was undertaken to document the diseases outbreaks of the *L. vannamei* in Kavali, Gudur and Nellore divisions of Nellore district in Andhra Pradesh, India (Fig. 1).

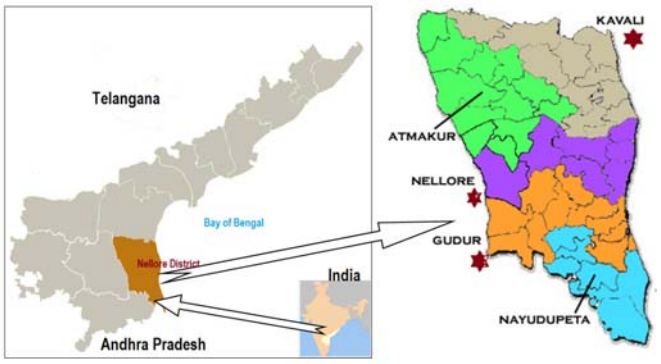


Fig 1: Location of Kavali, Gudur and Nellore study division areas in Nellore district of Andhra Pradesh.

2. Materials and Methods

Nellore district (officially: Sri Potti Sriramulu Nellore district), located in Coastal Andhra region, is one of the 13 districts of Andhra Pradesh. Nellore district is known as shrimp capital of Andhra Pradesh, it is located in Nellore District, lies between 13-30' and 15-6' of the Northern latitude and 70-5' and 80-15' of the Eastern Longitude and extending over an area of 13076 Sq. Km. This district is bordered by the Bay of Bengal to the east and consists of 5 revenue divisions. During the study period (January 2015 to December 2015), 287 culture ponds were observed in Kavali (Allure, Vidavalur mandals), Gudur (Kota, Gudur, Chittamur mandals) and Nellore (Indukurpet, Venkatachalam, Manubrolu, T. P. Gudur mandals) divisions comprising of 32 villages where the *L. vannamei* culture is more predominant in Nellore district of Andhra Pradesh (Table: 1). Moribund and diseased *L. vannamei* were collected from the different culture shrimp ponds. Whole shrimps were immersed immediately in 95% ethanol in plastic containers and samples were maintained at 4 ºC until processing for disease identification in the laboratory.

Table 1: Study areas in 3 divisions of Nellore district.

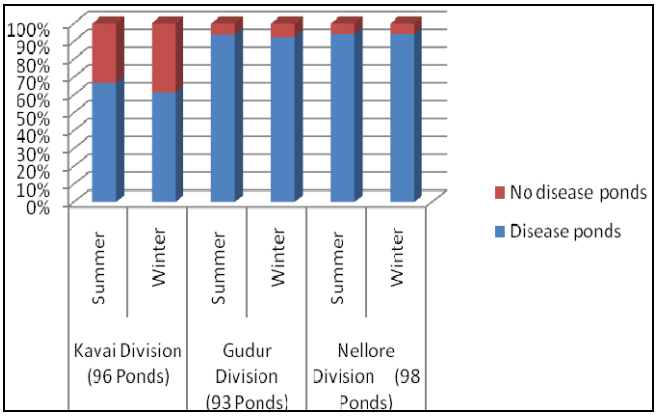
Sl.No.	Name of the Division	Number of Mandals	No. of Villages studied	Total ponds studied
1	Kavali	2	10	96
2	Gudur	3	16	93
3	Nellore	4	6	98
	Total	9	32	287

3. Results and Discussion

Seven major diseases viz. White Spot Syndrome Virus (WSSV), Black Gill Disease (BGD), Running Mortality Syndrome (RMS), Loose Shell Syndrome (LSS), White Faecal Syndrome (WFS), White Muscle Disease (WMD) and Infectious Hypodermal and Haematopoietic Necrosis (IHHN) were found in *L. vannamei* culture ponds of all the three divisions of the Nellore district. About 50% diseases occurred in the culture ponds of Kavali division, 94% in Gudur and Nellore divisions. It is observed more disease ponds in Gudur and Nellore divisions during both the seasons (Table: 2 & Histogram). It is found that, those farms which were implemented bio-security measures and without any Dissolved Oxygen (DO) problems were less infected.

Table 2: Division-wise disease outbreaks ponds particulars.

Name of the Division	Kavai Division (96 Ponds)		Gudur Division (93 Ponds)		Nellore Division (98 Ponds)	
	Summer	Winter	Summer	Winter	Summer	Winter
Disease ponds	64	59	87	86	92	92
No disease ponds	32	37	6	7	6	6



Histogram: Division-wise disease occurrence ponds particulars

All farmed marine (penaeid) shrimp species are highly susceptible to white spot disease, with mass mortalities commonly reaching 80–100% in ponds within a period of 3–10 days [6, 7]. White Spot Syndrome Virus (WSSV) has a wide host range among decapod crustaceans [8, 9, 3] and is potentially lethal to most of the commercially cultivated penaeid shrimp species (OIE 2003). It is observed that inspite of its SPF status of brood stock, WSSV infection is found in *L.vannamei*. White spots appear on the carapace and other parts of the body. Due to lack of bio-security at the farm, pumping water without disinfection and filtration were observed to be main reasons for the outbreak of WSSV (Fig. 2).

Fig 2: White Spots in carapace of the *L.vannamei.*

Although Infectious Hypodermal and Haematopoietic Necrosis (IHHN) does not cause mortalities in *P. vannamei*, it has been shown to reduce growth by up to 30% and cause deformities of the rostrum and anterior appendages in a condition called "runt deformity syndrome" [10, 11, 12]. The symptoms include certain deformities in cuticle and rostrum of infected shrimps, marked reduction in food consumption followed by behavioural changes and appearance of clinical symptoms. Good

management practices will help to prevent the entry of the virus into culture system. Generally the larvae produced from SPF parents will not get IHHNV infection. The presence of IHHNV infection in a pond can be an indication of using post larvae produced from brood stock raised from local shrimp farms (Fig. 3).

Fig 3: Tail necrosis in *L. vannamei.*

Poor water quality is the main factor for Black Gill Disease (BGD) disease (Fig. 4). This disease is common in penaeid shrimps. This disease generally due to abiotic factors like heavy organic load in pond bottom, presence of pollutants, low DO problems and high stocking densities. These environmental factors negatively influence the sustainability of shrimp farming and also directly affect to the growth rate and survival in the grow-out culture system [13]. This disease indicates that the pond bottom is not properly maintained. The growth of the animal is retarded in this disease. Change in the colour of the gill is the first clinical symptom of fungal infection. In the early stages, the gills appear opaque white to yellow or brown in colour and finally it appears as black [14]. The black gill diseases of shrimp causes substantial damage to shrimp culture system in grow out ponds during the end of the culture.

Fig 4: Shrimps infected with black gill disease.

Running Mortality Syndrome (RMS) is a new syndrome was found in Nellore district (Fig. 5). The farmed shrimp in the affected ponds show different mortality patterns which are the result of unusual symptoms with no co-relation to any other reported diseases. In early stages of this condition the affected shrimps show cut antennae, uropods turn red in colour and later the hepatopancreas begins will turn reddish yellow; finally entire body turns dark red in colour. Continuous internal mortality was noticed. The dead shrimp settle at the bottom of the pond and to not come to sides or surface. Mortality noticed only during inter- moult stage. Mortality rate is relatively more in low saline ponds. White or yellow faecal matter noticed in the gut. The mortality percentage is very high in most of the cases. Several farmers have lost their crops. In the beginning, the farmers managed the situation by immediately removing the dead shrimps. Some farmers reduced the stocking densities and were able to harvest the crop successfully without RMS.

Fig 5: Running Mortality Syndrome in *L. vannamei.*

Loose Shell Syndrome (LSS) has been reported in the cultured *Penaeus monodon* since 1998 in India [15]. In the present study, loose shell syndrome was also observed in majority of ponds in all the three divisions i.e., Kavali, Gudur and Nellore in the district (Fig. 6). The affected shrimp are with soft exoskeleton, lethargic, spongy, and flaccid, feed poorly; atrophy of hepetopancreas and show a gap between the muscle and the shell. Moribund, weak shrimp with muscular dystrophy appear on pond edges and results in progressive mortality. The growth of the shrimps is severely affected by the LSS infection in the culture ponds [16]. The mineral deficiency, poor water quality and high loads of vireo pathogens may be the causes of loose shell syndrome [17].

Fig 6: Loose Shell Syndrome (LSS) in *L. vannamei*

White Faeces Syndrome (WFS) is observed in all the divisions of *L. vannamei* culture ponds (Fig. 7). Incidences of WFS were observed after 50-60 days of stocking of the PLs [18]. WFS in shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies, which superficially resembles like with protozoan Gregarines. White faecal matter floats on the water surface in the culture ponds.

Gregarine protozoans along with huge amount of pathogenic Vibrio bacteria may be responsible for WFS. Vibrio species have been found in the fecal analysis from infected shrimps [19, 20]. Early disease indications appear in feed trays and at water surface, where abundant floating white feces are observed. Poor water quality, unhealthy seed, high loads *Vibrio* spp. and presence Protozoa gregarines like organisms in the intestine and hepatopancres are some of reasons for causes of the disease [21].

Fig 7: White faecal matter in *L. vannamei* culture pond.

White Muscle Disease (WMD) is also observed in all the three divisions of Nellore district (Fig. 8). Infected shrimp shows lethargy in movement, whitish tissue appearance and shrimp becoming opaque. Several authors reported white muscle disease in shrimps and prawns [22, 23, 24]. The disease starts with tail becoming whitish and also white colour appears from the middle of the back will result in loss of transparency.

Fig 8: White muscle disease in *L. vannamei*

4. Conclusion

The diseases are serious problems posing threat to sustainability of *L. vannamei* culture in Nellore district of Andhra Pradesh. The farmers should be vigilant and be sensitised on the knowledge about these diseases and their prevention. If the farmers implement better management practices at every stage of culture then only the sustainability of *L. vannamei* culture can be achieved. The biosecurity measures like crab fencing, bird fencing and maintaining a reservoir ponds are to be maintained for the minimising the diseases during the culture period. The drawn water for culture should be filtered properly and should be disinfected.

The farmers should purchase the quality seed from the Coastal Aquaculture Authority (CAA) permitted registered hatcheries only. Once the disease is found in culture pond, the water should be properly treated, disinfected and then only the water should be released into the creek. Small farmers can adopt cluster approach to check out the water quality and to control the diseases.

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