The present scientific work is an endeavour to fulfill the gaps in fishing sector through field survey, laboratory work and scientific assessment to achieve the goal of successful sustainable development.

PHYSICOCHEMICAL PARAMETERS:

The pond water sample from pelagic level was collected in triplicates in plastic containers between 10 A.M. to 12 Noon in the middle of each month during the study period of January 2011 to July 2016 and was analyzed partly in the field and partly in the laboratory as per the methods suggested by Welch (1982), Jhingron (1991) and APHA (1992).

Water samples were collected in triplicate from the marginal water layer (below 10 cm to surface) of the sample pond in plastic sampler and sterilized BOD bottle of 10 litre capacity. A number of physicochemical parameters including water colour, pH, free CO_2, dissolved oxygen, total alkalinity, salinity, nitrate, phosphate were performed in the field within 1 hour of sampling to minimize the error of delaying. Some samples were carried to the laboratory in black painted bottles after mixing with 2mg $l^{-1}$of chloroform as preservative for further chemical analysis.

Water colour

According to Kudesia (1980) the water colour was determined by visual comparison of sample with known concentration of colour solution which helps in determining the water colour (by using Platinum- Cobalt- Comparator).

Water Temperature

Temperature of the water body was determined with the help of Centigrade Thermometer, graduated in 0.1°C scales. Thermometer was dipped directly into the sample. Mercury level was read in the sample. Temperature of deep water system was read by means of a reversible thermometer (Gille, 2002).

pH (Hydrogen ion concentration)

A portable digital pH meter of ELICO make in laboratory (PORTABLE pH METER MODEL L 1-120) was used to measure the pH of water Michael (1984).

Free carbon dioxide

Using phenolphthalein as indicator the free carbon dioxide was measured titrimetrically against Sodium hydroxide solution. 100 ml of water sample was taken in a conical flask and 2 drops of phenolphthalein indicator was added to it. The change of colour was recorded (i.e. no change from normal colour). After that, immediately the solution was titrated instantly with Sodium hydroxide (N/44 NaOH) solution drop wise along with stirring with glass rod and at the end point a permanent pink colour was developed (Welch, 1962).

Calculation

$$\text{Free } CO_2 \text{ (mg l}^{-1}) = \frac{ml\ of\ NaOH \times normality\ of\ NaOH \times 44 \times 1000}{ml\ of\ sample\ taken}$$

Dissolved Oxygen (DO)

The dissolved oxygen was calculated by means of Winkler's volumetric method. Collection of 200 ml of sample was followed by immediate and careful addition of 1 ml of manganous sulphate ($MnSO_4$) solution by dipping the pippete to below the bottom and immediately followed by 1 ml of alkali potassium iodide (KI) in the same manner. Stopper was positioned tightly and thorough mixing of the content was done by repeated upside down shaking method. The bottle was then kept for about 10 minutes to settle down the precipitate if developed. Then by using 2 ml of A.R. grade concentrated sulphuric acid (H_2SO_4), the resultant brown coloured precipitate was dissolved.

Then titration of treated sample (100 ml of aliquote) was done carefully against the standard solution of Sodium thiosulphate solution (N/80 $Na_2S_2O_3$) by its drop wise addition till the attainment of faint yellow colour (straw colour). As an indicator, ml of freshly prepared starch was then added to it. Immediately a deep blue or less blue colour was appeared. Again titration of the content was carried out against Sodium thiosulphate solution (Titrant) till the initial blue colour changed to

colourless. Finally the ml of titrant used in getting the end point was noted (Modified volumetric Winkler's Method, Strickland and Parsons, 1972, Jhingran, 1991).

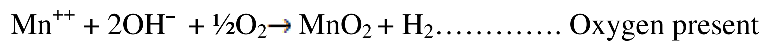
Chemical reactions involved:

$MnSO_4 = Mn^{++} + SO_4^{--}$

Alk KI = $K^+ + I^- + OH^-$

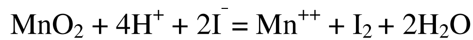
$Mn^{++} + 2OH^- \rightarrow Mn(OH)_2$……………………Oxygen absent

White ppt.

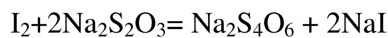


$Mn^{++} + 2OH^- + \frac{1}{2}O_2 \rightarrow MnO_2 + H_2$………… Oxygen present

Brown ppt.



$MnO_2 + 4H^+ + 2I^- = Mn^{++} + I_2 + 2H_2O$



$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$

Calculation:

1 ml of 0.0125 N $Na_2S_2O_3$solution = 0.1 ml of O_2

Dissolved Oxygen (mgl^{-1}) =

$$\frac{\text{ml of sample titrate} \times \text{Normality of Na2S2O3} \times 8 \times 1000}{\text{Volume of water in the conical flask}}$$

Precaution:

During sampling, at least double the volume of the sample bottle was allowed to overflow, with due care for avoiding air bubbles.

Soon after collection the sample was fixed and analysed within two hours of fixation in order to avoid the analytical errors.

Total Alkalinity

On titration against sulphuric acid solution using phenolphthalein and methyl orange indicator, the total alkalinity was found out. In each of the two conical flasks, 100 ml of the sample was taken and phenolphthalein indicator was added to one of

them. The solution became pink coloured. Again the solution was titrated with sulphuric acid (0.02 N H_2SO_4) upto disappearance of pink colour and amount of acid (in ml) used was noted. In this way the phenolphthalein alkalinity (PA) was obtained.

Then 0.5 ml of phenolphthalein indicator was added to the second conical flask. The PA value was taken as 0 (zero), in case the solution remained colourless. Thereafter, 0.5 ml of methyl orange indicator was mixed with the sample and titrated with 0.02 N H_2SO_4 until the disappearance of the pink colour. Again the amount of acid (in ml) used in titration was noted down. This value was the Total alkalinity (TA) (Welch, 1962 and Michael, 1984).

Calculation:

Let, A = ml of acid used for titration with phenolphthalein

B = ml of acid used for total titration (phenolphthalein and methyl orange)

Then:

In case A = B

Total alkalinity hydroxide mg $l^{-1} = \frac{A \times 1000}{ml\ of\ sample}$

In case A > ½ B

Total alkalinity hydroxide mg $l^{-1} = \frac{B \times 1000}{ml\ of\ sample\ hydroxide\ alkalinity}$

And

Hydroxide alkalinity mg $l^{-1} = \frac{(2A - B) \times 1000}{ml\ of\ sample}$

In case A = ½ B

Total alkalinity mg $l^{-1} = \frac{B \times 1000}{ml\ of\ sample}$

And

Carbonate alkalinity mg $l^{-1} = \frac{B \times 1000}{ml\ of\ sample}$

In case A <½ B

Total alkalinity mg l^{-1} = $\frac{B\times1000}{ml\ of\ sample}$

And

Carbonate alkalinity mg l^{-1} = $\frac{2A\times1000}{ml\ of\ sample}$

In case A =0

Total alkalinity mg l^{-1} = $\frac{B\times1000}{ml\ of\ sample}$

And

Bicarbonate alkalinity mg l^{-1} = $\frac{B\times1000}{ml\ of\ sample}$

Total hardness

In a conical flask 100 ml of water sample was taken. It was followed by addition of 1 ml of ammonia buffer, then 1 ml of inhibitor (Hydroxylamine hydrochloride) and at last addition of 2 drops of Eriochrome black T indicator to the solution. After a thorough shaking the solution was titrated with standard EDTA (0.021 M) until the wine red colour transformed to blue. The end point was recorded (Kudesia, 1980; NEERI, 1988; APHA, 2005).

Calculation:

Total hardness as $CaCO_3$ mg l^{-1} =

$$\frac{ml.\ of\ EDTA\ used\ by\ sample}{ml.\ of\ sample} \text{ x } 1000$$

Ammonia-nitrogen:

In order to measure ammonia-nitrogen a modified phenate method (Wetzel, 1983) was performed. Sample water was treated with a buffer solution of tri-sodium phosphate and the reagent was mixed to the solution. Final mixture was measured spectrometrtically (Shimadzu UV spectro-photometrically, Model UV 1601) at 665nm.

Calcium

In a conical flask 50 ml of sample, was taken and 2 ml of sodium hydroxide buffer solution was added to it. After a thorough shaking the solution was titrated immediately with the standard solution of EDTA with continuous stirring until blue

colour became visible. The volume of EDTA used was recorded. Reagent blank may be titrated first in the identical condition as far the sample. In a similar way as described for sample, EDTA titrant was standardized with 25 ml standard solution (NEERI, 1988)

Calculation:

Calcium (mgl^{-1}) = $\frac{(V^1-V^2)\times 100\times 1000\times 0.01}{ml.of sample}$

Where, V_1 = the volume of EDTA required for sample

V_2 = the volume of EDTA required for reagent blank

Manganese, Chloride, sulphate and phosphate are calculated by the following method of APHA (1985) by using spectrophotometric methods. Then mean annual physicochemical parameters are recorded for analysis.

PRIMARY PRODUCTIVITY:

The estimation of primary productivity is predicted for, was found to be dependent on the relationship between oxygen evolution and carbon fixation. As per the recommendation of Vollenweider (1969) and Jhingron and Pathak (1988), "Light and Dark bottle" with the Winklers – titration method of Gaarder and Gran (1927) was the most suitable as well as sensitive method for primary productivity analysis to provide meaningful differences in a few hours.

In the middle of each month, water samples between 10.00 am to 12 noon were collected in triplicate. The initial level of dissolved oxygen content was immediately determined by using the samples in first bottle on following modified Winkler's volumetric method (APHA, 1998). Black colour (dark bottle) was painted on the second bottle to prevent light and hence serve as control to measure respiration. The third bottle (light) was taken as test for measuring the net primary production. The rest two bottles were incubated under water by suspending them in euphotic zone for a period of three hours. Dissolved oxygen content (DO_2) of each bottle was estimated, after the incubation period. The factor 0.375 m was multiplied with all O_2 values obtained in the present study to convert them to Carbon values (Odum, 1956).Daily rates can be obtained from hourly rate by multiplying it with duration of

sunshine on that day. Convertion of Oxygen values (mgl^{-1}) to Carbon value was carried out by applying the equation suggested by Thomas et al. (1980).

$$\text{Primary production (g C)} = \frac{O2\ \boldsymbol{mgl^{-1}} \times \mathbf{0.375}}{\boldsymbol{PQ}}$$

Where PQ =1.25

PQ stands for respiratory quotient = Respiration / photosynthesis and a comprised value of 1.25 was used which characterizes metabolism of sugar, fat, and proteins. The values 0.375 represent a constant to convert Oxygen value to Carbon value (Thomas et. al., 1980).

PLANKTON ANALYSIS:

During the period from January 2013 to December 2014, the planktonic samples were collected on monthly basis by plankton net of standard bolting silk (Cloth no.-25, mesh size 0.03-0.04 mm) from 100 litre water sample on use of plastic bucket of 10 litre capacity. Finally the planktons were collected in net tube and preserved in 4% formaldehyde solution. Thereafter the samples were taken to the laboratory for quantitative and qualitative analysis in a Sedgewick rafter type counting cell (1 ml capacity) and then the planktons will be identified as per Allen (1930), Fritsch (1965), Filden (1968) and Willen (1976). After shaking vial containing the concentrated plankton sample a sub-sample of 1ml was quickly be drawn with the help of pipette and poured in the plankton counting cell. All organisms encountered were represented in absolute number. Three countings were made for each sample and the data represented in the text was the average value of counting. Counting was done by placing the counting cell under the microscope with a mechanical stage. Starting with one corner in each square in the row, the organisms were counted. Organisms were counted in each square in the row by moving the slide horizontally. After counting of one row, the mechanical device of the stage was used to bring up the next consecutive row. In this way, all the organisms in all the squares were counted. Then total no of planktons in a litre of water sample was calculated using the formula:

$$n = \frac{(a\ 1000)c}{l}$$

Where:

$n : is\ the\ number\ of plankton\ per\ litre\ of\ water$

$a : is\ the\ number\ of\ plankton\ in\ one\ ml\ of\ the\ sample$

$c : is\ the\ ml\ of plankton\ concentration$

$l : is\ the\ volume\ of\ original\ water\ samples\ in\ litres$

Community structure analysis

An assemblage of population in a prescribed area of physical habitat is called a biotic community (Odum, 1971). A biotic community may be defined in terms of species diversity from statistical viewpoint. Species diversity consists of a number of components which may respond differently in different environmental conditions. The major components are:

Species richness, which explains the variety and Eveness, gives details of equitability.

The simple ratio between total species and total number of individual provides species richness, while equitability is the allotment of individuals amongst species (Lioyd and Ghelardi, 1964).

The diversity and diversity indices are determined by various methods suggested by various workers. The index given by Shannon and Weinner, (1963) is used worldwide. The probability that the next individual will be the same species as the previous sample is measured by the S-W Index.

To study the community structure of the phytoplankton different indices were calculated.

Shannon and Weiner diversity index (Hs):

The Shannon and Weinner diversity index (Hs) is given by the equation (Shannon and Weinner, 1963)

$Hs = \sum PiInPi$

Where

H = Diversity index

i = Counts denoting the ith ranging from 1 – n.

P = Proportion that the ith species represents in terms of numbers of individuals with respect to the total number of individuals in the sampling space as a whole.

Pielou species Equality Index (j):

Pielou species Equality Index was represented by the equation (Pielou, 1969):

$$j = Hs/Log2s$$

Where

j = Equitability index

Hs = Shannon and Weiner diversity index

S = Number of species in a population

Margalef Species Richness Index (d):

The Margalef's Species richness (d), used to evaluate the community structure, is represented by the equation (Margalef, 1951) :

$$d = \frac{S-1}{LogeN}$$

Where

d = Specifies richness index

S =Number of species in a population

N = Total number of individuals in sample species

The value of 0.375 represents a constant to convert the oxygen value to carbon value.

PROCUREMENT OF FISH, DISSECTION AND ANALYSIS:

For the present study a total of 540 freshly caught matured female specimens within the weight ranging from 150-750 g were studied after being collected from a local fish pond located near Bhubanswar city and they were classified into three age groups ie. 180 young with weight ranging from 150g to 350g(90 male and 90 female),180 matured with weight ranging from 350g to 550g (90 male and 90

female)and 180 matured but adults with weight ranging from 550g to 750g (90 male and 90 female). After morphometric measurements (weight=200-750g, total length=31-42 cm, standard length=23.5-32 cm) dissection was carried out under 100 watt illuminations, the internal organs (stomach, liver, ovary and testis) were exposed and carefully detached from the main body. Then their specific weight were determined by Afcoset Electronic balance (Reheman et al. 2002)

A total of about 180 major carps from each species (with almost equal weight) both male and female, young and adult were studied to record the gastrosomatic index, hepatosomatic index, gonadosomatic index and condition factor. The fish were procured in dead but fresh condition from local culture pond located in Bhubaneswar city. While procuring, the vernacular (Oriya) names were noted down by discussing with experienced fish sellers, their weights were recorded in electronic weighing machine (Model: Excon Instruments, Hyderabad) and brought to the laboratory.

As per the description of Talwar and Jhingron (1991) and Ghosh (2006) the fish were correctly identified up to species level after brought to the laboratory. Before commencing dissection, the total length and standard length were recorded properly and then under 100 watt illumination, the internal organs (i.e. stomach, liver, ovary and testis) were exposed carefully detected from the main body and their specific weights were determined by the laboratory electronic balance and observations are to be made from various points of research. The measurements and calculations thus obtained were recorded under following abbreviations:

BW - Body weight in g

TL -Total length in cm

SL - Standard length in cm

LHF1-Length from head end to base of dorsal fin in cm

LAF1 - Length of area of dorsal in cm

LF1CP - Length from the end of dorsal fin to caudal peduncle in cm

LF1 - Length of dorsal fin in cm

LF2 - Length of pectoral fin in cm

LF3 - Length of pelvic fin in cm

LF4 - Length of anal fin in cm

LF5 - Length of caudal fin in cm

DF2F3 - Distance between pectoral fin and pelvic fin in cm

DF3F4 - Distance between pelvic and anal fin in cm

DF4F5 - Distance between pelvic and caudal fin in cm

DEF2 - Distance between eye and pectoral fin in cm

WF1MF2 - Width from dorsal to mid of pectoral fin in cm

WF1F3 - Width from dorsal to pelvic fin in cm

WF1F4 -Width from dorsal to anal fin in cm

HW – Weight of heart in g

STL - Length of stomach in cm

STW - Weight of stomach in g

LW - Weight of liver in g

GW - Weight of gonads (pair) in g

KW - Weigh of kidney in g

ABL1 - Length of airbladder in cm

ABL2 - Length of airbladder in cm

ABLW - Weight of airbladder in g

K - Condition factor

GaSI – Gastrosomatic index

HSI – Hepatosomatic index

GSI - Gonadosomatic index

Morphometric features:

The morphometric features like total length, standard length and biological parameters were measured and determined following the standardized protocols.

Total length: It is the maximum elongation of the body from end to end. Thus, from the most anterior projecting part of the head to the posterior most tip of the caudal fin was included in total length (Biswas, 1985).

Standard length: It is the distance from the anterior most part of the head to the end of the vertebral column.

Condition factor: It was represented by relating the standard length of the fish to its weight (Beckman, 1948; Evans, 2000)).It was calculated by the formula:

$$K = 100\left(\frac{W}{L^3}\right)$$

where,

'K' is the coefficient of condition

'W' is the weight of fish (in gram)

'L' is the standard length of the fish (in cm)

Anatomical peculiarities:

a) Gastrosomatic index: It is defined as the weight of gut(stomach + intestine) as percentage of the total body weight of fish (Desai, 1970).It was expressed as :

$$\text{wt of gut and its contains in gram} \times \frac{100}{wt\ of\ fish\ in\ gram}$$

b) Hepatosomatic index : It is defined as the ratio of liver wt to the body weight. It was expressed as :

$$\text{wt of liver in gram} \times \frac{100}{wt\ of\ the\ fish\ in\ gram}$$

c) Gonadosomatic index: The development of gonads was estimated by determining its weight relative to the body weight of the fish (Hopkins, 1979). This was expressed as :

$$\text{wt of the gonad (Testis and ovary) in grams x } \frac{100}{wt\ of\ fish\ in\ gram}$$

BIOCHEMICAL ANALYSIS:

As the source of protein is limited to keep pace with the rapid population growth, it is crucial that all of its available sources should be utilized by Indians. Fisheries stand for an alternative source of protein. Fish acts as a key component of a healthy diet.

Fish meal serve as a source of energy for human beings as it is full of most important nutritional components (Ojewola and Annah, 2006; Sutharshiny and Sivashanthini, 2011). Fish is essential young as well as old age people also need as a vitamin and mineral rich food (Edem, 2009; Moghaddam *et al.*, 2007).

Application of proper knowledge on the biochemical composition of fish is found in several areas. Today fish is finding more acceptances because of its special nutritional qualities as per ever-increasing awareness about healthy food. In this circumstance, it has become a primary requirement for the nutritionists and dieticians to have a proper understanding about the biochemical constituents of fish. Fish and fishery products are used in animal feeds. So, formulation of such products requires proper data of the biochemical composition (Html document,Vikapaedia, 2018).

Normally, the biochemical composition of the whole body in states the fish quality.For this reason,nutritional and edible value in terms of energy units of a species compared to other species are assessed by proximatebiochemical composition.Variation of also occur within same species. Depending upon the fishing ground, fishing season, age and sex of the individual and reproductive status biochemical composition of fish flesh may also vary within same species (Pradhan et al., 2012). The main factors responsible for this variation are the spawning cycle and food supply (Love et al.1968)

There is a great variation in chemical constituents of fish from species to species which undergo changes due to physiological factors such as feeding,

maturation, etc. Many species of fish experience the transfer of fat and protein to the gonads prior to spawning (Y.T. TAN, 1971). According to Sivakami et al. 1986, although several studies regarding the proximate composition of biochemical components of many commercially important marine fish were performed but comparatively insignificant work had been carried out on the basis of different weight group, age, sex and season of freshwater fish.

Investigation of the Indian major carps specimen had been done for the analysis of various biochemical constituents i.e. moisture, ash, protein, lipid and fatty acids of muscle and liver on the basis of wet weight to analyze the impact of season and sex.

OBJECTIVES

- Analysis of the biochemical profile of muscle and liver of certain Indian major carp.
- This study has emphasized on the correlation among biochemical parameters.
- Biochemical parameters were compared on the basis of growth, season, and sex.
- Body size category of 150-750 g
- Observed data analysis by using advance statistical package.

MATERIALS QND METHODS:

Fish were collected from local fish pond located in Bhubanswar city during the study period of January 2011 to July 2016. The specimens were collected, properly cleaned in the laboratory and the total length in centimeter, total weight in gram and sex were determined. Based on their total length and weight fish were classified into three age groups i.e. young (150-350 g), matured (350-500 g) and matured but adults (500-750 g). Body muscle and liver samples of each group were used for the analysis of biochemical components in every season.

Moisture content

Moisture was determined by drying samples in an oven (Egan et al. 1997). On a pre-weighed petridis known weight of the sample was taken. Then the petridis

containing sample was placed overnight in oven at 110ºC. After removing the sample from oven it was allowed to cool in desiccators.

Moisture (per cent) = $\frac{Wet\ weight\ of\ muscle - Dry\ weight\ of\ muscle}{Wet\ weight\ of\ muscle}$ x 100

The results were expressed as per cent.

Ash

Ash was determined by incineration of sample for 10 hours in a muffle furnace at 550ºC (Egan et al., 1997).

Ash is the total mineral content of the specimen sample. 5 g of the sample was taken in a dry pre-weighed crucible. The weighed crucible and sample was placed over a tripod stand and heated it over the flame to make it smoke free. When smoke subsides, the crucible with its content was transferred to the muffle furnace with the help of a pair of tongs. Crucible with sample kept at 550ºC for 6 hours at this temperature. The crucible was removed from furnace and weight was taken.

Percent of Ash = $\frac{W_3 - W_1}{W_2 - W_1}$ x 100

W_1= weight in gram of empty crucible

W_2 =weight in gram of crucible + sample

W_3 =weight in gram of crucible + ash

The results were expressed as percent on the wet weight.

Protein

Protein was estimated by the method of Lowry et al. (1951).

1 ml of 1N NaOH was added to a 10 mg of sample for protein extraction in water bath for 30 minutes. Then it was cooled at room temperature and neutralized with 1 ml of 1N HCl. After centrifugation at 2000 rpm for 10 minutes the extracted sample an aliquot of the sample (1ml) was further diluted with distilled water (1/9 V/V). Thereafter, 1ml of diluted sample was taken and treated with 2.5 ml of mixed reagent (Carbonate- tartarate copper) and 0.5 ml of 1N Folin's reagent. After 30

minutes, sample absorbency was red at 750 nm as measured using spectrophotometer. The results were expressed as percent on the wet weight.

Lipid

Lipid was estimated by the method of Folch et al., (1957). At first Ten mg of dried sample was homogenized in 10 ml of chloroform methanol mixture (2/1 V/V). Then the homogenate was centrifuged at 2000 rpm. In order to remove the non-lipid contaminants, the supernatant was then washed with 0.9 percent saline solution and allowed to separate. The upper phase was discarded by siphoning. The lower phase was dried in an oven and the weight was taken. The lipid content was expressed as percent on the wet weight by the following formula

$$\text{Lipid (per cent)} = \frac{Weght\ of\ lipid\ (mg)}{Weight\ of\ sample\ (mg)} \times 100$$

Fatty acid

Estimation of total lipid and fatty acid was done as per according to Folch et al., (1957), which takes place in Christle's (Christiansen et al., 1989), Kandaemir and Polat(2007).

After extraction, separation and weighing of the total lipid in crude extract was done. Then the total lipids were saponified and the unsaponified portion was disposed of. Then the 6 M HCl was added to the saponified for its acidification until it reached pH 1.50. Total fatty acids were obtained and then total amounts were fixed by weighing (An AND, HM-200 series, 0.0001 g sensitive balance were used to take all weights in mg.). The total lipid and fatty acids were expessed by percentage based on the wet weight. The results were shown as X±SE.