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Research Note

Organic Load Induced Black Gill Discoloration in Farmed *Litopenaeus vannamei* and its Mitigation Using a Pond Sanitizer

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White leg shrimp, *Litopenaeus vannamei* (Boone, 1931) is considered as a species that revolutonized shrimp farming industry in India in the recent past. There has been a marked shift from farming of indigenous black tiger shrimp, *Penaeus monodon* to culture of exotic white leg shrimp, *L. vannamei* in India from the beginning of this century. With its present performance in Asia, world production of this species will overtake all other cultivated shrimp species. Over 90% of shrimp culture in Western hemisphere is represented by white shrimp (Wurmann et al., 2004).

Intensification of shrimp farming poses several problems including the sporadic occurrence of diseases in farms. In addition to the most widely occurring viral and bacterial diseases a number of less prevalent disease manifestations are also observed in shrimp farming sector. The present study deals with the observation of black discolouration of gills of farmed *L. vannamei* and its mitigation using a pond sanitizer. Black gill syndrome is also known as black gill disease, burned gills, black spot disease, and branchiostegite melanization. Generally there are multifocal black or brown spots in gills, or general discoloration of gills due to melanization at the sites of tissue necrosis.

The cause of black discoloration in shrimps varies from environmental factors to even specific pathogens. The most commonly reported aetiology for black gill disease is fungal infection caused by *Fusarium* spp (Egusa & Ueda, 1972, Tareen, 1982). Excessive accumulation of toxic substances and higher levels of nitrite in culture water, high organic matter content and highly reduced organic condition in soil, presence of pathogenic bacteria (*Vibrio* spp, *Flexibactor* spp, *Cytophaga* spp) or virus (infectious hypodermal and haematopoietic necrosis virus) also can lead to black gill discolouration in shrimp (Alavandi et al., 1995).

The present observations were made during an experimental farming trial at the brackishwater fish farm of Central Institute of Fisheries Education, Kakinada centre from March to May 2010. The ponds were dried for one month and the top soil was scraped and removed, ploughed and the pre–chlorinated water (20 ppm available chlorine) was filled in the pond. Bio-security measures such as bird fencing over the pond using nylon twine and crab fencing using silpaulin sheets of 60 cm height on pond dykes were fixed. The pond was stocked with a stocking rate of 30 sq m^{-1} and shrimps were fed with a commercial pellet feed with 32% crude protein. Feeding was regulated according to check tray observations and the visual observations of the gut. Ponds were provided with two aerators (4 hp each) throughout the culture period.

Microscopic examinations of infected and moribund specimens were carried out with whole wet mounts (10x magnification) using a light microscope. To verify the possibility of deterioration of the pond bottom, soil quality parameters such as organic carbon level and organic matter (Walkley & Black,

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1934) soil pH (pH meter -ELICO, PE 131, India) and total soil nitrogen (FAO, 1970) of the affected pond and a normal pond were analysed for comparison by collecting samples from different corners of the pond. Water quality parameters also were analysed from both ponds following standard methods (APHA, 1998)

In order to decompose the soil organic matter rapidly, potassium permanganate ($KMnO_4$) was applied in the pond as a pond sanitizer. Initially $KMnO_4$ was applied @ 5 kg ha^{-1} by mixing with sand (sand: $KMnO_4$ at 2:1 ratio) and the mixture was broadcasted to the pond uniformly. After two days, a second dose of $KMnO_4$ was broadcasted @ 10 kg ha^{-1}.

From the 70^{th} day of culture, few moribund shrimps were observed on the periphery of the pond. The effected shrimps were moving on the surface of the pond. Clinical signs observed on the shrimps were dark melanization of gills with muddy appearance and also moribund shrimps exhibited organic debris accumulated at the gill surface (Fig. 1). Dead samples were also having pleopods with muddy appearance. Microscopic examinations of the gill did not reveal the presence of any fungal hypae indicating the absence of fungal pathogens such as *Fusarium* spp. Black gill condition in shrimp caused by *Fusarium* spp initially produces generalized 'gill discoloration' which gradually develops to 'blackened gill' condition and eventually leading to death of affected individuals. The fungal disease is characterised by many black spots on gills and all the infected parts of gills will carry septate hypae of the fungus (Egusa & Ueda, 1972). Such observations were not prominent in the present study, eliminating the possibility of a fungal contamination.

Physico-chemical parameters of water from the affected and normal ponds were analysed during the entire farming period. The range of water quality parameters during the month of May 2010 when infections had occurred has been given in Table 1. There was no prominent difference in water quality parameters between the infected and normal ponds.

High organic matter content due to residual feed, debris, and faecal matter on pond bottom and highly reducing conditions in soil also can cause black gills in shrimp (Alavandi et al., 1995). The organic carbon load (Table 2) in the affected pond (1.46%) was found to be double than the normal pond (0.76%). Abraham & Sasmal (2009) reported a total organic carbon level ranging from 0.22 to 0.70% in a *P. monodon* farm stocked at 10 sq m^{-1} and fed with a commercial feed. Results of the present study indicate excess accumulation of organic load in the affected pond. Higher levels of total soil nitrogen content in the affected pond also indicates the accumulation of organic load. Feed is the major contributor for organic load in shrimp farms and increase in level of total nitrogen in shrimp farms is mainly contributed by left-over feed (Boyd, 1992). Martin et al. (1998) reported that 38% of the total organic nitrogen input in a shrimp pond is accumulated at the pond bottom. Several authors reported that there is an association between the input of feed in aquaculture ponds and the accumulation of organic matter (Avnimelech & Ritvo, 2003; Avnimelech & Lacher, 1979). Excess feeding of the shrimp due to over estimation of the survival percentage during the feed calculations could be the reason for the accumulation of organic load in the pond.

(a) (b)

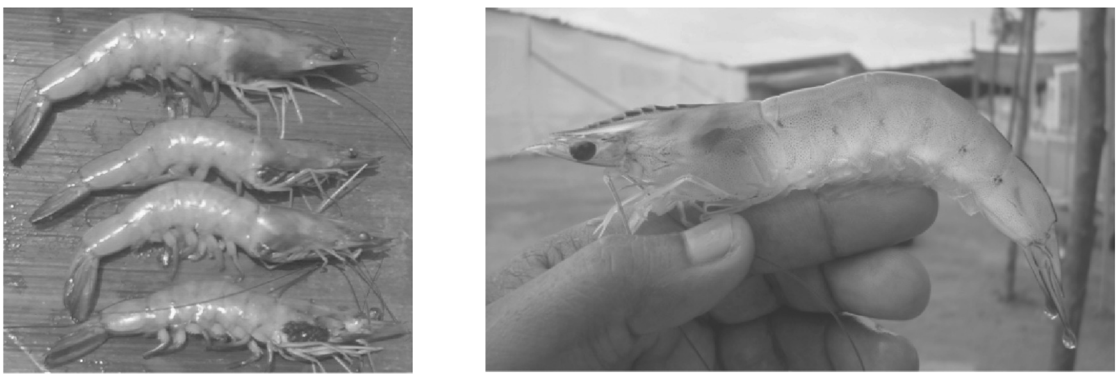

Fig. 1. (a) *L. vannamei* samples showing black discoloration of gills, pleopods and uropod & (b) normal specimen

Table 1. Comparison of water quality characteristics (average) of affected and normal ponds

Pond	pH	Dissolved oxygen (ppm)	Salinity (ppt)	Total alkalinity (ppm)	Nitrite (ppm)	Ammonia (ppm)
Infected	8.1 -8.2	4.4 - 5.2	29-30	192-208	0.02- 0.12	0 -0.02
Normal	8.2-8.4	4 - 5.8	29-30	198-200	0.01- 0.018	0 -0.01

Table 2. Comparison of soil quality characteristics (average) of affected pond and the normal pond

Pond	pH	Organic carbon (%)	Organic matter (%)	Available nitrogen (mg 100 g soil^{-1})
Affected pond	8.36	1.46	2.52	140
Normal pond	8.02	0.76	1.24	69

(a) (b) (c)

Fig. 2. Gills of shrimp from infected pond at different stages of the treatment. (a) infected dead shrimp, (b) shrimp on 3rd day of post treatment of $KMnO_4$ showing partial recovery, (c) fully recovered shrimp on 5th day of post treatment with pale brown gills

From the observations of clinical signs and pond conditions such as organic carbon and organic nitrogen levels in the soil, it was evident that the affected pond was polluted as a result of accumulation of organic load due to over feeding, leading to accumulation of debris on the shrimp gill surface. In order to decompose the soil organic matter rapidly, $KMnO_4$, an oxidising agent was applied to the pond as a pond sanitizer. Marked reduction in the black discolouration of gills was observed on 3rd day post treatment (DPT) and the muddy appearance of the gills was changed to pale black colour (Fig. 2). On 5th DPT, complete recovery of the gill discolouration was observed in the shrimps.

Oxidizing agents such as potassium permanganate, hydrogen peroxide, calcium hydroxide and calcium hypochlorite are used for controlling phytoplankton, eliminating disease causing organisms, or oxidizing bottom soils in aquaculture (Boyd, 1995; Wilkinson, 2002). Potassium permanganate oxidizes organic and inorganic substances and kills bacteria, thereby reducing the rate of oxygen consumption by chemical and biological processes (Lay, 1971). In water, permanganate quickly oxidizes labile organic matter and other reduced substances and is transformed to relatively non-toxic manganese dioxide which precipitates out and thus it is considered to be safe (Boyd & Massaut, 1999).

In a nutshell, the present study indicates that the excess accumulation of organic matter will lead to pollution which in turn may lead to the black/brown discolouration in shrimps. But this specific problem can be overcome by the application of oxidising agents that reduces the organic load to safer levels. Better feed management practices are the simplest way to prevent pollution in shrimp ponds.

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