3.0. MATERIALS AND METHODOLOGY

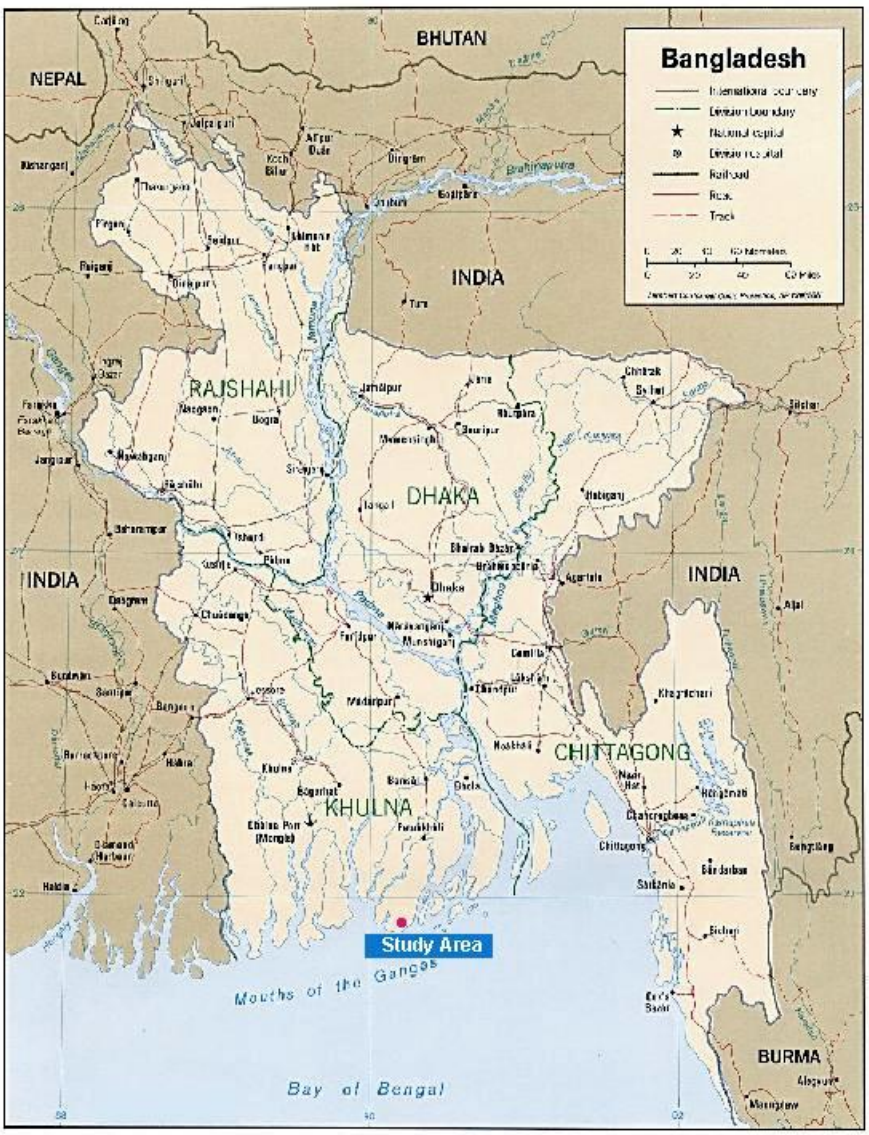
3.1. Location of the Study Area

The study area included Latachapali Union of Kalapara Upazila in the district of Patuakhali under Barisal division situated in the south-western part of Bangladesh (Fig-1). The Latachapali Union is comprised of 33 villages as shown in Fig-2. As the investigation emphasised on villages where majority of the population were considered as fishermen, an in depth survey indicated 11 such fishermen villages out of the 33 villages in the Latachapali Union (Fig-3). Fishermen villages were considered on the basis of occupation of the people of the different villages. Villages having greater than 60% people involved in fisheries activities were considered as fishermen villages.

For the identification of the studied area, the help of the Union Parishad and NGOs were taken. Maps of the study area were collected from the office of the Union Parishad of Latachapali Union. NGO workers, local people and Union Parishad members confirmed and reconfirmed the location of different study areas.

The investigation also focused on fish processing activities (fish drying, salting, smoking etc.). Therefore, such places where processing activities took place in Latachapali Union were taken into consideration for investigation. These processing areas were named as "**Shutki Point**". The field survey led to 6 "**Shutki Points**" in the Latachapali Union (Fig-4). Within each **Shutki Point**, a number of small houses were built to carry out fish processing activities (fish drying, salting, smoking etc.). These were named for the purpose of understanding in the present investigation as "**Shutki Mahal**". Again, a detailed survey resulted in the finding 31 such **Shutki Mahal** (Appendix-1).

A detailed investigation of the fishermen thriving on "**Shrimp Fry Collection**" was also under taken. Such fry collection area is illustrated in Fig-5.



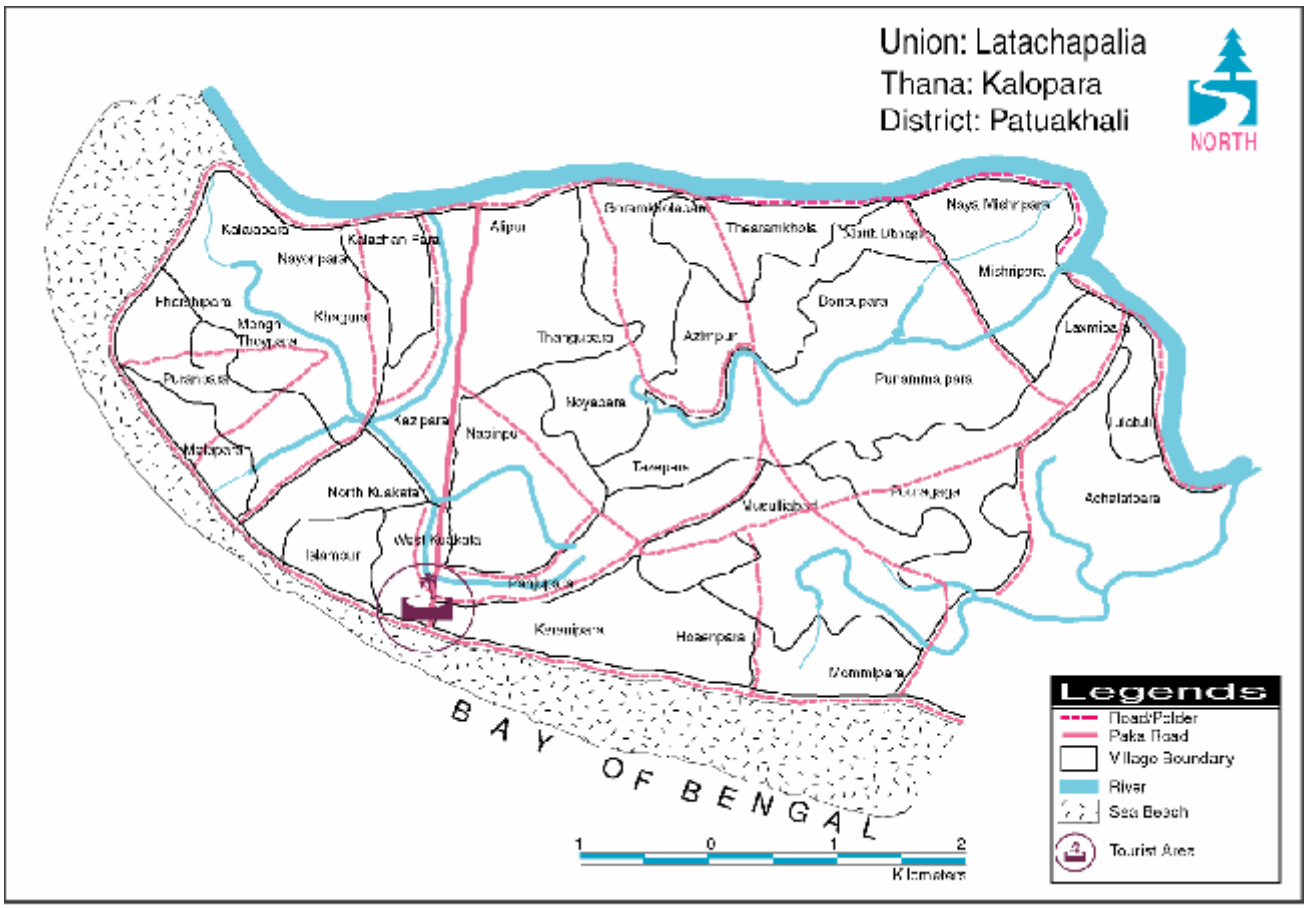


Fig-2: Total villages of Latachapali Union

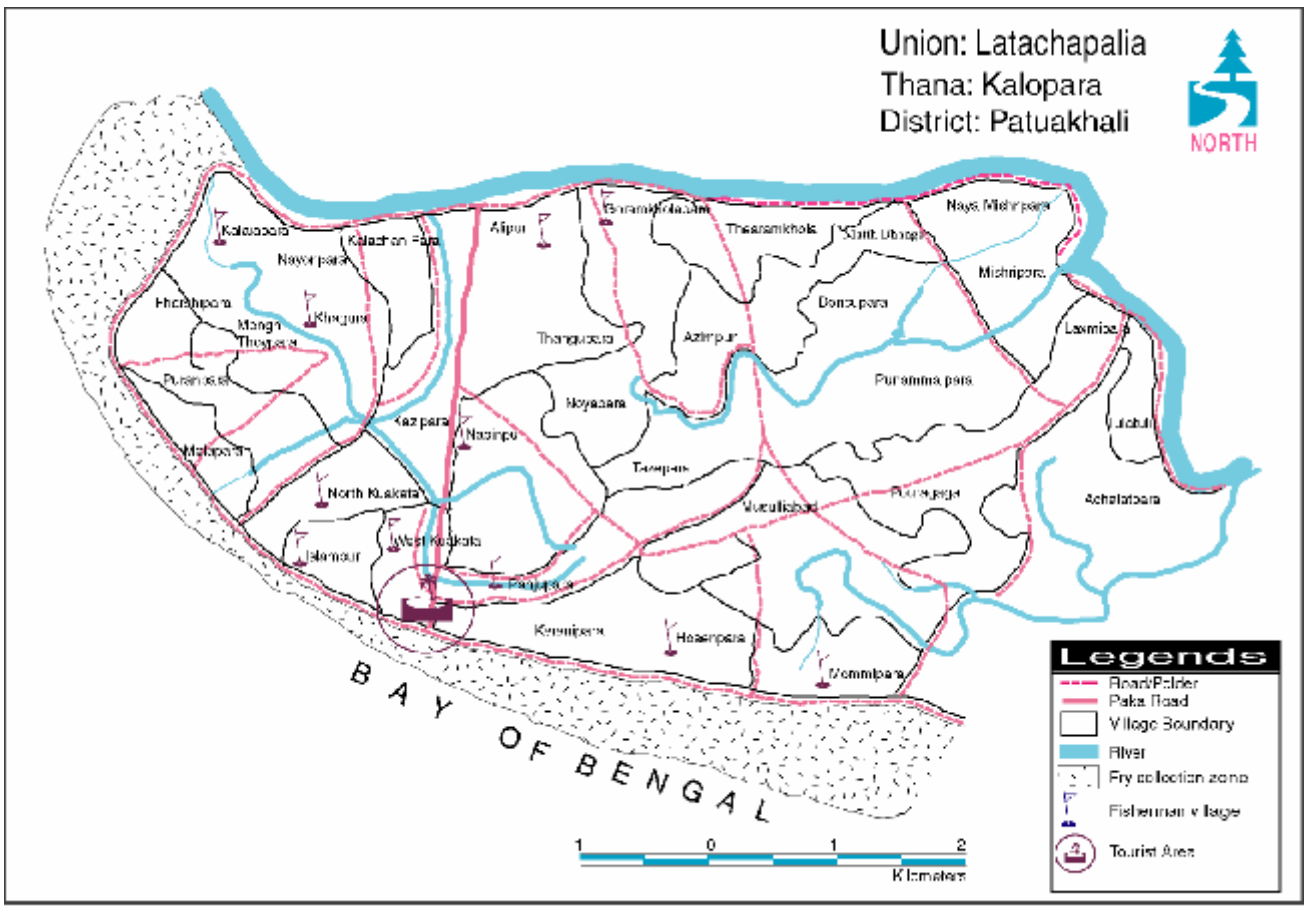


Fig-3: Fisherman villages of Latachapali Union

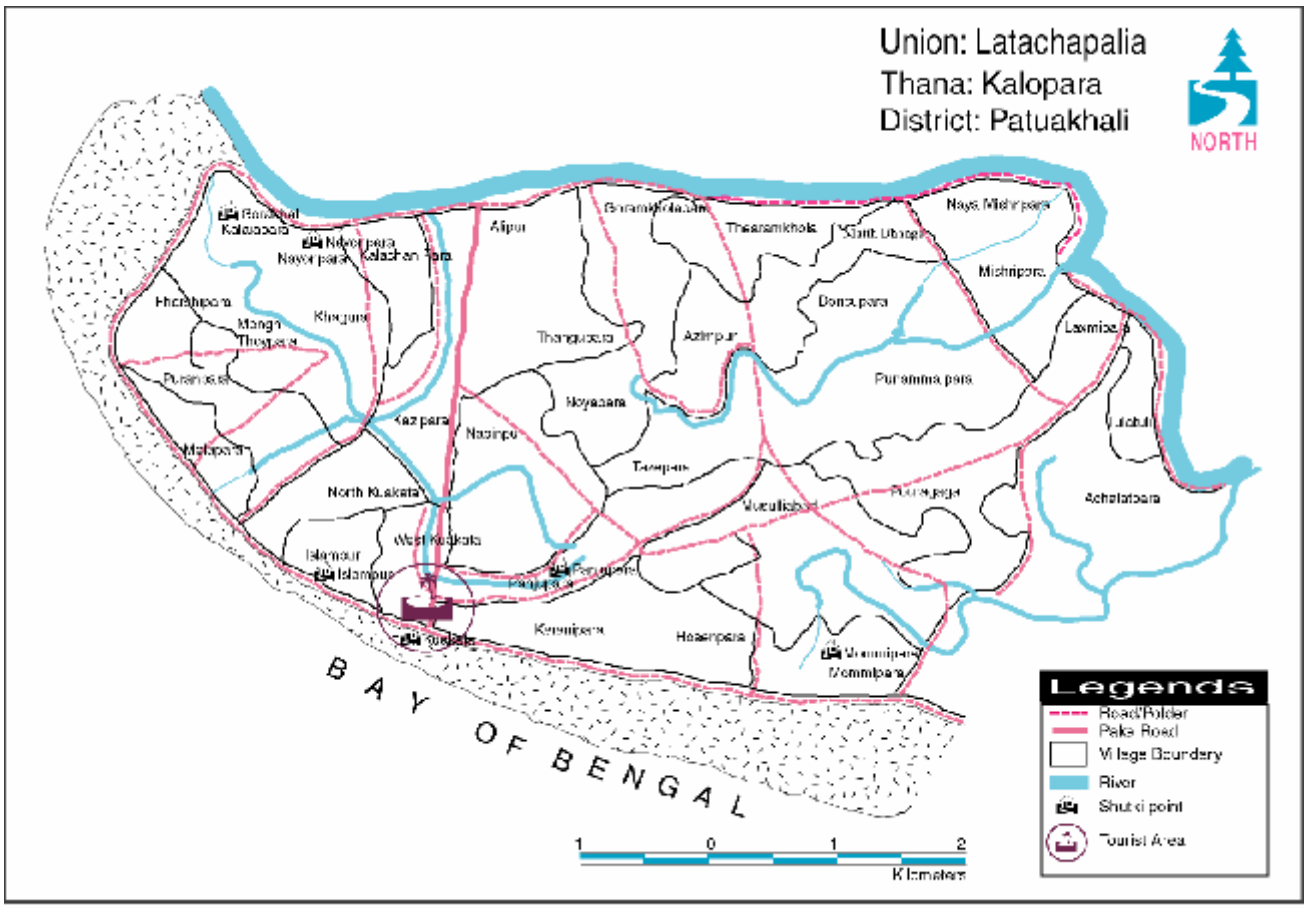


Fig-4: Processing point of Latachapali Union

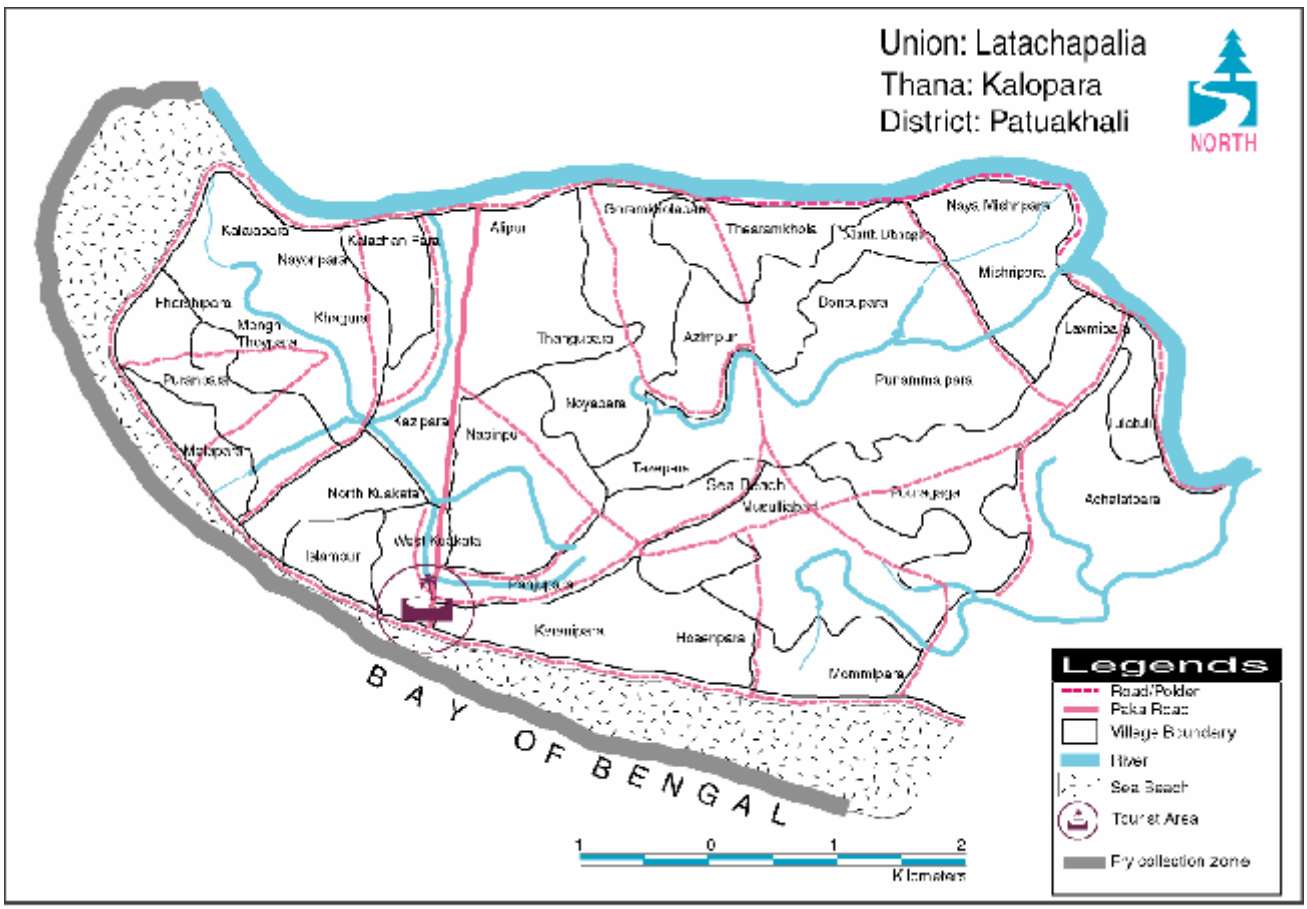


Fig-5: Shrimp fry collection zone

The survey also dealt with **Local Fish Market, Fish Landing Centres** and **Fresh Fish Arots** (depot). It was observed that the study area included two fish landing centres and thirteen fresh fish arots (Fig-6).

3.2 Field Study

3.2.1 Data Collection Method

Preliminary assumptions on the number of total people of the studied villages were done through reconnaissance survey. For the present investigation, 10-12% representative populations of the different fishermen villages have been considered to investigate the various parameters.

A range of **Rapid Rural Appraisal** (RRA) tools were applied with different degrees of effectiveness according to the procedure of Haque and Blowfield (1995) and slight modification was done on certain tools whenever required. The different RRA tools applied are listed below:

- Rapport Building:
- Group Discussion
- Personal observation
- Practical Activities
- Brain Storming
- Physical Mapping
- Structured Questionnaire (Appendix: 2)

3.2.2. Sampling

One hundred respondents were randomly selected from each village on the basis of selected parameters. Interview took place on the boat, field, respondent residence, and fish market and river and canal side. For each respondent, occupation was the primary criteria for taking further information. Those respondents only involved in fisheries activities were included for study purposes.

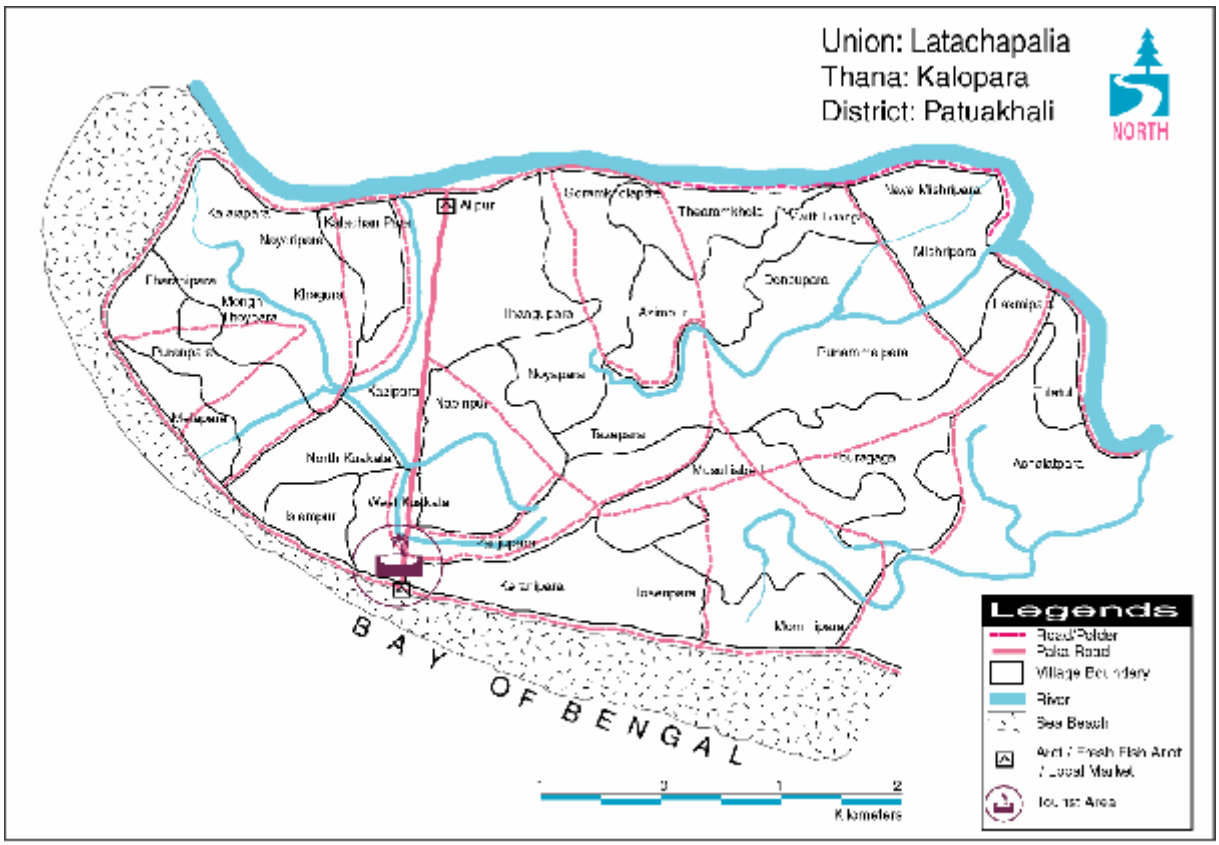


Fig-6: Local Market,Fish Landing Center and Fresh Fish Arot (Depot)

3.2.3. Shutki Point and Shutki Mahal

Rapid rural appraisal (RRA) tools were used to obtain data on shutki points and shutki mahals. (Appendix-3)
Indicators used:

- Cost of Building
- Manpower
- Chemicals and salt

- Processing methods
- Yearly production
- Yearly earning
- Profit analysis
- Prices
- Working status
- Credit

3.2.4. Shrimp Fry Collection

Shrimp fry collection data were obtained through RRA procedure. Preparation of structured questionnaire for this purpose is shown in Appendix-4.

Indicators used:

- Fry collection area
- Fry preservation and marketing

3.2.5. Fresh Fish Arot, Fish Landing Centre and Local Fish Market

Information on fresh fish arot, fish landing center and local fish market was done in accordance with structured questionnaire (Appendix-5)

Indicators used

- Rent
- Manpower
- Preservation methods
- Monthly production
- Yearly production
- Yearly earning
- Profit
- Price
- Working status
- Credit

3.2.6. NGOs Engaged in Fisheries Activities

Initial information on different parameters related to fisheries activities was collected from the different Non Governmental Organisation (NGO) such as Community Development Center (CODEC), Bangladesh Rural Advancement Committee (BRAC), Association for Social Advancement (ASA), and Grameen. Using informal group discussion, information was collected.

3.2.7. Case Studies

The selection of case study was taken on the basis of economical and social status of people in the study area. They were poor fisherman, rich fisherman, fry collector, businessmen etc. Following the interviews, a cross-section of people with whom rapport had been established was invited to take part in case studies. The case was built up over repeated visits to individual, their family members and others whose response could be used to verify the accuracy of the information. This technique provided in-depth information not only about the individual but the systems governing behaviour and the interrelationships between people and institutions. The questionnaire for case studies is given in Appendix-6.

3.3. Laboratory Analysis

3.3.1 Species Studied

For laboratory analysis 10 fresh fish of 10 different species were collected (Table-1) from the project site.

Table 1: List of fresh fish species collected from Kuakata

ID	Scientific Name	English Name	Local Name
FS_1	*Mugil cephalus*	Mullet	Parshe
FS_2	*Setipinna phasa*	Anchovy	Phasa
FS_3	*Coilia dussumieri*	Gold spotted anchovy	Boiragi
FS_4	*Scatophagus argus*	Skates	Bistara
FS_5	*Sillanopsis panijus*	Lady fish	Tular danti
FS_6	*Arius caelatus*	Engraved catfish	Gang Tengra
FS_7	*Hilsa ilisha*	Hilsha fish	Ilish
FS_8	*Polynemus paradiseus*	Thread fin	Tapashi
FS_9	*Platicephalus indicus*	Bartail flathead	Chat baila
FS_{10}	*Pelamys chiliensis*	Mackerel and Tuna	Kankon

In case of dried fish, 18 samples of 14 different species were investigated (Table 2).

Table 2: List of all dried fish species collected from Kuakata

ID	Scientific name	English name	Local name
S_1	*Mugil cephalus*	Mullet	Parshe
S_2	*Scoliodon shorrakowah*	Shark	Kamot
S_3	*Harpodon nehereus*	Bombay duck	Loytta
S_5	*Arius caelatus*	Engraved catfish	Gang Tengra
S_6	*Setipinna phasa*	Anchovy	Phasa
S_7	*Hilsa ilisha*	Hilsh fish	Ilish
S_8	*Scoliodon sorrakowah*	Shark	Kamot
S_9	*Polynemus paradiseus*	Thread fin	Tapashi
S_{10}	*Mugil cephalus*	Mullet	Parshe
S_{11}	*Trichuirus haumela*	Cutlass fish	Churi
S_{12}	*Harpodon nehereus*	Bombay duck	Loytta
S_{13}	*Pampus chinensis*	Chinese pompret	Rupchanda
S_{14}	*Setipinna phasa*	Anchovy	Phasa
S_{15}	*Himantura walga*	Stingray	Shaplapata
S_{16}	*Muraenesox bagio*	Common pike conger	Samudrik Baim
S_{17}	*Epinephelus lanceolatus*	Sea bass	Bole
S_{18}	*Cynoglossus bengalensis*	Tongue fish	Kukurjib
S_{19}	*Tetraodon patoka*	Box fish	Potka

However, it is to be noted that during the total period of investigation, it was difficult to collect common dried species. Therefore, after a detailed field survey, it was decided that 4 common species, which were available both in summer and winter in the project area, would be evaluated for comparison. These four common species are listed in Table-3. As a result, the investigation focused on the quality assessment of 10 fresh fish and 4 common dried fish available in summer and winter and all other dried species collected only once during the study period.

Table-3: List of four common dried fish available both in summer and winter

ID	Scientific name	English name	Local name	Season
S_1	*Mugil cephalus*	Mullet	Parshe	Summer
S_2	*Scoliodon shorrakowah*	Shark	Kamot	
S_3	*Harpodon nehereus*	Bombay duck	Loytta	
S_6	*Setipinna phasa*	Anchovy	Phasa	
S_8	*Mugil cephalus*	Mullet	Parshe	Winter
S_{10}	*Scoliodon shorrakowah*	Shark	Kamot	
S_{12}	*Harpodon nehereus*	Bombay duck	Loytta	
S_{14}	*Setipinna phasa*	Anchovy	Phasa	

3.3.2. Sample Collection, Transportation, Storage and Sampling

Samples investigated either dried or fresh (iced) was collected from Kuakata, the project site. Fresh fish caught from deep sea and landed in Kuakata shutki area were purchased from Arot. Dried fish were collected from the local shutki market of Kuakata. The fresh samples were kept in insulated icebox with sufficient ice while, the dried samples were packed individually with air tight polythin bag. All the collected samples were brought to the Quality Control Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna for investigation by road transportation.

As soon as the samples were received in the laboratory, they were first evaluated organoleptically by a three-member taste panel. Dried samples were stored in air tight polythene bag at 4^0C while fresh fish samples were stored at -20^0C in deep freeze for further investigations.

3.3.3. Organoleptic Assessment of Dried and Fresh Fish

The organoleptic assessment of the quality of dried and fresh fish samples were carried out according to the procedure of Payram and Pilgrim (1977). However, for the dried species studied in the present investigation, a slightly modification was done in order to fit in appropriately the characteristics provided by Payram and Pilgrim (1977). The modification made was related to scoring as shown in Table 5. The different characteristics for organoleptic evaluation is provided in Table-4. The organoleptic characteristics emphasised on odour, flavour, toughness, fibrousness and appearance. A three-member taste panel assessed the organoleptic quality of both dried and fresh fish.

Table 4: Organoleptic score sheet for dried Fish

Odor	flavor	Toughness	Fibrousness	Appearances	Score
Absent	Absent	Absent	No fibrousness	Very good appearance	10
Very slight	Very slight	Very slightly tough	Very slight	Very slight loss of appearance	8
Slight	Slight	Slightly tough	Slightly fibrous	Slight loss	6
Moderate	Moderate	Moderately by tough	Moderately	Moderate loss	4
Strong	Strong	Strongly tough	Strong fibrous	Strongly loss of appearance	2
Very strong	Very strong	Extremely tough	Extremely fibrous	Extremely poor	0

Source: Paryam and Pilgrin, (1977)

For fresh fish, organoleptic evaluation was carried out according to the procedure of Shewan and Ehrenberg (1977) as illustrated in Table-6. Quality had been defined by a 10 point scale. Characteristics assessed were eye, pupil, gill, body surface, texture, appearance, odour, etc. In addition, acceptability of the fresh fish was evaluated as per the characteristics presented in Table-6.

Table-5: Modification of the organoleptic score sheet

Acceptability	Score (Paryam and Pilgrim, 1977)	Modified Score (present investigation)
Like extremely (LE)	9	9 - 10
Like very much (LVM)	8	8 - 8.9
Like moderately (LM)	7	7 -7.9
Like slightly (LS)	6	6 - 6.9
Like neither like nor dislike (LNLND)	5	5 - 5.9
Dislike slightly (DS)	4	4 - 4.9
Dislike moderately (DM)	3	3 - 3.9
Dislike very much (DVM)	2	2 - 2.9
Dislike extremely (DE)	1	1-1.9

Table 6: Organoleptic score sheet for fresh fish (Shewan and Ehrenberg, 1977)

Eye		Pupil		Gill	
Characteristics	**Score**	**Characteristics**	**Score**	**Characteristics**	**Score**
Bright, clear and dull	10	Slight and transparent	10	Bright red	10
Slight dull	9	Slight dull / misty	8	Slight dull red / slight pale	9
Moderately dull	8	Dull / Slight milky white	7	Slight pale / pale	8
Dull	7	Watery color / whitish	6	Moderately pale / yellowish	7
Slightly sunken	6	Slightly misty / milky white	4	Pale / whitish	6
Moderately sunken	4	Pale white	2	Brownish / Grayish	4
Sunken	2	Extremely white / more white	0	Dark color	2
Completely sunken	0			Blackish	0
Body Surface		**Texture**		**Belly wall**	
Characteristics	**Score**	**Characteristics**	**Score**	Characteristics	Score
Shining bright	10	Frame and elastic	10	Normal fresh	10
Slight loss of brightness	8	Rigor stage	8	Slightly discoloration	8
Loss of brightness	6	Just post rigor stage	6	More discoloration	6
Slight dull	4	Slightly soft	4	Slightly digested	4
Dull & slight reddish of tail end	2	Moderately soft	2	More digested	2
Completely dull	0	Soft and loose	0	Extremely digested	0
Odour		**Overall Acceptability**			
Characteristics	**Score**	**Characteristics**	**Score**		
Fresh odour	10	Highly acceptable (HA)	10		
Sweety odour	8	Acceptable (A)	9		
Slightly spoilage odour	7	Moderately acceptable (MA)	8		
Moderately spoilage odour	6	Just acceptable (JA)	6		
Spoilage odour	5	Just unacceptable (JUA)	5		
Slightly off odour	4	Unacceptable (UA)	3		
Moderately off odour	3	More unacceptable (MUA)	1		
Off odour	2	Extremely unacceptable (EUA)	0		
Extremely off odour	0	Extremely off odour			

3.3.4. Microbiological Assessment

3.3.4.1. Control Strains

a) Escherichia coli
b) Salmonella typhi
c) Vibrio cholerae
d) Vibrio parahaemolyticus
e) Proteus

The above strains were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) Dhaka.

3.3.4.2. Media and Reagents

The following media and reagents were prepared for investigating all microbiological parameters (Standard Plate Count, Total Coliform, Faecal Coliform, *Vibrio cholerae* and *Salmonella sp.*) in fresh and dried fish samples.

§ Plate Count Agar (PCA)

23.5 gm of plate count agar (PCA) was added into a conical flask containing in 1000 ml. of distilled water and boiled to dissolve the medium completely. The medium was then sterilised by autoclaving at 15 lb. pressure (121ºC temperature) for 15 minutes.

§ Peptone Water (0.1%)

1.0 gm of peptone powder was added in 1000 ml. of distilled water. Then the medium was sterilised by autoclaving at 15-lb pressure (121ºC temperature) for 15 minutes.

§ Lauryl Sulfate Tryptose Broth (LSTB)

35.6 gm of LSTB was dissolved in 1000 ml. of distilled water in a flask. 9-ml aliquots of LSTB broth were distributed as 9-ml aliquots into sterile screw capped tubes containing Durham's tubes and sterilised by autoclaving at 15lb. pressure (121ºC) for 15 minutes.

§ Brilliant Green Lactose Bile Broth (BGLB):

40 gm of BGLB was suspended in 1000 ml. distilled water. It was mixed well, distributed in fermentation tubes, and sterilised by autoclaving at 15-lb. pressure (121ºC) for 15 minutes.

§ Eosin Methylene Blue Agar (EMB)

36.0 gm agar powder was suspended in 1000 ml. of distilled water and then heated to dissolve completely. After dissolving, the medium was sterilised by autoclaving at 15 lb. pressure (121ºC) for 15 minutes. Then it was cooled to 50 º C in order to oxidise the methylene blue.

§ Peptone Water (2%)

Dehydrated 1 gm peptone water base was added in 1000 ml. of distilled water. Then the medium was sterilised by autoclaving at 15 lb. pressure (121ºC temperature) for 15 minutes.

§ Simmons Citrate Agar

23 gm citrate agar was suspended in 1000 ml. of distilled water and boiled to dissolve completely. Then the medium was distributed as 7-8 ml. aliquots to each of the screw capped tubes and sterilised by autoclaving at 15 lb. pressure (121ºC) for 15 minutes and cooled to room temperature and allowed to solidify as slopes with 1 inch. butt leaning against a sterile glass pipette.

§ Alkaline Peptone Water (pH 8.6 –9.0)

20 gm dehydrated alkaline peptone water base was suspended in 100 ml. of distilled water in a conical flask and boiled to dissolve completely. Then the medium was dispensed as desired and sterilised by autoclaving at 15 lb. pressure (121ºC) for 15 minutes.

§ Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS)

8.9 gm of dehydrated TCBS agar base was added in a flask containing 1000 ml. of distilled water. The suspension was boiled for 10 minutes, and cooled to 50º C. This was then poured into petridishes and allowed to solidify.

§ Iron Agar (KIA)

55.6 gm of KIA powder was suspended in 1000 ml. of distilled water in a conical flask and boiled to dissolve completely. Then the medium was distributed as 7 ml. into required number of screw capped test tubes. The tubes were sterilised by autoclaving at 15 lb. pressure (121ºC) for 15 minutes. Then the medium was allowed to solidify as slopes with 1 inch. butt leaning against a sterile glass pipette.

§ Urea Agar
2.4 gm dehydrated urea agar base was suspended in 95 ml. distilled water in a conical flask and boiled to dissolve completely. Then the medium was sterilised by autoclaving at 115 ºC for 20 minutes. The sterilised medium was cooled to 50 ºC and aseptically introduced into 5 ml. of filter sterile 40% urea solution. Then the medium was mixed well. 2 ml. of this was distributed into sterile one drum vial.

§ Selenite Broth (SB)
23 gm of powder (SB) was suspended in 1000 ml. distilled water in a conical flask and warmed to dissolve completely. Then the medium was distributed in sterile tubes and sterilised in a boiling water bath or free flowing stream for 10 minutes.

§ Tetrathionate Broth (TB)
46.0 gm. of powder (TB) was suspended in 1000 ml. distilled water in a conical flask completely and heated to boiling and cool to 45ºC . Then 20 ml. of iodine solution were added into the medium and mixed well and distributed into the tube 10 ml. amount of sterile tubes. Iodine solution was prepared by dissolving 6 gm of iodine crystals and 5 gm potassium iodine in 20 ml. of water.

§ Xylose Lysine Deoxycholate (XLD)
56.5 gm of XLD agar was suspended in 1000 ml. of distilled water, heated with frequent agitation until the medium was dissolved and then transferred to a water bath at 50ºC. After cooling, the medium was poured into sterile petridishes.

§ MacConkey Agar (MCA)
40.0 gm of MacConkey agar powder was suspended in 1000 ml. of distilled water and heated if necessary to dissolve the medium completely. Then the medium was distributed into the sterile plates and allowed to solidify

§ Buffered Peptone Water (BPW)
20 gm dehydrated buffered peptone water base was suspended in 1000 ml. of distilled water and boiled to dissolve completely. Then the medium was dispensed as desired and sterilised by autoclaving at 15 lb. pressure (121ºC) for 15 minutes.

§ MR-VP broth
7 gm of peptone and 5gm of dextrose was completely dissolved in 5 ml of potassium phosphate and mixed well.

§ KOVAC'S Reagent
5 gm of p-dimethyalamino benzaldehyde was dissolved in 75 ml of iso-amyl alcohol. Then 25 ml concentrated hydrochloric acid was added into the reagent

§ Methyl Red Solution
0.1 gm methyl red was dissolved in the 95% ethyl alcohol and diluted to 500 ml distilled water.

§ Barrit's reagent

Solution A: 5.0 gm alpha-napthol was dissolved in absolute ethanol with constant stirring.

Solution B: 40.0 gm of potassium hydroxide was dissolved in 75 ml of distilled water. The solution became warm, and was allowed to cool to room temperature. 3.0 gm creatine was added and stirred to dissolve completely. After dissolving water was added to make 100 ml volume.

§ Oxidase reagent
Tettramethyl - P- phenylene dihydrochloride was dissolved in distilled water at a concentration of 1%.

3. 3. 4 .3. Enumeration of Standard Plate Count (SPC)
20.0 gm of pooled sample (dried fish) was weighed and taken into a sterile warring blender jar for blending. The sample was added into a flask containing 180 ml 0.1% sterile peptone water .The mixture was homogenised for two minutes. This provided a dilution of 10^{-1}. 1.0 ml. of this suspension was transferred into McCarty's bottles containing 9 ml of 0.1% peptone water to give a dilution of 10^{-2}. The process was repeated for the preparation of 10^{-3}, 10^{-4} and 10^{-5} dilutions respectively.

The appropriate dilutions were selected and for every dilution 1.0 ml aliquot was transferred into a sterile petridishes. 20.0 ml portion of molten Plate Count Agar (PCA) was poured into each of this sterile petridishes. The plates were then rotated 5 times clockwise, 5 times anticlockwise, 5 times back and forward. Care was taken not to splash agar on the lid of the dish. Plates were left to solidify The plates were inverted and incubated at 35^0 C± 2^0 C for 48 hours. Plates with 30-

300 colonies on the surface were only counted. Flow chart for the enumeration of Standard Plate Count (SPC) is provided in Figure 7.

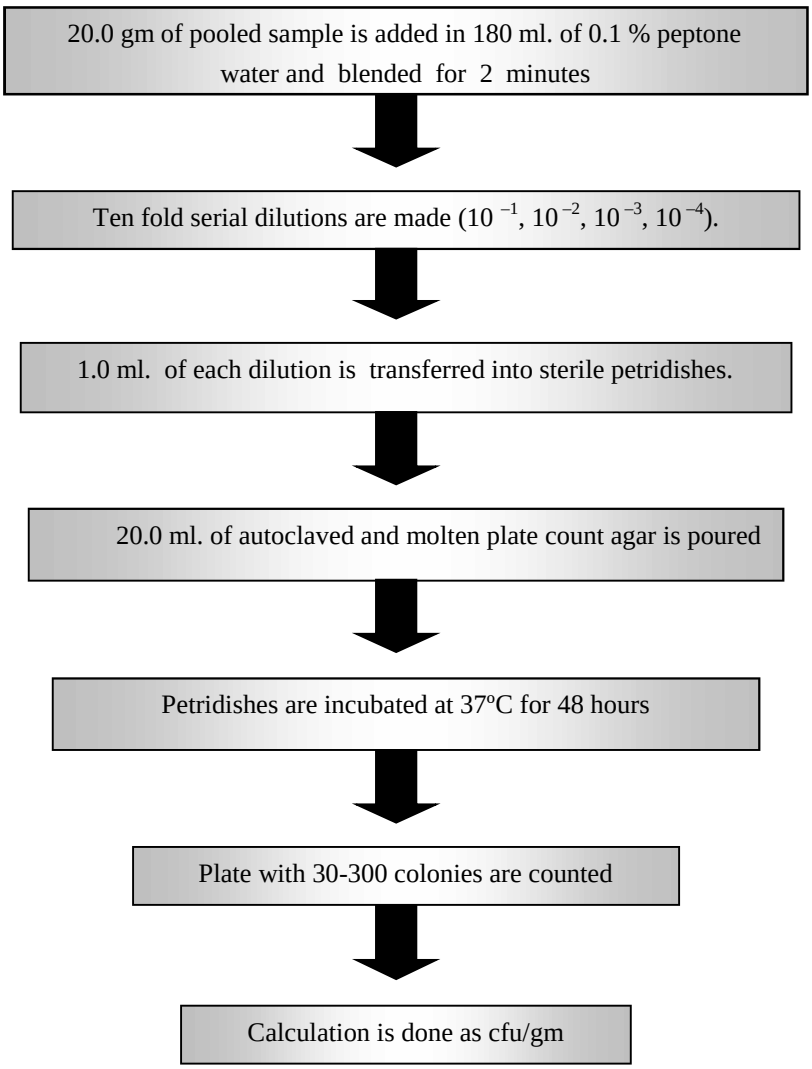


Fig-7: Enumeration of standard plate count (SPC)

3. 3. 4. 4. Quantification of Total Coliform (TC)

20.0 gm pooled sample (dried fish) was weighed and aseptically taken into a sterile waring blender jar and then blended. After blending, the sample was added into conical flask containing 180 ml. of 0.1% peptone water. Ten fold serial dilutions were made with this suspension in McCarty's bottles containing 9.0 ml. of 0.1% peptone water.

1.0 ml. of each dilution was transferred into three screw-capped test tubes containing autoclaved. Laryal Sulphate Triptose (LST) broth (10.0 ml) with Durhums tubes. The tubes were inverted to ensure that Durham tubes did not contain gas bubbles. These tubes were incubated at 370 C ± 10 C for 48 hours. The formation of gas within 24 or 48 hours was considered as evidence for the presumptive positive test for Coliform organisms.

A loopful of suspension from all positive LST broth tube was transferred into 10.0 ml volumes of Brilliant Green Lactose Bile (BGLB) broth. These tubes were incubated at 370 C ± 10 C for 48 hours.

Gas production in BGLB tubes considered as sufficient evidence for the confirmatory test for Total Coliform organisms. Number of positive tubes out of 3 was recorded for each dilution. Using the MPN Tables, the MPN of Total Coliform (TC) was calculated based on the portion of confirmed LSTB tubes (with gas production) for 3 consecutive dilutions in BGLB broth at 370 C ± 10 C.

Flow chart for the Quantification Total Coliform (TC) of dried and fresh fish is shown in Figure 8

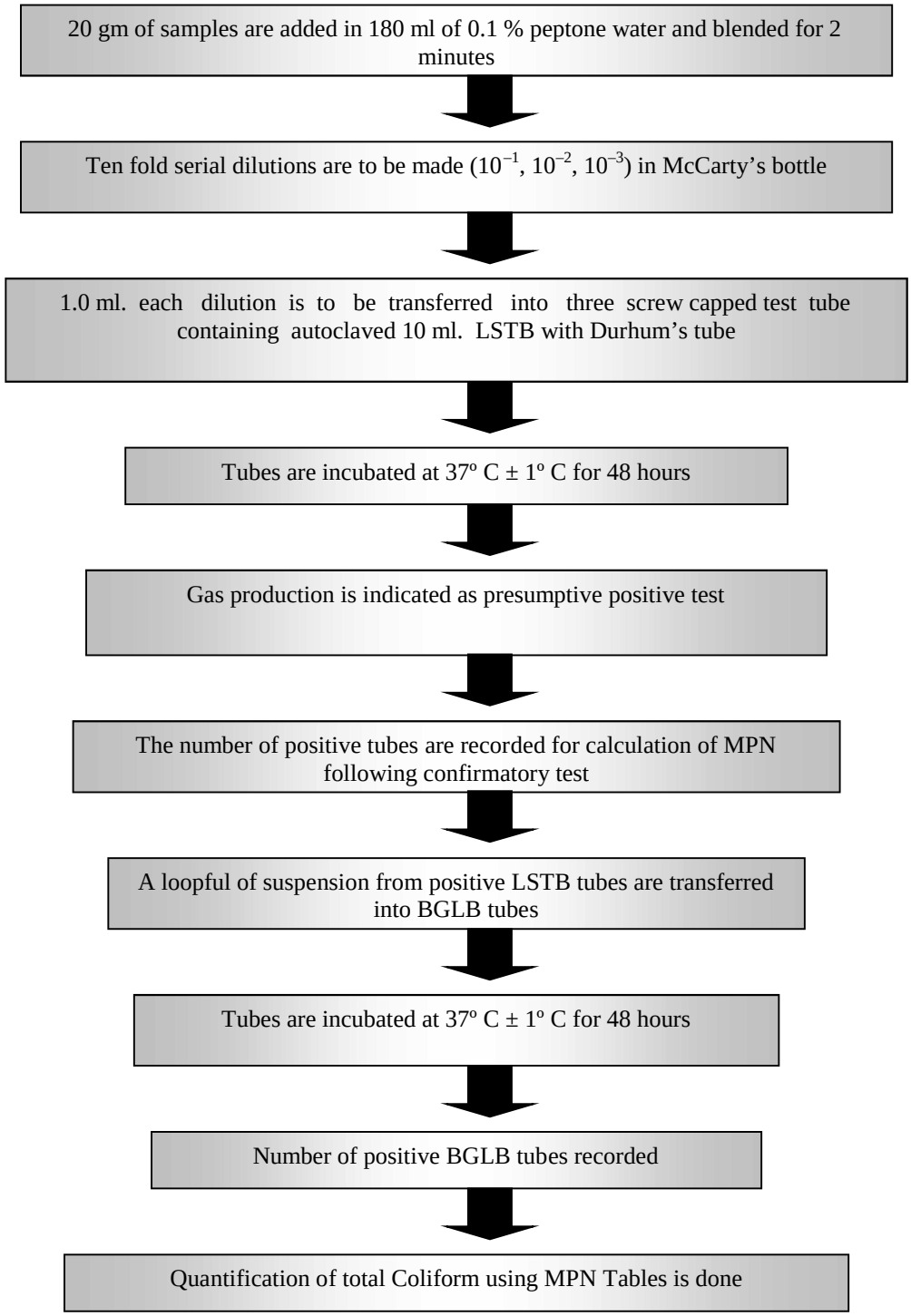


Fig. 8: Quantification of total coliforms (TC)

3.3.4.5. Quantification of Faecal Coliform (FC)

Those tubes of Lauryl Sulphate Tryptose Broth (LSTB) which were positive for gas production in Total Coliform (TC) estimation tests were considered. Subcultures were made from all positive tubes of Lauryl Sulphate Tryptose Broth (LSTB) into a 10 ml volume of Brilliant Green Bile Broth (BGLB) and incubated at 44.5 m 0.5 º C up to 48 hours. Any tube showing gas production was considered positive for the presence of presumptive Faecal Coliforms (FC).

All positive tubes were sub-cultured by streaking onto plates of EMB agar. Then these plates were incubated at 37º C for 18 - 24 hours. After incubation, the plates were examined for suspicious Faecal Coliform colonies i.e. black or dark centered with the greenish metallic sheen

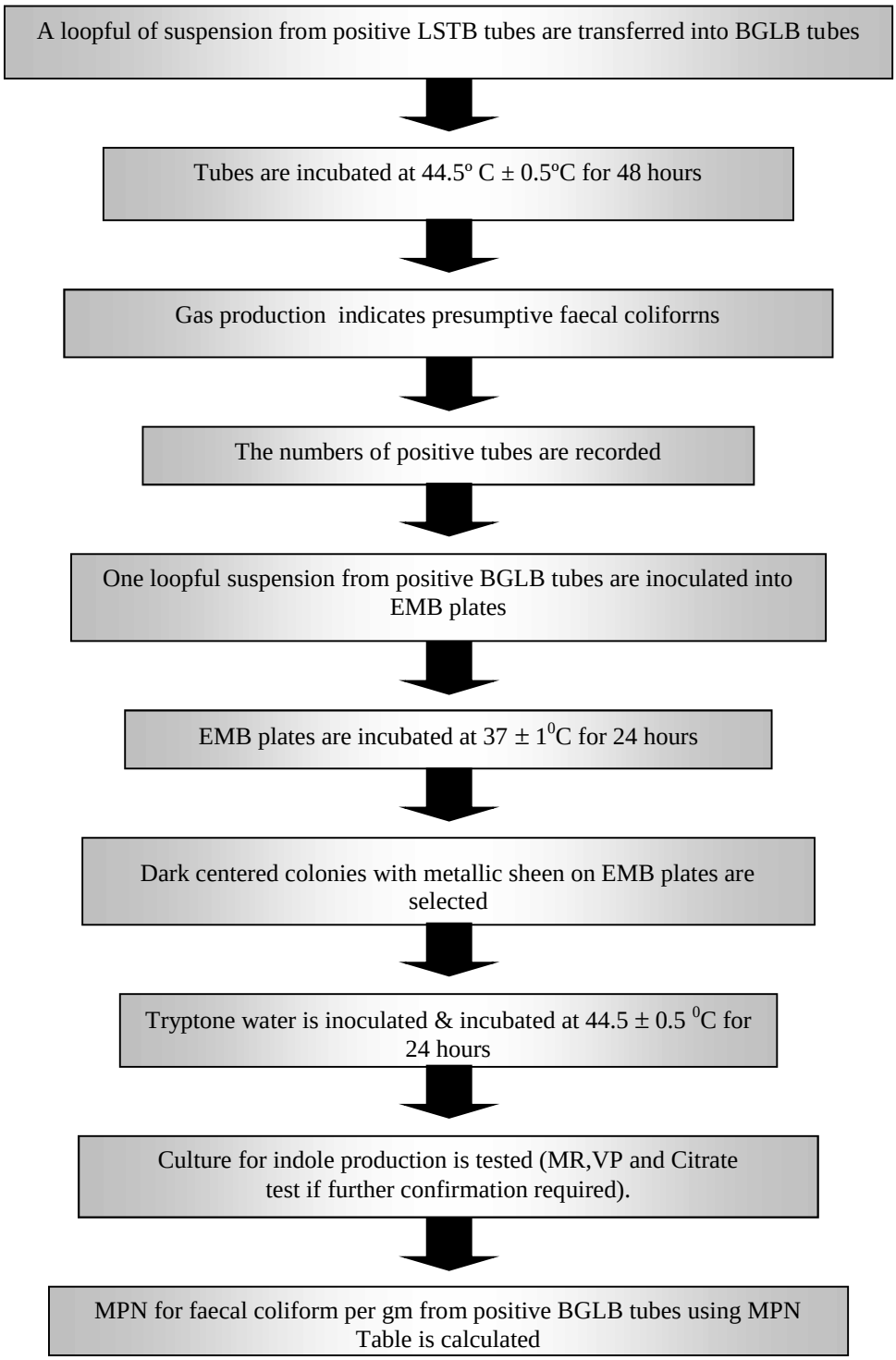


Fig-9: Quantification of faecal coliform (FC)

- **Indole**

Typical colonies were inoculated into individual tube of tryptone water and incubated at 44.5 ± 0.5 º C for 24 hours. Cultures were tested for indole production with KOVAC's reagent. Presence of indole indicated confirmation for the faecal coliform group.

The MPN of Faecal Coliform (FC) was calculated based on proportion of confirmed LSTB tubes (with gas production) for 3 consecutive dilution in BGLB broth at 44.5 ± 0.5 0C.

For further confirmation (if required) methyl red (MR), Voges – Proskauer and Citrate Utilisation tests were carried out.

a) Methyl Red test
Using sterile technique, each experimental organism was inoculated into tubes containing 5.0 ml of 0.1% peptone water. Then the inoculating tubes were incubated at 37 º C for 48 hours. One third of each culture was transferred into an empty test tube and set aside for the Voges – Proskauer tests. Five drops of the methyl red indicator were added to the remaining aliquot of each culture and observed for the colour of the culture turning red as an indicative of a positive test.

b) Voges – Proskauer (VP) test
To the aliquots of each broth culture separated during the methyl red test, 10 drops of Barritt's reagent A was added and culture was shaken. Immediately 10 drops of Barritt's reagent B was added and the culture was reshaken every 3 to 4 times. The culture was for 15 minutes for the formation of pink complex color as an indicative of positive VP test

c) Citrate Utilization test
Using sterile technique, the suspected isolate was inoculated into its appropriately labeled deep tube by means of loop inoculation and one tube was be served as a control. Then the inoculating tubes were incubated at 37 º C for 24 to 48 hours.

3.3.4.6. Isolation and Identification of *Vibrio cholerae*
25 g of the sample (dried fish) was added with approximately 225 ml. of alkaline peptone water in a " Warring" blender flask . Then the sample containing alkaline peptone water was blended for 1 min. The sample was incubated at 37 ± 1^0 C for 6-8 hrs.

At the end of the incubation period, a loopful of the alkaline peptone water was streaked onto Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates. The plates were then incubated at 37^0C $\pm 1^0$ C for 24 hrs. At the end of incubation, plates were checked for the characteristic colony of *Vibrio cholerae*. On TCBS agar colonies appeared as large (2–3 mm.), smooth and yellow, slightly flattened, with opaque centre and translucent peripheries.
Suspected colonies of *Vibrio cholerae were* selected from TCBS agar plates for the following biochemical tests.

§ Kligler's Iron Agar (KIA)
Using sterile technique, each experimental isolate was inoculated into its appropriately labelled tube by means of a stab-and–streak inoculation. Screw cap of the tubes were not fully tightened. One tube was be served as a control. Then the inoculating tubes were incubated at 37 ± 1^0 C for 18 to 24 hours. Vibrio colonies manifested on alkaline slant (red), acid butt (yellow) and produce neither gas nor blackening in the butt (i.e. H_2S negative).

§ Oxidase test
Two or three drops of the p- amino- dimethylalanine oxalate were added to the surface of the growth of each test organism. Then the growth was observed for the presence or absence of a colour change from pink, to maroon and finally to purple. Colour change in 10-30 seconds indicated positive test.

§ Indole production
The procedure is as described in 3.3.4.5.

- **Urease Test**

Using sterile technique, each experimental isolate was inoculated into its appropriately labelled urea agar tube by means of loop inoculation. Then the inoculating tubes were incubated at 37 ºC for 24 hours. Presence of deep pink colour due to splitting of urea, releasing ammonia indicated positive urease test. In the experiment, *Proteus vulgaris* and *V. cholerae* were included as positive and negative control respectively.

§ Citrate Utilization Test
The procedure is as described in 3.3.4.5.
V. cholerae shows following reaction in above mentioned biochemical tests:

Tests	Results
KIA	Acid slant, acid butt; no gas ; no H_2S
Indole	+
Urease	-
Citrate	+
Motility	+
Oxidase	+

If any isolate showed the above biochemical properties, it was further characterised by mannitol fermentation, sucrose fermentation, lysine decarboxylase, arginine dihydrolase and ornithine decarboxylase test to differentiate it from other members of vibrionaceae (*Plesiomonas, Aeromonas*) family.

Flow chart for the isolation and identification of *Vibrio cholerae* from dried and fresh fish is shown in Figure 10

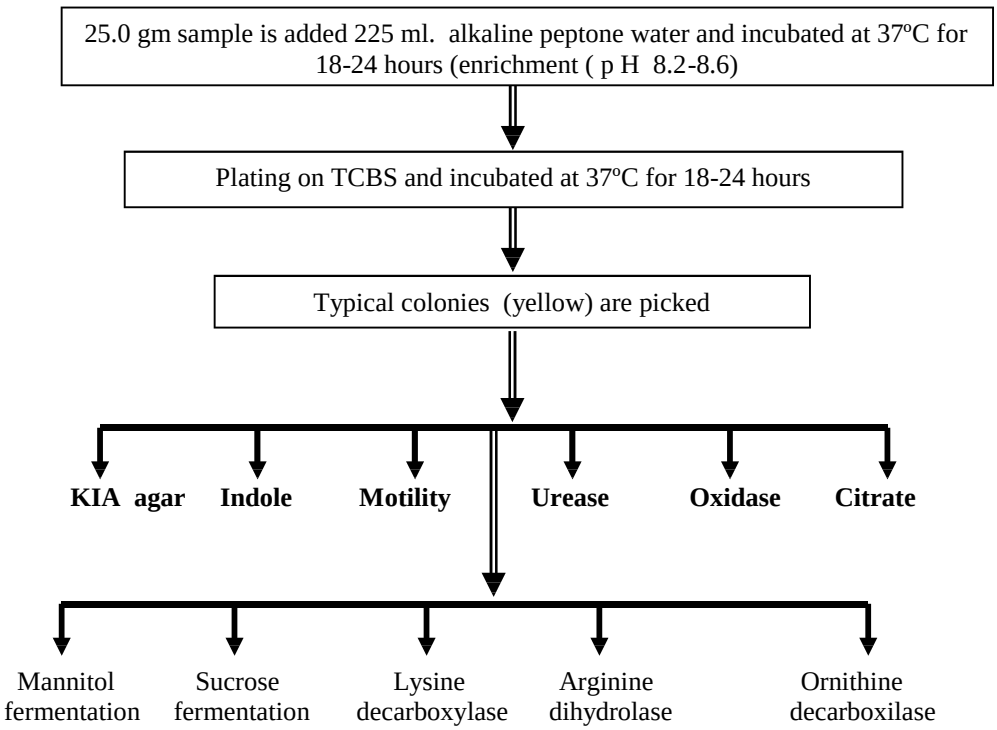


Fig 10: Isolation and identification of *Vibrio cholerae*

3.3.4.7. Isolation and identification of *Salmonella sp.*

25.0 gm of sample (dried fish) was weighed and taken into a waring blender flask. 225 ml. of buffered peptone water (BPW) was added into the sample and then homogenized for 1 minutes. Sample was incubated at 37º ± 1º C for 24 hours.

The resuscitated culture was gently mixed. 1.0 ml. of the mixture was added each to 10 ml of Selenite broth (SB) and 10 ml of Tetrathionate broth (TB). The selective enrichment broth were then incubated at 37º ± 1ºC for 24 hours. After incubation, a loopful of each of the two selective enrichment broth were transferred to the surface of the selective agar media, i.e. Xylose Lysine Deoxycholate (XLD) agar .The plates were then incubated at 37º ± 1º C for 24 hours and observed for characteristics colonies for ***Salmonella sp.*** After 24-48 hrs. ***Salmonella sp.*** shows pink colonies with black centre of H_2S on XLD.

Several suspected colonies were picked up for biochemical tests as follows:

a) Kligler's Iron Agar (KIA)

KIA tubes were prepared and inoculated as described in 3.3.4.6. *Salmonella* culture produce an alkaline slant (red) and acid butt (yellow) with or without production of H_2S.

b) Purification of isolate

Streaking McConkey agar purified KIA cultures. Typical colonies appear transparent and colourless.

c) Urease

Urease agar was prepared and inoculated as described in 3.3.4.6. *Salmonella* culture produces negative reaction in urease test.

d) Indole

Indole test was carried out as described in 3.3.4.5. *Salmonella* culture produces negative reaction in indole test.

d) Methyl Red (MR) Test

Methyl Red (MR) Test was carried out using culture grown in MR-VP broth as described in 3.3.4.5. *Salmonella* culture shows positive test i.e. acedic end product in methyl red test.

e) Voges – Proskauer Test

Voges – Proskauer **t**est was carried out using culture grown in MR-VP broth culture as described in 3.3.4.5. *Salmonella* culture is supposed to give negative VP test.

f) Glycerol Fermentation Test

Glycerol- Peptone water broth was prepared adding measured amount of peptone, sodium chloride, glycerol, bromothymol blues in distilled water and dispersed in 3 to 4 ml volume in test tube. A negative reaction can help to exclude the possibility of selecting *Hafnia* and *Citobacter*

g) Lysine Decarboxylase Test

Lysine decarboxilase broth is inoculated with a small amount of growth from a *Salmonella* suspicious KIA agar. Tubes are incubated at 37 ± 1^0 C for 96 ± 2 hours. Tubes are observed at every 24 hours interval. *Salmonella* causes an alkaline reaction, indicated by a purple coloration in the medium. A negative result is indicated by a yellow colour throughout the media.

h) Citrate Utilisation Test

Citrate test was carried out as described in 3.3.4.5. *Salmonella* usually give positive test shown by growth and colour change from green to blue.

Following are the biochemical properties of *Salmonella* isolates

Tests	Results
KIA	Alkaline slant, acid butt; with or without H_2S
Urease	-
Indole	-
MR	+
VP	-
Citrate	+
Glycerol fermentation	-
Lysine Decarboxilase	+

Flow chart of the isolation and identification of *Samonella sp* is shown in Figure 11

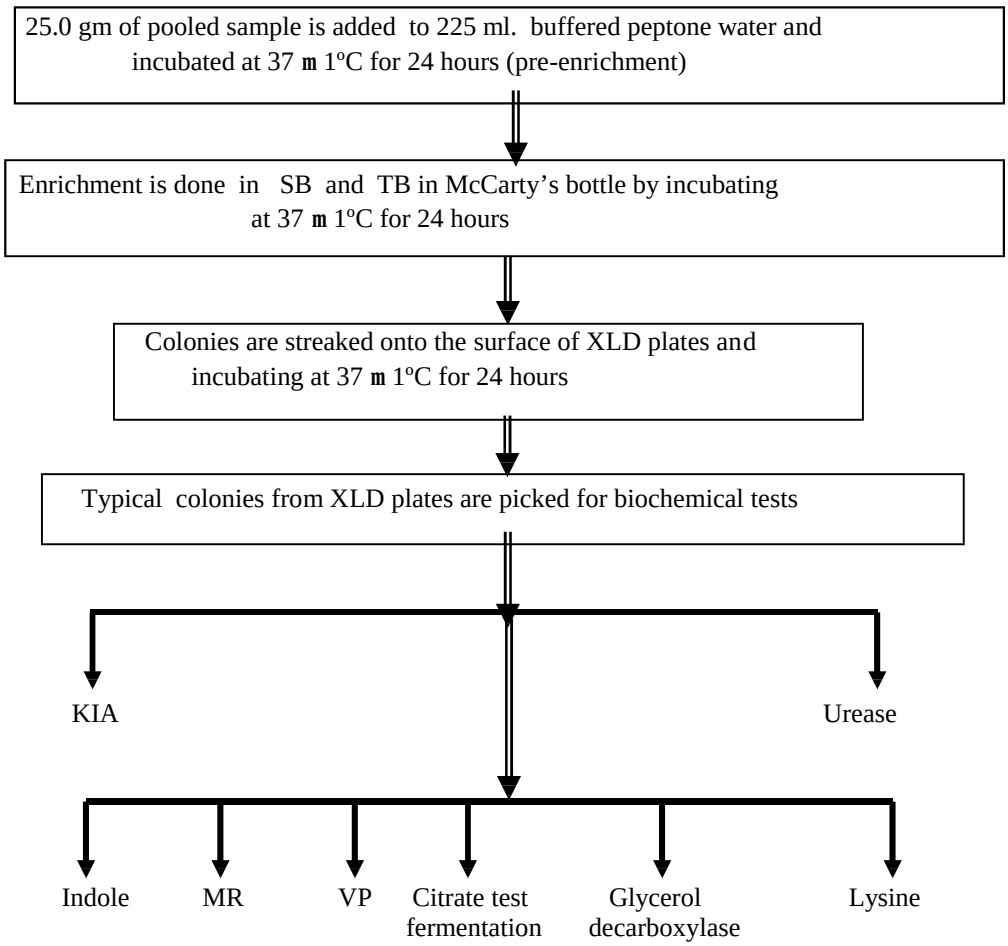


Fig –11: Flow chart for the isolation and identification of *Salmonella sp.*

3.3.5. Biochemical Assessment

3.3.5.1. Proximate Composition

Proximate composition (the percentage of moisture, protein, fat and ash) of collected samples both dried and fresh were analysed according to AOAC methods (1988).

3.3.5.1.1. Moisture

About 5 g. of the macerated fish sample was taken in porcelain basin of known weight. The sample was weighed accurately by using an electric balance and dried in an oven at 105^0c for 24 hours. Drying, cooling (in a desiccator) and weighing were continued for a constant final weight.

3.3.5.1.2. Ash:

About 2.0 gm of sample was taken in porcelain crucible. The crucible was placed on a hater and heated first over a low flame till the entire mineral was completely charred followed by heating in muffle furnace at 550^0 C for 5 hours till the residue became white. It was then cooled in a desiccator and weighed finally.

3.3.5.1.3. Crude Protein:

0.5 gm of sample was weighed and taken into a dry Kjeldhal flask. 5.0 gm of digestion mixture and 15 ml. of concentrated H_2SO_4were added to the sample. The mixture was then digested by heating in digestion chamber until the content becomes clear. The content of the Kjeldhal flasks were cooled and diluted with 70.0 ml. of distilled water. Diluted digested product was transferred in Kjeldhal distillation apparatus and then made it alkaline by adding 30 ml. of 40% NaOH solution. Distillate was collected in 2% boric acid solution with indicator (mixed indicator) and titrated against 0.1 N hydrochloric acid (HCl) solution.

The percentage of protein content in the sample was calculated.

3.3.5.1.4. Fat:

10.0 gm. of dried sample was grounded well and taken into a thimble paper. The thimble together with sample was then placed in Soxlet apparatus and fat was extracted with acetone. Acetone was heated to its boiling temperature and continuous water flow was maintained to condense evaporated acetone. Acetone extract was filtrated into a weighed cleaned beaker. It was then heated on an electric hot plate to evaporate acetone and moisture and weighed.

The percentage of fat content in the sample was calculated.

3.3.5.2. pH

Pooled dried fish and muscle of fresh fish was taken from anterior portion (avoiding the red meat portion). 10 gm sample was grinded and mixed well with 100 ml distilled water and grinding for 30 sec. The homogenate was measured in a pH meter with a glass electrode using an expanded scale.

3.3.5.3. *Determination of Total Volatile Basic Nitrogen (TVB-N) and Trimethyl Amine Nitrogen (TMA-N) through Conway Micro-Diffusion Technique*

Total volatile basic nitrogen and trimethyl amine nitrogen was determined according to Conway micro-diffusion technique (1977). Slight modification was done to meet the requirement of the present investigation.

3.3.5.3. 1. Solution and Reagents:

I. Inner ring solution (1% boric solution containing indicator)
II. Mixed indicator
III. Hydrochloric acid (0.02 N)
IV. Standard potassium carbonate solution (K_2CO_3) solution.
V. Potassium carbonate (K_2CO_3) solution (50%).
VI. Trichloroacetic acid (TCA) solution (4%).
VII. Neutralized formaldehyde solution (10%)

3.3.5.3. 2. Extract Preparation:

The extract was prepared by mixing 2 gm of the minced muscle with 8 ml of 4% TCA in a 10 ml beaker and grind well. It left for 30 minutes at ambient temperature with occasional grinding. After then, it filtered through filter paper (Whatman no. 1). The filtered solution was kept in Mackerty bottle and was labeled. The filtered solution was also stored in a freezer at -20^0C (to prevent any further chemical, bacterial or enzymic break down of the muscle).

3.3.5.3. 3. TVB-N Determination

Three Conway's unit were taken which had been thoroughly cleaned with a neutral detergent to remove any containment. To the edge of the outer rim of each unit was applied the sealing agent (Vaseline). Using a micropipette, 1.0 ml of inner

ring solution was pipetted into the inner ring of each unit. In to the outer ring of each unit, 1.0 ml of the sample extract was pipetted. 1.0 ml of saturated K_2CO_3 solution was carefully pipetted into the outer ring of each unit. Immediately the units were covered and closed with clip. The solutions in the units were then mixed gently, to prevent any solution mixing from one ring to the other. After then, the units were placed in an incubator at 45^0C for 45 minutes. Unit covers were removed and the inner ring solution, now a green colour, was titrated with 0.02N HCl using a burette (50ml) until green coloured solution, turned to pink.
A blank test was also carried out using 1ml of 1% TCA, instead of sample extract.

3.3.5.3. 4. TMA-N Determination
Trimethyl amine in fish muscle was determined by the Conway technique. Prior to addition of potassium carbonate, 1 ml of 10% neutralised formalin was added to the extract to react with ammonia and thus allow only the TMA to diffuse over the unit. The calculation was done by the same formula as used in the Conway micro-diffusion technique for TVB-N.

3.3.5.4. Water Reconstitution Behaviour
Five gm. of flesh was kept soaked in 1000 ml of water at room temperature for 150 minutes and in hot water at 80^0 C for 60 minutes with occasional stirring. Water was then drained off through a close nylon net. All the flesh was then transferred to the strainer, extraneous was wiped off by a piece of blotting paper, and flesh was weighed again. By the given soaking time, flesh could reabsorb maximum amount of water. Result in this respect has been expressed in terms of weight of water absorbed by five gm. of moisture free sample.

3. 4. Data Assembly, Presentation and Analysis

The data from questionnaire were grouped, categorised and interpreted according to the objectives as well as the indicators. Some data contained numeric while other contained narrative facts. For measurable and indicative answer data have been grouped in tabular forms.

All the collected information obtained from the eleven different fishermen villages of the studied area were accumulated and analysed. The results from these villages were then compared with each other. Comparative and overall study results were also prepared. All the collected data were analysed by using computer and presented in tabular and graphical form. Statistical analyses were done with the help of Microsoft Excel.

Cost-benefit ratio of Shutki Mahal and Fresh Fish Arot were calculated by the following formula:

Total sells = Production (out put)* Unit Selling Cost
Gross benefit = Sales – Cost of goods sold
Total cost = Fixed Cost + Cost of goods sold + Commercial expanses
Cost-benefit ratio = Total benefit / Total cost
Net benefit = Total benefit - Total cost

All the analyses such as mean, standard deviation, correlation coefficients, among different variables were calculated and graphically presented by using the Microsoft Excel, Version 97, Program in PC.