SPATIO-TEMPORAL VARIATION IN MACRO-INVERTEBRATES OF GHODAGHODI LAKE, FAR-WEST, KAILALI, NEPAL

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RECOMMENDATION

This is to recommend that the thesis entitled "Spatio-temporal variation of macroinvertebrates of Ghodaghodi Lake, Far West, Kailali, Nepal" has been carried out by Ms. Gita Rijal for the partial fulfillment of Master's Degree of Science in Zoology with special paper Fish and Fishery. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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ABSTRACT

Recently species associated with fresh water ecosystems have steeply declined due to pollution, overexploitation, habitat modification, environmental degradation and impact of other developmental activities. The physio-chemical parameters are also changing in these recent years which are also the cause for the loss in aquatic diversity. Macro-invertebrates forms important links in food chain and are considered as an indicator of changes in aquatic habitat. Since Ghodaghodi Lake contributes habitat for wide variety of aquatic species, the status of macro-invertebrates is yet to be known. Therefore, this study was designed to investigate the spatio-temporal variations of macro-invertebrates and describe the relationship between certain environmental variables and assemblage structure of macroinvertebrates. Macro-invertebrates samples were collected in seasonal basis from four sites of the Lake by using bin sampler and then analyzed. Macro-invertebrate community richness, Shannon-Wiener diversity, and evenness were calculated for each site.Data on the temperature, depth, pH, conductivity, dissolved oxygen, hardness, free carbon dioxide, alkalinity and total dissolved solid was recorded. Redundancy analysis (RDA) was performed to examine the relationship between the Macro-invertebrate assemblages and environmental variables. Altogether 42 families belonging 18 orders, 6 classes and 3 phylum's of macroinvertebrates were recorded during the study period. The highest diversity index value (3.16) was observed in winter season and lowest value (3.03) was observed in the autumn season. The highest diversity index value (3.22) was observed at site C and/owest value (3.10) was observed at site B. Culicidae family (9.84%) had highest frequency in all four season and four station. The Scolopendridae (0.22%) and Sphaeriidae (0.22%) families had lowest frequency. The water temperature was found to be highest at site D at summer season i.e., 30.2 °C. Depth of sampling sites of Lake washighest at site D in summer season i.e., 300 cm. TDS was maximum at site C in autumn season i.e., 196.1.

Conductivitywas recorded maximum at siteA in summer season i.e., 236.5 µs. The pH of the water was alkaline throughout the year i.e., greater than 7 pH. It was recorded 7.1-8.5. The maximum DO was found to be 5.7 mg/l at site A in winter season. The free CO2 was 3.6 mg/l highest at site B in winter season. Hardness was found to be highest at site A in summer

season i.e., 379 mg/l. The alkalinity of the water was found to be maximum at site C in winter season i.e., 228 mg/l. Redundancy Analysis (RDA) shows that: Family Culicidae, lycosidae, Thiardae were most strongly positively correlated with the temperature in summer season and strongly negatively correlated with alkalinity in autumn and winter season.Noteridae, Bulinidae, Achatinidae, Lumbricidae, Ephydridae, Hydrometridae are positively correlated with DO, depth, conductivity and hardness of water. Mutillidae, Curculionidae, Lymnaeoidae, Notonectidae, Scolopendridae, Pyralidae, Elmidae, Viviparidae, Corduliidae, Gerridaepreferred positively correlated with pH, total dissolved solid and CO₂. Seasonal variation and location influence the macro-invertebrates communities.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of Abbreviations
DO	Dissolved Oxygen
UNWTO	World Tourism Organization
NLBI	Nepal Lake Biotic Index
LWQC	Lake Water Quality Class
NEPBIOS	Nepalese Biotic Score
LQI	Lincoln Quality Index
WQI	Water Quality Index
RSI	Relative Strength Index
VDC	Village Development Committee
GPS	Global Positioning System
АРНА	American Public Health Association
TDS	Total Dissolved Solid
RDA	Redundancy Analysis
NDWQS	Nepal Drinking Water Quality Standard
WHO	World Health Organization
WWF	World Wildlife Fund
NAST	National Academy of Science and Technology
DNPWC	Department of National Parks and Wildlife Conservation
IUCN	International Union for Conservation of Nature

1. INTRODUCTION

1.1 Background

Nepal is rich in water resources with more than 6,000 rivers and 5,358 lakes,among the existing lakes, 2,719 lie in Terai-Siwalik, 545 in mid-hills and 2,094 in highlands(Shrestha 1990, Jha et al. 2010). Altitude-wise, over 2,700 lakes (51%) are distributed below 500 meters above sea level, 2,227 lakes (42%) above 3,000 m asl, and only 419 lakes (<8%) are in the mid-hills between 500 m and 2999 m(Shrestha 1990, Jha et al. 2010). These freshwater ecosystems experience a great pressure by human activities that alter the physical, chemical, and biological processes associated with water resources. Therefore, the protection and maintenance of high-quality lotic and lentic water have become an increasingly important issue in recent years.

1.2 Wetland of Nepal

Wetlandsaredefinedaslandstransitionalbetweenterrestrial and aquaticecosystemswherethewatertableisusuallyator nearthesurfaceorthe land discovered by shallow water and as in such state, supports considerable amount of biological diversity of the earth (Mitsch andGosselink1986).The Ramsar convention defines wetlands as " areas of marsh, fen, pet lands or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh,brackish or salt, including areas of marine water, the depth of which at low tide does not exceeds six meters" (Ramsar and Iran 2012).Wetlands, on a global scale, have offered significant opportunities for tourism and recreation, providing economic benefits to the governments, the tourism industry, and the local communities, and the income has been used as a basis for their conservation (Ramsar and UNWTO 2012).

Nepal has only Inland and Riverine types of wetlands. Shrestha and Bhandari (1992) have reported seven types of wetlands in Nepal with their habitat types as follows: i. Lakes, ii. Ponds, iii. Reservoirs, iv. River floodplains, v. Swamps, vi. Marshes vii. Rice Paddies.

1.3 Macro-invertebrates

Macro-invertebrates are those animals which can be seen with the naked eye; for e.g. snails, insects, cray fishes, flat worms, many aquatic insects, juveniles or larvae in the water. Aquatic macro-invertebrates are macroscopic fauna without a backbone (vertebra), visible to unaided eye, and retained by a standard sieve of mesh size 0.5-0.6 mm (APHA 1985,Reddy and Rao 1991,Wiederholm 1980). They include diverse group of organisms such as flatworms, roundworms, annelids, molluscs, and crustaceans. They are mostly found attached to the substratum under water.

A biodiversity hotpot is a bio-geographic region that is both a significant reservoir of biodiversity which is threatened with destruction (Myer et al. 2000). The ecological attributes of wetlands (river, lake, reservoir, marshy land, paddy field etc.) are highly diverse and provide especially important ecosystem services (Costanza et al. 1991). Macro-invertebrates are appropriate indicator of aquatic habitat because these taxa show much faster reproductive rate, higher species abundance than higher tropic fauna. Nowadays, benthic macro-invertebrates represent the most widely used group of organisms in freshwater biomonitoring, due to their different sensitivity to change in both chemical characteristics of the water column and physical properties of habitats (Rossaro et al., 2011, Szivák and Csabai 2012). Macro-invertebrates are a heterogeneous group of ubiquitous and abundant organisms, relatively easy to collect, identify and enumerate (Bonada et al. 2006).

Aquatic insects serve as food for fish as well as other aquatic fauna. Aquatic insect larvae which are rich in nitrogen contents may be very much useful as fish prey organism. Benthos play an important role in biological productivity of river and lake. Aquatic insects mostly feed on the detritus and other organic matter, debris, and humus thus constituting important pathways of energy flow and are thus very beneficial for the productivity of aquatic environment. Benthic macro-invertebrates are assemblage in bio-monitoring technique.

Spatio-temporal is related to the space and time having both spatial extension and temporal duration. Spatio-temporal variation means the variation of any things related to space and time. Most freshwater ecological studies have strongly suggested biological monitoring of water bodies along with the environmental characteristics (Dudgeon 1999).

1.4. Water Quality Parameters

Water quality parameters play a big role in planktons, benthos and other biodiversity productivity as well as the biology of the cultured organism and final yield. Water quality determines the productivity of water body. The physiochemical attributes of a water body are principal determinant of growth rates and development. The physiochemical factors and nutrient status of water quality play the most important role in governing the production plankton biomass and bear great importance for macro-invertebrates.

Water quality parameter like dissolve oxygen, temperature, pH, conductivity, transparency, total dissolved solid matter, free carbon dioxide, hardness of water, alkalinity, depth, etc affects directly and indirectly the whole water ecology.But little information is available in Nepal on the seasonal variation of macro-invertebrates and their relationship with the physiochemical parameters of water.

1.5. Ghodaghodi lake Complex

The Ghodaghodi Lake Complex is situated at an altitude of 205 m above mean sea level (with a latitude of 28° 41′ 03″N and a longitude of 80° 56′ 44″ E in the tropical lowlands of western Nepal, and is under the jurisdiction of the Government. The lake complex is connected to an extensive tropical mixed forest in the lower slopes of the Siwalik Hills to the north, and in between two protected areas: Bardia National Park and Suklaphanta Wildlife Reserve in the south. Therefore, the Ghodaghodi lake complex functions as an important corridor for wildlife movement between these two protected areas (Sah and Heinen 2001).

Ghodaghodi Lake complex is the largest interconnected natural lake system in the plain land of Nepal and was designatedaRamsarsitein2003duetoitshighbiodiversity value. The present uses of lake resources include fishing, livestock grazing and collection of fodder, firewood,andnontimberforestproductsincludingmedicinal plants. Tharu is, an indigenous ethnic group of the lake area, comprising more than 50% of the total population, arethe most dependent community on wetland resources. The uses of lake resources by local people, mainly fodder, aquaticmacro-phytes,livestockgrazing,andfishing,werealso mentioned by the total households and population of nearest village.

1.6 Objectives

1.6.1 General Objectives

To examine Spatio-Temporal variation in macro-invertebrates of Ghodaghodi Lake.

1.6.2 Specific Objectives

a. To investigate seasonal diversity of macro-invertebrates.

b. Todescribe the relationship between certain environmental variables and macroinvertebratesassemblage structure.

1.7 Rational of the study

The Ghodaghodi Lake Complex is one of the most important sources of water for many wild life fauna. This study area is the wetland of the Terai region which is important for the aquatic macro-invertebrates habitats. Aquatic macro-invertebrates are the bio-monitoring agent, source of food, and bio-control agents in an aquatic ecosystem. The aquatic ecosystem and diversity of macro-invertebrates of the lake affected by the many physiochemical parameters like water tempeture, pH, Dissolved Oxygen, Depth, Conductivity, Free Carbon-dioxide, hardness, and alkalinity etc.

This study of macro-invertebrates investigated the present status of macro-invertebrates of Ghodaghodi lake. Macro-invertebrates occupying bottom of the water body, form an important link in detrius based food chain. This includes the importance of invertebrate organisms in the natural water and promoted many investigators to make studies in the qualitative and quantative aspects of its biota (Gupta1979, Yadava et al. 1984, Shrestha and srivastav 1991). Invertebrates are good indicator of water quality (Resh 1995). Since fresh water macro-invertebrates are highly suitable for bio-monitoring for the natural and human impact to aquatic ecosystem (Barbour 2008, korte et al. 2010) and Ghodaghodi lake supportswide variety of aquatic species, the status of macro-invertebrates yet to be known. Thereforethis study is designed to investigate the variation in micro-invertebrates assemblage of the Ghodaghodi Lake, which will be first work in this study area.

1.8 Limitation of the study

The detail study was difficult and the study could not cover whole wetland area due to limited time, lack of sufficient budget and limited technical instruments. Macro-invertebrates sampling was not done in the center of the lake due to technical problem. The collected macro-invertebrates are difficult to identify to the genus and species level.

2. LITERATURE REVIEW

2.1 Spatial and temporal distribution of Macro-invertebrates

Aquatic macro-invertebrates comprise a diverse group of organisms that includes are diverse group of they comprise a rich and diverse group of organisms that includes insect larvae, crustaceans, worms and molluscs. At multiple spatial and temporal scales, the variation in the community structure of the macro-invertebrates are influenced by a combination of multiple abiotic environmental conditions, biotic processes (Malmqvist 2002., Heino et al.2003; Snelgrove and Butman 1994, Thrush 1999, Widdows and Brinsley 2002, Ysebaert and Herman 2002., Dye, 2006, Mackay et al., 2010). The community structure of macroinvertebrates is related with physiochemistry, physiography and geography. They show distinct patterns of distribution and abundance with altitude (Hynes, 1970; Williams and Hynes, 1971; Corkum, 1989). For example, the community structure of macroinvertebrates from the riffles of fifty-eight streams of three regions of the Himalaya (Anapuma, Langtang and Everest) in Nepal was related to chemistry, physiography (substratum composition, altitude and size), geographical location and the land use (Rundle et al. 1993). The influence of altitude and land-use changes on macro-invertebrate assemblages was also reported from riffles of forty-three streams in the Dolpo region of western Nepal (Suren 1994). Study of macro-invertebrates from tributaries of Buddhiganga river, Western Nepal by Bhandari et al., (2018) observed that the diversity varied substantially across altitudinal ranges. Macro-invertebrates from river systems in east-central Nepal showed pronounced altitudinal changes in assemblage composition (Ormerod et al. 1994).

The density of organisms is considered to be a useful index of water quality. An optimal density of organisms exists in undisturbed areas of most natural waterways, although the density of some benthic invertebrates fluctuates widely with changes in the seasons (Hynes, 1960). Strong seasonal variation among invertebrates was observed in streams of central Nepal (Brewin et al. 2001). The spatio-temporal variations in diversity and density of macro-invertebrates was found in riffles and pools of the Mardi and the Vijayapur streams, Pokhara,

Nepal (Pokheral 2013). Seasonal varioation of macro-invertebrate communities were also reported by Olomukoro and Oviojie (2015) from Lake in Benin City, Nigeria.

Aquatic macro-invertebrates are found on the substrata of an aquatic ecosystem (Dernie et al. 2003). The abundance and distribution of these organisms are related to habitat conditions such as substrate, organic detritus and other environmental factors (Cyr and Downing, 1988; Dvorák and Imhof, 1998; Weatherhead and James, 2001). Substrate specificity of aquatic macro-invertebrates was reported from tributaries of Buddhiganga River, Nepal by (Bhandari et al. 2018). Study on the relationship between macro-invertebrates and substrate types from littoral zone of the Jagadishpur Reservoir showed that soft substrate supports diversity of macro-invertebrates than non-soft substrate (Shah et al. 2011).

Aquatic macro-invertebrates have been widely used to measure biological integrity of aquatic ecosystems. They are sensitive to water quality, water quantity, habitat and availability of food (Dallas and Mosepele 2007). Macro-invertebrates were used to classify the Rampur Ghol, Nepal into different Water Quality Classes based on Saprobic Water Quality Classification (SWQC) approach (Bajracharya and Pant2017). A number of studies related to water quality using macro-invertebrates have been carried out in Western (Suren, 1994; Sharma et al., 2005; Gurung et al., 2013, Matangulu et al., 2017), Central (Shrestha et al., 2008; Rana and Chhetri, 2015) and Eastern (Jha et al., 2015; Gurung et al. 2016) parts of Nepal.

Spatial distribution of benthic macro-invertebrate assemblages in relation to environmental variables specifically pollution level was reported from Korean Streams(Jun, et al.2016).Shrestha and Adhikari(2016) investigated the effects of seasonal water quality variations in distribution and abundance of macro-invertebrates in Taudaha lake, Kathmandu, Nepal.

2.2 Relation between water quality parameters with macro-invertebrates

Growth of the invertebrates community in the river system is influenced by environmental factors and use them as indicators of pollution gradient (Giller and Helenra 1993).Due to organic pollution and water quality composition of macro zoo-benthic fauna wide variations in values have been observed (Sharma 1999).Availability of DO is an important factor that determines the composition and distribution of fresh water communities (Hynes, 1960, Giller and Malqvist, 1998, Dodds, 2002). The concentration ofDO depends on multiple factors such as photosynthesis carried by aquatic plants, respiration by aquatic organisms, changes in atmospheric temperature and pressure etc. (Allan, 1995 and Dodds, 2002).Macro-invertebrates from both upland and low land responded in a similar manner to DO content. Mayflies turned out to be highly sensitive to low oxygen conditions; mortality was observed in Chironomidae when oxygen concentration was below 8% saturation (Connoly et al.2004).

Thelake water quality class(LWQC) was positively correlated with temperature, conductivity, calcium hardness, magnesium hardness, nitrogen concentration, total phosphorus, concentration and negatively correlated with secchi depth and oxygen concentration in lakes and reservoirs in Nepal(Shah and Shah 2011). The macro invertebrates can be used as economic tools for determining water quality of streams and rivers as efficient water quality indicators (Rana and Chhetri 2015).

Taxon richness ofmacro- invertebrates was not significantly positively correlated with depth and organic matter content, taxa, on the other hand, was inversely and not significantly related with depth.The constant oxygenation of the whole water column allows the occurrence of organisms, independent of depth and organic matter content(Lima et al. 2013).

Despite a number of studies related with aquatic diversity from different parts of Nepal, there is still no study has been conducted on the aquatic macro-invertebrates in Ghodaghodi Lake. Therefore, this study is designed to investigate the spatio-temporal variation in aquatic macro-invertebrates of Ghodaghodi Lake, Western Nepal.

3. MATERIALS AND METHODS

3.1 Study area

The study area was in Ghodaghodi Lake lies in Ghodaghodi municipality, Kailali district occupying about 138 ha. area. It is perennial ox-bow lake of Far-Western provience with 28° 41' 03" latitude and 80° 56' 44" longitude and have topography roughly palm shaped with ramifications. Before election thewetlandcomplex manv local was borderedbythreelocalpoliticoadministrativeunitscalledVillageDevelopmentCommittees (VDC): Sandepani in the East, Darakh in the South, and Ramshikharjhala in the North and West. This lake is surrounded by tropical mixed forest in the lower slope of Siwalik Hills. It was designated as Ramsar Site on 13 August 2003. It provides habitat for 34 species of mammals, around 29 species of fish, 140 species of birds and 9 species of herpeto fauna (DNPWC and WWF Nepal, 2003). The lake is fed by atmospheric inputs, surface flows, springs. It has no inlet with two outlets along the Mahendra high way. It has sub tropical monsoonal type of climate with dry winter and rainy summer.

3.2Sampling sites

Site A: This sampling station was East which is surrounded by tree and small herbs. This side is disturbed from illegal grazing and fishing. A new green tower has been made at 20 m south from this sampling station.

Site B:This sampling station was South near the Mahendra highway,which has two water passing outlet. One is in highly human disturbed area near the gate and another is about 100 m east from first one.

Site C:Station C was West 150 m far from temple near Mohanyal community forest.

Site D:This sampling station situated in North which surrounded by large tree and herbs. There is illegal fishing in this station. Crocodiles can be seen in winter season at edge of the lake in this side, they do sunbath in winter season.

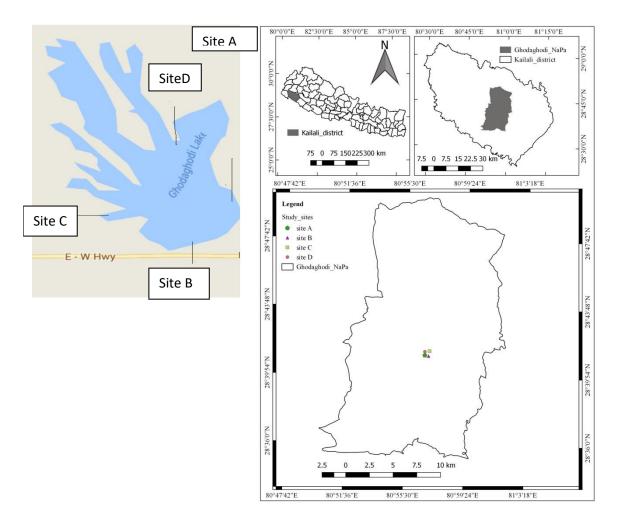


Figure 3.1: Map of study area

3.3 Materials used

Following materials was used during the field and lab work:

- a. GPS
- b. Camera (cannon)

- c. Titrating materials
- d. Thermometer
- e. Standard pH meter (combo)
- f. 70% alcohol
- g. Collecting Vials
- h. Bin sampler
- i. Aquatic net
- j. Compound microscope (10x magnification),
- k. Sechi disk
- l. DO meter (A28403)

3.4 Sampling method

Sampling was conducted in the months of March, July, October and December.Macroinvertebrates samplewere collected by using bin sampler (40 cm diameter) and a net with a mesh size of 150µm. Samples were collected from about 4 to 5 spot within the sample radius at each stations.Samples collected were sieved with a set of sieves of 20cmdiameter and mesh sizes of 2mm, 1mm, and 150µm respectively.Then photographs were taken by using the camera (cannon) and the samples weretransferred to vials and preserved in 70% ethanol and brought to the laboratory for identification.

3.5 Identification of macro invertebrates

Macro-invertebrates sampleswere identified to operational taxonomic unit on morphologically with the help of compound microscope and hand lens, using relevant keys (Budha 2016), (Dudgeon1999), (Nesemann et al., 2007; Nesemann et al. 2011), (Subraramanian and Sivaramakrishnan 2007), (Winterbourn and Gregson 1981).

3.6Analysis of physio-chemical parameters of water

Physio-chemical parameter of water i.e. water temperature, depth, pH, conductivity, dissolved oxygen, hardness, free carbon dioxide, alkalinity and total dissolved solid etc was measured by the standard methods (APHA 1995), and (Trivedy and Goel1984) for each site.

3.6.1 Water temperature

Temperature is important for its effect on the chemistry and biological reaction in organisms in water. A standard mercury thermometer was used in this research work by dipping the tip of thermometer directly into water surface and temperature was measured in Celsius (°C).

3.6.2 Depth of Water

Depth was measured by using nylon rope with weight and a measuring tape was used to record the depth in centimeter (cm).

3.6.3 Conductivity

Conductivity of water is the measurement of the ability of water to carry an electric current. Conductivity of water was measured by directly dipping the tip of pH meter (Hanna combo pH/EC/TDS/Temp tester Model HI18130 high range) in water surface. It is measured in µs.

3.6.4 Total Dissolved Solid (TDS)

The presence of various kinds of minerals that is dissolved in water is the total dissolved solid of water. TDS was measured by directly dipping the tip of pH meter (Hanna combo pH/EC/TDS/Temp tester Model HI18130 high range) in water surface in parts per million (ppm) unit.

3.6.5 pH

pH of water was measured by directly dipping the tip of standard portable pH meter (Hanna combo pH/EC/TDS/Temp tester Model HI18130 high range) in water surface. The readings shown by the pH meter were noted down in the field record sheet.

3.6.6 Dissolved oxygen

Dissolved oxygen in water determines water quality. Yhe low amount of DO in the water is the sign of organic pollution. It is an important parameter in assessing water quality because its influence on the organisms living within a body of water.

Dissolved oxygen was measured by the help of DO meter (A28403) in mg/l.

3.6.7 Free Carbondioxide

Free CO₂ was measured by using titration method in laboratory of Central Department of Botany. The water sample was titrating against strong alkali (NaOH). Phenolphthalein indicator was used in titration.For the measurement of CO2, 50 ml of water sample was taken in a beaker, 2-3 drops of phenolphalein indicator was used. If the color of sample didn't appear as red then it indicates that there was a presence of free CO2. Then it was titrated with 0.0227N NAOH (1 gm of NAOH in 1 liter of distilled water) until the pink colour of the solution appeared. It was calculated by the following formula of (Trebedi and Goal 9186).

Free CO₂ =
$$\frac{(ml \times N) \text{ of } NaOH \times 1000 \times 44}{V}$$

Where, V = Volume of water sample used in ml

N= Normality of NaOH 44= Atomic weight of co₂

3.6.8 Total Hardness

Water hardness is the amount of dissolved calcium and magnesium in the water. Hardness is express as calcium carbonate (mg/l), with soft water ranging from 0-75mg/l and hard water from 150-300 mg/l (Fisheries research center, 1994). Hard water lakes are more productive in comparision with soft water lakes. It was measured by EDTA titrametric method (Trivedy and Geol1786). For this 50 ml of water sample was taken in a conical flask and 1 ml of Ammunium buffer was added. Again 2 drops of Erichrome Black T indicator was added in that solution and shaken till the color changed to wine red. The solution was titrated against

EDTA (3.723gNa2EDTA.2H2O in 1liter distilled water) until the color changed into blue and the volume of EDTA used was recorded, and calculated by the formula:

Total hardness (mg/l) = $\frac{ml \ of \ EDTA \times 1000}{ml \ of \ sample}$

3.6.9 Alkalinity

Alkalinity of the collected water sample was measured by adding Sulphuric acid to decrease the pH of the sample. In 100 ml of sample 2 drops of phenolphthalein indicator was added. The solution either changed to pink or remains colorless. The total alkalinity was measured by adding methyl orange indicator to the colorless sample solution and titrated until yellow color changed to pink. The amount of acid used was recorded and converted to the equivalent weight for alkalinity (Swarup et al. 1981).

3.7 Data Analysis

The information thus obtained from field visit was analyzed statistically by exel, multivariant analysis from R softwareversion 3.5.3, species diversity index (Shannon and Weiner, 1949), Evenness index (E).

Species diversity index:

Shannon-Weiner diversity index(Shannon and Weiner, 1949) was designated as H', which was calculated as:

$$H' = -\Sigma (Pi) \times \ln (Pi)$$

Where, Pi = ni/N

ni = Number of individuals of species

N = Total number of all individuals in the sample.

ln = Logarithm of base (e).

Evenness index:

It was calculated by whether species are distributed evenly across seasons and across siteselements, evenness index was determined by the following equation:

$E=H'/\ln S$

Where, H' = Shannon-Wiener's diversity index.

S = Total number of species in the sample.

Multivariate analysis

Redundancy analysis (RDA), a direct multivariate ordination method (ter Braak 1988; ter Braak and Prentice 1988) based on a linear response of species to environmental gradients, was applied by using vegan library in 'R' (Oksanen et al. 2019).

4. RESULTS

4.1 Distributional pattern and frequency of occurrence of macro-invertebrates

Altogether 42 families belonging to 18 orders, 6 classes and 3 phylums were found in this study. The distribution patterns and frequency occurrence of different species of macro-invertebrates in Ghodaghodi Lake complex is shown in ANNEXES IV. The Culicidae family (9.84%), Gerridae (8.61%), Viviparidae (8.72%), Libellulidae (6.82%) and Physidae (5.59%) were found higher frequency in all four season and four station. TheScolopendridae (0.22%), Sphaeriidae (0.22%), Ampullariidae (0.34%), Noteridae (0.34%), Hydrophilidae (0.34%) and Ephydridae (0.34%), family were found lower frequency. Among lowest distribution Scolopendridae was absent in site B and site C and Sphaeriidae was absent in site A and site B in Ghodaghodi Lake Complex.

S. N.	Phylum	Class	Order	Family	Scientific name
1				Nepidae	-
2				Naucoridae	-
3				Notonectidae	-
4			Hemiptera	Gerridae	-
5				Hydrometrida	-
				e	
6	Arthropoda	Insecta		Pleidae	-
7		-	Trichoptera	Polycentropod	-
			iae		
8				Culicidae	<i>Culex</i> spp.
9			Diptera	Ephydridae	-
10				Tabanidae	-
11				Curculionidae	-
12				Gyrinidae	-
13				Dytiscidae	-
14			Coleoptera	Elmidae	-
15				Hydrophilidae	-
16				Hydraenidae	-
17				Noteridae	-

Table 1: Diversity of macro-invertebrates in Ghodaghodi Lake:

10			Diacontono	Perlidae	
18			Plecoptera		-
19			Ephemeroptera	Leptophlebiid	-
20				ae Baetidae	
20				Corduliidae	-
21			Odanata		-
22			Odonata	Libellulidae	
23				Chlorocyphid	-
24				ae	
24				Coenagrionid	-
25				ae	
25			Orthoptera	Tetrigidae	-
26				Gryllidae	-
27			Lepidoptera	Pyralidae	-
28			Hymenoptera	Mutillidae	-
29		Archinida	Araneida	Lycosidae	-
30		Chilopoda	Scolopendrom	Scolopendrida	-
			orpha	e	
31	Annelida		Gnathobdellida	Hirudidae	-
32		Clitellata	Oligochaeta	Lumbricidae	
33				Ampullariidae	Pila globosa(Swainson,
		Gastropoda	Mesogastropod		1822)
34			a	Thiardae	Melanoides tuberculata
	Mollusca				(Mueller, 1774)
35				Viviparidae	-Bellamya dissimilis
					(Mueller, 1774)
					-Bellamya bengalensis
					(Lemarck, 1882)
36				Bithynidae	-Bithynia cerameopoma
					(Benson, 1830)
					-Bithynia ghodaghodiensis
					(Gloer Bosineck, 2013)
37				Lymnaeoidae	-Lymnaea acuminate
			Basommatopho		(Lamarck 1822)
			ra		-Redix persica (Issel, 1865)
38				Planorbidae	-gyraulus convexisculus
					(Hutton, 1874)
					- gyraulus euphraticus
					(Mousson, 1874)
39				Bulinidae	-
40				Physidae	-
41			Pulmonata	Achatinidae	_
42		Bivalvia	Eulamellibranc	Sphaeriidae	Sphaerium indicum
			hiata	r	(Deshayes,1854)
		1			(200111)

4.2 Diversity status of macro-invertebrates fauna of Ghodaghodi Lake

The diversity index and evenness index of the aquatic macro-invertebrates was found to be varied from season to season and site to site as shown in fig. 4.1 and 4.2 respectively. The highest diversity index value (3.16) was observed in winter season and lowest value (3.03) was observed in the autumn season. The highest diversity index value (3.22) was observed at site C andlowest value (3.10) was observed at site B.

The Evenness index was recorded as highest (0.90) in spring and autumn season and lowest (0.88) in summer season. The Evenness index was recorded as highest value (0.90) at site C and lowest value (0.88) at site A.

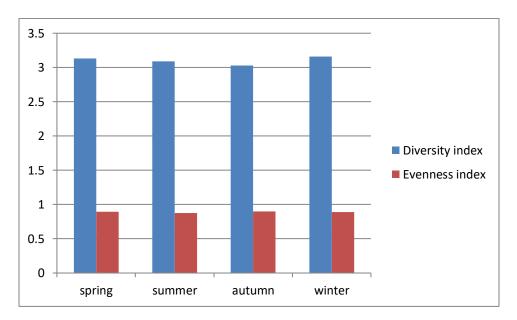


Figure 4.1: Season wise Diversity index and Evenness index

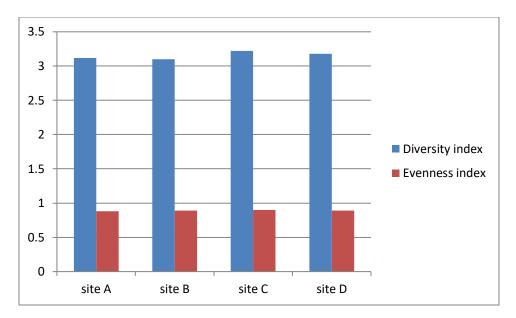


Figure 4.2: Site wise Diversity index and Evenness index.

4.3 Physiochemical parameters

4.3.1 Water temperature

The water temperature was found to be highest at site D at summer season i.e., 30.2 °C and lowest temperature was recorded at site A i.e., 16.4 °C at the winter season. The variation in temperature during various seasons at different station was presented in figure 4.3.

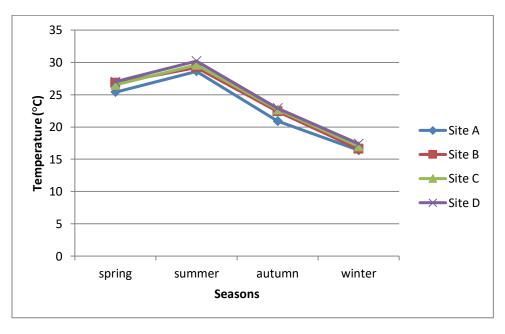


Figure 4.3: Variation in temperature at differentsampling sites and season.

4.3.2 Water depth

The depth of Ghodaghodi Lake varies from season to season. The depth was found to be highest at site D in summer season i.e., 300 cm where as it was recorded lowest at site A in spring season i.e., 95 cm.

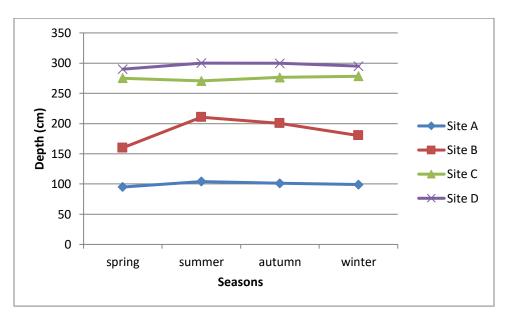


Figure 4.4: Variation in depth at differentsampling sites and season

4.3.3 Total Dissolved Solid (TDS)

The total dissolved solid was found to be maximum at site C in autumn season i.e., 196.1 ppm and lowest total dissolved solid was found to be at site C in summer season i.e., 75 ppm.

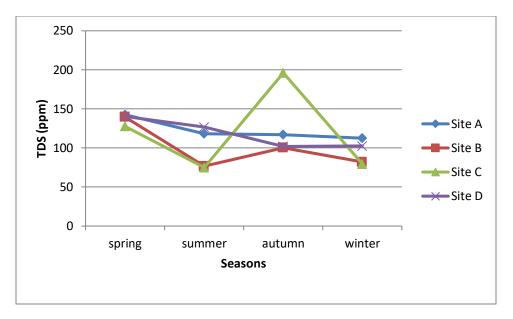


Figure 4.5: Variation of Total Dissolved Solid at differentsampling sites and season.

4.3.4 Conductivity

The conductivity was found maximum at siteA in summer season i.e., 236.5 μ s where as it was recorded minimum at site A in spring season i.e., 142 μ s.

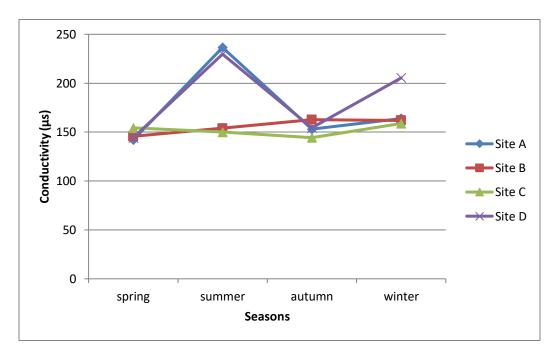


Figure 4.6: Variation of Conductivity at differentsampling sites and seasons.

4.3.5 Hydrogen ion concentration (pH)

The pH of the water shows whether the water is alkaline or acidic. The pH of the Ghodaghodi lake was found to be alkaline throughout the year i.e., greater than 7 pH. The pH was found to be maximum at site C and site D in spring season i.e., 8.5 and it was recorded minimum at site A and site C in summer and autumn season i.e., 7.1.

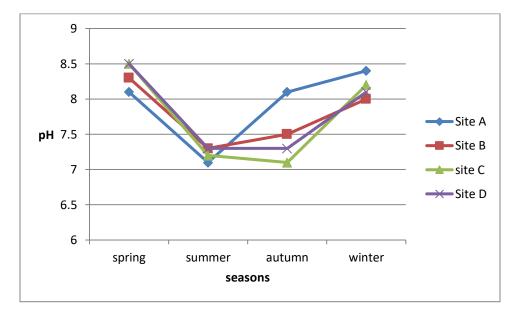


Figure 4.7: Variation of pH at different sampling sites and seasons

4.3.6 Dissolved oxygen (DO)

The dissolved oxygen plays an important role in the development of the aquatic lives. The maximum dissolved oxygen was found to be 5.7 mg/l at site A in winter season and minimum dissolved oxygen was found to be 2.8mg/l at site C in summer season.

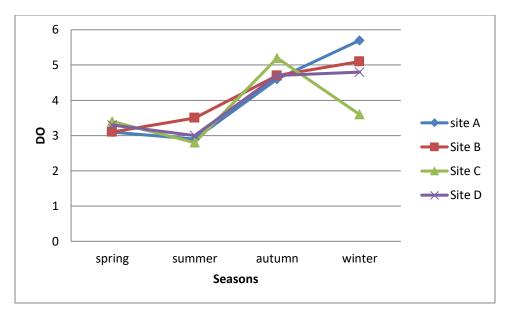


Figure 4.8: Variation of DO at different sampling sites and seasons.

4.3.7 Free Carbon dioxide (CO₂)

The free CO2 ranged from 2.3 mg/l to 3.6 mg/l. The highest free CO₂ was recorded at site B in winter season i.e., 3.6 mg/l and lowest free CO₂ was recorded at site D in summer season i.e., 2.3 mg//l.

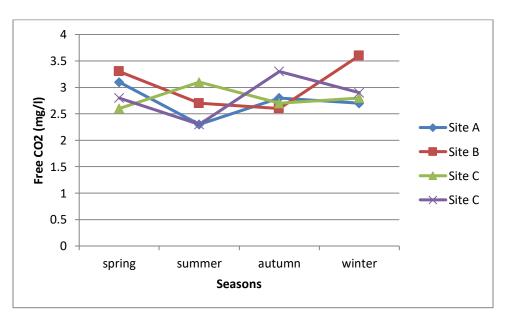


Figure 4.9: Variation of CO₂ at different sampling sites and seasons

4.3.8 Total Hardness

The hardness of the water ranged from 51 mg/l to 379 mg/l. The hardness of the water was found to be highest at site A in summer season i.e., 379 mg/l and lowest hardness was found to be at site D at autumn season i.e., 51 mg/l.

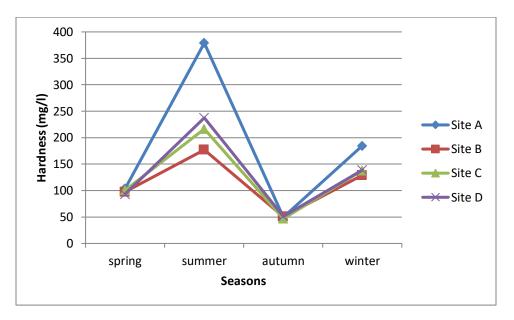


Figure 4.10: Variation of Total Hardness at different sampling sites and seasons

4.3.9 Alkalinity

The alkalinity of the water of the water was found to be maximum at site C in winter season i.e., 228 mg/l where as minimum alkalinity was found to be minimum at site B in spring season i.e., 146 mg/l.

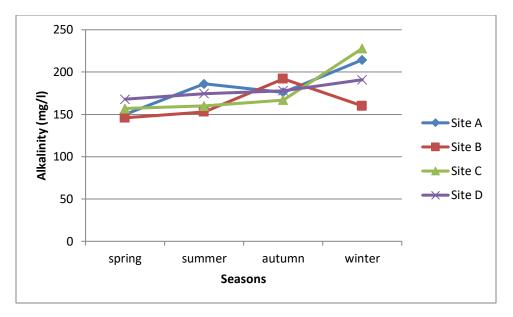


Figure 4.11: Variation of alkalinity at differentsampling sites and seasons.

4.4. Macro-invertebrates-environmental variable relationships

A total of 42 families of macro-invertebrates belonging to 18 orderswere recorded from the Ghodaghodi Lake Complex (table 1). Triplot scores for constraining variables and the axes are given in figure 4.12. The first two axes (RDA1 and RDA2) accounted for macro-invertebrates variation and physico-chemical parameters. Family Culicidae, lycosidae, Thiardae were most strongly positively correlated with the temperature and strongly negatively correlated with alkalinity.Noteridae, Bulinidae, Achatinidae, Lumbricidae, Ephydridae, Hydrometridae are negatively correlated with temperature and positively correlated with DO, depth, conductivity and hardness of water. Mutillidae, Curculionidae, Lymnaeoidae, Notonectidae, Scolopendridae, Pyralidae, Elmidae, Viviparidae, Corduliidae, Gerridaepreferred positively correlated with pH, total dissolved solid and CO_2 and negatively correlated with temperature and alkalinity.

Table 2:DCA summary of RDA triplot

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.18	0.08	0.06	0.06

Decorana values	0.18	0.08	0.04	0.02
Axis lengths	1.65	1.05	1.17	0.78

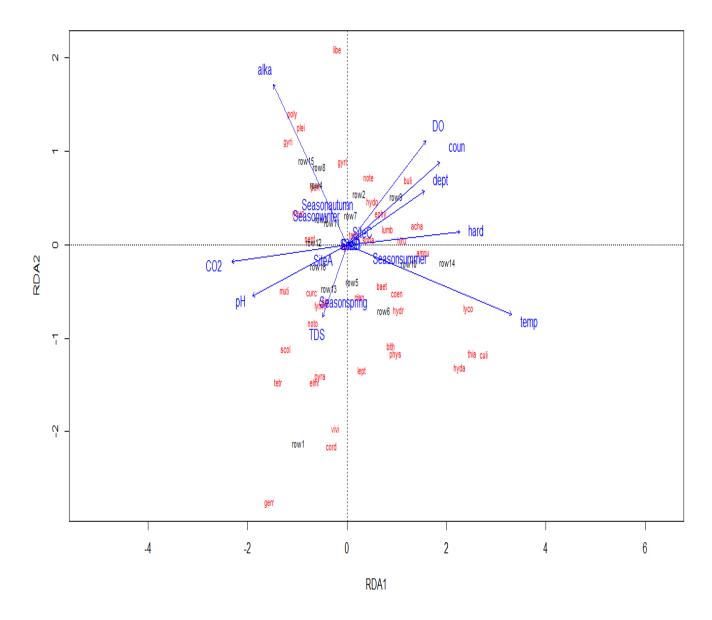


Figure4.12:RDAordination of macro-invertebrates family inrelation to physio-chemical parameter in the Ghodaghodi Lake Complex. (DO=dissolved oxygen, temp=tempeture, dept=depth, coun=conductivity, TDS=total dissolved solid, Hard= total hardness, Alka=alkalinity)

5. DISCUSSION

This study was conducted for a period of about 10 months, from March 2018 to December 2018 to cover four different seasons. The aim of study was to explore the aquatic macro-invertebrates fauna, their diversity and their relation with the environmental parameters of the lake. A total of 894 individual aquatic macro-invertebrates (mainly aquatic insects and mollusca), of 42 family were collected from four sampling site at four season of Ghodaghodi Lake which belongs to 18 orders 6 classes and 3 phylum. There is a lack of studies on macro-invertebrate from Ghodaghodi Lake.Few studies were conducted on mollusks (Subba, 2003 and Budha 2016). This study recorded 10 families 4 orders of Molluscs from Ghodaghodi Lake.

Pokharel (2013) found that ephemeroptera was the dominant order among the macroinvertebrates in hill-streams having natural environmental conditions, followed by trichoptera, plecoptera, diptera, coleoptera, odonata, hemiptera, oligochaeta, megaloptera.However, present study found thatCulicidae the dominant order among the macro-invertebrates in Ghodaghodi Lake Complex followed by Gerridae , Viviparidae , Libellulidae and Physidae family. It may be due to water level increases in the rainy season and hydrological condition varied in the month to month (schafer, lundstrom and petersson, 2008).

In this study, the highest diversity index value (3.22) was observed in site C and 3.16 in winter season. The lowest value (3.10) was observed in site B and 3.03 observed in the autumn season. In consistence with our observation, higher taxa richness and population density were observed during autumn/winter and lower during summer/monsoon season in various lotic water-bodies (Sunder, 1997, Brewin et al., 2000; Sharma et al., 2004). Similarly, temporal variations including higher richness and frequency values in winter/spring season and lower values in during autumn were observed in the present study, which could be due to seasonal climatic conditions, lake ecology abiotic characteristics and biotic communities including the human activities. This may be due to rainfall as it influences the water depth and as well as water quality to the diversity in extreme conditions (Mohd Rasdi et al., 2012).

The total macro-invertebrate composition was not significantly linkedtothe water quality composition. Temperature is one of the major environmental factors that have a significant effect on the other water quality variables and aquatic species (Niraula, 2012, Abdo, 2005). Seasonal changes in water temperature are directly affected by the air temperature (Allan, and Castillo, 2007). Similar trend of seasonal water temperature variation was observed in Ghodaghodi Lake. Organic and inorganic particulate matters were important factors that affect the sechi depth of the lake (Thiemann, and Kaufmann, 2002). The lower sechi depth of the lake during spring season was mainly due to the transport of sediments from the agricultural land in the watershed (Gregory, Swanson et al., 1991). The value of depth was more in rainy season while low in spring season. Seasonal change in water was found to be related with corresponding change in atmospheric temperature (Kundanger et al. 1996). In the present result temperature was found 16.4°C to 30.2°C.

The measurement of hydrogen ion concentration (pH) in water is the negative alogarithm of the concentration of hydrogen ion and acidic water has pH less than 7 and basic water has pH greater than 7. The mean pH of river was approximately neutral i.e. 6.7 and 100% of the samples were within the permissible limit prescribed by Nepal drinking water quality standard (NDWQS, 2005)and (WHO, 2011). Normal pH range should be between 6.2-8.5according to the WHO (2003). In present study pH of the Ghodaghodi lake was found to be alkaline throughout the year i.e., greater than 7 pH. It was recorded 7.1-8.5.

Cnductivity is determined by decomposition of organic matters, input of ions from temperature of the water and total solids (Ghimire 2010). The higher conductivity observed during summer was possibly due to the agricultural activities, leaching of fertilizers from the cropland (Chenet al., 2016) and (Maret, et al., 2010), and a significant amount of dissolved salts in the lake (Kazi, et. al. 2009) Various studies(Lacoul and Freedman, 2005;Gautam and Bhattarai, 2008; and Bhatt et al., 2012) have reported higher lake conductivity during summer, which is a strong indicator of agricultural intensity and its associated impacts. Similarly, in this study higher conductivity was recorded in summer season i.e., 236.5 μ s.

The concentration of DO in natural water depends on the physical, chemical, and biochemical activities of in the water body (Kazi, et. al., 2009, Masters, 2003). As expected, the concentration of DO fluctuated during the study seasons. Since the concentration of DO

is inversely related to the temperature (Allan and Castillo, 2007;Masters, 2003).Decreased levels of dissolved oxygen influence benthic macro-invertebrate assemblages (Ward, 1992). The current study revealed the increased DO concentration 5.7 mg/l at site A in winter season. In current study lowest concentration of DO was in 2.8 mg/l at site C in summer season, DO shows positive correlation with macro-invertebrates. The decrease in DO in summer season may be due to the increase in temperature (Agrawal, 1999).

Alkalinity is the measure of waters ability to neutralize acidity. Alkalinity values of 20-200 mg/L are common in fresh water ecosystems. Alkalinity below 10 mg/L indicates poorly buffered water system (Biggs, 1995). The present study showed alkalinity of water was found to be 146-228 mg/l. Therefore, alkalinity values are common in fresh water ecosystems in the Ghodaghodi Lake Complex. Shah et al. (2011) investigated Temperature, pH, dissolved oxygen, free CO2, total alkalinity, total hardness and significantly varied between seasons. Alkalinity in present study was only due to bicarbonates according to (Jhingran, 1975). High alkalinity in winter season may be due to less water volume and more bicarbonates (Panday and Devkota 2016).

Free carbondioxide depends on the respiration of biota, photosynthesis as well as decomposition (Choudary et al. 2014). The high value of CO2 found in winter season while low in summer season. In the present study increase in free CO2 can be attributed to the rate of favorable temperature (sukhija 2007). The more value of CO2 may be due to decrease in productivity leading to decomposition forming more CO2 in water (Rajan and Samuel 2016).

Hardness in water is caused by metallic ions dissolved in water which include calcium and magnesium ions. It is expressed as calcium carbonate (mg/l). According to WHO (2003) limits the range between 0-40 considered as soft, 40-100mg/l as moderately hard and 100-300mg/l considered as extremely hard. The average value of hardness is found higher in ...season and lower in... season. It is due to high flow rate and less amount of Ca++ and Mg++ (Bhattarai 4004).

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

A total of 894 individuals species were found in the different four sites of the Ghodaghodi Lake Complex; belonging to 18 orders and 42 families. The Culicidae family showed the highest frequency in all four seasons and sites but Scolopendridae and Sphaeriidae showed the lowest frequency in all four seasons and sites. The Scolopendridae was absent in summer and winter seasons at site B and C whereas the Sphaeriidae was absent in site A and B and it was present in spring season only. The Diversity index and Species richness was recorded highest in winter seasons at site C and the Evenness index was recorded highest in spring and autumn seasons at site C. Some macro-invertebrates were positively correlated with water quality parameters and some macro-invertebrates were negatively correlated with water quality parameters. The family Culicidae, Lycocidae and Thiardae were strongly positively correlated with temperature and strongly negatively correlated with alkalinity.

6.2 Recommendations

Based on this research, following are the important recommendations:

- Future studies should investigate taxonomy up to genus and specieslevel and distribution of larvalworks related to macro-invertebrates should be encouraged.
- The pollution must be controlled to increase the diversity and maintenance of Lake ecosystem.
- Population of freshwater mollusca are declining day by day; so strong rules for mollusca habitat conservation should be implemented.

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ANNEXES III:Distribution of macro-invertebrates in sites and seasons (Spr=Spring, Sum=Summer, Aut=Autumn and Win=Winter)

S						Site	в			Site C				Site D			
Ν		S	Su	А	W	S	Su	А	W	S	S	А	W	S	S	А	W
		Р	m	u	in	Р	m	u	i	Р	u	u	i	Р	U	u	i
		r		t		r		t	n	r	m	t	n	r	m	t	n
1	Nepidae	3	5	0	6	2	0	4	1	2	2	0	1	9	0	3	1
2	Naucoridae	2	0	0	0	3	0	6	2	2	0	3	4	4	2	6	4
3	Notonectidae	2	3	1	2	3	2	0	0	0	2	4	0	6	0	2	2
4	Gerridae	1															
		7	0	6	3	6	4	2	2	0	3	5	7	7	2	3	10
5	Hydrometridae	0	0	0	0	0	0	0	0	0	1	0	0	2	1	0	0
6	Pleidae	0	0	0	2	0	0	0	3	0	0	0	0	0	0	0	1
7	Polycentropodia																
	e	0	1	2	1	0	0	0	1	0	0	1	1	0	0	0	0
8	Culicidae	5	6	3	2	7	9	5	0	9	11	3	2	5	16	0	5
9	Ephydridae	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
10	Tabanidae	0	0	1	0	0	1	2	0	0	0	0	0	0	0	0	0
11	Curculionidae	2	3	0	2	1	2	0	1	0	0	1	0	1	0	1	0
12	Gyrinidae	0	0	6	7	5	0	0	2	3	0	2	3	0	0	2	5
13	Dytiscidae	1	2	1	0	0	0	2	1	0	2	3	1	1	1	0	2
14	Elmidae	1	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0
15	Hydrophilidae	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0
16	Hydraenidae	1	0	0	0	1	2	0	0	2	1	0	0	0	2	0	0
17	Noteridae	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
18	Perlidae	0	0	0	2	0	1	0	1	0	0	0	0	0	0	0	1
19	Leptophlebiidae	1	1	0	0	0	2	0	0	0	1	0	0	1	0	1	0
20	Baetidae	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	2
21	Corduliidae	2	0	0	0	1	2	0	0	0	0	1	1	3	1	1	0
22	Libellulidae	2	5	4	3	1	1	4	7	4	5	5	2	1	4	7	6
23	Chlorocyphidae	2	1	1	1	1	0	1	1	0	3	3	0	2	0	1	0
24	Coenagrionidae	1	0	0	1	1	0	0	1	2	1	1	1	1	3	0	3
25	Tetrigidae	4	2	0	2	0	2	1	2	1	0	0	2	3	0	1	1
26	Gryllidae	0	5	3	3	0	2	2	0	0	1	3	1	0	1	0	3
27	Pyralidae	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0
28	Mutillidae	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0
29	lycosidae	0	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0
30	Scolopendridae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
31	Hirudidae	0	1	1	0	0	2	1	0	0	0	1	2	0	3	1	1
32	Lumbricidae	1	2	0	0	0	0	0	0	0	1	1	0	0	2	2	0
33	Ampullariidae	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0
34	Thiardae	2	0	0	0	3	3	2	1	4	5	2	1	3	7	0	1
35	Viviparidae	1															
		1	6	4	4	6	10	7	6	3	5	4	3	2	2	2	3

36	Bithynidae	2	1	0	1	0	0	0	0	2	1	0	2	1	2	0	0
37	Lymnaeoidae	5	5	2	2	3	2	1	2	4	3	0	4	2	1	4	2
38	Planorbidae	3	5	4	2	2	3	3	1	3	0	4	1	4	3	0	2
39	Bulinidae	2	3	2	4	2	2	2	2	4	4	2	2	1	2	1	1
40	Physidae	3	2	3	4	5	6	3	2	2	7	3	2	2	2	1	3
41	Achatinidae	0	0	0	0	2	1	0	2	3	4	0	0	0	0	0	0
42	Sphaeriidae																
		0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
	Total	78	60	45	55	57	61	51	43	56	66	52	44	67	60	40	59

ANNEXES IV Frequency distribution calculation of macro-invertebrates

S.N.		Family		S	ites	Total	Frequency	
	Order		А	В	С	D		%
1		Nepidae	14	7	5	13	39	4.36
2		Naucoridae	2	11	9	16	38	4.25
3	1	Notonectidae	8	5	6	10	29	3.24
4	- Hemiptera	Gerridae	26	14	15	22	77	8.61
5		Hydrometridae	-	-	1	3	4	0.45
6		Pleidae	2	3	-	1	6	0.67
7	Trichoptera	Polycentropodiae	4	1	2	-	7	0.78
8	_	Culicidae	16	21	25	26	88	9.84
9	Diptera	Ephydridae	-	2	1	-	3	0.34
10		Tabanidae	1	3	-	-	4	0.45
11		Curculionidae	7	4	1	2	14	1.57
12		Gyrinidae	13	7	8	7	35	3.91
13		Dytiscidae	4	3	6	4	17	1.90
14	Coleoptera	Elmidae	1	-	3	-	4	0.45
15		Hydrophilidae	2	-	-	1	3	0.34
16		Hydraenidae	1	3	3	2	9	1.01
17		Noteridae	-	2	1	-	3	0.34
18	Plecoptera	Perlidae	2	2	-	1	5	0.56
19	Ephemeropte	Leptophlebiidae	2	-	2	3	7	0.78
20	ra	Baetidae	-	1	2	2	5	0.56
21		Corduliidae	2	3	2	5	12	1.34
22	Odonata	Libellulidae	14	13	16	18	61	6.82
23		Chlorocyphidae	5	3	6	3	17	1.90
24		Coenagrionidae	2	2	5	7	16	1.79
25	Orthoptera	Tetrigidae	8	5	3	5	21	2.35
26		Gryllidae	11	4	5	4	24	2.68
27	Lepidoptera	Pyralidae	1	-	2	1	4	0.45
28	Hymenoptera	Mutillidae	3	2	-	-	5	0.56

29	Araneida	lycosidae	-	1	1	2	4	0.45
30	Chilopoda	Scolopendridae	1	-	-	1	2	0.22
31	Gnathobdelli	Hirudidae	2	3	3	5	13	
	da							1.45
32	Oligochaeta	Lumbricidae	3	-	2	4	9	1.01
33		Ampullariidae	-	-	1	2	3	0.34
34	Mesogastrop	Thiardae	2	9	12	11	34	3.80
35	oda	Viviparidae	25	29	15	9	78	8.72
36		Bithynidae	4	-	5	3	12	1.34
37		Lymnaeoidae	14	8	11	9	42	4.70
38	Basommatop	Planorbidae	14	9	8	9	40	4.47
39	hora	Bulinidae	11	8	12	5	36	4.03
40		Physidae	12	16	14	8	50	5.59
41	Pulmonata	Achatinidae	-	5	7	-	12	1.34
42	Eulamellibra	Sphaeriidae	-	-	1	1	2	
	nchiata							0.22
	Total		239	209	221	225	894	
								100.00