

Chapter-5

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Kalpani Beel is the only fresh water Beel (wetland) under the Chirang district of Assam, India. The Beel has inlet and outlet channels with the river Manas and it is an important breeding site of the fishes and other aquatic animals

5.1 Seasonal Variation in Water Quality of Kalpani Beel

Water is the most vital resource for life (Sharma, 2004). The floodplain lakes (commonly called Beel) comprise important inland aquatic resource of north eastern India and deserve special attention for their significant biogenic production potential (Sugunan, 1997). Knowledge of the hydrology of lakes is essential for their proper use and conservation. Water quality and aquatic flora and fauna of lakes are closely linked to the water (Mishra *et al.* 2009 and Odum, 1971). The monthly variations of water were well-known in physical and chemical properties, physical and chemical parameters were analyzed and assessed to know and understand the variations of the various parameters at different sites in the monthly intervals of the Beel. Four seasons were considered, including pre-monsoon (March to May), Monsoon (June to August), post-monsoon (September to November) and winter (December to February).

Air temperature (AT)

Air temperature (AT) is one of the most important factors in an aquatic environment. Changes in the air temperature naturally affect the water temperature (Kumar, 1997). In general, the rates of chemical and biological reactions are doubled with every 10°C increases in temperature (Boyd, 1984). In the present study monthly variability of atmospheric temperature has been observed. It was maximum 37°C and minimum 19°C (Table 6). In the present investigation seasonal variability of atmospheric temperature have been observed. It was maximum during monsoon (34.33±1.24°C) comparatively less during pre-monsoon (28.00±1.41°C), post-monsoon (27.33±2.49°C) and minimum during winter

($21.33 \pm 1.24^\circ\text{C}$) (Table 7). Laishram and Dey (2014) also found similar results as observed in the present study.

Water temperature (WT)

Surface water temperature (WT) is one of the most significant parameters which control inborn physical qualities of water (Laishram *et al.* 2014). The water temperature in the present study ranged between 17°C to 35°C (Table 6). The highest WT was recorded during monsoon session ($32.33 \pm 1.24^\circ\text{C}$) and lowest during winter ($19.33 \pm 1.24^\circ\text{C}$) which is a normal feature in fresh water bodies. More penetration of sunlight and longer duration should be for receiving sunlight in a day is the reason for higher temperature during monsoon season. In the Indian sub-continent, the temperature in most of the water bodies range between 7.8 and 38.5°C (Singhal *et al.* 1986). Sinha *et al.* (2011) also found similar results as observed in the present study. The WT across the four sites under study is within the limit of WHO standard of 30°C - 35°C (WHO, 1995). Water temperature of Kalpani Beel was found to be within normal limits.

Water p^{H}

Hydrogen ion concentration (p^{H}) gives an idea of the type and intensity of pollution (Hussainy, 1967). p^{H} is an important factor in determining the productivity of an ecosystem (Singh *et al.* 2009). p^{H} in the present study ranged from 7.8 to 11.00 (Table 6). Relatively lower p^{H} was observed in pre-monsoon and monsoon (8.66 ± 0.4714) and higher in winter (9.33 ± 0.4714) (Table 7). The variation in p^{H} is due to dilution of saline water caused by fresh water inflow (Tripathi, 1985). It might be due to human activities, decrease in the water level and presence of suspended particulate matter. Most of bio-chemical and chemical reactions are influenced by the p^{H} . The reduced rate of photosynthetic activities reduces the assimilation of carbon dioxide and bicarbonates which are ultimately responsible for increase in p^{H} (Deb *et al.* 2012). The p^{H} of Narmada river water samples was found to be in the range 7.6 to 9.9 (Shraddha *et al.* 2011). The p^{H} of the water under study is exceed the WHO standard of 6.50 - 8.50 .

Free CO₂

Free CO₂ in water is derived from many sources such as atmosphere, respiration by the organisms, bacterial decomposition of organic matter etc. Rain also absorbs small amount of gas and delivers it to the water on which it falls (Kaushik *et al.* 1999). The Values of FCO₂ were found to be in the range of 8.00- 40.00 mg/L and mean value of 17.36 mg/L (Table 6). It was found to be higher in monsoon (19.50 ±14.17 mg/L) and low in winter (15.13±2.44 mg/L) (Table 7). The higher value of FCO₂ in monsoon may be due to high rate of composition of organic matters by bacteria, resulting in rapid production of CO₂ (Pandey *et al.* 1999). A high value of free CO₂ was observed in monsoon and low in winter. Earlier workers also observed similar seasonal variations for production of free carbondioxide (Pandey *et al.* 1999 and Kosygin *et al.* 2007). The observed values were above the WHO standards of 22 mg/L.

Total hardness (TH)

Total hardness in water is the sum of the concentration of alkaline earth metals cations like Ca²⁺ and Mg²⁺. Hardness is generally governed by calcium and magnesium salts which largely combines with bicarbonates and carbonates giving temporary hardness and with sulphates, chlorides and others anions of a minerals acids causing permanent hardness. Water hardness up to 60 mg L⁻¹ was considered as soft water, from 121 to 180 mgL¹ as hard water and above 180 mg L⁻¹ as very hard water (Kannan, 1991). Hardness is usually expressed in terms of equivalent quantity of calcium carbonate. Water quality association has provided a general scale of hardness demarcating the limits for soft and hard water (Lehr *et al.* 1980). Total hardness (TH) of water was found to range from 80.00 mg/L to 212.00 mg/L during the study period and average value 163.33 mg/L (Table 6). Its peak value was observed in post monsoon (198.66 ± 9.84 mg/L) and minimum hardness was recorded in monsoon (124.66 ± 42.12 mg/L) (Table 7). High value of hardness during post monsoon attributed to decrease in water volume and increase of rate of evaporation of water. Based on average hardness levels, Kalpani Beel can be considered as hard water Beel. The hardness value was found above the highest desirable limit of 100 mg/L as prescribed for drinking water (WHO,1971).

Total alkalinity (TA)

The alkalinity in water is usually interpreted as the quantity and kinds of compounds such as bicarbonates, carbonates and hydroxides present which collectively shift the p^H to the alkaline side of neutrality. Alkalinity of water is its capacity to neutralize acid and is characterized by the presence of hydroxyl (OH^-) ions capable of combining with hydrogen (H^+) ions in solution (Kaushik and Saksena, 1999). The TA ranged between 123.00 mg/L to 262.00 mg/L in the present study (Table 6). A decline in the alkalinity was observed during the winter (150 ± 20.41 mg/L) and TA was found to be higher in post-monsoon (240 ± 21.60 mg/L) (Table 7). The high value of TA in post-monsoon may be due to the dissolution of calcium carbonate from the sediments and use of detergent and soap (Meetei *et al.* 2011). The observed values is to be found in the middle point when compared to one reported by Singh *et al.* 2010 in Kharungpat Lake, Manipur (38.00 to 284.0 mg/L) (Singh *et al.* 2010). Similar study was observed that the bicarbonate and total alkalinity in both the water bodies vary from 98.0 mg/L to 185.4 mg/L and 117.0 mg/L to 167.6 mg/L (Pathak *et al.*, 2012). The observed values were found above the WHO permissible limit of 120 mg/L.

Phosphates (P)

The importance of phosphates (P) in water bodies is well established (Hosetti *et al.* 2001). The P along with other salts is the prime contributors for the degradation of water quality. The formation of algal blooms because of high phosphorous concentration leads to eutrophication. The phosphates were found to be in the range between 0.00 mg/L to 1.50 mg/L (Table 6). In the present study seasonal variability of phosphates have been observed. The highest phosphate was recorded during post-monsoon 0.5 ± 0.40 mg/L and lowest during Pre-monsoon 0.33 ± 0.23 mg/L (Table 7). The high value of phosphates in post-monsoon may be due to the discharge of domestic sewage, detergents and agricultural run-off. The presence of high phosphate concentration is harmful for fish and aquatic life and also harmful for plant growth since they affect the water absorption indirectly and other metabolic process of the plants (APHA, 2005). The standard is 0.2 mg/L for surface Inland water. Inorganic phosphorous play an important role in aquatic

ecosystem, when present in low concentration; it is one of the most important nutrients, but in excess along with nitrates and potassium, cause algal blooms in water (Chakrapani *et al.* 1996). Phosphates of Kalpani Beel was found to be within normal limits.

Chlorides (Cl⁻)

Chlorides (Cl⁻) occur naturally in all types of water. Cl⁻ is one of the most important parameters in assessing the water quality. Higher concentration of chlorides indicates higher degree of organic pollution (Munawar, 1970). This opinion has been supported by Deshmukh (1964) and Ramakrishnarao (1990). In the present study, Cl⁻ is generally considered as nutrients. Cl⁻ is toxic to fresh water life at high levels. In fresh water its concentration remains quite low. However, concentrations above 150 mg/L are toxic to crops and generally unsuitable for irrigation (Brahma *et al.* 2012). The concentration of chlorides in water samples was found in a range of 9.00 mg/L to 21.00 mg/L (Table 6). Its minimum concentration was observed during pre-monsoon (13.33 ± 4.71 mg/L) while it was the maximum during winter (20 ± 0.00 mg/L) (Table 7). In the drinking water samples, chloride will be around 20 mg/L. In contrast to this, the observed value is slightly more than drinking water levels. Such high levels of chloride perhaps originated from agricultural activity. Cl⁻ around 20 mg/L is considered to be favorable for the fresh water community.

Nitrate (NO₃⁻)

NO₃⁻ represents the highest form of nitrogen. The main source for nitrates is biological oxidation of organic nitrogenous substances which come from sewage wastes. Atmospheric nitrogen is fixed into nitrate by the nitrogen fixing bacteria also and is a significant contributor to the budgets of nitrates. The concentration of nitrate in water samples was found in a range of 0.00 mg/L to 10.00 mg/L (Table 6). Maximum concentration was recorded during monsoon (6.66 ± 4.71 mg/L) and its minimum was noticed in post-monsoon and winter (0.00 ± 0.00 mg/L) (Table 7). The high value of NO₃⁻ in monsoon may be due to chemical fertilizers from cultivated lands, drainage from livestock feeds and as well as domestic sources. High amounts of NO₃⁻ in the water generally indicate polluted situation (Mason,

1989). When organic matter is decomposed in water, the complex proteins were converted into nitrogenous organic matter and finally to nitrates by bacterial action (Zafar, 1964). Beside the above, the regeneration of nitrates from sediment to surface water also plays an important role for higher values. The standard of inland surface water is 0.1 mg/L (Ramtake *et al.* 1989). This parameter is very significant from the point of view of productivity in lakes.

Nitrite (NO₂⁻)

NO₂⁻ is the partially oxidized form of nitrogen found in very low concentration in natural water. It has no mineral source in water but occurs as an intermediate form during denitrification and nitrification reactions (Gupta, 2009). The values of NO₂⁻ were found to be in the range 0.00 mg/L to 2.00 mg/L (Table 6). It was found to be higher in monsoon 1.00 + 0.81 mg/L and low in post monsoon and winter 0.00 + 0.00 mg/L (Table 7). NO₂⁻ in water is formed either by oxidation of ammonia by aerobic nitrifying bacteria, e.g. *Nitrosomonas* or by reduction of nitrates by facultative anaerobic denitrifying bacteria, e.g. *Pseudomonas* (Gupta, 2009). The observed values were found above the WHO permissible limit of 1.00 mg/L. Presence of minute quantity of nitrite in water is indicative of organic pollution.

Ammonia (NH₃)

NH₃ in the water is released as an end product of decomposition of organic matter and also as an excretory product of some aquatic animals. It dissolves in water to form ammonium hydroxide which further dissociates into ammonium (NH₄⁺) and hydroxyl (OH⁻) ions (Gupta, 2009). The concentration of ammonia in water samples was found in a range of 0.00 mg/L to 0.50 mg/L (Table 6). NH₃ was found to be higher in pre-monsoon 0.33±0.31 mg/L and low in post monsoon and winter 0.00 ± 0.00 mg/L (Table 7). Domestic wastes being often rich in nitrogenous organic matter and many industrial effluents add to the ammonia load in water leading to toxic levels at certain times. Aquatic autotrophs are capable of utilizing ammonium ions at a fast rate (Gupta, 2009). The NH₃ of the water under study is within the WHO standard of 0.25 to 5.0 mg/L.

Iron (Fe)

Iron is an essential trace element to both animals and plants (Brahma *et al.* 2012). Normally Fe is only slightly toxic but excessive intake can cause siderosis and damage to organs through excessive Fe storage (Huheey *et al.* 2001). Fe may be in the dissolved state or in a colloidal state that may be peptized by organic matter (Brahma *et al.* 2012). The Fe in this study ranges from 0.00 mg/L to 0.30 mg/L (Table 6). FE was found to be higher in post monsoon season 0.2 ± 0.1414 and low in winter 0.00 ± 0.00 (Table 7). The BIS and WHO limit in drinking water is 0.3 mg/L in natural water. Water containing iron at a concentration greater than 2.00 ppm causes straining of clothes and plumbing fixtures and imparts bad taste and colour to water (De, 2016). The Fe of the water under study is within the WHO limit of 0.30 mg/L.

5.1.1 Correlation Analysis

The objective of correlation analysis is to analyse the relationship between two variables in order to predict or estimate the value of one variable from the known value of the other variable. The correlation may be either positive or negative. If a change in one variable (X) produces a change in the other variable (Y) in the same direction, this correlation is said to be positive or the variables are said to be positively correlated. If a change in one variable (X) produces a change in the other variable (Y) in the opposite direction, then the variables are said to be negatively correlated or inversely related. The correlation coefficient shall generally range between -1 and +1 (Vijayalakshmi and Sivapragasam, 2008).

In the present study Air temperature showed high significant positive correlation with water temperature ($r = 0.997$), significant positive correlation with Nitrate ($r = 0.567$) and Nitrite ($r = 0.500$) and showed negative correlation with p^H ($r = - 0.491$) and Chloride ($r = - 0.456$). Direct relationship between Air temperature and Water temperature was also reported by Tamuli *et al.* (2018) in Morakolong Beel, Assam. Water temperature directly followed the changes in the Air temperature in the Beel as exposed by the significant and positively correlation ($r = 0.997$) with Air temperature. Similar study was also recorded by Yadavi *et al.* (1987) in Dighali Beel, Assam. Water temperature also showed positive correlation with Nitrate ($r =$

0.549) and Nitrite ($r = 0.483$) and negative correlation with p^H ($r = -0.490$) and Chloride ($r = -0.467$). p^H showed negative correlation with Free carbon dioxide ($r = -0.619$), Nitrate ($r = -0.555$), Total hardness ($r = -0.379$) and Nitrite ($r = -0.326$). Tamuli *et al.* (2018) also reported that the p^H is negatively correlated with free carbon dioxide and Nitrate. In the present study Free carbon dioxide was positively correlated with total hardness ($r = 0.424$) and Nitrate ($r = 0.429$) and negatively correlated with Iron ($r = -0.358$) which is supported by the result of Bordoloi (2012). Total hardness showed significant positive correlation ($r = 0.574$) with total alkalinity. Similar result was also recorded by Abir (2014) in the Rudrasagar wetland-A Ramsar site, Tripura. It is seen that total alkalinity shows significant positive correlation ($r = 0.602$) with Iron and negative correlation ($r = -0.382$) with ammonia and chloride ($r = -0.315$). Phosphate was inversely related to ammonia ($r = -0.292$) which is supported by the findings of Pathak and Mankodi (2013) in the Danteshwar pond, Gujarat. Chloride showed insignificant positive correlation ($r = 0.254$) with Nitrite. Nitrate had a direct relation ($r = 0.898$) with nitrite of water and positive correlation ($r = 0.399$) with ammonia but Barman *et al.* (2015) also reported that the nitrate is positively correlated with nitrite. In the present study nitrite showed significant positive correlation with ammonia ($r = 0.620$) and positive correlation ($r = 0.303$) with Iron. Ammonia showed insignificant positive correlation ($r = 0.408$) with Iron. Iron showed no any correlation with other parameters.

5.2 Fish Biodiversity

A total 55 fish species listed from the Beel. The rich fish fauna of the lake indicates the high productivity of the lake. Taxonomic authentications were performed under supervision of skilled experts from Zoological Survey of India (ZSI) in Shillong and also following standard literature. From the investigation 55 fish species that belonged to 38 genera, 21 families and seven orders was recorded from Kalpani Beel. Fish species of Kalpani Beel belonged to order *Cypriniformes*, *perciformes*, *Osteoglossiformes*, *Siluriformes*, *Synbranchiformes*, *Syprinodoniformes*, *Tetradontiformes*. Through our survey it was confirmed that order *Cypriniformes* was most dominant group presenting 26 species pursued by *perciformes* with 15 fish species, followed by seven species from *Siluriformes*, four from

synbranchiformes and 1 species each from *Syprinodoniformes*, *Tetradontiformes* and *Osteoglossiformes*. Among 21 families order *perciformes* comprised of eight families contributing 38.09% followed by five from *Siluriformes* (23.80%), three from *Cypriniformes* (14.28%), two from *Synbranchiformes* (9.52%) and *Osteoglossiformes*, *Tetradontiformes* and *Syprinodoniformes* had one family each (4.76%). However, the catch list was dominated by family cyprinidae with 23 fish species distantly followed by five species of *Channidae*, three species each of *Bagridae* and *Mastacembelidae* families. *Ambassisidae*, *Belontiidae*, *Osphronemidae* and *Cobitidae* were represented by two families and the last remaining 13 families consisted of single species.

Fishes represent half of the entire number of vertebrates living in the world. They are found in almost all feasible aquatic habitats. Around 21,723 living fish species are recorded out of the recorded 39,900 species of vertebrates. Of the 21,723 living fish species 8,411 are freshwater fish species and around 11,650 are marine (Jayaram, 1999). India is regarded as one of the renowned mega biodiversity countries of the world. India is placed in the ninth position in provisions of freshwater mega biodiversity (Mittermeier and Mitemeir, 1997). There are approximately 2,500 species of fish species in India of which 930 are freshwater species and 1,570 species are marine (Kar *et al.* 2003). Fresh water fishes are very much useful for the assessment of water quality as bio indicators, river network connectivity or flow regime (Chovane *et al.* 2003).

Several works in lakes relating to fish fauna have been carried out by several workers in various parts of India. Seethal Lal *et al.* (2014) studied Ichthyofaunal diversity of Vattakkayal, kollam district, Kerala, South India and listed 22 species of fishes that belonged to 10 orders and 17 families. Among them the order *perciformes* was found to be dominant and was represented by 9 species with 40.90% contribution of the total species. Kumar (2014), reported 20 species of indigenous fish species belonging to 11 genera and 5 families were identified from Kumaun Himalaya, Uttarakhand, India. Nayaka (2018) during the study period 10 species of fresh water fishes belonging to 5 families were recorded from the Mallasandra lake of Karnataka state, India. Percentage of composition and species richness, order *cypriniformes* was dominant (5 species) followed by *perciformes* (3

species), siluriformes (2 species). Ahirrao and Mane (2000) listed 32 species of fishes that belonged to 25 genera and eight families from Parbhani district in Maharashtra. Pawar *et al.* (2003) listed 11 species belonging to 05 orders from Dam near Mukhed, Nanded district (M.S.). Sakhare (2007) reported 29 fish belonging to 20 genera falling in 4 orders from Yeldari reservoir of Parbhani district. Sonaware and Barve (2015) recorded 23 species of 20 genera, 10 families and 08 orders in which order cypriniformes was dominant with 9 species from the Lower Dudhana dam district-Parbhani (M.S.) India. Chilgar and Jagtap (2018) recorded 63 species of fresh water fishes belonging to 6 orders, 17 families from Marathwada region, Maharashtra and cypriniformes was dominated with 34 species. Results, reported by above mentioned workers are more or less similar.

North East India is one of the hotspots of freshwater fish biodiversity and is known in the world for it (Kottelat and Whitten, 1996). Of the approximately 930 species inhabiting in freshwater bodies in India, North eastern India alone represents 267 species that belongs to 114 genera, 38 families and 10 orders respectively (Sen, 1982), which is 33.13% (approximately) of total freshwater fishes of India (Sen, 2000). Vishwanath *et al.* (2010), recorded 520 species of fishes from different water bodies of North-east India, whereas Bhattacharjya *et al.* (2003) recorded 217 fish species from the water bodies of Assam.

The natural lentic water bodies locally known as 'Beel' representing flood plains wetlands including the marshes and swamps, which are locally known as 'jalah', 'doloni' or 'pitoni' in Assam (Goswami, 2009). A total 3513 numbers of wetlands are identified in Assam by Assam Remote Sensing Application Centre, Assam (ENVIS, 2016).

In Northeast India several workers have studied fish diversity in aquatic ecosystem. Nath and Deka (2012) studied on fish diversity of Chandubi Lake of Assam, India and recorded 63 species, of which one species (*Cyprinus carpio*) was found to be exotic and the other species were found to be indigenous to Assam. Bora and Biswas (2015) listed 40 fish species belonging to 13 families from Moridikhow Beel in Sivasagar district of Assam. Kalita *et al.* (2016) studied on Ichthyofaunal diversity of Motapung-Maguri Beel of Tinsukia district of Assam. A

total of 48 fish species including 5 exotic fish species belonging to 35 genera under 18 families from 7 orders recorded. Cyprinidae is the most leading family with 19 numbers of species contributing about 40% of the 18 recorded families. Borah *et al.* (2017) studied on the Ichthyofaunal diversity of Kankati Beel of Biswanath district, Assam and recorded 51 fish species, belonging to 21 families and 10 orders. Cyprinidae family was the most dominant which include 18 species contributing 35%. Singha *et al.* (2017) recorded 67 number of fish species including 4 exotic fish belonging to 49 genera under 25 families from 8 orders from Diplai Beel of Kokrajhar district of Assam, India.

The study conducted on the Kalpani Beel, recorded a total 55 fish species that indicate rich diversity of fishes in the study site. The rich fish diversity in the Beels of Assam has been reported by number of earlier workers from Deepor Beel, 54 species (Goswami and Kalita, 2012); Tamranga Beel, 63 species (Agarwala, 1996); Charan Beel, 64 species (Rahman *et al.* 2012); Era Kopili Beel, 47 species (Chhetry and Deka, 2016); Sone Beel, 70 species (Kar and Dey, 1993) and Sarma *et al.* (2012) reported 77 species recorded from Goronga Beel of Morigaon district of Assam.

Present study recorded one endangered species (EN) (*Clarias batrachus*) and five near threatened (NT) species (*Hypophthalmichthys molitrix*, *Colisa sota*, *wallago attu*, *C. fasciatus* and *Parambassis lala*,) which is a very informative and important finding. The presence of species *Colisa* is significant to Kalpani Beel but this species is positioned in near threatened (NT) category in IUCN (2011). Of these two vulnerable (VU) species (*Catla catla*, *Cyprinus carpio*), 43 least concern (LC) species and three not evaluated (NE) species are recorded. The percentage of occurrence 1.81% were found endangered (EN), 3.63% found vulnerable (VU), 9.09% found nearly threatened (NT), 78.18% was found to be least concern (LC), Besides all these 1.81% were found data deficient (DD) and 5.45% were not evaluated (NE).

The conservation status of the recorded fish species as per IUCN have been reported from Mallasandra lake (Nayaka, 2018), *Oreochromis mossambica* was given the near threatened (NT) status, Diplai Beel (Singha *et al.* 2017), recorded 1

species as vulnerable, 2 species is data deficient, 7 species is near threatened, 55 species are least concern, 1 species is endangered and other 2 species are not evaluated, Motapung-Maguri Beel (Kalita *et al.* 2016), recorded no species as vulnerable, 1 species is endangered, 1 species is data deficient, 2 species are lower risk-near threatened, 39 species are lower risk- least concern and other 5 species are not evaluated, Moridikhow (Bora and Biswas, 2015), categories the recorded fish species, major fishes of this lake are of least concern (LC) category, 4 species are nearly threatened (NT) and 5 species are not evaluated (NE). Nath and Deka (2012) recorded 7 endangered species and 8 vulnerable species from the Chandubi lake of Assam.

Result obtained from the study indicated that fish diversity of Kalpani Beel is rich. The rich fish fauna of the Beel indicates the productivity of the Beel.

5.3 Aquatic Macrophytes

Hydrophytes or aquatic plants are grow in wet places or in water, either partly or wholly submerged. On the basis of life forms hydrophytes are classified into phytoplankton and macrophytes. Macrophytes are predominantly vascular plants (Shukla and Chandel, 2012; Ambasht, 1990). The study was carried out in the Kalpani Beel to evaluate the macrophytic diversity of the wetland for a period of one year i.e. from February, 2017 to January, 2018. Aquatic macrophytes are referred to as water plants, as well as amphiphytes and / or amphibian plants (Das, 2013). Macrophytes are important factors for helping in maintaining ecological balance. Aquatic macrophytes play a pivotal role in maintaining primary productivity of water ecosystem (Bhute and Harney, 2017).

During the study, a total of 67 macrophytic species belonging to 56 genera and 35 families have been reported from the Kalpani Beel of the Chirang district of Assam, India as shown in Table 12. Dominant families were Ariaceae with 6 species followed by Nymphaeaceae (5 Species), Hydrocharitaceae (4 Species), Poaceae (4 Species), Asteraceae (3 Species), Cyperaceae (3 Species), oxalidaceae (3 Species), Scrophulariaceae (3 Species), Polygonaceae (3 Species). Seven families namely Apiaceae, Convolvulaceae, Lemnaceae, Lentibulariaceae,

Menyanthaceae, Pointederiaceae, Trapaceae (2 species each) whereas eighteen families represents 1 species each (Table 13).

The studies on aquatic macrophytes are important to limnologist in order to understand the fluctuating of the aquatic ecosystem, to fisheries personnel; as inventory of fish food and to pollution control personnel, for their nutrient removing capacity (Murkute and Chavan, 2016). Macrophyte helps in stimulating growth of phytoplankton and in recycling of various organic matters. Macrophytes also provide suitable breeding and sheltering place for macroinvertebrates and fishes (Meshram, 2003). They serve as bioindicators for the possible degree of damage in aquatic ecosystem (Pieczynska and Ozimek, 1976).

The aquatic macrophytes are important sources of food, fodder and herbal medicine for domestic household materials. They also serve as food and provide dwelling place for many nutritionally rich consumable wild edible insects (Narzari and Sarmah, 2015a, 2015b). Macrophytes as a component of fresh water ecosystems play important roles in the structure and functioning of aquatic ecosystem (Pandit, 1984 and Wetzel, 2001). Water plants, including macrophytes are universally recognized as important participants in the natural processes of water self-purification (Dembitsky *et al.* 1992 and Gayevskaya, 1966).

Aquatic plants can reduce biological oxygen demand and these plants are now exploited for biofiltration of organic waste in the wastewater treatment systems (Ghosh, 2005). They are also pollution indicators of the water bodies (Kaul *et al.* 1980). Aquatic macrophyte diversity plays a significant role by helping to understand the dynamics of wetland ecosystem (Sarma and Deka, 2014). Decline in macrophyte community clearly indicates problems and changes in water quality and ecological status of water body (Bhute and Harney, 2017).

During the last few decades several works relating to aquatic macrophytes have been carried out by researchers throughout the world (Sculthope, 1967; Cowardian *et al.* 1997; Denny, 1985; Cook, 1996; Whigham, 1993; Mitsch *et al.* 1993; Gopal, 1995; Boulton and Brock, 1999; Keddy, 2000). Burlakoti and Karmacharya (2004) recorded 61 macrophytic species from Beeshazar Tal (BeeshazarLake) of Chitwan,

Nepal. Islam *et al.* (2017), identified a total of 39 aquatic weeds species from the Bangladesh Agricultural University Campus, Mymensingh, Bangladesh.

India has a rich biodiversity and is one of the 12 'Mega Diversity' centers of the world. It has the two of the 25 renowned biodiversity 'hot spots of the world the Western Ghats and Eastern Himalayas (Santra, 2012). Several works in Lakes relating to macrophyte flora have been carried out by several workers in various parts of India. Sharma and Singhal (1988) recorded 11 species of macrophytes from tropical lake, Sarronagar Lake, Hyderabad, Andhra Pradesh. 25 species of macrophytes were listed from Gujrat Tal in Jaunpur Township of North India Ambasht (2005). Narayana *et al.* (2005) studied the aquatic macrophytes of Husain Sagar, Karnataka. Kiran *et al.* (2006) recorded 15 macrophyte species in the fish culture ponds in Bhadra fish farm of Karnataka. Shahid *et al.* (2007), a total of 18 species belonging to 11 families were recorded from Nilnag Lake, Kashmir, India. Game and Salaskar (2007) recorded the macrophytes on Makhmali Lakes, Thane, Maharashtra. Wani *et al.* (2007) has recorded 16 species of emergent macrophytes in Nilnag Lake in western Himalaya (Kashmir). Game and Salaskar (2007) recorded the macrophytes on Malchmali lakes, Thane, Maharashtra. Harney *et al.* (2013) reported 19 species of macrophytes in three water bodies of Bhadrawati of Chandrapur District, Maharashtra state. Harney (2014) recorded 16 species belonging to 15 families from Dudhala lake of Bhadrawati town in the Chandrapur district of Maharashtra. Dhore and Luchare (2014) recorded 15 species of macrophytes in Yevatmal district, Maharashtra. Murkute and Chavan (2016) revealed that total 24 species of macrophytes were reported from three fresh water pond at Brahmapuri, Chandrapur (M.S.). Bhute and Harney (2017) recorded 15 species of macrophytes from Nagrala Lake of Bhadrawati, Chandrapur (M.S.). Palit *et al.* (2017) recorded 46 species belonging to 23 families from Lalbandh wetland, Birbhum, West Bengal.

In North East India several workers have studied macrophyte diversity in the wetlands. Assam is a part of a global biodiversity hotspot (Myers, 1988). Studied aquatic and wetland angiospermic macrophytes in the Kamrup district of Assam and recorded 128 species, belonging to 100 genera and 50 families (Das, 2013). Dutta *et al.* (2014) recorded 68 macrophytic species belonging to 49 genera and

28 families from Kapla beel, Barpeta, Assam. Deka *et al.* (2014), a total of 137 macrophytic species belonging to 114 genera and 53 families have been listed from wetlands of Nalbari district of Assam. All total 228 plant species were recognized under the 153 genera and 57 families from the five water bodies of Sonitpur district of Assam, India (Sarma and Borah, 2014). A total of 42 macrophytes species recorded under 34 genera belonging to 28 families from the Maijan wetland, Assam. Out of recorded species, the Asteraceae family was found to be maximum (6 spp.) and followed by the Amaranthaceae, Araceae (Abujam *et al.* 2014). Kalita and Choudhury (2016) recorded 74 macrophytic species belonging to 54 genera and 30 families from the Urpod Beel, Goalpara district of Assam. A total of 58 species of aquatic macrophytes listed belong to 30 families, 17 species are listed in the global invasive species database and 4 species were reported to be invasive in India (Prasad and Das, 2018). Kalita and Sarma (2019) reported 186 aquatic macrophytes and ecotone plant species from three selected wetlands of Kamrup district of Assam.

Aquatic macrophytes are mainly vascular plants. They are divided on the basis of their habit and location in ponds or lakes. The macrophyte species were categorized into six categories such as Free floating (FF) submerged suspended (SS), Submerged anchored (SA), Rooted with floating leaves (RFL), Emergent anchored (EA), Swampy and marshy (SM) following the system of Weaver and Clement (1929) and Daubenmire (1947). In terms of number of plant species, Swamp and marshy with species showed the largest number (25 species) followed by Rooted with floating leaves (11 species), submerged anchored (10 species), emergent anchored (10 species), Free floating (8 species) and submerged suspended (3 species). The data is tabulated in the Table 14. Harney (2014) recorded 16 species of macrophytes from Dudhala lake of Chandrapur district of Maharashtra state, belonging to 5 groups such as 3 submerged floating weeds, 3 rooted floating leaves weeds, 1 rooted emergent with heterophile weeds, 6 free floating suspended submerged and 3 Rooted submerged hydrophytes. Bhute and Harney (2017) categorized the recorded 15 species of macrophytes from Nagrala lake of Bhadrawati, Chandrapur district of Maharashtra belonging to 5 groups such

as 5 Free floating suspended submerged, 4 Rooted floating leaves weeds, 3 Rooted submerged hydrophytes, 2 Submerged floating weeds and 1 Rooted emergent.

The result of the study indicates that a total of 67 species of macrophytes belonging to 6 groups were recorded during the study. The aquatic macrophyte diversity of the Kalpani Beel is very rich. It also provides useful information about water quality and ecosystem health.

5.4 Mineral Content of Six Selected Macrophytes

Aquatic plants contain high amount of vitamin C, vitamin E and minerals that are essential for fish nutrition and for the normal growth and developmental activities in fishes (Kalla *et al.* 2004). Mineral salts are essential for normal growth and other metabolic activities of the body. Animals cannot synthesise them in their body, and hence mineral salts are acquired through food. Mineral salts do not supply energy to the body, but they are essential for protection against diseases and repairing of damaged organs. Inadequate supply of mineral salts leads to various deficiency-related diseases (Das and Chakraborty, 2007). Minerals are associated with several uses for animal health.

Calcium (Ca)

Pistia stratiotes exhibit a higher concentration of Ca (0.121 mg) and *Hydrilla verticillata* exhibit low concentration of Ca (0.045 mg). Ca is a key trace element in fish nutrition because of its role in bones, teeth, muscle system, and heart function (Brody, 1994). The interrelation of Ca and P is crucial for the development and proper functioning of bones, teeth, and muscles (Dosunmu, 1997 and Turan, 2003).

Copper (Cu)

The concentrations of Cu were found to be in the range of 0.001 mg/100g to 0.012 mg/100g. It was found to be lowest concentration in *Pistia stratiotes* (0.001 mg) and the highest concentration of was estimated in *Hydrilla verticillata* (0.012 mg). Umar *et al.* (2007) it was found that the concentration of Cu in *Ipomoea aquatica* is 0.36 ± 0.01 mg/100g. The permissible limit of Cu set by FAO/WHO (1984) in edible plants is 0.3 mg. After comparison, Cu limit in the studied aquatic plants

with those proposed by FAO/WHO (1984) it is found that all plants accumulate Cu below this limit. Cu is an ultra-trace element essential for animals Cu is critical for animals as it is involved in the activity of enzymes, such as cytochrome oxidase, superoxide dismutase, lysyl oxidase dopamine hydroxylase, and tyrosinase. In addition, Cu proteins and chelates also have metabolic roles. One of the few investigations on Cu metabolism in fish by some researchers (Syed and Coombs, 1982) revealed similarities to mammals in the distribution of Cu and Cu-dependent enzymes. The optimal level of Cu in diet as determined for several fish ranges from 3 to 5 mg Cu Kg⁻¹ of their diet (Gatlin *et al.* 1986).

Cobalt (Co)

Among the investigated aquatic plants, *Hydrilla verticillata* exhibited a high concentration of Co (0.026 mg), whereas *Pistia stratiotes* exhibited nil concentration of Co (0.000 mg). Most of the studied taxa contain Co below the permissible level set by FAO/WHO (0.043 mg). Co is a key element for fish nutrition. The presence of Co is critical because Co is a component of cyanocobalamin (Vitamin B₁₂), which constitutes nearly 4.5% of its molecular weight. Most animals need the element for the synthesis of the vitamin by intestinal microflora and such bacteria have also been isolated from the intestinal tract of fishes (Kashiwada *et al.* 1970). Co, as part of vitamin B₁₂, is associated with nitrogen assimilation and synthesis of haemoglobin and muscle protein. In addition, Co influences certain enzymes. Co binds to insulin (Cunningham *et al.* 1955) and also reduces plasma glucose levels (Roginski *et al.* 1977). Co deprivation reduced the intestinal synthesis of vitamin B₁₂ in catfish (Limsuwan *et al.* 1981).

Chromium (Cr)

In the present study, the Cr content of the studied plant taxa varied from 0.003 mg (*Eichhornia crassipes*) to 0.011 mg (*Nymphaea rubra*). Cr is essential for normal carbohydrate and lipid metabolism (Anderson, 1981). The influence of dietary Cr on glucose metabolism of carp was investigated by earlier researchers (Hertz *et al.* 1989). It occurs in food as part of a biologically active molecule and as inorganic trivalent Cr.

Iron (Fe)

The range of Fe in the studied plants varies from 0.016 mg in *Hydrilla verticillata* to 2.711 mg in *Nymphaea rubra*. The permissible limit set by FAO/WHO (FAO/WHO, 1984) in an edible plant was 20 ppm. Fe plays an active part in oxidation/reduction reactions and electron transport associated with cellular respiration (Watanabe *et al.* 1997). Fe is necessary for normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins, and fats (Adeyeye *et al.* 1999). The Fe content of fish is very low compared with that of mammals (Van, 1975). Information on absorption and metabolism of Fe in fish is limited; however, the process of absorption and metabolism is generally the same as in other vertebrates (NIH, 2013).

Potassium (K)

The mineral contents in these six aquatic plants showed that K is the most abundant secondary macro element present, ranging from 37.362 mg in *Pistia stratiotes* to 0.335 mg in *Hydrilla verticillata*, which is higher than the standard level (0.11%) in the living cell. K helps in the transportation of CO₂ by blood, contributes to the contraction and expansion of muscles and the heart. K helps in the metabolism of carbohydrates and proteins; K is essential for normal growth and body defence. Deficiency of K in fish may adversely affect the permeability of the cell membrane and the osmotic pressure of the cells and irregularity in the muscle and heart contractions (Das *et al.* 2007).

Magnesium (Mg)

The Mg content of the studied plant taxa varied from 6.759 mg in *Nymphaea rubra* to 0.004 mg in *Hydrilla verticillata*. Mg concentration in this sample was expected to be high because Mg is a component of Chlorophyll (Dosunmu 1997). Mg acts as a co-enzyme in different enzymatic reactions and contributes to the normal functioning of muscles and nerves (Das *et al.* 2007 and Fleek, 1981).

Manganese (Mn)

The range of Mn varied from 0.428 mg in *Nymphaea rubra* to 0.004 mg in *Hydrilla verticillata*. Except *Nymphaea rubra* and *Trapa natans*, all other

investigated taxa accumulate Mn below the permissible limit set by FAO/WHO (1984). Mn is a key component for bone structure, reproductive ability, and normal function in the nervous system (Fleek, 1981). Mn is essential for fish and is widely distributed in fish and animal tissue. Mn is necessary for the normal functioning of the brain and for optimal lipid and carbohydrate metabolism (Clark *et al.* 1987).

Molybdenum (Mo)

The concentration of Mo in the studied aquatic plant range from 0.016 mg in *Eichhornia crassipes* to 0.001 mg in *Trapa natans*. The role of Mo in fish nutrition is not well known. Signs of Mo deficiency have not been established (Watanabe *et al.* 1997).

Sodium (Na)

Among the investigated aquatic plants, *Hydrilla verticillata* exhibited the lowest concentration of Na (0.035 mg) and *Trapa natans* exhibited the highest concentration of Na (4.299 mg). Among trace minerals, Na is an important nutrient as it is the main monovalent ion of extracellular fluids; Na ions constituting 93% of the ions (bases) found in the blood stream. Although the principal role of sodium in the animal is connected with the regulation of osmotic pressure and the maintenance of acid-base balance, sodium also has an effect on muscle irritability, and plays a specific role in the absorption of carbohydrate (Tacon, 1987). Na is a key constituent of blood, tissue fluid, and some enzymes. Na is associated with K in the body in maintaining optimal acid-base balance and nerve transmission (Setiawan, 1996).

Zinc (Zn)

Among the studied taxa, *Ipomoea aquatic* exhibits the highest concentration of Zn (0.041 mg), whereas *Hydrilla verticillata* exhibits the lowest concentration (0.011 mg). Among trace minerals, Zn is a key trace element in fish nutrition as it is involved in various metabolic pathways. It serves as a specific cofactor for several enzymes. In addition, Zn is an integral part of approximately 20 metalloenzymes, such as alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase. Research on Zn-gene interactions has revealed a basic role of this element in

growth control (Chesters, 1991). Fish can collect Zn in their bodies from the water they swim in and from the food they eat. Zinc, a trace mineral nutrient and as such, small amounts of Zn are needed in all animals (Al-thagafi *et al.* 2014). However, dietary Zn, requirement has not been determined until now for *L. rohita* juveniles (Akram *et al.* 2019).

5.4.1 Correlation Coefficient Test

A correlation analysis performed to investigate the relationship between different element concentrations in aquatic plant samples showed a positive correlation, whereas some analyses showed a negative correlation. In the present study copper (Cu) was positively correlated with calcium (Ca) ($r = 0.771$). Similar positive relationship between Cu and Ca was observed by Pattar *et al.* (2017) in some traditional medicinal plants. Cobalt (Co) showed high positive correlation with copper (Cu) ($r = 0.885$). Relationship between cobalt and copper was also reported by Zafar *et al.* (2010). Chromium showed insignificant positive correlation with copper (Cu) and negatively correlated with calcium and cobalt, which is supported by the findings of Khan *et al.* (2011). Iron showed positive correlation with chromium ($r = 0.883$) and copper ($r = 0.071$) and negatively correlated with calcium and cobalt. Similar observation was also recorded in copper by Pattar *et al.* (2017). In the present study potassium was positively correlated with calcium, chromium and iron, showed significant negative correlation with copper ($r = -0.971$) and cobalt ($r = -0.880$). Jiang *et al.* (2007) recorded similar observation in Cu ($r = -0.971$). Magnesium showed positive correlation with Ca ($r = 0.678$), K ($r = 0.507$), chromium and iron and Significant ($p \leq 0.05$) negative correlation with Co ($r = -0.761$) and Cu ($r = -0.563$). Similar observation were also recorded in chromium and iron by Bhowmik *et al.* (2012) in some aquatic plants of Tripura, India. Manganese showed positive correlation with calcium, chromium, iron, potassium and magnesium and negative correlation with copper and cobalt ($r = -0.693$). Jiang *et al.* (2007) also recorded, magnesium, iron and manganese contents were significantly correlated with most of the other mineral element contents. Molybdenum had positive correlation with copper, cobalt, chromium, iron, potassium and magnesium. Molybdenum showed insignificant negative correlation ($r = -0.514$) with calcium. Sodium showed positive correlation with calcium,

chromium, iron, potassium, Magnesium, manganese and molybdenum and copper and cobalt showed insignificant negative correlation. Similar result was also recorded in sodium and magnesium by Khalil *et al.* (2012). In the present study zinc showed positive correlation with calcium, chromium, iron, potassium, magnesium, manganese, molybdenum and sodium and negative correlation with copper and cobalt. Bhowmik *et al.* (2012) also observed similar type of positive correlation with calcium, magnesium and manganese and negative correlation with copper.

5.5 Photosynthetic Pigments of *Hydrilla verticillata* Plant

Photosynthesis is a photobiological reaction and needs absorption of light by photosynthetic pigments. The most important pigments required in the process are the chlorophylls and in addition to chlorophylls and carotenoid pigments also actively participate in photosynthesis (Pandey and Singha, 2013). Pigments content can be taken as an indicator of photosynthetic productivity of the plants (Vasu *et al.* 2009). The compounds most important in the absorption and conversion of radiant energy to chemical energy are the pigments that exist within the chloroplasts or chromatophores, of plants. Light initiates the process of photosynthesis through these chemicals and organelles (Glass, 1961). The major photosynthetic pigments of higher plants are, the chlorophylls and the carotenoids (Lichtenthaler, 1987). Chlorophylls, the green pigments of plants, are the most important pigments active in the photosynthetic process. *Chlorophyll-a* and *b* the best known and most abundant and are found in all autotrophic organisms (Allen, 1966 and Devlin *et al.* 1971). *Chlorophyll-a* is known as the main pigment which convert light energy into chemical energy. *Chlorophyll-b* as accessory pigments acts indirectly in photosynthesis by transferring the light it absorbs to *chlorophyll-a* (Costache *et al.* 2012). The second group of plant pigments, the carotenoids are two different types, (1) the *carotenes* which contain only carbon and hydrogen, and (2) the *xanthophylls* which contain carbon, hydrogen and oxygen atoms in the form of hydroxyl or epoxide functional groups (Goodwin, 1958). The roles of carotenoids are they protect against the photooxidation of chlorophyll and they absorb and transfer light energy to *Chlorophyll-a* (Devlin and Witham, 1986).

5.5.1 Chlorophyll pigments

In the present study it was revealed that the absorption peaks of photosynthetic pigments were found between the red and blue region of the visible spectrum (figure 7). The position of peak-6 in the blue region against wavelength 430-470 nm and peak- 1 in the red region against wavelength 650-760 nm was indicated presence of *chlorophyll-a* (662 and 435 nm in acetone solution). The position of peak-5 in the blue-green region against wavelength 470-500 nm and the position of peak-2 in the orange region against wavelength 600-650 nm was indicated the presence of *chlorophyll-b* (642 and 453 nm in acetone solution) in the *Hydrilla verticillata* plant. Photosynthetic pigments absorb light energy in the visible part of the spectrum ranging usually between 400-700 mμ(nm). Chlorophylls chiefly absorb in the violet-blue and red parts of the spectrum (Jain, 2007). The absorption spectra for *chlorophyll- a* and *b*, is determined with the help spectrophotometer. Both *chlorophyll- a* and *b* show absorption maxima in the blue-violet region and orange-red region of the visible spectrum. The absorption spectrum of chlorophyll provides indirect evidence of the wavelengths of light that are absorbed for the process of photosynthesis (Devlin and Witham, 1986). One of the striking properties of chlorophylls is that they can absorb certain wavelength of light, when the chlorophyll solution is kept between ordinary light and a spectrometer, it is found that certain wavelength of light are absorbed more than others. Maximum absorption of both *chlorophyll-a* and *chlorophyll-b* takes place in the region of blue-violet, with peaks at about 429 mμ and 453mμ respectively and the next maximum is in the red region of about 642 mμ and 660 mμ respectively (Mitra, Guha and Chaudhuri,1985). In ether solution *chlorophyll-a* shows maximum absorption peak at 662 nm in the red region and at 435 nm in the violet region while *chlorophyll-b* has prominent absorption peak at 642 nm and 453 nm respectively (Pandey and Sinha, 2013).

5.5.2 Carotenoid pigments

In contrast to the chlorophylls, which absorb light in the two region of visible spectrum of light. The carotenoids exhibited absorption in the green to yellow region against wavelength 533 to 570 nm (Table 18). The position of peak-4 in the

green region against wavelength 533 nm and peak-3 in the yellow region against wavelength 570 nm indicated the presence of carotene and xanthophylls respectively in the *Hydrilla verticillata* plant. Carotenoid pigments absorb light energy in blue, blue-green and green parts of the spectrum (Jain, 2007). Carotenoids absorb radiant energy in the mid region of visible light spectrum in between 449 and 490 nm. Carotene shows absorption peaks at 449 and 478 nm whereas xanthophylls at 440 and 490 nm (Pandey and Singha, 2013). Another property of β -carotene is that it can absorb the blue-violet portion (449 nm-478nm) the visible spectrum. Xanthophyll however absorbs light of 440nm and 490nm (Mitra, Guha and Chaudhuri, 1985).

Ritchie (2006) have described the absorption spectra of chlorophylls differ slightly when in different solvents. Acetone gives very sharp chlorophyll absorption peaks and has great advantage as the solvent for analyse of chlorophylls. *Chlorophyll-a* and *b* have absorption maxima in the 600-675 nm and in the 400-475 nm range. The absorption maxima for each peak are very dependent upon solvent polarity. The absorption peaks for *chlorophyll-a* in diethyl ether are 660 nm and 428 nm, whereas, more polar in methanol, the peaks are shifted to 665 nm and 432 nm. As with the chlorophylls, the absorption maxima of the carotenoids vary with polarity of the solvent. β - Carotene in diethyl ether has a λ max of 449.8 nm, but in the more polar acetone, the λ max is 454 nm (Goodwin, 1983).

Devlin and Witham (1986) observed the wavelength positions of the peaks may vary by a few nanometres with chlorophyll extracted from different plant species. The content of foliar pigments varies depending on species. Variation in leaf pigments (chlorophylls and carotenoids) and its relation can be due to internal factors and environmental conditions (Nayek *et al.* 2014). Shaikh and Dongare (2008) reported that the chlorophyll and carotenoids content varied with microclimatic conditions in *Adiantum* species.

Similarly, Michael and Nicholas (1998) also observed that the chlorophyll and carotenoids content in submerged angiosperms which varied in broad range due to ecological conditions such as light and temperature. Gulmira *et al.* (2006) had shown the differences in photosynthetic activity, chlorophyll and carotenoid levels

and chlorophyll parameter in green sun and shade leaves of *Ginkgo biloba* and *Fagus sylvatica*. Nagendra Prasad (2008) analysed the spectral data and isolated, antioxidant compound from aquatic plant, *I. aquatica*. Further, Chen and Chen (1992) determined the carotenoids and chlorophylls in water convolvulus by liquid chromatography. Vasu *et al.* (2009) studied biomolecular and phytochemical of *Eichhornia crassipes*, *Ipomoea aquatica* and *Nymphaea pubescens*, from these three aquatic angiosperms.

The results of the study indicate that photosynthetic pigments of the *Hydrilla verticillata* plant collected from Kalpani Beel is very rich. The rich photosynthetic pigments present in *Hydrilla verticillata* indicates the productivity of the plant. Aquatic plants produce a variety of health compounds and can be utilized as food and feed. Results also clearly indicate that extraction of photosynthetic pigments by different solvents are depends on chemical nature of bio-molecules (*chlorophyll-a*, *chlorophyll-b* and carotenoids). Temporal and seasonal changes and local ecological condition can also be the reason for variations of pigment in plants; therefore further study in this context is suggested.

5.6 Effect of *Hydrilla verticillata* Formulated Fish Feeds on Growth and Muscle Composition in *Labeo rohita* Fingerlings

Crude protein in experimental diets ranged from 26.50 g/100g to 34.65 g/100g while one of the commercial feed showed higher protein content (47.5g/100g). Among the three experimental diets H40 had the highest protein content. On the other hand a low amount of protein which was even lesser than that of the control diet was observed in H50. Fish feed with 26-28% protein, is considered to be sufficient in a semi-intensive system of fish culture (Debnath, *et al.* 2017). Venugopal *et al.* (1984); Renukaradhya *et al.* (1986) and Mohanty *et al.* (1990) reported better growth with fish feed containing approximately 30% protein. Our study indicated that all the experimental diets had protein content ranging from 26.50 g/100 g to 34.65 g/100 g which as indicated by Debnath, *et al.* (2017) is adequately reasonable quantity for better fish growth.

For successful fish culture, a systematic knowledge of nutritional requirement of individual fish species is needed (Rath, 2000). Lower fat content was found in the

experimental diets compared to the control diet and commercial feeds. Fishes require no more than 3-9% of total fats from their daily diets. Therefore lower fat content in the experimental diets indicates that it can be an ideal fish feed. Fiber content in all the experimental diet was high. High fiber content promotes growth and production in fishes and crude fiber is beneficial in improving the utilization of certain nutrients in fishes (Steffens, 1981). Comparing all the nutritional parameters of the three experimental diet it was hypothesized that H40 with protein (34.35 g/100g), fat (1.78 g/100g), ash (0.22 g/100g), carbohydrate (45.78 g/100g) and caloric value (336.54 K cal/g) would be the most ideal fish feed with suitable proportion of nutrients in comparison to the H30 and H50.

Growth parameter studies in fishes are important for effective study of their nutritional requirements. Maximum weight gain, length gain and specific growth rate was observed in fingerlings fed with H40. Better utilization of H40 formulated feed was observed in H40 diet fed group which may be due to the presence of favorable organoleptic properties preferred by fishes in the H40 diet. The present study clearly indicated that feeding fish with high levels of *Hydrilla* incorporation meal (H50) has not yielded better results in terms of growth. Optimum levels of incorporation 40% (H30, H40) yielded better results in terms of growth. Similar observations were obtained when fishes were fed with experimental diet incorporated with *Nymphaea* leaf (Fynn-Aikins *et al.* 1992 and Sivani, *et al.* 2013).

Another reason for good growth of fish fingerlings in H40 treatments may be high fiber content. The addition of fiber in feed enhances the fish growth by providing increasing retention time of feed in the gut for the enzyme to act (Kono *et al.* 1987). Earlier worker (Fischer, 1972) reported that large grass pellets containing fairly large amount of Crude fiber affected digestibility and food conversion. Not only high fiber content but also high ash content (12%) of *Nymphaea* leaf powder in the test diets might have contributed to a better conversion. It has been reported that reduced digestibility with increased carbohydrate content is related to actual reduction in gland stimulation and enzyme reduction (Falge *et al.* 1978). Protein and energy should be kept in balance in formulated diets. Any deficiency or excess of digestible energy can reduce growth rates of fish. Protein is used for fish growth

if adequate levels of fats and carbohydrates are present in the feed; otherwise protein may be used for energy rather than growth.

Survivability of fish fingerlings in all the experimental units was exceptionally good. The poor performance in control diet fed group may be due to the absence of *H. verticillata* in that diet. Banarjee, (1967) indicated that water quality parameter did not produce any stress of fish during experiment. In our experiments no effect of water quality on the fishes was observed.

5.6.1 Cost analysis of formulated feeds

Cost analysis of the formulated experimental feeds and commercial feeds indicated a significant cost reduction with decrease in other nutrient additives in the formulated experimental feeds. The results, showed that cost of the commercial feeds range from Rs.4,300.00 per kg (Tetra Bits complete) to Rs.799.00 per kg (Optimum) and the production cost of experimental diets were CD for Rs. 101.33 per kg, H30 for Rs. 94.28 per kg., H40 for Rs.91.93 per kg and H50 for Rs.89.58 per kg. The cost of formulated feeds showed a remarkable cost reduction with increase in *H.verticillata* meal content in the test feeds. The best feed H40, in which 40% of ingredients were replaced by *H.verticillata* meal, seems to be the cheapest among all the feeds except H50 feed, as there is no commercial value for *Hydrilla verticillata* plant at present. However, the cost will be similar to that of *H.verticillata* integrated feed when it is freely obtainable. *H. verticillata* is a preferred natural food by fishes in many aquatic ecosystems. Fish farmers regard them as aquatic weeds and often remove them from the ecosystems in huge amounts. *H. verticillata* grow profusely in lakes all over country. Also there is no any commercial value for *H. verticillata* at present. So, there will be a considerable reduction in production cost of fish feed if *H. verticillata* is utilized.

5.6.2 Muscle composition in *Labeo rohita*

Carcass quality is a matter of great importance from the perspective of consumer acceptance. Increase in muscle protein and lipid content of fish fed on experimental diets could be ascribed to the efficiency of the fishes to digest plant ingredients as an energy supplement for growth (Sivani *et al.* 2013).

The muscle protein of fish fed diet ranged between 19.80 to 20.5 g/100 g which is slightly lower than the value (22.86 g/100g) of protein for carp fishes in USDA Food Composition Database but it can meet the daily recommended intakes of protein for infants and children between ages 0-8 years (Food and Nutrition Board, 2005). Very less difference were observed in the fat and ash contents of fishes fed the experimental diet and control diet. Due to low lipid content in the fish muscle, lower calorific value was obtained. Low fat content is not nutritionally disadvantageous, because nutritional quality of fats can be judge by the quality of fatty acids it possesses. Among the three experimental diet fed groups, the nutrient content of muscles of H40 diet fed group exhibited better nutritional attributes.

5.6.3 Invention through Biotechnological approaches

Biotechnology is technology that utilizes biological systems, living organisms or parts it to develop or create different products. New technologies and products are developed every year within the areas e.g. medicine, agriculture or industrial biotechnology. Fish is highly nutritive and rich source of animal proteins. For the enhancement of fisheries and to attain maximum yields from resources of fresh water, it is necessary to provide artificial feed, by which fish grows speedily and attains maximum weight in shortest possible time. In the present study, experimental diet incorporated with *H.verticillata* is an ideal and palatable feed for *L. rohita* fishes. Adequate amount of protein, fats, fiber and energy in the experimental diets is suitable for fulfilling the daily dietary requirements of fishes. Among the three experimental diets H40 diet yielded the best dietary requirements of fishes. Among the three experimental diets H40 diet yielded the best positive results compared to H30 and H50. This indicates that optimum levels of incorporation which is 40 percent (H40 diet) is an ideal amount of incorporating *H.verticillata* in fish diet for obtaining proper growth and development. Fingerlings of *L. rohita* grow well on diets containing 27% to 35% protein as consequence of optimum percentage of protein and efficient utilization of diets. Cost of the feed could be cut down up to 71% by incorporating *H.verticillata* in the fish feed. The feed prepared with *H.verticillata* have a good acceptance and palatability among *L.rohita* fishes. The results suggest that *H.verticillata*

incorporated diet can be beneficially used as feed for fishes to obtain better production.

5.7 Significance of the study

Water quality in Kalpani Beel, is a suitable breeding ground for river fishes. It is also a suitable territory for aquatic creatures and plants and the production of fishes as well. The Kalpani Beel is host to fresh water fish species which even includes globally threatened fish species. It is an ecologically important ox-bow lake. Fifty five fishes species recorded in the studied Beel indicate rich diversity of fishes in the Beel and also the good health of the Beel ecology. Macrophytes diversity of Kalpani Beel is very rich. Aquatic macrophytes respond to the changes in water quality and have been used as signal of pollution.

Aquatic plants are important source of minerals required for normal growth of aquatic animals. The study suggests that *Hydrilla verticillata* can be used for nutritional purposes due to the amount and variety of nutrients it contains. Photosynthetic pigments analyzed in *Hydrilla verticillata* plant indicated that it contains chlorophyll-a, chlorophyll- b, carotene and xanthophylls. The chlorophyll content can be taken as an index of photosynthetic productivity of a plant.

Formulation of organic fish food by biotechnological approach using *Hydrilla verticillata* plant powder as ingredient at different levels and only optimum levels of incorporation 40 percent (H40 diet) yielded better results in term of growth. The study suggests that *Hydrilla verticillata* meal could be used as a source of dietary protein for *Labeo rohita* (rohu). The study also revealed that the cost of the feed could be cut down up to 71% using locally available aquatic weeds as source of nutrients. The muscle protein and lipid content study indicated that high protein percentage was found in the H40 feed fed fish (rohu) muscle. The feed with *Hydrilla verticillata* plants have a good acceptance and palatability

5.8 Benefit to the society

The invention of low cost artificial fish feed will be highly beneficial for the small scale poor fish farmers. Fish feed generally constitutes 60-70% of the operational cost in intensive and semi-intensive aquaculture system. Natural food is available in the water body alone is not sufficient to achieve fast growth of fish. The most

commonly used artificial feed in India is a mixture of rice bran and oil cake. Earlier workers, has reported that this conventional feed is nutritionally insufficient. The fish feed used in aquaculture is quite expensive, nutritionally inadequate, irregular and is insufficiently produced in supply in many third world countries. Therefore, it is important to produce low cost, nutritionally balanced fish feed that can increase production levels and can be easily purchase from market even by the poor fish farmers. Among the formulated fish feeds, H40 feed yielded the best positive outcome compared to H30 and H50 feeds. This indicates that H40 (40 percent *Hydrilla verticillata* meal incorporating formulated feed) is a nutritionally balanced and cost-effective fish feed that can easily obtained by the deprived fish farmers which will support inimproving fish production.

5.9 Prospect of the study

Among the formulated feed ingredients, protein is an expensive ingredient. The protein content of *Hydrilla verticillata* powder incorporated experimental fish food were 30.60% in H30, 34.35% in H40 and 26.50% in H50, future study on certain potential aquatic weeds offer excellent scope as the nutrient loaded materials, aquatic plants also contain high amount of vitamin C, vitamin E and minerals that are essential for fish nutrition and for the normal growth and development of fish are naturally grow in the whole country without much agronomic care. Future of research, in this field will help in forming data bank of nutrients and minerals from aquatic weeds. Database for a regional bio-resource can contribute in accelerating further research. Aquatic weeds if used effectively for formulation in fish feed preparation; will be a solution in management of aquatic weeds. In near future it will be help in setting up of feed manufacturing industry in the state which will produce a low cost and nutritionally balanced fish feed by use of aquatic weeds.