CHAPTER 2 - GENERAL MATERIALS AND METHODS

2.1. Collection of fish sample from natural habitat:

A total of 700 live fish specimens were randomly collected from different parts of West Bengal and Assam (**Table 1 and figure 2**) during May, 2015 to April, 2017. Fishes were collected by local fisherman with the help of small bamboo trap and cast net from rivers and hill streams (**Figure 3**). After collecting the fishes were packed in oxygenated packet with methylene blue and the packets were arranged in thermocol box for further transportation. Then the fishes were safely transferred to CIFE, Kolkata centre.

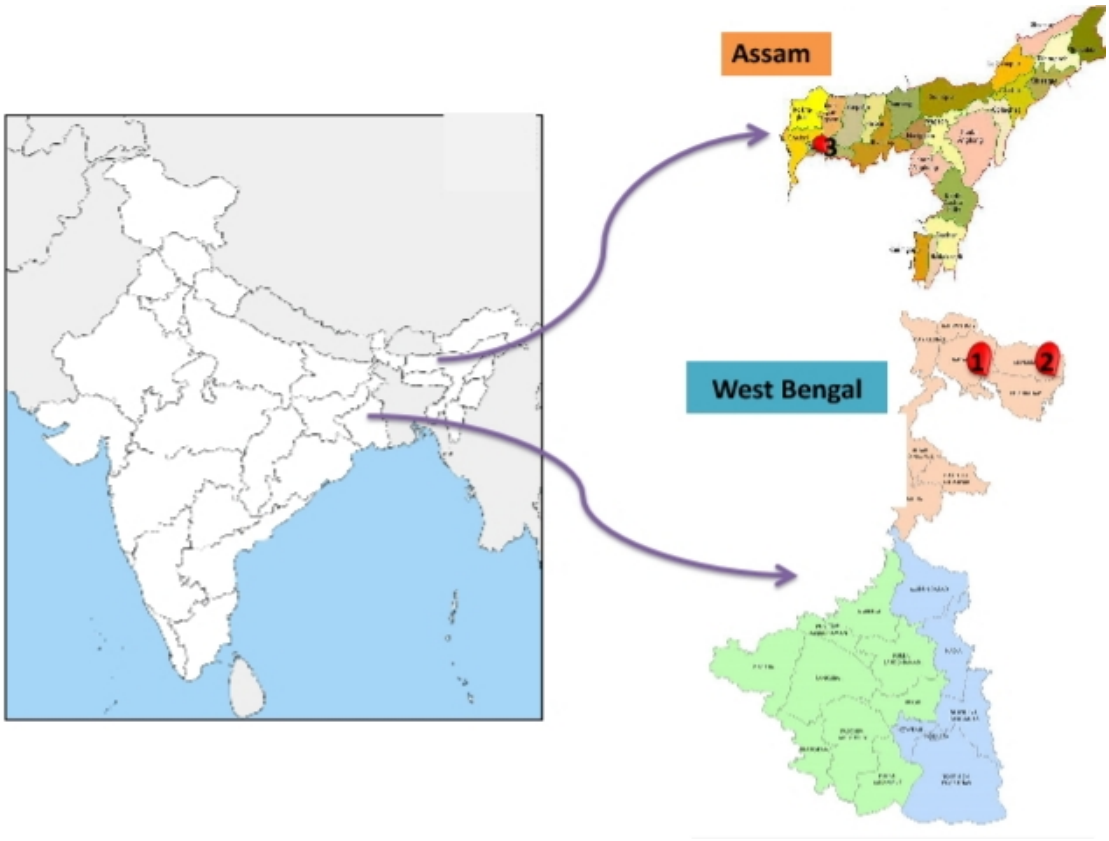


Figure 2. Sampling site of *C. nobilis*

(See colour photo in Plate No. II, Fig. 2)

Table 1. Details of collection sites of *C. nobilis*

Site	State	District	Area	River	Location	
1	West Bengal	Jalpaiguri	Daspara	Teesta	26.2842° N	88.4427° E
2	West Bengal	Alipurduar	Barobisha	Raidak	26.4679° N	89.8048° E
3	Assam	Goalpara	Pahartoli	Brahmaputra	26.1845° N	90.6340° E

Figure 3. Fish catching with bamboo trap and cast net

(See colour photo in Plate No. II, Fig. 3)

2.2. Acclimatisation of procured fish:

After transporting, the fishes were disinfected by giving bath treatment with 5 PPM $KMnO_4$ for 1 minute. After discarding dead and weak fishes the healthy fishes were transferred to fibre tanks of 1000 L capacity with continuous aeration. After one day the fishes were fed on Tubifex @ 2% of total body mass twice in a day at 10:00AM and

5.30PM. Fishes were acclimated for 15 days in laboratory conditions before starting of the further experiments.

2.3. Sites of experiments:

Laboratory works were carried out in CIFE, Kolkata centre. Biological study, feeding and ecological study were implemented at Ornamental Research Wet laboratory of CIFE, Kolkata centre. Breeding and larval rearing experiments were done at Ramkumar Fishery Farm, Domjur, Howrah. (**Figure 4**)

a) Ramkumar Fishery Farm **b) Wet Laboratory at CIFE, Kolkata**

Figure 4 (a-b). Experimental sites of the research work

(See colour photo in Plate No. II, Fig. 4)

2.4. Regular monitoring and cleaning of the experimental aquariums:

The unconsumed feed was removed by pipette after 2 hours of feed given. The filter medium was cleaned before feeding once in 3 days. Only 25-30% water was exchanged thrice a week to remove the accumulated faces. The fish were kept under natural photopyriod throughout the experiments. Bottom substrate and aquarium plant

wash once in a week and treated with 5 PPM $KMnO_4$ solution. Water temperature recorded daily twice at 9:30AM and 2:30PM. The other water quality parameters such as pH, DO and Alkalinity were tasted once in a week. A one minute bath treatment with 5 PPM $KMnO_4$ was done biweekly. Methylene blue was used once in 2 weeks as antifungal treatment.

2.5. Analysis of Hydrobiological parameters:

DO, Alkalinity, Total Hardness and Free CO_2 were studied following standard procedures (APHA, 2005) (**Figure 5**). Nitrates and Ammonia were tasted by using test kits developed by Seachem Laboratories, USA.

i. Water temperature: The water temperature was recorded with the help of a mercury centigrade thermometer with 0.5^oC accuracy.

ii. pH: pH was analyzed by using of digital pH meter.

iii. Dissolved Oxygen: Modified Winkler's method was used to detect the dissolved oxygen level of water. The value was expressed in PPM.

iv. Free CO_2: Phenolphthalein indicator with N/80 Sodium Hydroxide method was used to detect the free Carbon di-oxide level of water. The value was expressed in PPM.

v. Alkalinity: Alkalinity of the water was estimated by titration with N/50 Sulphuric acid using Methyl orange and Phenolphthalein indicator. The value was expressed in PPM.

vi. Total Hardness: Total hardness i.e. total multivalent cations' concentrations were measured by 0.01M EDTA using EBT indicator. The value was expressed in PPM.

a) pH meter

b) Mercury thermometre

c) Water testing

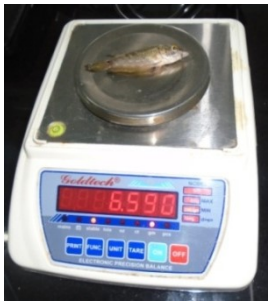
Figure 5 (a-c). Testing the hydrobiological parameters in laboratory

(See colour photo in Plate No. III, Fig. 5)

2.6. Collection of length and weight data of fish:

The length and weight of the fish were taken with the help of digital Vernier calliper and digital weighing balance respectively (**Figure 6**). The total length was recorded to the nearest mm from the tip of the snout to the tip of the caudal fin and standard length was also recorded to the nearest millimetre from the tip of the snout to the base of the caudal fin. The weights were taken to the nearest g.

a) Length of fish



b) Weight of fish

Figure 6 (a-b). Length and weight data collection of the fish

(See colour photo in Plate No. III, Fig. 6)

2.7. Live fish food culture:

Live fish food is a valuable protein resource and most important component for fish growth and maturation. For constant supply of live food culture practice can play a vital role. Live fish food like Tubifex, Mosquito larvae and Daphnia are cultured in CIFE, Kolkata (**Figure 7**). Artemia nauplii also hatched from dry cyst in laboratory.

i. Tubifex culture: The culture method followed as per Marian and Pandian, 1984. In cemented channel with continuous running water flow Tubifex was cultured. 75% raw cow dung and 25% soft sand were used as substratum. The tubifex worms were incorporated @ 10g/cm^2 after 2 days of substrate preparation. Fresh raw cow dung was added @ 25g/m^2 once in 4 days. Harvesting was done in every 15 days @ 50mg/cm^2.

ii. Mosquito larvae culture: For mosquito larvae culture milk solution was added with rain water in a fibre tank of approx 500Lt. capacity and leave undisturbed. Medium mash sized net used as cover to avoid the accumulation of unwanted leaf and dirt. After 2-3 days mosquito larvae were harvested @ 20-30 Nos./tank.

iii. Daphnia culture: Daphnia was culture by the process mentioned by Mahaptara, 2017. At first 4 cm deep soft soil base was added in 500Lt. cemented tank. Then $Cacl_2$ and raw cow dung were added @ $4g/m^2$ and $1kg/m^2$ respectively. Filtered water added after 2 days and after 1 week Daphnia inoculated @ 10 Nos./Lt. Phytoplankton was added @ 1mg/Lt. as food for Daphnia at every alternative day. After 1 month daphnia was harvested by plankton net.

iv. Artemia nauplii hatching: Filtered NaCl solution (@ 12-15g/Lt) was added in a cylindrical jar for hatching of Artemia cyst. In room temperature (26-30^oC) pH maintained at 7.5-8.2 and continuous vigorous aeration was given. Artemia cysts were added @ 0.5-0.75g/Lt. and electric bulb light was added for faster the hatching process. After approx 26 hours hatched Nauplii were harvested.

a) Tubifex **b) Mosquito larvae** **c) Daphnia** **d) Artemia**

Figure 7 (a-d). Live fish food culture

(See colour photo in Plate No. III, Fig. 7)

2.8. Proximate analysis of fish food organisms:

The proximate biochemical composition of live fish feed organisms i.e. moisture, ash, crude protein and crude lipid were evaluated by following the AOAC, 1995 method (**Figure 8**).

i. Moisture (%):

The moisture content of live food organisms were determined by taking known weight of sample in a petridish and drying it in a hot air oven at 80-90^{o}C temperature for approx 6 hours and then again weighed the dried sample. The difference between the sample weights gave the moisture content.

$$\textbf{Moisture}\ (\%) = \frac{\textbf{Weight of wet sample} - \textbf{Weight of dried sample}}{\textbf{Weight of wet sample}} \times \textbf{100}$$

ii. Total Ash (%):

Ash content of the dried samples were estimated by taking a known weight of dried samples in a silica crucible and placing it in a muffle furnace at 550 ^{0}C for 2 hours after reaching the temperature.

$$\textbf{Total Ash}\ (\%) = \frac{\textbf{Weight of crucible + ash} - \textbf{Weight of empty crucible}}{\textbf{Weight of the sample}} \times \textbf{100}$$

iii. Crude Protein (%):

Crude protein content of the dried samples were estimated by semi-automatic nitrogen digestion and distillation unit (Pellican, Kelplus-KES06L, India) followed by titration. The nitrogen content was calculated by the following formula.

$$\textbf{Nitrogen}\ (\%) = \frac{\textbf{V x 0.0014 x D}}{\textbf{A x W}} \times \textbf{100}$$

Where, V = Volume of N/10 H_2SO_4 used for titration; 0.0014 is the amount of nitrogen content in 1 ml of N/10 H_2SO_4; D = Dilution Factor (total volume made); A = Aliquot (diluted sample) taken for distillation; W = Weight of the sample taken for digestion.

The crude protein percentage was = $\textbf{Crude protein}\ (\%) = \textbf{N2}\ (\%)\ \textbf{x 6.25}$

iv. Crude Lipid (%):

Lipid percentages of the dried samples were estimated by lipid extraction unit (Socsplus-SCS06R, Pellican, India) using petroleum ether (Boiling point 40-60 ^{0}C) as the solvent.

$$\textbf{Crude lipid}\ (\%) = \frac{(\textbf{Weight of beaker} + \textbf{lipid}) - \textbf{Weight of empty beaker}}{\textbf{Weight of the sample}} \times \textbf{100}$$

a) Hot air oven b) Muffle furnace c) lipid extraction d) Nitrogen digestion

Figure 8 (a-d). Proximate analysis of live fish food organisms

(See colour photo in Plate No. III, Fig. 8)

2.9. Analyses of some biological parameters of the fish:

i. Condition factor: $K = \frac{W}{aL^3}$ is used for the calculation of condition coefficient as stated by Le Cren (1951).

ii. Weight gain:

$$\text{Weight gain (g)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}$$

iii. Length gain:

$$\text{Length gain (mm)} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}}$$

iv. Survival rate:

$$\text{Survival}(\%) = \frac{\text{Total number of harvested fish}}{\text{Total number of stocked fish}} \times 100$$

v. Food Conversion Ratio:

$$\text{FCR} = \frac{\text{Total amount of feed given (g)}}{\text{Total weight gain (g)}}$$

vi. Specific Growth Rate (%):

$$\text{SGR \%} = \frac{\text{Log}_n \text{ Final weight} - \text{Log}_n \text{ Initial weight}}{\text{Number of days}} \text{ x } 100$$

2.10. Methods used for statistical analyses:

The collected data were subjected to different statistical analysis; like:

i. **Standard deviation:** $SD = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})}{n-1}}$

Where, 'n' denoted the number of data, X_i denoted each values of the data and $\bar{X}$ denoted the mean value of X_i. The formula was given by Galton in the year 1860 (Bulmer, 2003)

ii. **Standard error:** $SE = \frac{SD}{\sqrt{n-1}}$

Where, SD is standard deviation and n is the number of data.

iii. **Student's 't' test:** $t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{(SE_1)^2 + (SE_2)^2}}$

Where, $\overline{X_1}$ and $\overline{X_2}$ represents the mean compared and SE_1 and SE_2 are their respective standard errors. The formula was given by William Sealy Gosset in the year 1908 (Richard, 2004)

iv. **Correlation coefficient:** $r = \frac{n(\sum XY) - (\sum X)(\sum Y)}{\sqrt{[n\sum X^2 - (\sum X)^2]\,[n\sum Y^2 - (\sum Y)^2]}}$

Where, n denotes the number of total observation, x denotes independent variable in x-axis and y denotes dependent variable in y-axis. The formula was given by Pearson in the year 1895 (Pearson, 1895).

v. **Linear regression:** Y = a + bX

Where, X is the independent variable, Y is the dependent variable, a is the Y-intercept and b is line slope. At first Galton used the word regression (Galton, 1885) but the modern form of the equation was given by Fisher in the year 1922.

$$a = \frac{(\sum y)(\sum x^2) - (\sum x)(\sum xy)}{n\sum x^2 - (\sum x)^2}$$

$$b = \frac{n\sum xy - (\sum x)(\sum y)}{n\sum X^2 - (\sum X)^2}$$

vi. **Analysis of variance (ANOVA):** $F = \frac{MST}{MSE}$

Where F is ANOVA coefficient, MST is mean squares due to treatment and MSE is mean squares due to error.

$$MST = \frac{\sum n(X - \bar{X})^2}{p - 1}$$

$$MSE = \frac{\sum (n-1)S^2}{N - p}$$

Where, n is the number of samples in population, p is the number of population, X is mean of treatment, $\bar{X}$ is mean of all observations, S is standard deviation of samples and N is total number of observations. The procedure described by Zar in the year 1974.

All statistical analyses were done with the help of Microsoft Excel, 2010 and SPSS version 16.