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# Effect of polyculture of shrimp with fish on luminous bacterial growth in grow-out pond water and sediment

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#### PEER REVIEW

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#### Comments

This is an important study as this is the novel idea for diseases control in aquaculture. The study recommends a vigilant monitoring of shrimp ponds towards the end of second month of cultivation. Details on Page 441

## ABSTRACT

**Objective:** To study the distribution of marine luminous bacteria in shrimp culture systems of West Bengal and the effect of polyculture of shrimp with fish to reduce luminous bacteria. Methods: Luminous bacterial counts were enumerated by spread plating on seawater complex agar from shrimp grow-out pond water and pond sediment samples of West Bengal, India. Results: About 31.16% and 51.44% of pond sediment and pond water samples respectively had detectable levels of luminous bacteria. It was noticed that in normal ponds a shift happened in bacterial profile of water from the day of flooding up to 60 d, with the dominance of luminous bacteria among vibrios, reaching counts 10<sup>4</sup> cells/mL or more. While in diseased ponds, luminous bacterial abundance within the ponds was noticed in the first 6 weeks of culture. Marked reduction in luminous bacterial counts of water and sediment was observed through out the culture period in polyculture ponds compared to monoculture ponds. There was no incidence of white spot syndrome viral disease and luminous vibriosis in both controlled and experimental

## ponds.

Conclusions: The results suggest vigilant monitoring of ponds for luminous bacteria abundance and polyculture of shrimp with fish in ecofriendly sustainable aquaculture can reduce the impact of shrimp disease outbreak.

#### **KEYWORDS**

Shrimp culture, Luminous bacteria, Biological treatments, Polyculture

#### **1. Introduction**

West Bengal is bestowed with the largest impounded brackish water area in India, covering an area of about 2.10 lakh ha besides the coast line of 158 km, spreading over three districts namely East Midnapore, North 24 Parganas and South 24 Parganas. Development of coastal aquaculture in West Bengal is centered on shrimp Peneaus monodon (P. monodon) farming. Scientific culture of shrimp started in West Bengal during mid 1980s and by 2010 more than 54000

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ha area has been brought under culture through traditional, improved traditional, extensive, improved extensive, semiintensive and intensive methods<sup>[1-3]</sup>. Traditional/improved traditional farming is normally practiced at North 24 Parganas and South 24 Parganas districts; while extensive, modified extensive and semi-intensive farms are mainly concentrated at East Midnapore district. The rapid growth of the shrimp farming industry halted suddenly in 1996-1997. The setback to the industry was attributed mainly to the environmental and health problems resulting in the outbreak



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of diseases<sup>[1,4,5]</sup>. In India, the gross economic losses due to shrimp diseases were estimated at Rs. 10221 million in 2006– 2008 and loss continues even now<sup>[6]</sup>. Integration of algae, fish and settling pond for an effective biological process for the treatment of shrimp farm effluents and reduction of disease outbreaks has been proposed<sup>[7,8]</sup>. This article describes the distribution of marine luminous bacteria (LB) and polyculture of shrimp with fish and simple biological treatments for ecofriendly sustainable aquaculture.

## 2. Materials and methods

Samples of grow-out pond water and pond sediment from shrimp culture systems (n=138) were collected along the coastal belt of West Bengal, India between 2000 and 2006 for quantitative and qualitative studies on LB. Luminous bacterial counts (LBC) were enumerated by spread plating on seawater complex (SWC) agar[9]. Briefly, the sediment samples, taken at four places from each pond, were pooled together and mixed thoroughly in a homogenizer before analyses. Surface water samples were collected in 300 mL capacity sterilized containers. Ten-fold serial dilutions of water and sediment samples were prepared and appropriated dilutions of the samples were plated on to SWC agar. All plates were incubated at (30±2) °C for up to 24 h. Luminescence on SWC agar was observed in a dark room after 16-20 h of incubation. Luminous colonies with distinct colony characteristics were aseptically picked, repeatedly streaked on SWC agar until pure and maintained on SWC agar slants. Luminous bacterial isolates were identified as described elsewhere<sup>[10,11]</sup> following the scheme of Abraham et al<sup>[12]</sup>. The details of shrimp farming and management practices followed are summarized in earlier reports<sup>[4,5]</sup>.

Attempts were also made to reduce the outbreak of diseases through biological means such as use of biological products and polyculture of shrimp with fish in Soula, Contai region (Lat 21°48'N; Long 87°45'E), East Midnapur district for one crop following modified extensive culture. The details of species used, stocking density and the use of chemical and biological products are furnished in Table 1 and Table 2, respectively. The polyculture (shrimp and fish) and monoculture (shrimp alone) ponds received biological products as per the directions provided by the manufacturers and consultants.

## Table 1

Details of stocking and production of fish/shrimp in culture ponds.

Name of the species	Stocking density		Hamman at a star (a) (Maran I	Production in kg/crop	
	Pond A	Pond B	SD)	Pond A	Pond B
	(0.6 ha)	(0.68 ha)		(0.6 ha)	(0.68 ha)
P. monodon	$15/m^2$	17.5/m <sup>2</sup>	29.0-31.0 (29.5±1.50)	1 806.00	2650.0*
L. parsia	1 000/pond	1000/pond	100.0-245.0 (155.0±50.00)	100.75	97.0
M. gulio	1 000/pond	1 200/pond	30.0-69.0 (50.0±15.00)	32.50	39.0

<sup>\*</sup>Shrimp production in monoculture (control) ponds with the stocking density of 17.5/m<sup>2</sup> was 2043 and 2448 kg/crop. No white spot syndrome virus infection and luminous vibriosis were observed in shrimp.

#### Table 2

Chemical and biological products used in the ponds with and without fish during culture.

Product	Brand/Commercial name
Probiotics	Super PS, Bottom Lac, Farm yeast juice $^*$
Feed additives	Nucleotin + PNP complex
Herbal sanitizer	Formula 369
Other chemicals	Zeolite, lime, lime stone powder

<sup>\*</sup>Composition of farm yeast juice are ground nut oil cake: 5.0 kg, polished rice: 5.0 kg, molasses: 5.0 kg, bakers yeast: 200 g, super PS: 750 mL.

## 3. Results

The results on the distribution of LB in shrimp culture system are depicted in Figures 1–3. About 31.16% and 51.44% of pond sediment and pond water samples, respectively, had detectable levels of LB (Figure 1). In normal ponds, the LB counts increased up to 60 d of culture (DOC) in growout ponds and decreased thereafter. Although there were fluctuations, the average LB counts were found to be higher (log 2.91±0.57/mL) between the DOC 30 and 60 (Figure 2). The sediment samples also recorded the highest average LB counts (log 3.49±0.58/g) between the DOC 30 and 60 (Figure 3). The present study identified three species of LB, *viz.*, *Vibrio fischeri* (0.57%), *Vibrio harveyi* (94.86%) and *Vibrio splendidus* biotype 1 (4.57%) from the shrimp farming systems of West Bengal.







Figure 2. Log LBC in pond water.



Figure 3. Log LBC in pond sediment.

The results of polyculture of shrimp with fish are furnished in Table 1. The aquatic drugs and biological products used in various stages of production are presented in Table 2. The pond A yielded 1806 kg *P. monodon* (stocking density: 15/ m<sup>3</sup>), 100.75 kg *Liza partia* (*L. parsia*) and 32.5 kg *Mystus gulio* (*M. gulio*). While the pond B yielded 2650 kg *P. monodon* (stocking density: 17.5/m<sup>3</sup>), 97.0 kg *L. parsia* and 39.0 kg *M. gulio*. The average size of *L. parsia* and *M. gulio* at harvest was (155±50) g and (50±15) g, respectively. The shrimp productions in the monoculture ponds (stocking density: 17.5/m<sup>3</sup>) were in the range of (2043-2448) kg/pond (Table 1). The water and sediment samples of polycultrue ponds recorded comparatively low LBC than in monoculture ponds (Figure 4).



Figure 4. Log LBC in *P. monodon* ponds with and without fish.

## 4. Discussion

The results indicated that shrimp culture systems of West Bengal are characterized by abundant availability of nutrients derived from source water, excess feed, shrimp excreta, dead and decaying organic matter, which caused significant luminous bacterial growth. The water entering the pond was identified as the main source of luminous vibrios in free– living planktonic forms. *Vibrio harveyi* was the dominant LB both in water and sediment samples, and also in the earlier studies<sup>[11,12]</sup>. Further, the results clearly revealed a shift in bacterial profile of water from the day of flooding up to 60 d with the dominance of LB, reaching counts 10<sup>4</sup> cells/mL or more. Similar results have been observed in the Philippines shrimp ponds<sup>[13]</sup>. According to their study<sup>[13]</sup>, the mortalities of cultured juvenile shrimp, P. monodon, were associated with dominance of luminous vibrios in the rearing environment. During the study period, white spot syndrome viral disease and red disease were the predominant among the infectious diseases, followed by vibriosis, luminous vibriosis and shell disease<sup>[1]</sup>. In diseased ponds, a shift in luminous bacterial abundance within the ponds was noticed in the first 6 weeks of culture. The LB counts during that period ranged from log 3.00 to log 4.04/mL water. At this stage, cultured shrimp were affected by luminous vibriosis because of the shift in bacterial association, *i.e.*, free living planktonic to pathogenic association. Such ponds experienced 100% mortality within 60-72 DOC<sup>[14]</sup>. The occurrence of mortalities in P. monodon juveniles affected by luminous vibriosis was preceded by the dominance of LB in the pond rearing water. The study, therefore, recommends a vigilant monitoring of shrimp ponds towards the end of the second month of cultivation.

The farmers used variety of aquadrugs and biological products in various stages of production as measures of health management. There was no incidence of white spot syndrome viral disease and luminous vibriosis in both polyculture and monoculture ponds, and also in adjacent farms. The results corroborate the observations of Priyadarsani and Abraham<sup>[15]</sup>, who recorded no incidence of diseases in traditional (bhery) system culturing finfish and shellfish. Marked reduction in LBC of pond water and pond sediment was observed through out the culture period in polyculture ponds. The reduction was, however, less compared to SEAFDEC[7], who reported that biological treatments can reduce LB from 7.5×10<sup>2</sup> CFU/mL in pond water and 1.0×10<sup>1</sup> CFU/g in pond sediments to zero, and reduce total nitrogen and phosphorus concentrations in the culture water from 33% to 9%. The fact is that mullets and other detritivorus fishes consume waste detritus and assimilate a portion of the nutrients as flesh; in doing so, organic sludge accumulation in pond sediment is greatly reduced, thus, make the treatment process ecofriendly. The finfish also enhance microbial biofilm operation through grazing, which keeps biofilm thickness, and therefore, operation optimal. Additional nutrients, from the biofilm itself, are also incorporated into fish flesh via grazing[8]. Based on the results of this study, vigilant monitoring of ponds towards the end of the second month of cultivation and polyculture of shrimp with fish to reduce the impact of disease outbreak are recommended.

## **Conflict of interest statement**

I declare that I have no conflict of interest.

## Acknowledgements

The study was financed by the Indian Council of Agricultural Research, Government of India, New Delhi under the National Agricultural Technology Project.

## Comments

## Background

The rapid growth of the shrimp farming industry halted suddenly in 1996–1997. The setback to the industry was attributed mainly to the environmental and health problems resulting in the outbreak of diseases. In India, the gross economic losses due to shrimp diseases were estimated at Rs. 10221 million in 2006–2008 and loss continues even now.

### Research frontiers

Integration of algae, fish and settling pond for an effective biological process for the treatment of shrimp farm effluents and reduction of disease outbreaks has been proposed.

#### Related reports

Samples of grow-out pond water and pond sediment from shrimp culture systems (n=138) were collected along the coastal belt of West Bengal, India between 2000 and 2006 for quantitative and qualitative studies on LB. LBC were enumerated by spread plating on SWC agar.

## Innovations and breakthroughs

The present study identified three species of LB, viz., Vibrio fischeri (0.57%), Vibrio harveyi (94.86%) and Vibrio splendidus biotype 1 (4.57%) from the shrimp farming systems of West Bengal.

#### Applications

Based on the results of the present study, vigilant monitoring of ponds towards the end of second month of cultivation and polyculture of shrimp with fish to reduce the impact of disease outbreak are recommended.

## Peer review

This is an important study as this is the novel idea for diseases control in aquaculture. The study recommends a vigilant monitoring of shrimp ponds towards the end of second month of cultivation.

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