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2. MATERIALS AND METHODS

2.1. Physiography and Climate

- Physiography

The major part of north eastern Bay of Bengal coast of India forming the coastal zone of West Bengal constitutes, the lower part of Bengal basin and geographically encompasses three southern districts of state, viz. Midnapur (East), in the western part (covering about 27% of the coast line) and the districts South 24 Parganas and North 24 Parganas on the central and eastern parts (covering about 73% of the coastline). This zone spreads over 10,158.22 sq. km. and is situated within the latitude 21°30'N to 22°30'N and longitude 87°25'E to 89°10'E. It has been subdivided into three principal zones from east to west in the following sequence (West Bengal State Biodiversity Strategy and Action Plan, 2002):

- From the mouth of Haribhanga River delineating the India-Bangladesh border to the mouth of river Hugli being essentially the Sundarbans delta area along the sea (eastern sector).

- Tidal sea water traversing along river Hugli upto south of Diamond Harbour Municipality and on the west upto Haldia port (central sector).

- Digha – Junput coastal plain along the estuary and Bay of Bengal (western sector).

Geographically, the zone comprises of rural CD Blocks (full as well as in part) and some urban concentrations / municipal areas covering the districts of North 24 Parganas, South 24 Parganas and Midnapur (East). Based on the Environment (Protection) Act, 1986 and the subsequent notification to the coastal zone regulation in February, 1991, the total coastal region of West Bengal has been divided into three zones which are CRZ-I, CRZ-II and CRZ-III. The Coastal Regulation Zone (CRZ) Notification and its amendments and the Coastal Zone Management Plan (CZMP) of West Bengal have clearly stated the use of the resources of this zone in a sustainable manner. The various areas included under these zones are listed here.
CRZ – I: Areas that are ecologically sensitive and important like National Parks (e.g. proposed Sagar Marine Park), Santuaries (Sajnekhali, Lothian, Haliday island), Reserve Forests, Wildlife habitats, Mangroves (Sundarbans, Nijkasba, Khejuri in Medinipur), areas close to breeding and spawning grounds of fish and other marine life (Sagar, Newmoore, Sandhead islands), areas of outstanding natural beauty, historical / heritage area (e.g. Kapil Muni temple at Ganga Sagar, etc.) and areas between LTL and HTL. The total area of the CRZ – I is 8184.91 sq.km.

CRZ-II: Areas already been developed upto or close to the shoreline. ‘Developed area’ for this purpose would refer to areas within the municipal limits (e.g. Haldia Dock Complex) or in other legally designated urban areas which are substantially built up and provided with drainage, approach roads, water supply, sewerage mains etc. Haldia town and the eastern part of Digha have been included within this zone. The total area under CRZ – II is 3.41 sq. km.

CRZ – III: Areas, which are not particularly developed and do not fall under CRZ – I and CRZ – II. These will include coastal zone in rural areas (developed and undeveloped) and areas within the municipal limits or in other legally designed urban areas that are not substantially built-up. The total area under CRZ – III is 1969.90 sq. km.

The district-wise breakup of the area under CRZ-I, II and III is presented in Table 3 below.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>District</th>
<th>No. of C.D. Blocks / Legally Designated Urban Areas</th>
<th>Coastal zone of West Bengal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CRZ-I</td>
</tr>
<tr>
<td>1.</td>
<td>North 24 Parganas C.D. Block</td>
<td>6</td>
<td>840.54</td>
</tr>
<tr>
<td>2.</td>
<td>South 24 Parganas</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>i) C.D. Block</td>
<td>14</td>
<td>3052.18</td>
</tr>
<tr>
<td></td>
<td>ii) Sundarbans Reserve Forest</td>
<td>-</td>
<td>245.05</td>
</tr>
<tr>
<td>3.</td>
<td>Midnapur</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>i) C.D. Block</td>
<td>21</td>
<td>30.19</td>
</tr>
<tr>
<td></td>
<td>ii) Legally Designated Urban Areas</td>
<td>3</td>
<td>142.83(2)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>8184.91</td>
</tr>
</tbody>
</table>

[15]
Note: (1) 3.41 sq. km. comprises Haldia Dock Complex (1.625 sq.km.) and part of Digha Township (1.780 sq.km.) both of which are included in Haldia Urban Area and Digha Planning area respectively.

(2) Excluding Haldia Dock Complex and part of Digha Township area which are included in CRZ-II.

Source: Pilot study on Integrated Coastal Zone Management Plan for West Bengal. July 2000, Central Pollution Control Board, Zonal Office (East), Calcutta; Research and Produced by: Creative Research Group, Kolkata.

The coastal zone of West Bengal encompasses the famous mangrove ecosystem in the Indian Sundarbans part, which is restricted in the North and South 24 Parganas districts. With a considerable degree of marine and estuarine characteristics in major part of the coastal zone, the important morphotypes of coastal West Bengal are beaches, estuaries, mangrove swamps, tidal flats, tidal creeks, coastal dunes and backdune areas (Chaudhuri and Choudhury, 1994). Rocky shores are not found in this tide dominated estuarine system. Jetties, dykes, concrete piles or scattered infrastructures like lighthouse, mangrove tree trunks and pneumatophores act as various hard substrata on which specific benthic communities thrive.

Indian Sundarbans is situated at the apex of the Gangetic delta is one of the most biologically productive and taxonomically diversified, low line, mangrove detritus based, open, dynamic, heterogeneous coastal ecotone. Indian Sundarbans is bordered by Rivers Hooghly and Haldi on the West, Bay of Bengal on the South, Rivers Ichhamati, Kalindi and Raimangal on the East and, Northern limits adjusted to entire Police Stations of Diamond Harbour, Kakdwip to Basirhat boundary along the Dampier and Hodges line (1831), which coincides in the recent satellite imageries. Total area of Sundarbans was 36,000 sq. km., up to the year 1770 and presently it is 25,000 sq. km. Out of which Indian part 9630 sq. km. and balance lies with Bangladesh.

Out of 9630 sq. km, 4264 sq. km. wetland mangrove/forest known as reserve forests. In which, 2195 sq. km - wetland mangrove and 2069 sq. km. - tidal river system only. Reclaimed area 5,366 sq. km. retained for human settlements in 19 blocks (13 in 24-Parganas(S) and 6 in the North 24-Parganas,) and one municipality in each district (Taki and [16]
Jaynagar) and one town-Canning (1093 mouzas and 1060 villages). 41.8 percent water spread areas and salt marshes, 44.3 percent forests, 13.9 percent sand flats and sea beach. This mangrove forest has been declared as the World Heritage Site by IUCN in 1987, Biosphere Reserve under Man and Biosphere Programme by UNESCO in 1989 and is a proposed RAMSAR site.

The rivers are the live matrix of deltaic complex, on which the unique spectrum of biological diversity is embedded. In Indian Sundarbans, approximately 2069 sq. km area is occupied by tidal river systems which finally end up in the Bay of Bengal. The seven main estuaries, from west to east are highlighted in Table 4 along with their salient features.

<table>
<thead>
<tr>
<th>River</th>
<th>Description</th>
</tr>
</thead>
</table>
| Hugli      | ➢ It forms the western border of Indian Sundarbans  
            ➢ It is the main river of West Bengal and is a direct continuation of the river Ganges.  
            ➢ Most of the coastal industries of West Bengal are concentrated along the western bank of this river. |
| Muriganga  | ➢ It is a branch of the Hugli river.  
            ➢ It flows along the east of Sagar Island, the largest island in the deltaic complex.  
            ➢ Unique mangrove vegetation is found along the bank of this river. |
| Saptamukhi | ➢ It has its origin at Sultanpur.  
            ➢ It is connected with the Muriganga (Bartala) branch of the Hugli river through Hatania-Duania canal. |
| Thakuran   | ➢ It begins near Jayanagar in South 24 Parganas and has a number of connections with the Saptamukhi.  
            ➢ It has perhaps earlier connected with the Kolkata canal through the Kultali and the Piyali rivers, which exist today in a dying state. |
Matla

- This river originates at the confluence of Bidyadhari, Khuratya and the Rampur Khal close to the town of Canning in south 24 Parganas.
- Matla is connected to Bidya and ultimately flows to the Bay of Bengal. The fresh water connection and discharge to this river has been lost in the recent times.
- Salinity of the river water is relatively high (in comparison to Hugli or Muriganga) owing to fresh water cut-off from the upstream region.

Bidyadhari *

- This was flourishing branch of the Bhagirathi during the 15th or 16th century, but now serves only as a sewage and excess rainwater outlet from the city of Kolkata.
- The river bed is completely silted and presently it is almost in dying condition.

Gosaba

- The waters of Matla and Harinbhanga (Raimangal) through a large number of canals from it.
- The river flows through the reserve forests.

Harinbhanga

- The river begins from Sahebkhali in North 24 Parganas and is connected with the Rampura khal by Barakalagachi river and with the Gosaba river through the river Terobhanki.
- The Harinbhanga (also known as Ichamati and Raimangal) forms a natural demarcation between India and Bangladesh.

*Presently a dyeing estuary and not considered within the seven major types.

The presence of 34 mangrove species and some 62 mangrove associate species (Mitra, 2000) in the zone is the only mangrove based home ground of Royal Bengal Tiger (*Panthera tigris tigris*) in the planet Earth. The deltaic complex sustains 102 islands, out of which 48 are inhabited and 54 are uninhabited. The flow of Ganges (Bhagirathi) River through Hugli estuary in the western sector of Indian Sundarbans to end up at Bay of Bengal has made the geographical situation totally different from the eastern sector, where five major rivers lost their roots with Ganga – Bhagirathi system due to heavy situation. The water chemistry has changed accordingly. The low-lying tidal flats of Indian Sundarbans during the quaternary period have been developed from alluvial deposits of river Hugli, Saptamukhi and Matla.
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together with tidal incursions. The soil consists of clayey loam or different black clay and there is no rock. The areas are about 1 m above the mean sea level and are submerged under saline estuarine waters for several hours in the spring tide twice a day.

The delta complex of Indian Sundarbans is also noted for its seasonality in terms of climatic condition and wind action as highlighted here in brief. Frequent Nor’ Westers is also common in the premonsoon season.

• Seasonality and Climate

The deltaic lobe of Indian Sundarbans ecosystem experiences a moderate type of climate because of its location adjacent to the Bay of Bengal as well as due to regular tidal flushing in the estuaries. Coastal processes are very dynamic and accelerated by tropical cyclone which is locally called ‘Kal Baisakhi’ (Nor’ Wester). The seasonal climate in Sundarbans may be conveniently categorized into premonsoon (March to June), monsoon (July to October) and postmonsoon (November to February). Each season has a characteristic feature of its own, which is very distinct and unique. The oscillations of various physico-chemical variables in different seasons of the year are discussed here in brief.

2.1.1. Wind

The direction and velocity of the wind system in the coastal West Bengal are mainly controlled by north-east and south-west monsoons. The wind from the north and north-east commences at the beginning of October and continues till the end of March. The month of January and February are relatively calm till with an average wind speed around 3.5 km/hrs. Violent wind speed recommences from the south-west around the middle of March and continues till September. During this period, several low pressure systems occur in this region, a number of which takes the form of depressions and cyclonic storms of varying intensity.

2.1.2. Waves and Tides

The wind is the basic driving force for generating surface waves in the coastal zone of West Bengal. Surface waves in the coastal zone of West Bengal are mainly due to wind actions. Sea waves in the region rarely become destructive except during cyclonic storms. During Nor’ Westers, the wind speed rises above 100 km/hr and is usually accompanied by huge tidal waves. When the cyclonic incidences coincide with the spring tides, wave height can
rise over 5 m above the mean sea level. Ripples waves appear in the month of October, November and December when wind generated wave height varies approximately between 0.20 to 0.35 m. In the month of April to August, large wavelets are formed in the shelf region and they start breaking when they approach towards the coastal margin. Wave height rises up to 2 m during this period, which causes maximum scouring of land masses. Wave actions, micro and macro-tidal cycles and long shore currents are recorded in most of the islands in this ecosystem. With the change in seasons, tidal pattern in the estuarine systems of coastal West Bengal also changes (Pillay, 1958). During the monsoon month, the effect of flood tide is more or less countered and nullified by freshets and there is a strong predominance of ebb tide. The strength of flood tide over ebb tide is at a minimum during the postmonsoon season. Conversely, during the premonsoon season, the effect of flood tide is considerably stronger than that of the ebb tide.

2.1.3. Surface Water Temperature
In coastal West Bengal, the seasonal variation of surface water temperature is not so drastic between premonsoon and monsoon seasons. The premonsoon (March to June) is characterized by a mean surface water temperature around 34°C. The monsoon period (July to October) shows a surface water temperature around 32°C (mean) and the post monsoon period (November to February) is characterized by cold weather with a mean surface water temperature around 23°C (Mitra, 2000).

2.1.4. Rainfall
The average annual rainfall in deltaic Sundarbans region is 1920 mm. Rainfall is usually maximum during the month of August / September and the monsoon period lasts from July to October. The south-west wind triggers the precipitation in the monsoon period with an average rainfall of about 165 mm. The postmonsoon period (November to February) is characterized by negligible rainfall and the premonsoon period (March to June) is basically dry, but occasionally accompanied by rain and thunderstorms.

2.2. Selection of species:

The giant fresh water prawn (*Macrobrachium rosenbergii*) (Fig. 3) is a highly valued delicious food and commands very good demand in both domestic and export market. *Macrobrachium rosenbergii* culture in Indian Sundarbans is gradually gaining momentum in
the present era owing to its price, taste, fast growth rate, less susceptibility to diseases and its compatibility to grow with carps. The species was selected for the present programme in three places of West Bengal namely Kalidaspur, Jharkhali and Basirhat.

**Systematic Position:**
Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Mandibulata
Class: Crustacea
Subclass: Malacostraca
Order: Decapoda
Family: Palaemonidae
Genus: *Macrobrachium*
Species: *rosenbergii* (De Man. 1879)

**Salient features**
1. Carapace covers the entire thorax.
2. Eyes on movable stalk.
3. Nineteen pairs of appendages, 13 pairs are thoracic and 6 pairs are abdominal appendages.
4. First-three pairs of thoracic appendages are modified as maxillipeds and serve for feeding.
5. Remaining five pairs of thoracic appendages used for walking hence called decapoda.
6. 2\textsuperscript{nd} walking leg is modified as large chela.

**Importance**
- Faster growth rate.
- Higher tolerance to wider range of temperature and salinity.
- Less cannibalistic tendency.
- Very good demand in both domestic and export market.
2.3. Selection of stations:

2.3.1. For collection of seaweeds and salt marsh grass

Three stations were selected at Indian Sundarbans region (within the latitude 21°30'N to 22°30'N and longitude 87°25'E to 89°10'E) during 2007-2009 [Fig. 4 (a)] for collection of seaweeds (*Enteromorpha intestinalis, Ulva lactuca, Catenella repens*) and salt marsh grass (*Porteresia coarctata*) in order to study the biochemical composition (protein, carbohydrate, fat, ash and moisture). Species were collected through three seasons viz. premonsoon, monsoon and postmonsoon. The names and coordinates of the stations are given in Table 5.

<table>
<thead>
<tr>
<th>Table 5. Names and geographical location of sampling stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Station</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

2.3.2. For culture ponds

The trial experiment on application of floral based feed was carried out in three stations of West Bengal [Fig. 4 (b)]. The study area comprises of both north and south 24 parganas district of West Bengal with varied salinity regime. In order to monitor the variation in water quality parameters in these two districts, three stations were selected: two in south 24 parganas (Kalidaspur and Jharkhal) and one in north 24 parganas (Basirhat). The detail description with geographical location is given in Table 6.
### Table 6. Detail description of the location of culture ponds

<table>
<thead>
<tr>
<th>Stations</th>
<th>Location</th>
<th>Co-ordinates</th>
<th>Pond size</th>
<th>Pond Salinity</th>
<th>Culture Period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kalidaspur</strong></td>
<td>Situated at Chhotomollakhali Island in the fringe area of Sundarban Tiger Reserve (STR) (River Salinity – 18.46 ±0.85 psu).</td>
<td>22°10'21&quot;N 88°53'55&quot;E</td>
<td>Experimental pond - 780 m²</td>
<td>Experimental pond - 1.99 ± 0.89 psu</td>
<td>2nd February, 2009 to 20th September, 2009</td>
</tr>
<tr>
<td></td>
<td>Control pond - 395 m²</td>
<td></td>
<td>Control pond - 1.97 ± 0.93 psu</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Jharkhali</strong></td>
<td>Situated in Sundarban Biosphere Reserve (SBR) on the bank of River Matla adjacent to Herobhanga Island (River Salinity – 22.50 ±0.68 psu).</td>
<td>22°01'16&quot;N 88°41' 2&quot;E</td>
<td>Experimental pond - 8200 m²</td>
<td>Experimental pond - 5.21 ± 0.95 psu</td>
<td>1st February, 2009 to 15th September, 2009</td>
</tr>
<tr>
<td></td>
<td>Control pond - 12000 m²</td>
<td></td>
<td>Control pond - 5.19 ± 1.00 psu</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Basirhat</strong></td>
<td>Situated in North 24 Parganas district of West Bengal which is connected to the Ichhamati River (River Salinity – 10.55±0.35 psu).</td>
<td>22°48'19.0&quot;N 88°54'21.5&quot;E</td>
<td>Experimental pond - 7500 m²</td>
<td>Experimental pond - 4.58 ± 1.28 psu</td>
<td>5th February, 2010 to 30th September, 2010</td>
</tr>
<tr>
<td></td>
<td>Control pond - 10,000m²</td>
<td></td>
<td>Control pond - 4.61 ± 1.44 psu</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4. Working phases:

For convenience, the entire working procedure has been divided into three phases:

2.4.1. Seasonal collection of selective seaweeds *Enteromorpha intestinalis, Ulva lactuca, Catenella repens* and salt marsh grass *Porteresia coarctata* and proximate analysis.

2.4.2. Preparation of floral based fish feed, proximate analysis of feed and application of this formulated feed for culturing *Macrobrachium rosenbergii*.

2.4.3. Monthly estimation of physico-chemical and zootechnical parameters.

2.4.1. Seasonal collection of selective seaweeds *Enteromorpha intestinalis, Ulva lactuca, Catenella repens* and salt marsh grass *Porteresia coarctata* and proximate analysis

2.4.1.1. Maceralgal and salt marsh grass collection

The mangrove associates include various salt marsh grasses, sea grasses, sand binders and macro algae. In Indian Sundarbans, common seaweed are *Catenella repens, Enteromorpha intestinalis, Enteromorpha compressa, Ulva lactuca, Caloglossa sp., Rhizoclonium hookeri, Rhizoclonium riparium, Chaetomorpha aerea, Vaucheria sp., Bosrichia sp., Caloglossa leprieurie*, etc. Among 16 species of seaweed *Enteromorpha intestinalis, Ulva lactuca* and *Catenella repens* are the dominant seaweed found in Indian Sundarbans.

Taxonomic position, salient features and economic importance of three dominant seaweeds are listed here.

- *Enteromorpha intestinalis*

**Systematic Position:**

Division – Chlorophyta  
Class – Chlorophyceae  
Order – Ulvales  
Family – Ulvaceae  
Genus – *Enteromorpha*  
Species – *intestinalis* (Link)

Figure 5. *Enteromorpha intestinalis* [24]
Salient features:
1. Plant body is tubular, more or less compressed, constricted, and coiled in the form of intestine.
2. Thallus dark green in colour and found attached to the substratum with the help of primary attaching cell.
3. Presence of numerous multinucleated rhizoids growing from lower cell of the thallus.
4. Cells of the thallus are small and elongated.

Importance:
- Rich in astaxanthin and hence used in fish feed preparation.
- Rich in protein (10-19%).
- Accumulator of heavy metal (Zn, Cu and Pb).
- Used as manure due to its rich content of trace elements.

*Ulva lactuca*

Systematic Position:
- Division – Chlorophyta
- Class – Chlorophyceae
- Order – Ulvales
- Family – Ulvaceae
- Genus – *Ulva*
- Species – *lactuca* (Linnaeus)

Figure 6. *Ulva lactuca*

Salient features:
1. Plant body is tubular, more or less compressed, flattened leaf like.
2. Thallus dark green in colour and found attached to the substratum with the help of primary attaching cell.
3. Presence of numerous multinucleated rhizoids growing from lower cell of the thallus.
4. Cells of the thallus are small and ovoid.
Importance:
- Rich in astaxanthin and hence used in fish feed preparation.
- Rich in protein (8-15%).
- Accumulator of heavy metal (mainly Cu).
- Edible from the point of view of human consumption.

* Catenella repens *

Systematic Position:
Division – Chlorophyta  
Class – Rhodophyceae  
Order – Gigartinales  
Family – Rhabdoniaceae  
Genus – Catenella  
Species – repens (Batters)

Figure 7. *Catenella repens*

Salient features:
1. Plants with repent and assurgent branches.
2. Branching is ditrichotomous below, but clearly pinnate above.
3. The axis and branches divided into dorsiventrally compressed ellipsoid to ovate segment 3 – 5 times longer than broad.
4. The haptera terminating into uncorticated flagellar outgrowth chiefly formed at forking points and not in the branching plane of the thallus.

Importance:
- Rich in astaxanthin and hence used in fish feed preparation.
- Rich in protein (9-17%).
- Accumulator of heavy metal (mainly Cu).
- Used as manure due to its rich content of trace elements.

*Porteresia coarctata* (salt marsh grass) is the dominant plant in a salt water marsh area being maintained in a natural condition in the southern part of Bay of Bengal, West Bengal, India. In Indian Sundarbans, salt marsh grass *Porteresia coarctata* is very common, which can
tolerate a wide range of salinity. Because of the luxuriant growth, higher biomass and high protein content, *Porteresia coarctata* has also been considered as animal fodder.

> *Porteresia coarctata*

**Systematic Position:**
- Domain: Eukaryota
- Kingdom: Plantae
- Subkingdom: Viridaeplantae
- Phylum: Magnoliophyta
- Subphylum: Euphyliophytina
- Infraphylum: Radiatopses
- Class: Liliopsida
- Subclass: Commelinidae
- Superorder: Poanae
- Order: Cyperales
- Family: Poaceae
- Genus: *Porteresia*
- Species: *coarctata* - (Roxb.) Tateoka

**Salient features:**
1. Perennial grass found growing on mudflats of lower Gangetic delta (Indian Sundarbans).
2. This species can tolerate high range of salinity.
3. Leaves linear, leathery with spinulose margins.

**Importance:**
- Pioneer species of mangrove ecological succession.
- Efficient in binding soil particles and prevents soil erosion.
- Rich in protein (18%), which is used as animal fodder.
- Rich in iodine
The algal samples on block jetties and hard substrata (like boulder, mangrove trunk etc) were hand-picked from shallow littoral water and salt marsh grasses (*Porteresia coarctata*) were carried out at ebb within 500 meter coastal stretch at three different selected stations; washed twice in the field with ambient seawater followed by fresh water to remove epiphytes, sediments and organic matter; rinsed with distilled water, dried with tissue paper and brought to the laboratory to store at -20°C.

### 2.4.1.2. Analytical methods:

#### 2.4.1.2.1. Analysis of biochemical parameters:

The collected species were subjected to biochemical analysis as per the standard protocol. For each species, triplicate analyses were averaged for each of the samples for soluble carbohydrate, total protein, total lipid, ash and moisture.

**a) Estimation of protein:** The Lowry method was used for protein determination (Lowry *et al.*, 1951). The samples were digested in 1 N NaOH, and then allowed to react with an alkaline copper citrate solution and Folin-Ciocalteau phenol reagent to measure protein concentration colorimetrically based on absorptions at 660 nm in a Beckman Coulter DU 640 spectrophotometer, and compared to a bovine serum albumin standard.

**b) Estimation of carbohydrate:** The total carbohydrate content was assayed by the phenol-sulphuric acid method (Dubois *et al.*, 1956). The dry organic matter was reacted with 2.5N HCl on water bath for 3 hour to convert into 5 hydroxymethyl furfural. This gives green coloured product with anthrone reagent with an absorption maximum at 630 nm. The carbohydrate was estimated by the standard curve prepared by different concentrations of glucose using the formula below and expressed as mg.g⁻¹ DW = Mg of glucose / Volume of the test sample × 100.

**c) Estimation of lipid:** Total lipid was determined by Soxhlet method as described by Folch *et al.* (1957).

**d) Estimation of ash:** The ash contents were estimated by heating the sample overnight in a furnace at 525°C (AOAC, 1995).
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e) Estimation of moisture: Moisture content was determined by weight difference method keeping the sample at 100°C overnight (AOAC, 1995).

Protein, carbohydrate, lipid, ash and moisture contents were expressed as the percentage dry weight (DW). The results are given as a mean with standard deviation (±SD) as quality assurance to the data.

2.4.1.2.2. Analysis of physico-chemical parameters:

Surface water were collected simultaneously during high tide from all the three sampling sites to monitor salinity, temperature and nutrient (nitrate, phosphate and silicate) of the ambient water as per the method of Strickland and Parsons (1972) to pinpoint the hydrological parameters to which the vegetation are exposed in natural condition.

**Surface water temperature** was measured by using 0°–100°C mercury Celsius thermometer. The **surface water salinity** was measured by means of an optical refractometer (Atago, Japan), and cross-checked in laboratory by employing Mohr-Kundson method (after Strickland and Parsons, 1972). The correction factor was found out by titrating the silver nitrate solution against standard sea water (IAPO standard sea water service Charlottenlund, Slot Denmark, Chlorinity 19.376 %).

Surface waters for **nutrient** was collected in clean TARSON bottles and transported to the laboratory in ice-freezed condition. Triplicate samples were collected from the same collection site to maintain the quality of the data. The standard spectrophotometric method of Strickland and Parsons (1972) was adopted to determine the nitrate concentration in surface water. **Nitrate** was analysed by reducing it to nitrite by means of passing the sample with ammonium chloride buffer through a glass column packed with amalgamated cadmium filings and finally treating the solution with sulphanimamide. The resultant diazonium ion was coupled with N - (1-naphthyl)- ethylene diamine to give an intensely pink azo dye. Determination of the **phosphate** was carried out by treatment of an aliquot of the sample with an acidic molybdate reagent containing ascorbic acid and a small proportion of potassium antimony tartarate. Dissolved **silicate** was determined by treating the sample with acidic molybdate reagent. The resultant silico-molybdic acid was reduced to molybdenum blue.
complex by ascorbic acid and incorporating oxalic acid prevented formation of similar blue complex by phosphate. SYSTRONICS UV-VIS spectrophotometer (Type 117, Sr. No. 690) was used for nutrient (NO₃, PO₄ and SiO₂) analysis at their respective wavelengths.

2.4.1.3. Statistical analysis:

All data were expressed in terms of mean ± SD (standard deviation) and range. In addition, data concerning the environmental parameters and content of proteins, carbohydrates, lipids, ash and moisture for the species were analyzed by Duncan’s multiple range test at p<0.05 (SPSS 9.0, 1999) for identification of significant statistical seasonal differences during the study period. The Pearson correlation coefficient (r) was also computed between the biochemical composition of the species and environmental parameters.
2.4.2. Preparation of floral based fish feed, proximate analysis of feed and application of this formulated feed for culturing *Macrobrachium rosenbergii*

### 2.4.2.1. Preparation of floral based fish feed:

Three different feeds (diet I, diet II and diet III) were formulated using different percentages of *Enteromorpha intestinalis* and *Porteresia coarctata* dust (Table 7) using ‘Pearson square’ method. The feed ingredients were chosen on the basis of its nutritional status, price and year round availability in the local market. After weighing, the feed ingredients were mixed together manually (Fig. 9) until a homogeneous mixture was obtained. Then wet ingredients and water were added into the mixture to obtain dough. Following this, mixture was manually pressed through a locally manufactured feed pelletizer. The pellets were dried in well aerated place under the shade for 2 days until became sufficiently dry. Finally, the pellets were sacked in plastic bags and kept in a cool, dry place until used.

<table>
<thead>
<tr>
<th>Components</th>
<th>Diets</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>10</td>
</tr>
<tr>
<td>Rice bran</td>
<td>17</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>4</td>
</tr>
<tr>
<td>Fish meal/ Shrimp meal</td>
<td>-</td>
</tr>
<tr>
<td>Mustard oil cake</td>
<td>34</td>
</tr>
<tr>
<td>Vitamin + Mineral mixture</td>
<td>5</td>
</tr>
<tr>
<td><em>Enteromorpha intestinalis</em> dust</td>
<td>30</td>
</tr>
<tr>
<td>Mixture of <em>Enteromorpha intestinalis</em> and <em>Porteresia coarctata</em> dust</td>
<td>30</td>
</tr>
<tr>
<td><em>Porteresia coarctata</em> dust</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Ingredients (%) of experimental diets (I-III)
Figure 9. Preparation of floral based fish feed
2.4.2.2. Biochemical analysis of feed:

a) **Estimation of protein:** The Lowry method was used for protein determination (Lowry *et al.*, 1951). The samples were digested in 1 N NaOH, and then allowed to react with an alkaline copper citrate solution and Folin-Ciocalteau phenol reagent to measure protein concentration colorimetrically based on absorptions at 660 nm in a Beckman Coulter DU 640 spectrophotometer, and compared to a bovine serum albumin standard.

b) **Estimation of carbohydrate:** The total carbohydrate content was assayed by the phenol-sulphuric acid method (Dubois *et al.*, 1956). The dry organic matter was reacted with 2.5N HCl on water bath for 3 hour to convert into 5 hydroxymethyl furfural. This gives green coloured product with anthrone reagent with an absorption maximum at 630 nm. The carbohydrate was estimated by the standard curve prepared by different concentrations of glucose using the formula below and expressed as \( \text{mg.g}^{-1} \ \text{DW} = \frac{\text{Mg of glucose}}{\text{Volume of the test sample}} \times 100 \).

c) **Estimation of lipid:** Total lipid was determined by Soxhlet method as described by Folch *et al.* (1957).

d) **Estimation of ash:** The ash contents were estimated by heating the sample overnight in a furnace at 525°C (AOAC, 1995).

e) **Estimation of moisture:** Moisture content was determined by weight difference method keeping the sample at 100°C overnight (AOAC, 1995).

Protein, carbohydrate, lipid, ash and moisture contents were expressed as the percentage dry weight (DW). The results are given as a mean with standard deviation (±SD) as quality assurance to the data.
2.4.2.3. Application of this formulated feed

Commercial diet purchased from local market was given to the control pond containing fish meal. Diet I, diet II and diet III were given to the experimental ponds of Kalidaspur, Jharkhali and Basirhat respectively.

As a part of scientific culture, feed chart (Table 8) was maintained on the basis of days of culture (DOC) during the culture period in the experimental pond (WWF, 2006).

<table>
<thead>
<tr>
<th>Table 8. Feed chart</th>
</tr>
</thead>
<tbody>
<tr>
<td>First month</td>
</tr>
<tr>
<td>Second month</td>
</tr>
<tr>
<td>Third month</td>
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<tr>
<td>Fourth month</td>
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<tr>
<td>Fifth month</td>
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<tr>
<td>Sixth month</td>
</tr>
<tr>
<td>Seventh month</td>
</tr>
<tr>
<td>Eighth month</td>
</tr>
</tbody>
</table>

![Figure 10. Culture ponds](image-url)
2.4.3. Monthly estimation of physico-chemical and zootechnical parameters

2.4.3.1. Pond preparation

Two ponds were selected on which one pond was treated as control and the other was treated as experimental. At the very initial stage of the 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.

2.4.3.2. Stocking

Prawn seed collection is a major practice in coastal West Bengal, which is presently discouraged by all sections of the society due to its linkage with several environmental issues like ecological crop loss, uprooting of mangrove seedlings, health problems of seed collectors etc. To step aside all these dark environmental issues seeds were procured from a commercial hatchery of Nellore district of Andhra Pradesh, and stocked with initial size 1.00 cm and 0.02 gr body weight in each pond. The mean stocking weight was determined from a sample of 100 prawn seeds that were blotted to free from water. Before stocking all the prawn seeds are well acclimatized to avoid temperature and pH shocks (Sarver et al., 1982).

2.4.3.3. Analysis of physico-chemical parameters

Monthly observations of surface water temperature (°C), surface water salinity (psu), surface water pH, dissolved oxygen (mg/l), nutrient (nitrate, phosphate and silicate) concentration (µg/l), Hardness (mg/l), alkalinity (mg/l), ammonia (µgat/l), BOD (mg/l), COD (mg/l), Phytopigment (Chlorophyll a) concentration (mg/m³) in the pond water and Soil pH and organic carbon (%) of sediments were done as per the following mentioned protocols.
a) **Surface water temperature:** Measured by using 0° - 100°C mercury Celsius thermometer.

b) **pH:** Measured by using a portable pH meter (Sensitivity = ±0.02).

c) **Surface water salinity:** Measured by means of an optical refractometer (Atago, Japan), and cross-checked in laboratory by employing Mohr-Kundson method (after Strickland and Parsons, 1972). The correction factor was found out by titrating the silver nitrate solution against standard sea water (IAPO standard sea water service Charlottenlund, Slot Denmark, Chlorinity 19.376 %o).

d) **Dissolved Oxygen (DO):** Measured by DO meter in the field and subsequently crosschecked in the laboratory by Winkler's method.

e) **Alkalinity:** Alkalinity is an electrometric measurement which is performed by the computer aided titrimeter (CAT) and the pH electrode. A potentiometric titration is taken to an end-point reading of pH 4.5. The amount of acid required to reach a pH of 4.5 is expressed in millimetres. The calcium ions (CO$_3^{2-}$) neutralize the acid in this reaction, and show the buffering capacity of the sample. From the amount of acid used, a calculation indicates the amount of carbonate (CO$_3^{2-}$) involved in the reaction. This is finally expressed as mg of CaCO$_3$ / L even though actually part of the alkalinity may be contributed by MgCO$_3$, Na$_2$CO$_3$ or K$_2$CO$_3$.

f) **Hardness:** Hardness is generally caused by calcium and magnesium ions present in the water. Calcium and Magnesium form a complex of wine red colour with Erichrome Black T at pH of 10 to 1. The EDTA has got a stronger affinity towards Ca$^{2+}$ and Mg$^{2+}$ and therefore by addition of EDTA, the former complex is broken down and a new complex of blue colour is formed.

g) **Dissolved nutrients (Nitrate, Phosphate and Silicate):** Surface waters for nutrient was collected in clean TARSON bottles and transported to the laboratory in ice-freezed condition. Triplicate samples were collected from the same collection site to
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maintain the quality of the data. The standard spectrophotometric method of Strickland and Parsons (1972) was adopted to determine the nitrate concentration in surface water. Nitrate was analysed by reducing it to nitrite by means of passing the sample with ammonium chloride buffer through a glass column packed with amalgamated cadmium filings and finally treating the solution with sulphanilamide. The resultant diazonium ion was coupled with N-(1-naphthyl)-ethylene diamine to give an intensely pink azo dye. Determination of the phosphate was carried out by treatment of an aliquot of the sample with an acidic molybdate reagent containing ascorbic acid and a small proportion of potassium antimony tartarate. Dissolved silicate was determined by treating the sample with acidic molybdate reagent. The resultant silico-molybdic acid was reduced to molybdenum blue complex by ascorbic acid and incorporating oxalic acid prevented formation of similar blue complex by phosphate. SYSTRONICS UV-VIS spectrophotometer (Type 117, Sr. No. 690) was used for nutrient (NO$_3$, PO$_4$ and SiO$_2$) analysis at their respective wavelengths.

h) Biochemical oxygen demand:

i) Two 300 ml BOD bottles were half filled with dilution water. With a large tipped pipette, the pre-calculated amount of sample was dispensed into each of the two 300 ml of BOD bottles. Then each bottle was filled with dilution water and the stopper was inserted and all air bubbles were excluded.

ii) An additional two 300 ml BOD bottles with only dilution water was filled and the stopper was inserted as step 1.

iii) At 20°C, one bottle containing diluted samples and one containing only dilution water was incubated.

iv) A DO determination on the remaining BOD bottles from step 1 and step 2 were run and initial DO content was recorded.

v) After 5 days, DO determination tests were done with the incubated bottles. The DO content of the incubated bottles was recorded. There should not be an increase or decrease of more than 0.2 mg/l of DO between initial dilution water and final dilution water. Large changes may be caused by improper techniques or contaminated dilution water.

\[
\text{BOD} = (D_1-D_2) - (B_1-B_2) \times P \times mgL^{-1}
\]

Where $D_1$ is dissolved oxygen of diluted sample immediately after preparation, mg/l; $D_2$ is dissolved oxygen of diluted sample after 5 days incubation at 20°C, mg/l; $P$ is
decimal volumetric fraction of sample used; \( B_1 \) is dissolved oxygen of seed control before incubation, mg/l; \( B_2 \) is dissolved oxygen of seed control after incubation, mg/l; and \( f \) is ratio of seed in sample to seed in control = (% seed in \( D_i \) ) / (% seed in \( B_i \)).

i) **Chemical oxygen demand:**
Organic substances in the sample were oxidized by potassium dichromate in 50% sulphuric acid solution at reflux temperature in presence of mercuric sulphate to neutralize the effect of chlorides and silver sulphate (catalyst). The excess potassium dichromate was titrated against ferrous ammonium sulphate using ferroin as an indicator. The amount of potassium dichromate used is proportional indicator to the oxidizable organic matter present in the sample.

j) **Ammonia:** The samples were mixed with phenol-alcohol solution and sodium nitroprusside solution, and then allowed to react with an Oxidising solution (sodium citrate solution and sodium hypochloride solution) to measure ammonia concentration colorimetrically based on absorptions at 640 nm in a Beckman Coulter DU 640 spectrophotometer, and compared to ammonium sulphate standard.

k) **Phytopigment (Chlorophyll a) concentration:** For pigment analysis, 1 liter of surface water, collected from each of the sampling stations during high tide condition was filtered through a 0.45 μm Millipore membrane fitted with a vacuum pump. The residue along with the filter paper was dissolved in 90% acetone and kept in a refrigerator for about 24 hours in order to facilitate the complete extraction of the pigment. The solution was centrifuged for about 20 min under 5000 rpm and the supernatant solution was considered for the determination of the chlorophyll pigment by recording the optical density at 750, 664, 647 and 630 nm with the help of SYSTRONICS UV 102 spectrophotometer. All the extinction values were corrected for a small turbidity blank by subtracting the 750 nm signal from all the optical densities, and finally the phytoplankton pigments were estimated as per the following expression of Jeffrey and Humphrey (1975).

\[
\text{Chl} \ a = 11.85 \ OD_{664} - 1.54 \ OD_{647} - 0.08 \ OD_{630}
\]
All the extinction values were corrected for a small turbidity blank by subtracting the 750 nm signal from all the absorbance values. The values obtained from the equations were then multiplied by the volume of the extract (in ml) and divided by the volume of the water (in litter) filtered to express the chlorophyll content in mg.m$^{-3}$. All the analyses were done in triplicate on the basis of collection of three samples from the same site in order to ensure the quality of the data.

l) Soil pH: Measured by using a portable pH meter (Sensitivity = ±0.02) after dilution (1:10) in a distilled water.

m) Soil organic carbon: Organic carbon content of soil was determined following a modified version of Walkley and Black method. Sediment samples were collected at the surface (0-5 cm) and air-dried. Organic carbon in air-dried sediment samples is oxidized by dichromate-sulphuric acid and the amount of remaining dichromate is determined by titration with a standard ferrous solution. One-gram sample was taken into a clean, dry 500ml conical flask. Exactly 10ml 1 N $K_2Cr_2O_7$ and 20 ml conc. $H_2SO_4$ was added and mixed by gentle swirling at first and then vigorously for a total time of 1 minute. The flask was kept to react the mixture for about 30 min.

After the reaction was over the content was diluted with 200 ml distilled water and then 10 ml conc. $H_3PO_4$ added, mixed and let cool. 1 ml of Diphenylamine as oxidation-reduction indicator was added and titrated with 0.4N Ferrous ammonium sulphate $[Fe(NH_4)_{2}(SO_4)_{2}]$ solution. At the end point colour changes from dull green through turbid blue to a brilliant green. A blank was run with same quantity of the chemicals but without sediment. Calculation was done by the following expression:

$$\% C = \frac{3.951 \times (1 - \frac{T}{S})}{G}$$

Where, $g =$ weight of sample in g
$S =$ ml ferrous solution with blank titration
$T =$ ml ferrous solution with sample titration
2.4.3.4. Analysis of zootechnical parameters

Individual weights and lengths of prawns (n=100) were taken at fortnightly interval for 8 months culture period and the relevant response variables were determined for each control and experimental ponds. The length-weight relationship of the cultured species was determined to evaluate the proportionality in growth for both control and experimental ponds. The following biotic parameters were measured for the culture species in both experimental and control ponds:

a) **Condition Index (CI):** Analyzed at fortnightly interval during the culture period as per the expression; CI = W/L^3 x 100, where W = weight of the cultured species (in g) and L = length of the cultured species (in cm) (Gayanilo et al., 1997).

b) **Specific growth rate (%):** Calculated after the harvesting of prawns as per the expression: SGR (%) = ln (final weight) – ln (Initial weight) / days of experiment x 100 (De Silva and Anderson, 1995).

c) **Feed Conversion Ratio (FCR):** Analyzed after the harvesting of prawns as per the expression: FCR = feed consumed (dry weight) / live weight gain (wet weight) (De Silva and Anderson, 1995).

d) **Survival rate:** Final no. of prawns / initial no. of prawns x 100 (De Silva and Anderson, 1995).

e) **Protein efficiency ratio (PER):** Estimated according to the following equation: PER = increase in mass of animal produced / mass of protein in feed (Weight gain / Protein intake) (De Silva and Anderson, 1995).

f) **Length-weight relationship:** The biometric measurements of the cultured species were made with graduated scale. The measurements taken were total length (TL) from the tip of the rostrum up to the tip of the telson. The measurements were made to centimeter (cm) as described by FAO species identification sheets for fishery purposes (Fischer et al., 1981). The specimens were also weighed using a pan balance.
for taking the total weight (TW). The length-weight relationship (Fig. 11) was estimated using the equation:

$$W = aL^b$$

Where, $W =$ Total weight, $L =$ Total length, $a =$ regression constant, $b =$ regression coefficient.

Figure 11. Estimation of weight and length of cultured species

2.4.3.5. Harvesting

Prawns were harvested at the end of 8 months. One day prior to harvest, the water levels in each pond were lowered to approximately 0.5 m at the drain end. On the following day, water was removed completely and each pond was seined three times with a 1.3-cm squaremesh seine (3.5 m long-1.0 m deep) and then completely drained. Remaining prawns were manually harvested from the pond bottom, and all prawns were purged of mud by holding in tanks with flowing water. Total bulk length, weight and number of prawns from each pond were recorded.

2.4.3.6. Statistical analysis

Analysis of variance (ANOVA) was computed between all the selected parameters (indicators of the experiment) considering both control and experimental ponds to evaluate the differences caused by inclusion of *Porteresia coarctata* and *Enteromorpha intestinalis* dust in the feed. All statistical calculations were performed using SPSS 9.0 for Windows.