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RESEARCH ARTICLE

BLACK GILL INFECTION IN *LITOPENAEUS VANNAMEI* AND ITS IMPACT ON PRODUCTION

Selvakumar, P. and *Murugasen, P.

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences Annamalai University, Parangipettai – 608 502, India

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ABSTRACT

Black gill disease in *Litopenaeus vannamei* was studied during 2014 in the grow-out ponds of Tamilnadu. The disease was characterized by black coloration and destruction of gills due to which lamellae became atrophic and necrosis. In severe cases, the disease led to chronic mortality of shrimps during the culture. The fungal stain *Aspergillus* sp. was isolated from the diseased gill. The prevalence of infection reached upto 80 % on 95th days. The *b* value of the diseased shrimps was slightly lower than normal one. The present study clearly shows that the poor water quality management caused the black gill disease and the stocking density also one of the factors which trigger the black gill disease in *L. vannamei* grow-out ponds.

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INTRODUCTION

Penaideae is a common shrimp family used in aquaculture by Indians. The culture of various aquaculture candidate species has an economical importance in many parts of the world as well as in India. The culture shrimps grown in the confinement environment, its vulnerable to disease caused by the microorganisms, which dramatically affects productivity. Rapid development together with the intensification of farming practices since last two decades has created several problems in the shrimp culture industry over the worked and also in India. Disease outbreaks in cultured shrimps are the major concern which resulted in severe economic losses. In addition to existing disease problems, several new diseases of unknown etiology remain to be studied to determine the causative agent and mechanisms of infection to manage the problem sustainably. Diseases in the shrimps aquaculture caused by bacteria, fungal, protozoan, culture environment and feed (Lightner, 1993).

The shrimps diseases caused by the biotic and abiotic factors are dependent the types of culture system (semi-intensive and intensive), water quality parameters and the management practice they follow. The shrimp industries facing one of the critical problem is disease outbreak which lead to heavy economic loses. In the present attempt, the gill disease is focused that too the black gill disease of the captive shrimps. Gill is the respiratory organ of the shrimps and also regulates the haemolymph osmatic process. Since the gill always link with surrounding waters, it's offer the pathogen and toxinx contact with the gill, hence, the gill is one of the most important and vulnerable organ in diagnosing health of a shrimp by gill (Shakuri, 2015). The black gill disease in the penaid prawns caused by the genus *Fusarium*. Ishikawa (1968) reported first time the *Fusarium* caused black gill disease in *Penaeus japonicas* from Japan. Followed by Lightner & Fontaine (1973) reported the block gill in the lobster (*Homarusamericanus*) from USA Within the species of *Fusarium*, *Fusarium solani* cause of black gill disease in *Penaeus japonicas* (Hatai & Egusa, 1978). Furthermore, *Fusarium moniliforme* and *Fusarium tabacinum* caused black gill in *Penaeus japonicas* (Rhoobunjongde *et al.,* 1991). Apart from the fungus the ciliates also induce the black gill syndrome. The black gill disease by the presence of ciliates found in the many of the shrimp species. The normal gill became dark coloration response to the ciliates occurrence which is the responsible causative agent for the melaninization of the gill leads to impair respiratory surfaces.

MATERIALS AND METHODS

The black gill infected *L. vannamei* were collected from the ponds from (the study area) then carefully transfer into the

***Corresponding author: P. Selvakumar**
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences Annamalai University, Parangipettai – 608 502, India

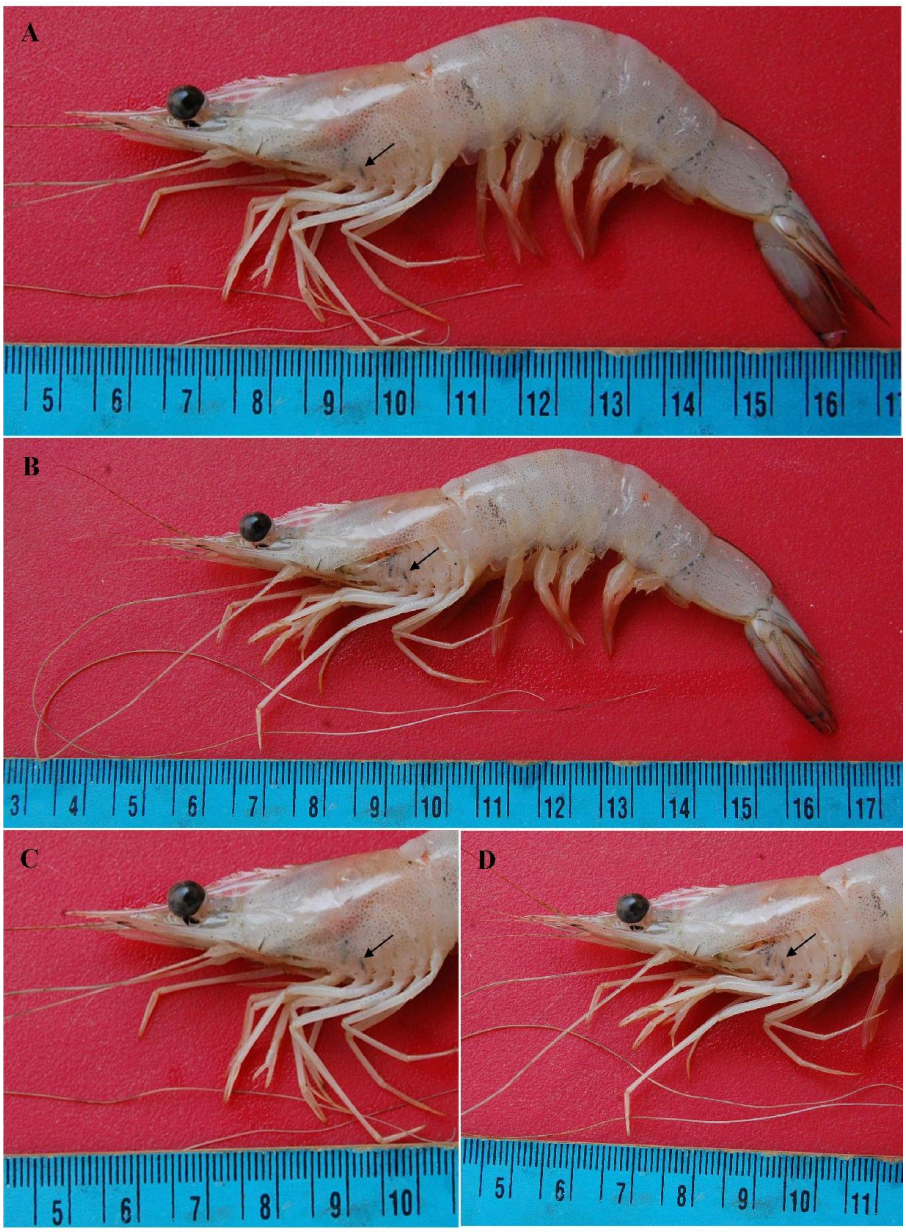

Figure 1. Beginning stage of black gill disease in *L. vannamei*

Figure 2. Sever infection of black gill disease in *L. vannamei*

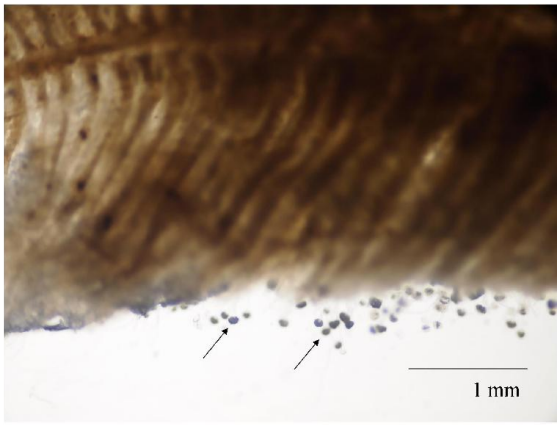

Figure 3. Wet mount black gill disease in *L. vannamei,* fungal spore (arrow)

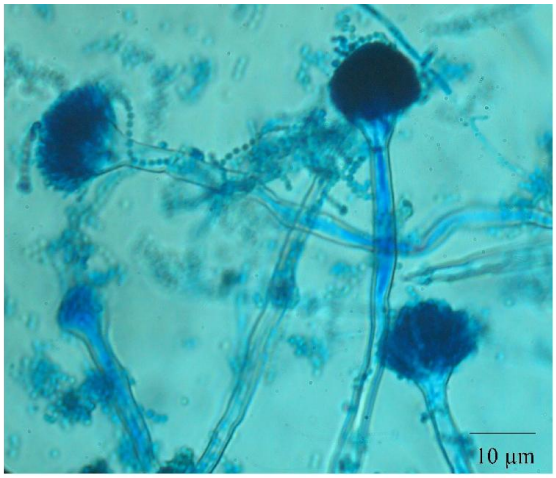

Figure 4. *Aspergillus* sp. Isolated from gill of black gill diseased shrimp

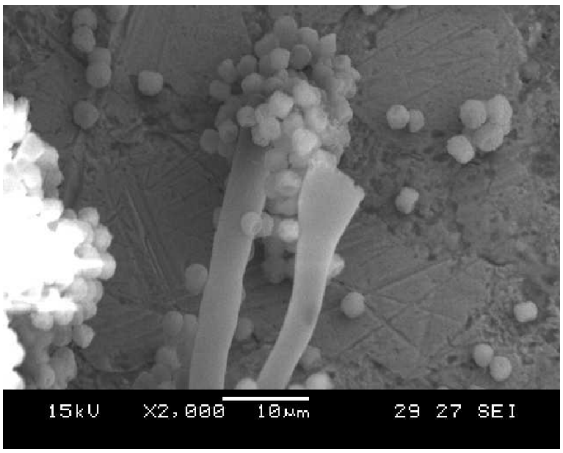

Figure 5. SEM image; *Aspergillus* sp. Isolated from gill of black gill diseased shrimp

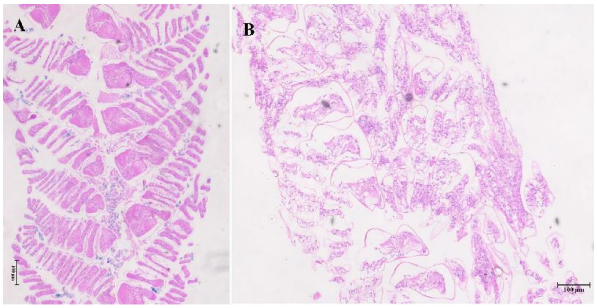

Figure 6. Cross section of *L. vannamei* gill; normal (A), black gill infected (B).

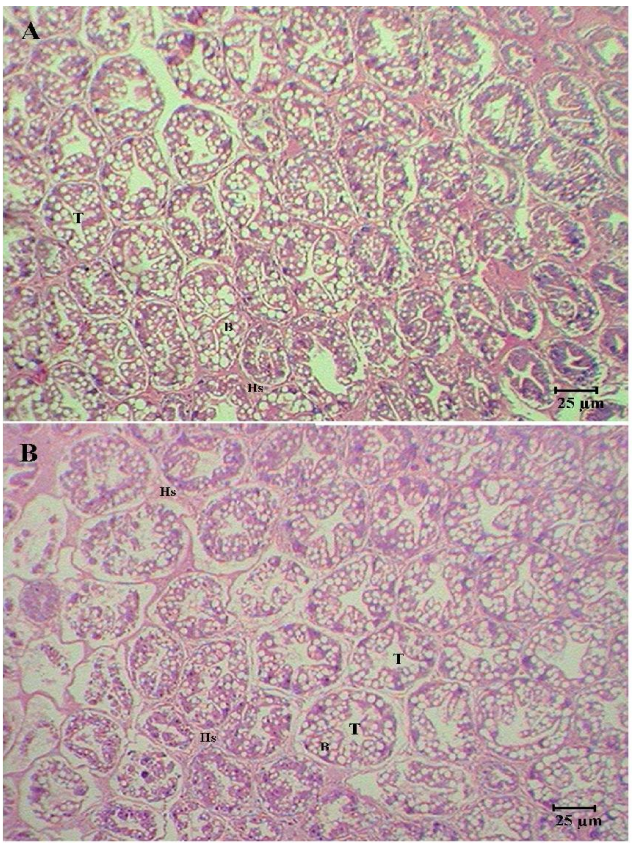

Figure 7. Cross section of *L. vannamei* hepatopancrease; normal (A), black gill infected (B).

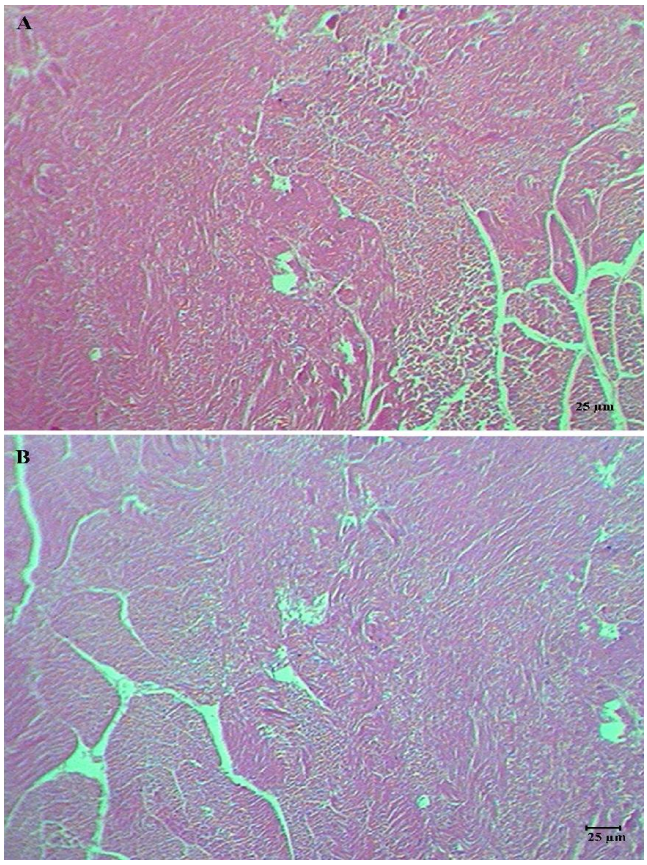

Figure 8. Cross section of *L. vannamei* muscle; normal (A), black gill infected (B).

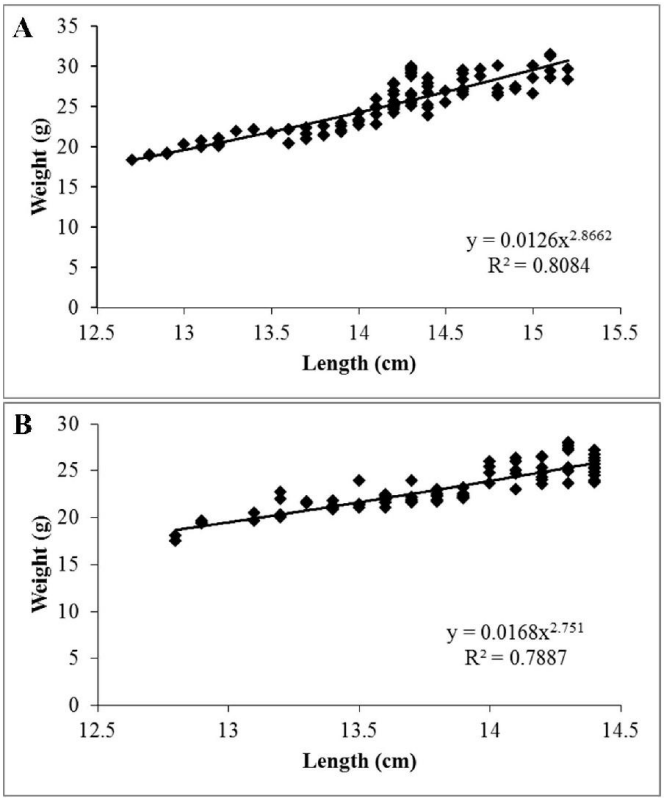

Figure 9. Length and weight relationship of L. vannamei; normal (A) black gill infected (B).

laboratory in live condition in a bucket with sufficient aeration. Twenty Infected shrimps were collected and the length and weight ranged between 17 cm and 20 to 25 g respectively were stocked. Water temperature, pH, Dissolved oxygen and salinity were measured using digital thermometer, pH pen, Winkler's method and refractor meter respectively. The length–weight relationship was calculated using the conventional formula $W = a.L^b$ where W is the derived weight of shrimps (g) (a), L is the standard length (cm) coefficient, 'a' is the intercept in the-axis, and the regression coefficient b is an exponent indicating isometric growth when close to 3.

The normal and black gill infected shrimps were transported to the laboratory in live condition for histopathological investigation. The samples were fixed in the Davidson's fixative and the sections were done according to the methodology described by (Inthisham Nassar *et al.,* 2010).
Black gill infected gills *L. vannamei* was washed three times in sterile physiological saline (0.85% NaCl) and inoculated on plates with Potato Dextrose Agar (PDA) medium (Himedia lab). Ampicillin and streptomycin sulphate (25µg/ml) were added to the medium to inhibit bacterial growth. Plates were incubated for 2–4 days at 25°C and sub-cultured on to fresh PDA plates. The single spore culture method was applied to obtain pure isolates. The isolates were maintained at 25°C on PDA slants for subsequent experiments. Fungal identification was done by lacto phenol cotton blue mount method to observe fungal morphology under light microscopy.

RESULTS

Prevalence of black gill in the infected pond reached up to 80 % on the 95th days of culture. The gill colour of the normal shrimps dusky white. In the earlier stage of the black gill disease caused black spot on the gill (Fig. 1). The external sign of the severe infected shrimps showing the black colored gill when compare with normal one. The entire gill became black in colour and few of them were in brown, which were the earlier stages of the black gill infection. The gill lamellae of the black gill infected shrimps showing the necrotic, atrophic and collapsed (Fig. 2). Further, the gill lamellae of the black infected shrimp's wet mount showed the presence of fungal spore in between gill lamellae (Fig. 3). After five days of inoculation fungal growth was observed in the black gill diseased inoculation, the fungal strain was bright to light green colonies, conidiosphores were coarsely rough and 1 mm in length and loosely radiated (Fig. 4 and 5).

Thehistological investigation of the black gill infected shrimps showed necrotic (Fig. 6). The muscle cross section of the normal and black gill infected shrimp showed no variant in the muscle fibers arrangements (Fig. 7). The cross section of the hepatopancreas shows the normal arrangement of hepatopancreatic cell, no variation found in the heamal space of the both normal and black gill infected shrimps (Fig. 8). The length of the normal shrimps varied from 12.7 to 15.2 cm and weight varied from 12.7 to 31.54 g. The length of the black gill infected shrimps varied from 12.8 to 14.4 cm and weight varied from 17.53 to 27.68 g. The length and weight relationship of the normal shrimps collected from the normal pond *b* value was $b = 2.866$ and the b value of the black gill infected pond was $b = 2.75$ (fig. 9).

The live black gill diseased and normal pond shrimps were transported to the laboratory with live condition. They were stocked in the tank (5, 10 and 20 animals \100 Littre) three tanks were maintained for each density. The water temperature ranged between 27.8 and 30.2 °C (28 ± 1.8 °C), salinity varied between 26 to 29 ppt (27 ± 1.8 ppt), pH ranged between 7.8 to 8.1 (7.9 ± 0.8), dissolved oxygen varied between 4.1 and 6.2 ml/l (4.8 ± 1.7 ml/l). On 4th days of culture the shrimps gill slowly became normal in colour on 7th day of culture the shrimps gill became normal in colour in the density of 5 animals/ 100 L tank. In the density 10 animal/ 100 L the animals begin to turn in the normal colour on 5th day and completely normal gill to be observed on the 10 days of culture. The same trend was found in the 20/ 100 L, where the animal begin to get normal on 10th days of culture and completely cleared the block colour on 16th days of culture. Almost similar results were noted in the dublicate tanks of the each density of experiment.

DISCUSSION

The prevalence of black gill disease is maximum in the present study than the earlier reports. Gadar (2013) reported the black gill disease in many shrimp species caused by the ciliates and the prevalence reached upto 43.27% in October from the Georgia coast. The disease was characterized by black coloration and destruction of gills due to which lamellae became atrophic and collapsed. In severe cases, the disease led to death of shrimps affecting the production and economy. The similar trend also found in the *Penaeus monodon* grow-out pond (Debansu *et al*., 2015)

The present study confirmed that the black gill disease caused by the fungal infection *Aspergillus* sp. Previously many author reported that fungal caused black gill in shrimps, Brock and Lightner (1990) also observed that damage caused by *F. solani* lead to black or melanin and is limited to head, tail, gills and muscles. Reza *et al.* (2014) reported that the black gill disease in marine shrimps due the bad environmental conditions, certain bacteria and fungi attack and nutritional deficiencies. Bian *et al*. (1981) found that the black gill infected *Penaeus japonicas* by fungus *fusarium solani* showed the pathological effect such as hemosits accumulation, hyperplasia and the histopathological investigation showed melanin Kotikoli lesions, granulomatous nodules in the black gill diseased shrimps. Khoa *et al.* (2015) reported the black gill diseased *Penaeus monodon* caused by *Fusarium incarnatum* from grow-out pond in Vietnam. The black gill diseased shrimps showed typical signs of black gill disease and mortalities about a month prior to harvest.

The cross section of the hepatopancrease and muscle showed no variation when compare with normal one, its clearly confirm that the black gill disease does not affect the hepatopancrease and muscle fibers. The longitudinal section of the black gill infected shrimp confirm the presence of fungal hypae which cause the necrotic lesion, which directly affect the growth of the shrimp it again confirmed by length and weight relationship analysis. The results of length and weight relationship in the present study showed that the b values of the normal and black gill diseased shrimps are lower than 3 which indicate the negative isometric growth. However, the b value of the black gill diseased shrimp showed slightly lower value than normal

one. Finally the length and weight relationship analysis revealed that the black gill disease slightly affect the normal growth of the shrimps. The present study clearly shows that the poor water quality management caused the black gill disease and the stocking density also one of the factors causing the black disease in *L. vannamei*. It is difficult to say that the fungal (black gill) caused mortality in the grow-out pond but the impact of fungal infection followed by black gill disease affect the respiratory system which lead to death.

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