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# Are the Sundarban Prawns Contaminated with Microbes ?

**[1]Pritam Mukherjee, [2]HareKrishna Jana, [1]Roopali Roy Chowdhury, [1]Bulti Nayak, [1]Madhumita Roy, [*3]Abhijit Mitra**

*[1]Department of Biotechnology, Techno India University, West Bengal, Salt Lake Campus, Kolkata, India*

*[2]Department of Microbiology, Panskura Banamali College, Purba Midnapore, Indiamitr*

*[*3]Department of Marine Science, University of Calcutta, Kolkata, India*

## Abstract

Prawns are the delicious food items with high export value. Freshwater prawns, *Macrobrachium rosenbergii* were reared in four ponds selected in the eastern part of Indian Sundarbans at a density of 2 individuals/$m^2$ for 90 days during 2016. The average initial weight of the seed was 0.08gm. Culture of this species in contaminated water system has resulted in the deterioration of the flesh quality. The present paper reflects the contamination of prawn tissue with microbial strains like *Salmonella, Shigella, Vibrio cholerae, Vibrio parahaemolyticus,* total coliform and fecal coliform.

## Introduction

Asia plays a leading role in farmed shrimps with almost 80% of world shrimp culture production [1]. Thailand, China, Indonesia, India rank among top five producers with Equador in place fourth in 1999. Though major improvements have been made within the last 20 years (including mass production of post larvae in industrial hatcheries and production of pellet feeds with high protein levels, which enabled increases in pond stocking densities and yields), but in the late 80s and early 90s several Asian countries also suffered drastic collapse. The main reason for decrease in production was identified as deterioration of water quality and emergence of numerous pathogens. Excessive and unplanned farm development and poor pond management have exaggerated this trend. In addition, the spread of bacterial and viral diseases was facilitated by the use of wild breeders and the transfer, without sanitary and health precautions, of exotic species trigger to serve as vectors of pathogens between different regions. The freshwater prawn (*Macrobrachium rosenbergii*) culture is gradually gaining a momentum in the present era owing to its price, taste and less susceptibility to diseases. During last few years, the demand of prawn in the world market is consistently increasing due to its export potential. Polyculture of the freshwater prawn with channel catfish, grass carp and silver carp [2]. has also been studied in details. The polyculture approach usually involves comparatively medium stocking densities of prawn and is economically attractive, since no special management is required especially for prawns.

Species of the freshwater prawn (*Macrobrachium rosenbergii*) are distributed throughout the tropical and subtropical zones of the world. They are found in most inland freshwater areas including lakes, rivers, swamps, irrigation ditches, canals and ponds, as well as in estuarine areas. Most species require brackish water in the initial stages of their life cycle and therefore they are found in

***Corresponding author:** Abhijit Mitra, Department of Marine Science, University of Calcutta, Kolkata, India. E-mail: abhijit_mitra@hotmail.com **Tel No:** 9831269550

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water that is directly or indirectly connected with the sea, although some complete their life cycle in inland saline and freshwater lakes. The present paper highlights the invasion of harmful microorganisms that also is quiet frequent in the ponds of freshwater prawns, *Macrobrachium rosenbergii.* The programme was carried out at Kalidashpur of Chotomollakhali Island in the eastern sector of Indian Sundarbans during 10th July to 10th October, 2016.

## Materials and Methods

The experiment was conducted during 10th July, 2016 to 10th October, 2016 at Kalidashpur area of Chotomollakhali Island in the eastern sector of Indian Sundarbans. One pond was treated as control (Pc) and other three (Pe1, Pe2 and Pe3) were treated as experimental. At the very initial stage of our experiment, attention was given on pond preparation properly. For this purpose, ponds were dried sufficiently in order to decompose all organic matters, to oxidize different toxic compounds present in the pond bottom soil and also to eliminate undesirable filamentous algal mat and eggs of different predatory fishes, crab *etc.* Then lime was applied accordingly to maintain soil pH and neutralize the organic acid, pyrite *etc.* present in the pond bottom. Slender mangrove twigs and concrete tubes were kept at different points of the ponds to provide shelter to the post larvae. Post larvae of *Macrobrachium rosenbergii* were obtained from a private hatchery of Andhra Pradesh and stocked at a rate of 2 individuals/$m^2$ with mean initial size of 1.00 cm and 0.08 gm of body weight. The feed composition of the control pond (Table 1) shows plant-based origin for all ingredients. To the above mentioned feed ingredients, garlic and *Porterasia coarctata* extracts were added only in case of experimental ponds as per the inclusion rate stated in Table 2. Weekly observations of surface water temperature, surface water salinity, surface water pH, dissolved oxygen, nutrient (nitrate, phosphate and silicate) concentration, phytopigment (Chl *a*) concentration, water transparency and organic carbon content in the pond bottom soil were done as per the protocols stated in Table 3. In this experiment, prawns were harvested at the end of 90 days. Survival rate and production of the cultured species were determined as per the standard method.

## Analysis of microbial load (Total coliform and fecal coliform) in ambient media (water and sediment)

The entire methodology of microbial analysis consists of the following steps:

**Table 1:**Feed ingredients (%)

| Ingredients | Percentage |
|---|---|
| Soya oil cake (SOC) | 30 % |
| Gram oil cake (GOC) | 16.6 % |
| Wheat flour | 3.3 % |
| Maize flour | 16.6 % |
| Coconut oil cake (COC) | 33.3 % |
| Vitamin + Mineral (mix) | 3000 ppm |

**Table 2:** Additives amount (kg/ton)

| Pond code | Additive used | Inclusion rate (kg/ton) |
|---|---|---|
| Pe1 | Garlic extract | 2.00 |
| Pe2 | Garlic extract | 4.00 |
| Pe3 | Garlic extract + Porterasia extract | 2.00 + 200 |

**Table 3:** Protocol followed to measure different parameters

| Parameters | Protocol followed |
|---|---|
| Surface water temperature (°C) | Monitored by Celsius thermometer. |
| Surface water salinity (psu) | Checked in the field by Refractometer and crossed checked in the laboratory by argentometric method. |
| Surface water pH | Checked in the field by portable pH meter (Sensitivity = ±0.1). |
| Dissolved oxygen (mg/l) | Checked in the field by DO meter and crossed checked in the laboratory by Winkler's method. |
| Nutrient (nitrate, phosphate and silicate) concentration (µgat/l) | Spectrophotometrically measured. |
| Phytopigment (Chl a) concentration (mg/m3) | Spectrophotometrically measured after acetone extraction. |
| Organic carbon (%) | Titration method. |

### A. Sampling

### Sampling of the Water

Water samples were collected monthly aseptically in sterilized glass container (autoclaved) with utmost care from the selected stations during 2016. The collected samples were immediately transferred in ice box and brought to the laboratory for further analysis.

### Sampling of the Sediment

Sediment samples from the respective areas were collected by scrubbing with sterile scalpel (upper 2 cm layer) in the sterile container and these were immediately put into ice box for further analysis in the laboratory.

**B. Preparation of the culture media for the microbial analysis**

**Preparation of the Lauryl Tryptose Broth for Presumptive test**

In order to prepare the Lauryl tryptose broth, at first required amount of dehydrated ingredients for single and double strength were dissolved separately in each 1 L of sterilized distilled water and it was thoroughly mixed and slightly heated by proper swirling. The pH was adjusted up to 6.8 $\pm$ 0.2 by either 0.1 (N) NaOH or 0.1 (N) HCl After that, it was distributed as required (10 ml DS & 5 ml SS) in test tubes containing inverted Durham's tubes and then placed in the autoclave for sterilization at 121°C and 15 lbs for 15 minutes.

The general ingredients of the prepared Lauryl Tryptose Broth are as follows:

| | |
|---|---|
| Tryptose | 20.00 g |
| Lactose | 5.00 g |
| Dipotassium hydrogen phosphate ($K_2HPO_4$) | 2.75 g |
| Potassium dihydrogen phosphate ($KH_2PO_4$) | 2.75 g |
| Sodium chloride (NaCl) | 5.00 g |
| Sodium lauryl sulfate | 0.10 g |
| Reagent grade water | 1 L |

**Preparation of the Brilliant Green Lactose Bile Broth for Confirmed test**

At first, required amount dehydrated ingredients were dissolved in 1 L of distilled water which was thoroughly mixed and slightly heated by proper swirling and then pH was adjusted up to 7.2 $\pm$ 0.2 by either 0.1 (N) NaOH or 0.1 (N) HCl. After that it was distributed in the test tubes (5 ml each) containing inverted Durham's tubes & then placed in the autoclave for sterilization at 121°C and 15 lbs for 15 minutes.

The general ingredients of the prepared Brilliant Green Lactose Bile Broth are as follows:

| | |
|---|---|
| Peptone | 10.00 g |
| Lactose | 10.00 g |
| Oxgall | 20.00 g |
| Brilliant Green | 0.0133 g |
| Reagent grade water | 1 L |

**A. Preparation of the collected samples**

**Preparation of the water samples**

The collected water samples were mixed thoroughly before analysis.

**Preparation of the sediment samples**

At first, 10 gm well-mixed sediment samples were weighed and serial dilution of $10^{-1}$ & $10^{-2}$ was made with sterile distilled water.

10 gm of sediment sample + 90 ml of sterile distilled water ($10^{-1}$ dilution)

ml of $10^{-1}$ dilution + 9 ml of sterile distilled water ($10^{-2}$ dilution)

**A. Microbial analysis of the samples**

**Microbial analysis of the Water sample**

For microbial analysis of surface water in terms of Total Coliform load, the Most Probable Number (MPN) procedure by Multiple Fermentation Technique was followed as stated in standard literature. The technique involves inoculating the sample and/or its several dilutions in a liquid medium of Lauryl Tryptose Broth. After expiry of the

incubation period, the tubes were examined for acid and gas production by the coliform organisms. This test is known as Presumptive test. Since the organisms other than the coliforms may also produce this reaction, the positive tubes from the Presumptive test were subjected to a Confirmatory test. The density of bacteria was calculated on the basis of positive and negative combination of the tubes. In case of water samples the results were expressed in MPN/100 ml and in case of sediment and biological samples the results were expressed in MPN/gm.

**Presumptive Phase**: **for Total Coliform**

For the Presumptive total coliform test, Sodium Lauryl Tryptose media was used as culture medium For analysis of water five test tubes each of 10 ml, 1 ml and 0.1 ml sample portion were used for the Presumptive test. For Presumptive Phase of total coliform analysis in sediment three sets of test tubes were used. First set containing five / three numbers of 10 ml Double Strength (DS) broth and second and third sets containing ten/six numbers of 5 ml Single Strength (SS) broth. Each tube in a set of five (10 ml, 1 ml and 0.1 ml of water sample) was inoculated in respective tubes and mixed thoroughly. In case of sediment sample each tube in a set of three 10 ml and 1 ml of $10^{-1}$ dilution and 0.1 ml of $10^{-2}$ dilution was inoculated to respective media tubes and mixed thoroughly. In each case a control set was also run parallaly. The inoculated test tubes were incubated at 36 $\pm$ $1^0$C. After 24 $\pm$ 2 hrs.

and examined for growth, gas and acidic reaction. If there is no gas & acid production then the tubes are reinsulated and reexamined at the end of 48 ± 2 hrs. Within each tube, Durham's tube was invertedly placed to show the bacterial growth with emission of gas. Production of gas bubbles and acids in the tubes within 48 ± 2 hours contribute a presumptive reaction. After the incubation period of 48 hours, the number of positive tubes was counted and the results were obtained in MPN/100 ml and MPN/gm.

## Confirmatory Phase: for Total Coliform

For confirmatory Total Coliform test, culture medium used was Brilliant Green Lactose Bile broth. The presumptive tubes were gently shaken and with a sterile loop (3.0 – 3.5 mm in diameter), one or two loop full of culture was transferred to a fermentation tube containing 5 ml Brilliant Green Lactose Bile broth with an invertedly placed Durham's tubes. The inoculated Brilliant Green Lactose Bile broth tubes were incubated at 36 ± $1^0$C. Formation of any gas with growth within 48 ± 2 hours constituted the Confirmed Phase. The results were obtained in MPN/100 ml and MPN/gm.

## Statistical Analysis

The collected data were finally subjected to statistical analysis. Simple correlations were performed between various ecological indices and physico-chemical parameters of different stations and correlation coefficient (r) value is determined by the following expression:

$$r = \frac{\sum xy - \bar{x}\sum y}{\sqrt{\left[\left(\sum x^2 - \bar{x}\sum x\right)\left(\sum y^2 - \bar{y}\sum y\right)\right]}}$$

Where, $x$ = Variable No. one

$y$ = Variable No. two

Correlation values are important to understand the strength of relationship between various parameters. If the value is significant (p = <0.01) then the two variables are strongly related. This relationship is direct if the 'r' value is positive and inverse if the 'r'value is negative.

The spatial and temporal variations with respect to biological and physico-chemical variables was evaluated through a Two-Way ANOVA and Multiple Regression Analysis was carried out using microbial load as dependent variables and physico-chemical variables of surface waters and sediment quality parameters as independent variables.

## Results

The total duration of the culture period of *Macrobrachium rosenbergii* was 90 days at Kalidashpur of Chotomollakhali Island in the eastern sector of Indian Sundarbans. The monthly mean surface water temperature, surface water salinity, surface water pH, dissolved oxygen, nutrient (nitrate, phosphate and silicate) concentration, phytopigment (Chl *a*) concentrations, water transparency and organic carbon content in the pond bottom soil of each ponds are documented in Table 4. The various parameters related to freshwater prawn culture in the selected ponds are highlighted in Table 5.

**Table 4:**Monthly variation of physicochemical variables of freshwater prawn culture ponds at Chotomollakhali Island during the culture period

| Parameters | July | | | | August | | | | September | | | | October | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pc | Pe1 | Pe2 | Pe3 | Pc | Pe1 | Pe2 | Pe3 | Pc | Pe1 | Pe2 | Pe3 | Pc | Pe1 | Pe2 | Pe3 |
| Temperature (°C) | 33.0 | 32.9 | 32.8 | 32.8 | 32.9 | 32.8 | 32.8 | 32.7 | 32.8 | 32.8 | 32.7 | 32.7 | 32.6 | 32.6 | 32.5 | 32.6 |
| Salinity (psu) | 1.03 | 1.01 | 0.98 | 0.97 | 0.65 | 0.89 | 0.65 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.59 | 0.44 | 0.00 | 0.00 |
| pH | 6.98 | 6.97 | 6.98 | 7.02 | 6.50 | 6.93 | 6.96 | 7.00 | 6.27 | 6.90 | 6.94 | 7.00 | 6.22 | 6.86 | 6.90 | 6.98 |
| D.O.(mg/l) | 5.12 | 5.10 | 5.19 | 5.12 | 4.23 | 5.17 | 5.32 | 5.39 | 4.04 | 5.08 | 5.44 | 5.61 | 3.91 | 4.89 | 5.05 | 5.29 |
| Nitrate (µgat/l) | 29.56 | 31.03 | 29.89 | 29.54 | 32.08 | 28.58 | 26.18 | 24.20 | 33.89 | 24.82 | 24.01 | 21.08 | 38.60 | 23.66 | 20.55 | 18.55 |
| Phosphate (µgat/l) | 4.14 | 3.97 | 4.04 | 3.85 | 5.03 | 3.81 | 3.63 | 3.09 | 4.98 | 3.08 | 2.99 | 2.16 | 5.17 | 2.94 | 2.84 | 1.97 |
| Silicate (µgat/l) | 50.18 | 53.49 | 49.85 | 51.79 | 66.23 | 48.28 | 42.16 | 49.28 | 59.89 | 46.34 | 40.79 | 43.44 | 62.42 | 45.08 | 39.68 | 38.65 |
| Chl*a*(mg/$m^3$) | 2.94 | 3.02 | 2.82 | 3.15 | 2.65 | 3.14 | 2.94 | 3.96 | 3.04 | 3.68 | 3.12 | 4.05 | 3.98 | 3.83 | 3.35 | 4.15 |
| Transparency (cm) | 21.8 | 20.8 | 23.4 | 22.9 | 19.5 | 20.5 | 26.1 | 26.4 | 16.8 | 22.3 | 27.0 | 27.8 | 15.2 | 26.0 | 29.0 | 28.9 |
| Organic carbon(%) | 3.28 | 4.05 | 3.94 | 3.97 | 4.16 | 3.51 | 3.42 | 3.66 | 5.05 | 2.96 | 2.85 | 2.53 | 6.11 | 2.99 | 2.40 | 2.19 |

**Table 5:** Details of Macrobrachium rosenbergii culture during 10.07.16 to 10.10.16

| Details | Pc | Pe1 | Pe2 | Pe3 |
|---|---|---|---|---|
| Pond area ($m^2$) | 780.38 | 588.42 | 2487.48 | 395.77 |
| Date of stocking | 10.07.2006 | 10.07.2006 | 10.07.2006 | 10.07.2006 |
| Date of harvest | 10.10.2006 | 10.10.2006 | 10.10.2006 | 10.10.2006 |
| Culture period (days) | 90 | 90 | 90 | 90 |
| Stocking density (No.s of $PL_5/m^2$) | 2 | 2 | 2 | 2 |
| Number stocked ($PL_5$) | 1561 | 1177 | 4975 | 792 |
| Initial weight (gm) | 0.08 | 0.08 | 0.08 | 0.08 |
| Initial size (cm) | 1 | 1 | 1 | 1 |
| Final weight (gm) | 10 | 17 | 22 | 29 |
| Final size (cm) | 8.2 | 10.1 | 13.2 | 15 |
| Daily growth rate (gm/day) | 0.077 | 0.110 | 0.244 | 0.321 |
| Survival rate (%) | 60 | 65 | 72 | 78 |
| Feed quantity given (kg) | 40 | 30 | 126 | 20 |
| Quantity of prawn harvested (kg) | 9.366 | 13.00 | 78.804 | 17.915 |
| Feed Conversion Ratio | 4.27 | 2.30 | 1.60 | 1.12 |
| Total Yield ($kg/m^2$) | 0.012 | 0.022 | 0.032 | 0.045 |

**Figure 1:** Monthly variation of DO level in different ponds

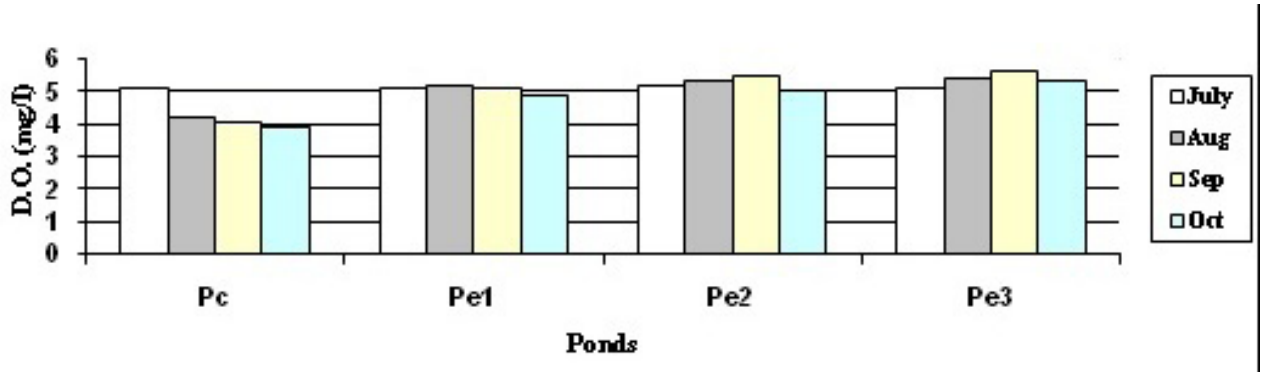



**Figure 2:** Monthly variation of transparency in different ponds

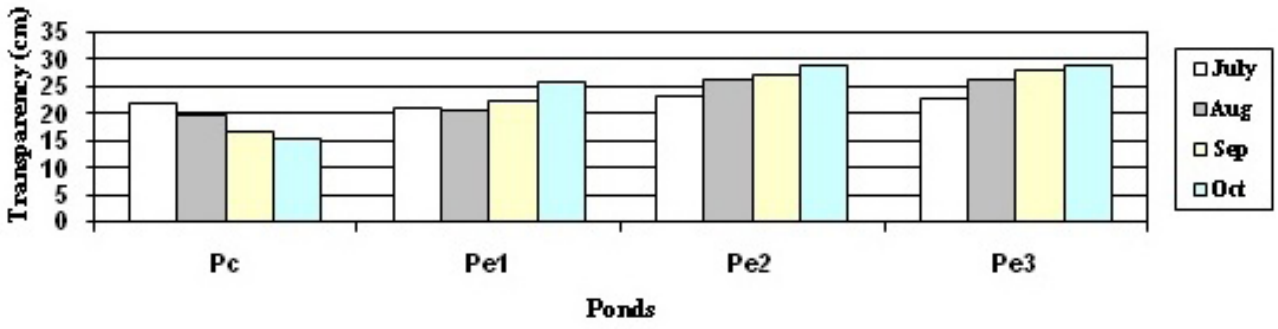
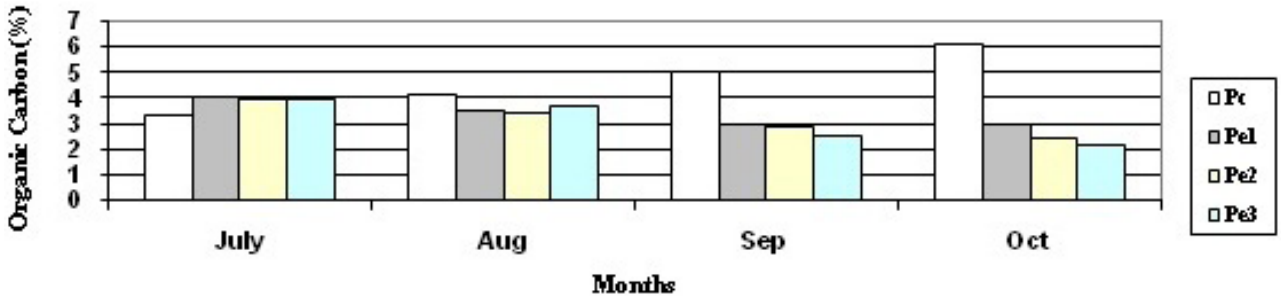


**Figure 3:** Comparative study of organic carbon load in different ponds




## Discussion

The four culture ponds investigated in the present programme exhibited variations with respect to production, survival rate, FCR value and physico-chemical variables. The three experimental ponds showed better aquatic environment in comparison to the control pond. The better aquatic environment of the experimental ponds is reflected through lowering of nutrient level and subsequent increment of phytopigment (Chl *a*) level. The significant inverse relationships between nutrient levels and phytopigment in all the three experimental ponds ($rNO_3 \times chl_a = 0.8685$, $p < 0.01$ in Pc; $rNO_3 \times chl_a = -0.9846$, $p < 0.01$ in Pe1; $rNO_3 \times$chl $a = -0.9829$, $p < 0.01$ in Pe2 and $rNO_3 \times chl_a = -0.9424$, $p < 0.01$ in Pe3; $rPO_4 \times chl_a = 0.3849$, p-value= insignificant in Pc; $rPO_4 \times chl_a = -0.9990$, $p < 0.01$ in Pe1; $rPO_4 \times chl_a = -0.9501$, $p < 0.01$ in Pe2; $rPO_4 \times chl_a = -0.9034$, $p < 0.01$ in Pe3; $rSiO_3 \times chl_a = 0.0820$, p-value = insignificant in Pc; $rSiO_3 \times chl_a = -0.8830$, $p < 0.01$ in Pe1; $rSiO_3 \times chl_a = -0.8284$, $p < 0.01$ in Pe2; $rSiO_3 \times chl_a = -0.7914$, $p < 0.01$ in Pe3) indicate healthy growth of phytoplankton at the cost of pond water nutrients. Transparency and DO values exhibited an increasing trend with time in the experimental ponds (Table 4 and Figures 1 and 2) indicating their positive role for the growth of phytoplankton. The organic carbon load at the pond bottom soil was also less in case of experimental ponds in comparison to the control pond during the entire culture period (Table 4 and Figure 3), which is an indication for lower FCR value through less wastage of feed matter. The overall health of the ambient media (pond water and pond bottom soil) in the experimental ponds speaks in favour of the efficacy of the herbal additives that were added to the feed in case of experimental ponds. The feed with *Porterasia coarctata* extract (protein percentage 31%) and garlic extract (applied in Pe3) showed better result than the other ponds. *Porterasia coarctata* popularly known as saltmarsh grass is a very common mangrove associate of Indian Sundarbans, belonging to Poaceae family. The average protein percentage of the plant ranges from 11% to 31% depending on the season. Garlic is a rich source of astaxanthin, which is a powerful, bioactive *anti-oxidant* that has demonstrated efficacy in animal or human models for addressing several health problems like muscular degeneration, Alzheimer's [3]. And Parkinson's diseases [4]. Cardiovascular diseases, stroke and several types of cancer. This carotenoid pigment plays many important functions in fishes, crustaceans and several aquatic animals like improved protection against oxidation and photo-oxidation, reproduction and development, immune response, resistance to diseases and communication system [5].

The benefit of any aqua cultural venture is reflected through the ultimate production figure, which showed significant variation amongst the selected ponds. In Pc the production was 0.012 kg/m$^2$. However, the productions were 0.022 kg/m$^2$, 0.032 kg/m$^2$ and 0.045 kg/m$^2$ in Pe1, Pe2 and Pe3 respectively. The variation may be attributed to the specially formulated plant based feed (rich in astaxanthin) that not only boosted up the growth of prawns, but also upgraded the ambient aquatic health (in terms of nutrient load, DO, transparency, organic carbon *etc.*). The highest production in Pe3 confirms the positive dependency of the prawn growth on *Porterasia coarctata* originated protein.

In Indian Sundarbans, a considerable fraction of the population depends on the forest resources for their livelihood. This often creates a negative impact on the natural resource base of the deltaic complex at the apex of Bay of Bengal. The present programme may open an avenue of developing ecofriendly prawn feed through

involvement of the local people. This will not only defray the people from illegal entry into the forest but will also improve the animal and fish nutrition sector through setting up of small-scale feed units.

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