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Macro-invertebrate Diversity and its Relationship with Environmental Variables in Adha Lake between Monsoon and Post-monsoon Seasons

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Abstract

Lentic water bodies are amongst the most threatened wetland habitat types as anthropogenic disturbances have significantly influenced the structure and function of aquatic ecosystems. This study compared the seasonal variations of macro-invertebrate diversity and analysed the physiochemical parameters to study the influence of surrounding land use on the lentic ecosystem of Adha Lake. Macro-invertebrate abundance in the lake was used as an indicator to assess the effect of surrounding land use. The lake was categorised into four major zones namely agriculture zone, forest east zone, catchment zone, and forest west zone. Sampling was carried out along the littoral zone of the lake. Physiochemical variables were collected for both the seasons. *Chironomidae* and *Baetidae* families were the most dominant macro-invertebrates in the lake. The least families encountered were *Acrididae, Aeshnidae, Tabanidae, Hydrophilidae,* and *Libellulidae* during monsoon season, and *Simuliidae* and *Culicidae* for post-monsoon season. There was no significant difference in Shannon Wiener's Diversity Index for monsoon and post-monsoon seasons, $p > .05$. pH, salinity, conductivity, total dissolved solid, and water temperature had negative correlation with diversity and richness; however, total dissolved solid, water temperature, and pH had positive association with taxon evenness. The HKH-bios index and NHBL index indicated that the lake is polluted which could be attributed to discharge from the paddy fields. Restoration and protection of Adha Lake as White-bellied heron's habitat may need significant conservation and advocacy measures.

Keywords: macro-invertebrate, pH, salinity, temperature, water conductivity, White-bellied Heron

Introduction

Freshwaters are among the most threatened habitat types in the world (Kasangaki *et al*., 2008). It is observed to have declined by 50% between 1970 and 2000 in the Living Planet Index for freshwater ecosystems (Millennium Ecosystem Assessment, 2005). The aquatic scientists and the conservationists are challenged on how to determine the influence of human activities on the structure and function of aquatic ecosystems (Sutherland *et al*., 2002). Potential solution to the above problem relies on understanding the functional relationship between changing landscape, physiochemical conditions, and biotic assemblages of rivers.

Adha Lake is a typical low altitude (1300 m asl) water body having socio-cultural and conservation significance, and is located in Jigme Singye Wangchuck National Park (JSWNP). The lake is surrounded by paddy terraces in the north, forests on the east and west, and forest covered catchment area feeding the lake in the

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southeast. It falls in a semi-arid forest zone comprising of chirpine forest (*Pinus roxburghii* Sarg.) and cool broadleaved forest; and functions as a water hole for wild animals during migratory season.

Adha Lake is contaminated during monsoon when paddy cultivation discharges nutrient rich water overflows into the lake. The lake area adjoining the terraced paddy field shows signs of eutrophication with rich algal assemblages. Livestock grazing in the catchment area adds additional nutrient load, mainly from the animal droppings. These processes, if not intervened, are likely to have significant impact on the lake ecosystem as a whole, especially on the survival of the Critically Endangered White-bellied heron (*Ardea insignis* Hume) and keystone species such as the Mountain hawk-eagle (*Nisaetus nipalensis* Hodgson) inhabiting the lake (JSWNP, 2014). The lake also has two species of fish, the Copper Mahseer (*Neolissochilus hexagonolepis* McClelland) and Common Carp (*Cyprinus carpio* Linneaus). Paddy cultivation discharge, cattle grazing around the lake, and fish population is likely to affect the water quality of the lake. In this study, water quality of the lake was investigated using macro-invertebrate communities as biotic indicator of water quality.

Materials and Method

Study area

Adha Lake (90° 6' 32.49E and 27°17' 32.92N) is a low altitude lake (1300 m asl) located in Wangdue Phodrang District under the jurisdiction of Jigme Singye Wangchuck National Park (Figure 1). The lake is 216 m long and 99.3 m wide with a surface area of 2.38 hectares.

Major zones around the lake

Macro-invertebrate habitats are strongly influenced by vegetation characteristics, depth, and complexity of the substrates present (Efitre *et al*., 2001; Korte *et al*., 2010). Degradation of vegetation affects hydrological discharge regimes in the lake, which increases temperature, sedimentation process, and affects the existence of benthic rheophilic and their habitats

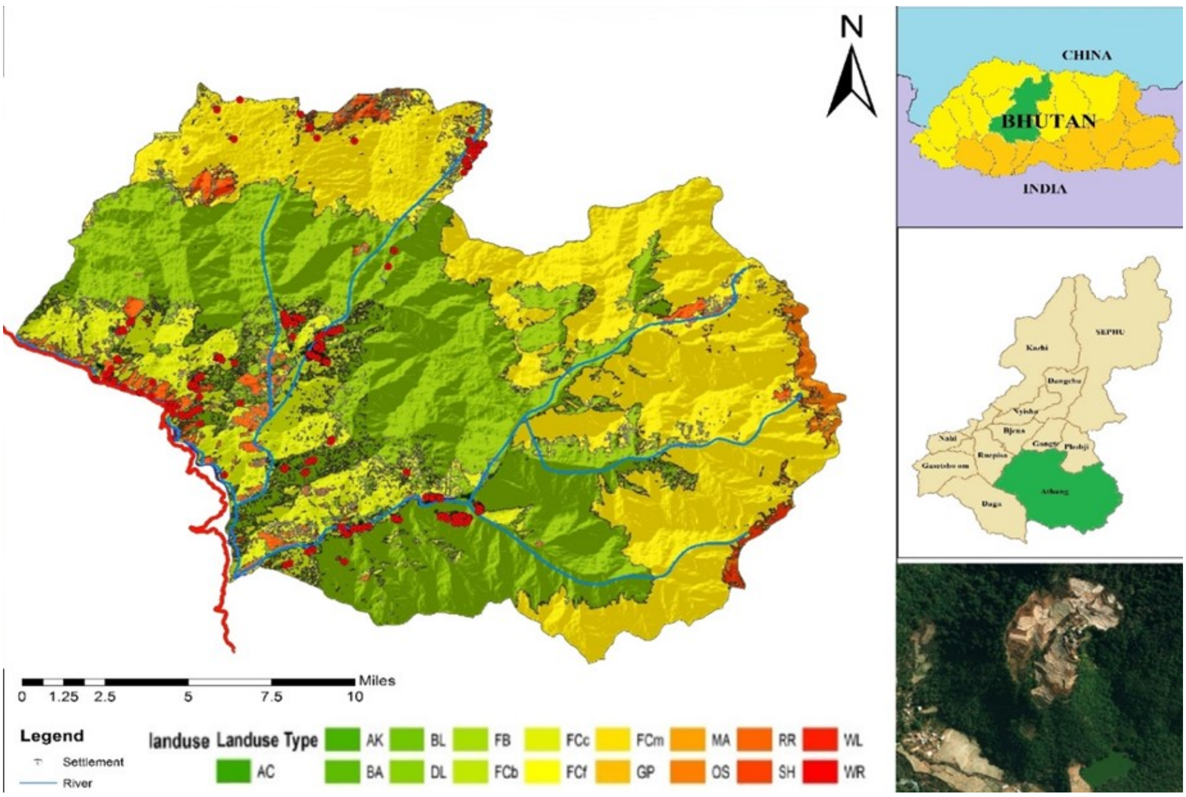


Figure 1: Map showing study area (Adha village)

(Sutherland *et al*., 2002). For the study purpose, based on the physical land use characteristics, the lake was divided into four major sampling zones as presented in Figure 2.

Agriculture zone

This zone consists of paddy terraces located in the north, where surface runoff from the uphill terraces drains into the lake during monsoon. At the site, the substrates composed of coarse gravels, sandy bottom, boulder rocks, cobbles, and aquatic flora. Agriculture is the main land use in the vicinity, though there are some grassland areas.

Forest east zone

This zone is located along the eastern side of the lake. Leaf litters and woody logs dominated the lake substrates with patchy areas covered by emergent and submerged aquatic flora. The surrounding riparian vegetation had closed canopy with trees and woody shrubs. The soil primarily comprised of humus and loam.

Catchment zone

Feeding water into the lake, the catchment zone is located in the southeast. The soil is sandy loam dominated by *Ageratina adenophora* (Spreng.) King & H. Rob. and the surrounding riparian vegetation is dominated by *Alnus nepalensis* D. Don. The area was degraded due to uncontrolled cattle grazing.

Forest west zone

The riparian vegetation is dominated by *Quercus griffithii* Hook. f. & Thomson ex Miq. and other cool broadleaved species. The lake substrate is dominated by leaf litters. This zone has the lake outlet and is relatively influenced by human activities such as worshiping and religious ceremonies, and has tradition trail connecting villages.

Sampling of macro-invertebrates

In this study, the major zones were assigned with transects along the littoral zone of the lake

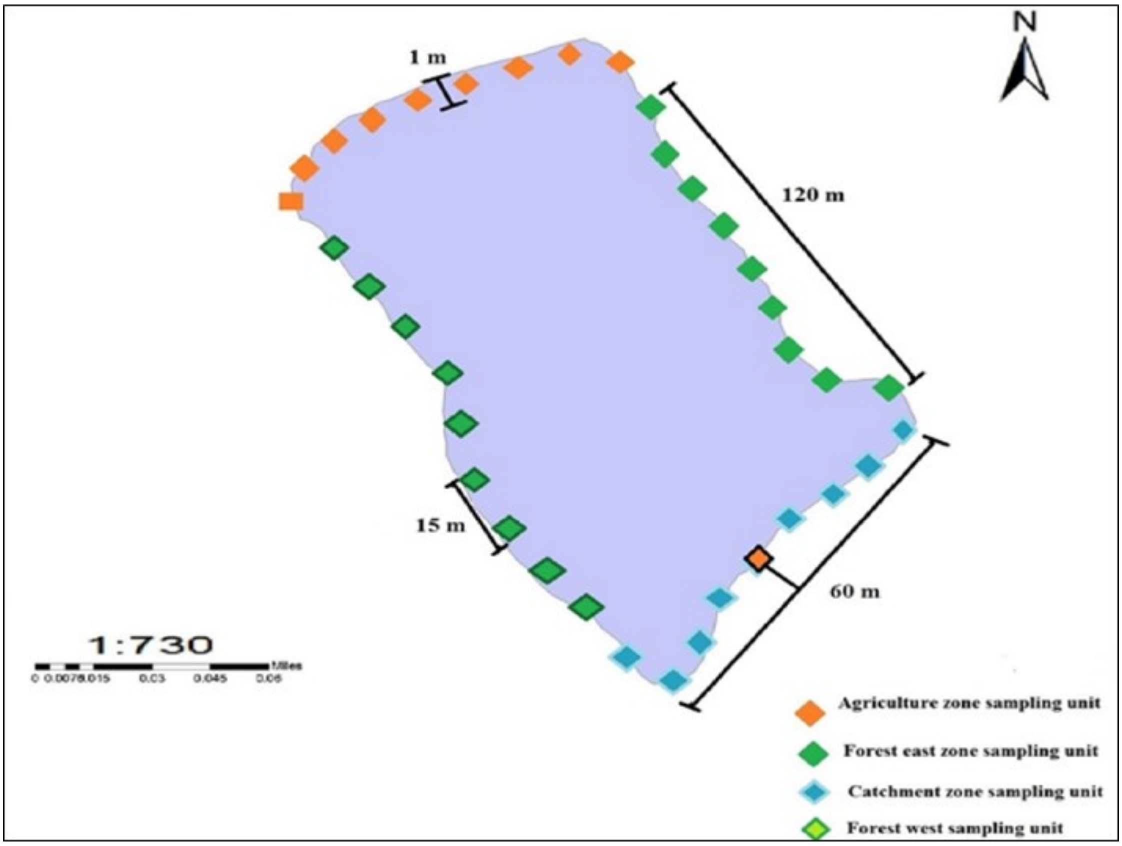


Figure 2: Sampling layout in Adha Lake (study area)

(Figure 2). Sampling plots were systematically laid at an interval of 15 m. Samplings were carried out from July 2016 to September 2016 (monsoon) and from December 2016 to February 2017 (post monsoon). Kicking/lifting, brushing, and rubbing of bedrocks and boulders were done; and the dislodged macro-invertebrates were swept into the 500-µm mesh D frame net. In woody debris and leaf litter sites, the samples collected were washed thoroughly into the net and specimens recovered using forceps. Macro-invertebrates were transferred into labelled containers containing 70% ethanol. The sampled macro-invertebrates were identified to operational taxonomic level by using HKH field keys (Hartmann, 2006) and other available guides such as Bosquet (1990); Choate (1999); Pescador *et al*. (2000); and Nesemann *et al*. (2011). Physiochemical parameters such as temperature, pH, conductivity, total dissolved salt and salinity were measured in the field with the help of soil and water testing kits (*PCS* tester) at the sampling sites before sampling macro-invertebrates.

Data analysis

Macro-invertebrate diversity between seasons as well as between major zones was computed using Shannon Wieners Diversity Index ($H' = -\Sigma Pi*\ln Pi$). The taxon richness was computed using $R = (S-1)/\text{LogN}$ (Wilson, 1992) and Evenness using Pielou evenness formula $E = H'/H\max$ (McGinley, 2014). Diversity, richness, and evenness were compared using Mann-Whitney test between seasons and Kruskal Wallis test among major zones using SPSS programme. Excel spreadsheet and *PC ORD* software were used to analyse various parameters of major zones of the monsoon and post-monsoon season. Spearman's rho correlation test was used to see the association between macro-invertebrate diversity, evenness and richness with physiochemical parameters. The water quality at each sampling site was assessed using Nepal Lake Biotic Index (Shah *et al*., 2011) which is computed as:

$$NLBI = \frac{\Sigma^{n}{}_{i} TTSi}{n}$$

Results and Discussion

Macro-invertebrate diversity

A total of 1,009 macro-invertebrates belonging to 8 taxonomic orders and 2 sub-orders comprising of 20 families (Table 1) were recorded in the study area. Taxa belonging to the *Chironomidae* (n = 413, *RA* = 40.93) was the most common family observed in all the sites followed by *Baetidae* (n = 173, *RA* = 17.15). This could be because the lake's littoral zone was degraded and polluted with high disposal of leaf litters, twigs, and logs from surrounding vegetation. Also, surface runoff during rainy season from the surrounding agriculture land use added silts and nutrient loads. These probably might have led to low oxygen level, which supported *Chironomidae* and *Baetidae* taxa in the study area. Coffman and Ferrington Jr. (1996) also confirmed that these families are found in habitats with low oxygen level and in relatively heavily polluted areas, feeding on small particles of organic debris. Members of these families are common in mud, silts and soft sediments where they build tubes or tunnels as refuges (Foote, 1987). *Baetidae* are scrapers, feeding on algae and detritus. They are also tolerant to temperature fluctuation (Morihara and McCafferty, 1979); which seems to be the case in Adha Lake since discharges from agriculture paddies seem to change the water temperature frequently.

Cluster analysis

The cluster analysis based on the relative abundance of taxa found in eight major zones for two seasons showed two major habitats. The forest east zone and forest west zone of monsoon season and west forest of post monsoon had similar family composition with 50% similarity. The dominant families were *Chironomidae, Synlestidae, Naididae,* and *Gerridae* (Figure 3). The similarity among these zones could be due to similarity in riparian vegetation, mostly domi-

Table 1: Taxa and relative abundance of macro-invertebrates in the study area

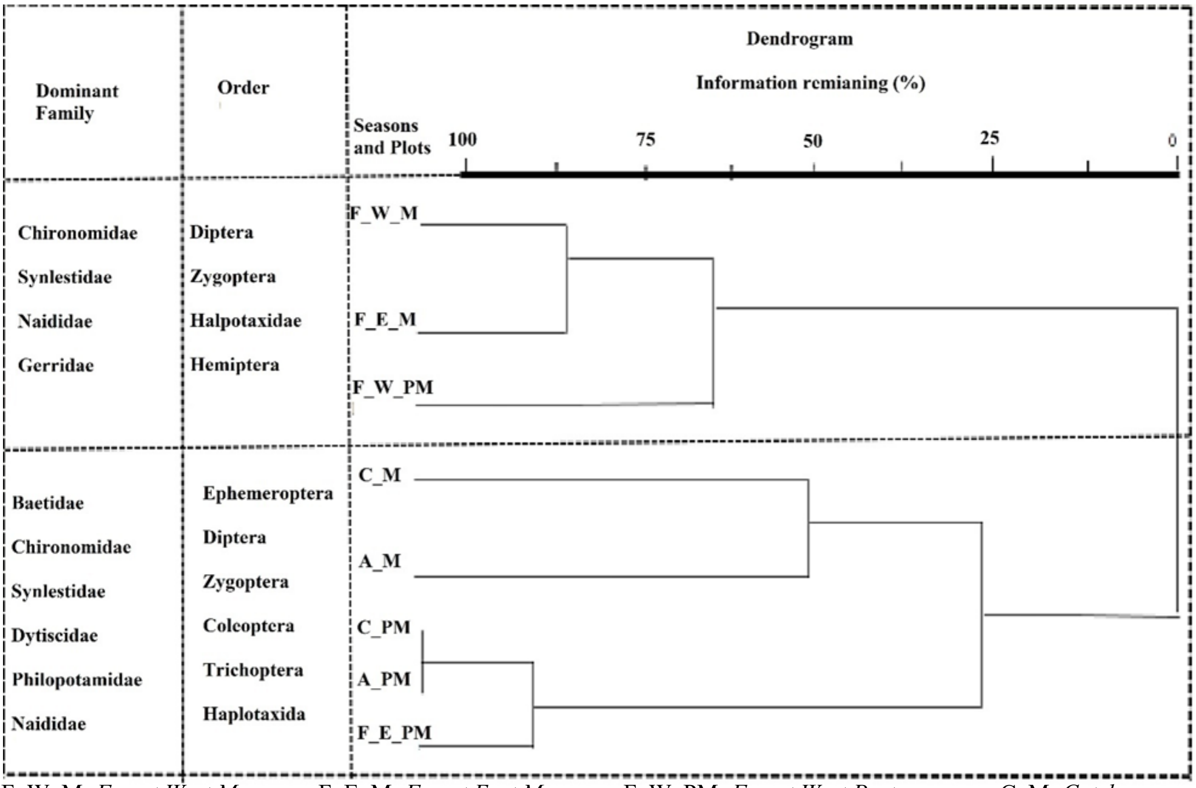
Order	Sub-order	Family	Count	RA%
Odonata	Zygoptera	Protoneuridae	17	1.68
		Synlestidae	150	14.87
	Anisoptera	Gomphidae	10	0.99
		Libellulidae	1	0.1
		Macromiidae	4	0.4
		Aeshnidae	1	0.1
Haplotaxida		Naididae	117	11.6
Ephemeroptera		Baetidae	173	17.15
Hemiptera		Gerridae	44	4.36
Coleoptera		Noteridae	7	0.69
		Hydrophilidae	1	0.1
		Dytiscidae	30	2.97
Diptera		Tabanidae	1	0.1
		Chironomidae	413	40.93
		Culicidae	2	0.2
		Simuliidae	1	0.1
Trichoptera		Helicopsychidae	11	1.09
		Lepidostomatidae	18	1.78
		Philopotamidae	7	0.69
Orthoptera		Acrididae	1	0.1
		Total	**1,009**	**100**

nated by old growth trees, and same land use influence received throughout the seasons. The surrounding vegetation cover provides similar substrate composition in the lake, where macro-invertebrate distribution is strongly influenced by complexity of substrate, depth and vegetation characteristic (Efitre *et al*., 2001).

Similarly, catchment zone monsoon and agriculture zone monsoon were indicated as sister clade with 50% similarity in relative macro-invertebrate abundance and catchment zone post monsoon and agriculture zone post monsoon had close to 100% similarity (Figure 3). This was probably because of similar influences received from surrounding land use. The nutrient rich surface runoff from the paddy field during monsoon and surface runoff from cattle grazing over areas near catchment zone could have resulted in similar habitats in these two zones, having similar macro-invertebrate abundance.

Macro-invertebrate diversity

There were small differences in macro-invertebrate diversity between the two seasons as indicated by Shannon Diversity Index (H' = 1.72), Evenness ($E_{H=}$.62), and Richness (S_R= 5.63) for monsoon season and Diversity (H' = 1.73), Evenness ($E_{H=}$.67) and Richness (S_R= 4.38) for post monsoon season (Figure 4). However, Mann-Whitney test indicated no significant seasonal variations for Diversity (U = 6, p = .567), (Med = 1.51, SE = .051, CI = 95%), Evenness (U = 8, p = .1), (Med = 1.66, SE = .084, CI = 95%) and Richness (U = 8, p = .1), (Med = 8.00, SE = .828, CI = 95%) for monsoon and post monsoon season. In Garhwal Himalaya, Uttarakhand, diversity of macro-invertebrates was more during post monsoon season (Negi and Mamgain, 2013). The diversity of macro-invertebrates is influenced by surrounding land use that affects wa-



F_W_M: *Forest West Monsoon*, F_E_M: *Forest East Monsoon*, F_W_PM: *Forest West Post monsoon*, C_M: *Catchment Monsoon*, A_M: *Agriculture Monsoon*, C_PM: *Catchment Post Monsoon*, A_PM: *Agriculture Post Monsoon*, F_E_PM: *Forest East Post Monsoon*.

Figure 3: Cluster analysis of taxa based on major zones

ter pH, temperature, salinity, and dissolved oxygen (Dhakal, 2006). Whereas in the current study, the lake is fed by perennial water sources so the water volume remains almost constant throughout the year. This might explain why the diversity between monsoon and post monsoon remains similar.

Relationship between macro-invertebrates and physiochemical parameters

Macro-invertebrates have certain optimum ranges to survive and breed; however, in Adha Lake, the ordination (non-matrix multidimensional scaling) graph showed negative correlation between diversity indices and physiochemical parameters (Figure 5); pH (r_s = -.17, p = .69), temperature (r_s = -.55, p = .16), salinity (r_s = -.69, p = .06), TDS (r_s = -.55, p = .594), and conductivity (r_s = -.19, p = .65) (Table 2). The surrounding land use pattern may have influenced the optimum range of physiochemical parameters required for macroinvertebrates throughout the seasons. Similarly, there was a weak negative correlation between taxon richness and physiochemical parameters (Figure 5); pH (r_s = -.21, p = .61), temperature (r_s = -.29, p = .49), salinity (r_s = -.45, p = .26), TDS (r_s = -.45, p = .26), and conductivity (r_s = -.05, p = .91) (Table 2).

The surface runoff during monsoon season from surrounding land could have enriched nutrients in the lake, increased its turbidity, and changed the optimum range of physiochemical parameter requirements. This may

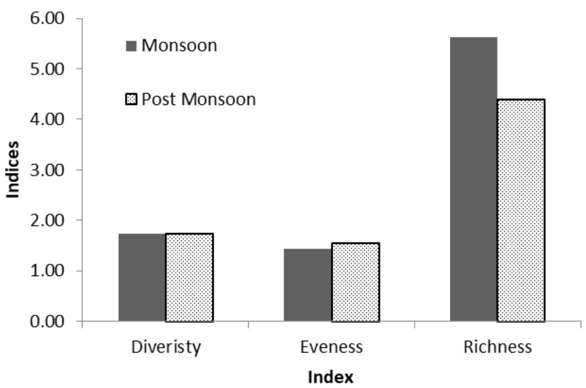


Figure 4: Shannon Wiener Diversity, Evenness and Richness of macro-invertebrates

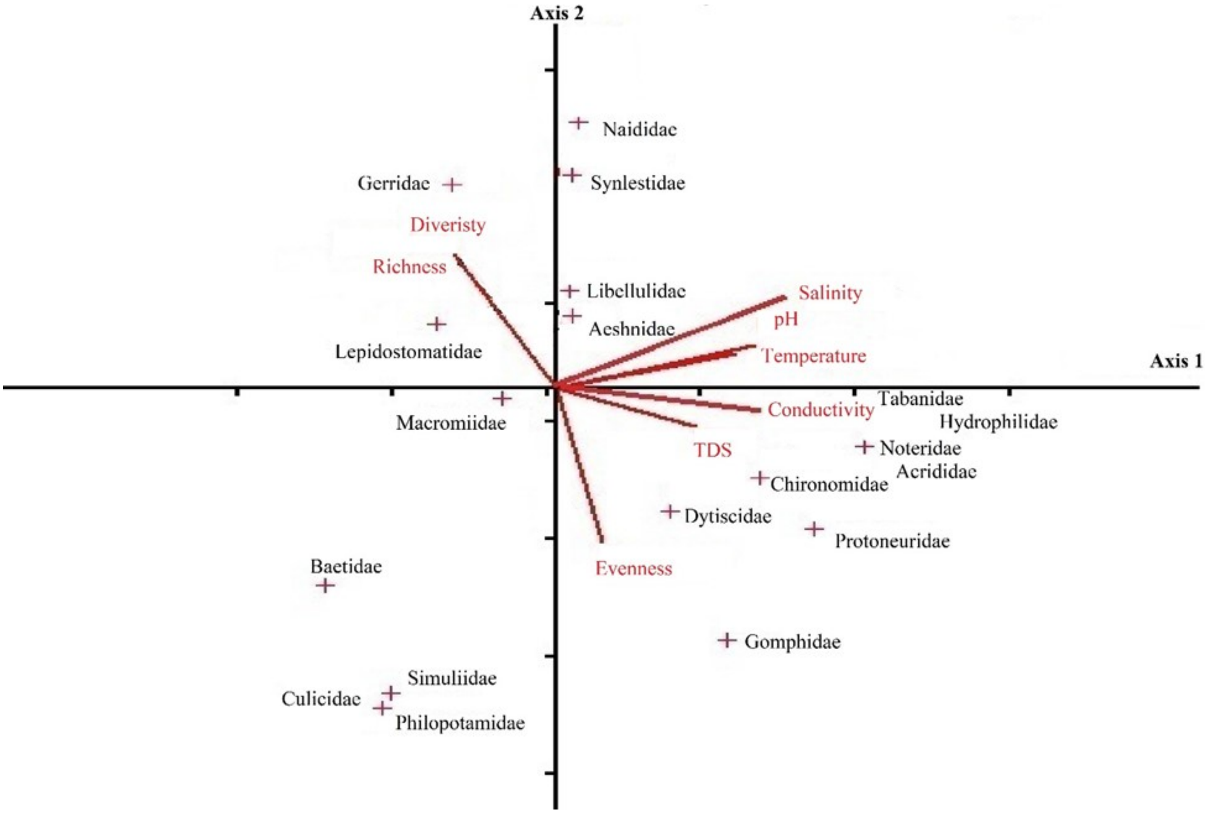


Figure 5: Ordination graph (NMS) showing relationship between Shannon Diversity Index and physio-chemical parameters

The ordination graph (NMS) indicated that there was non-significant ($p > .05$) negative correlation between the taxa diversity and the salinity, TDS and conductivity of the study area (Figure 5). This may be due to accumulating osmotic stress from salt concentration which wipes out taxa from the habitat. According to Dunlop *et al*. (2005), salt and turbidity have pronounced direct toxic effects and indirect ecological effects on freshwater biota above certain thresholds. The current findings support the study by Brraich and Kaur (2017) in Nangal

Table 2: Spearman correlation between diversity indices and physiochemical parameters

	pH	Temp.	Salinity	TDS	Conduct.	Diversity	Evenness	Richness
pH	1	.762*	0.67	0.33	-0.24	-0.17	0.19	-0.21
		0.03	0.07	0.42	0.57	0.69	0.65	0.61
Temp.		1	.786*	0.24	-0.17	-0.55	0.05	-0.29
			0.02	0.57	0.69	0.16	0.91	0.49
Salinity			1	0.57	0.29	-0.69	-0.02	-0.45
				0.14	0.49	0.06	0.96	0.26
TDS				1	0.48	-0.55	0.05	-0.45
					0.23	0.16	0.91	0.26
Conduct.					1	-0.19	-0.26	-0.05
						0.65	0.53	0.91
Diversity						1	0.21	0.43
							0.61	0.29
Evenness							1	-.762*
								0.03
Richness								1

wetland, India, where water temperature, electrical conductivity, TDS, alkalinity, and salinity are negatively correlated with macro-benthic organisms.

The pH (r_s = .19, p = .65), temperature (r_s = .05, p = .91), and TDS (r_s = .05, p = .91) have positive association with taxa evenness while salinity (r_s = -.02, p = .96) and conductivity (r_s = -.26, p = .53) showed negative association (Table 2). The positive relationship could be because these physiochemical parameters suppress the diversity and richness of taxa leading to uniform and even distribution. The salinity and conductivity have negative correlation with taxa evenness, as the distribution of taxa within the habitat are likely to be hampered by salt concentration.

Physiochemical variables

There was significant differences in temperature (U = .001, p = .001), (Med = 20.75), pH (U = .001, p = .001), (Med = 7.65), salinity (U = 271, p = .001), (Med = 46.5), TDS (U = 304.5, p = .005), (Med = 49), and conductivity (U = 258, p = .001), (Med = 69.1) between monsoon and post monsoon seasons. Similar findings were reported by Alagoa and Aleleye-Wokoma (2012), where they found that there is a significant seasonal difference in physiochemical parameters of temperature, turbidity, and pH. The difference in these physiochemical parameter variables, especially pH and salinity in the study area, could be due to high turbidity attributed to surface runoff from paddy field into lake during monsoon, where nutrient rich water is drained making water slightly alkaline.

In addition, the edges of lake in two other zones (forest zone east and west) were covered with debris like leaf litters, twigs and rotten logs fallen directly into the lake. Besides this, the strategic location of the lake at the bottom of a valley collects all the organic surface runoff from surrounding land uses. During post monsoon, the agriculture land remained uncultivated and received less to no precipitation, thereby reducing surface runoff. The water is slightly acidic during post monsoon.

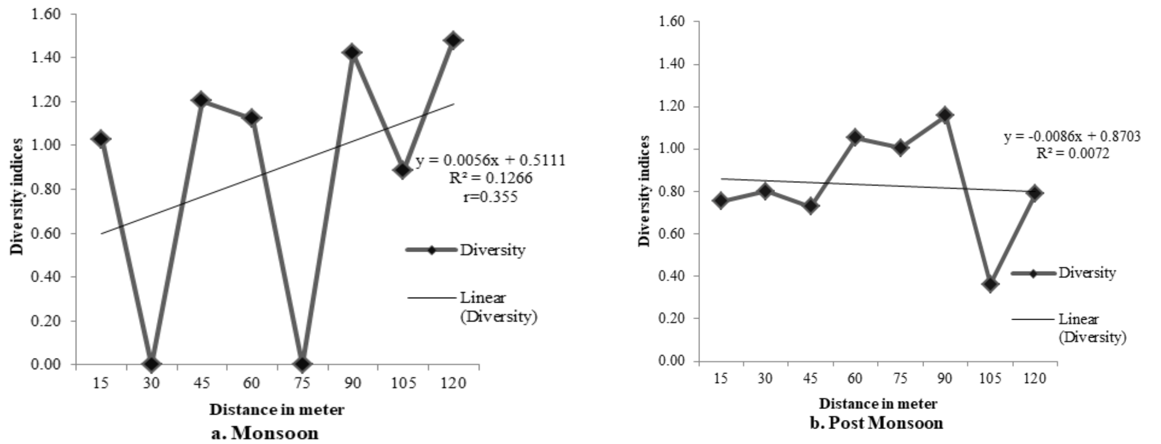


Figure 6: Association between diversity and distance to agriculture zone

Chatzinikolaou and Lazaridou (2007) mentioned that constant constructions together with contamination from agriculture runoff have direct relation with the water pH and siltation. Besides contamination from anthropogenic activities, the vegetation along the water bodies have maximum influences on physiochemical parameters including water pH (Fernandez-Diaz and Benetti, 2008; Dorji, 2014). The study by Zinabu (2002) also made a similar finding where salinity and total ions seemed to increase during the wet season in some lakes.

Macro-invertebrate diversity and agriculture

Regression analysis showed weak association between macro-invertebrate diversity and distance from agriculture zone; R^2 = 0.1266 for

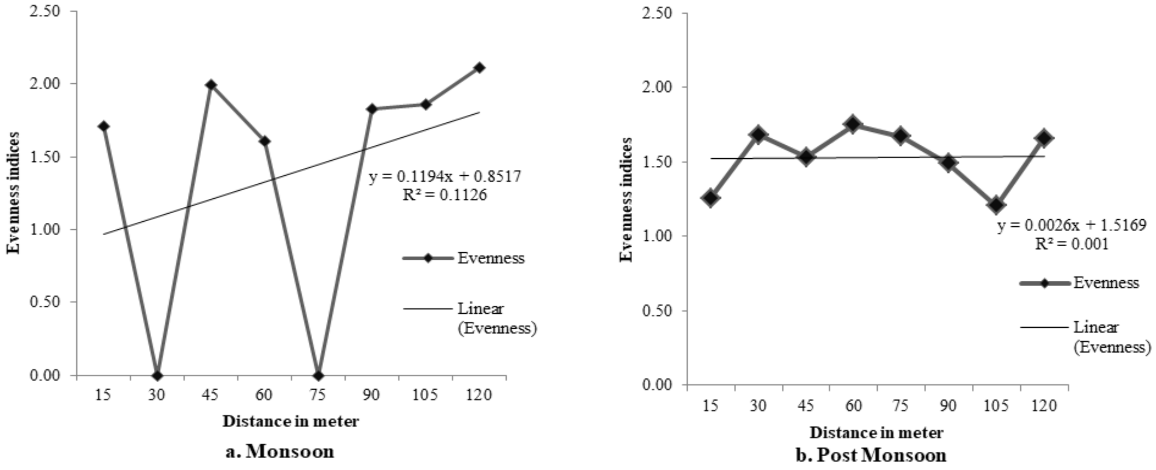


Figure 7: Association between evenness and distance to agriculture zone

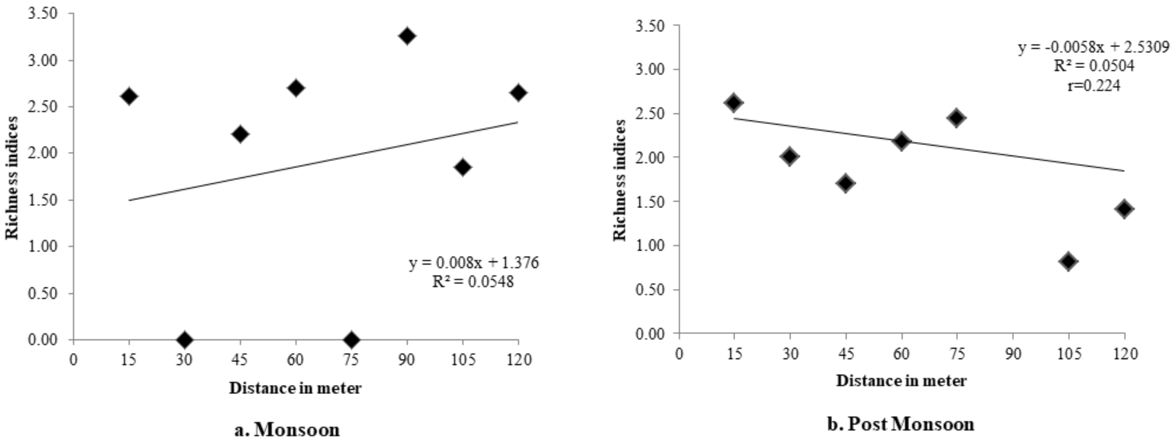


Figure 8: Association between richness and distance to agriculture zone

monsoon and R^2 = 0.0072 for post monsoon (Figure 6). There was also weak association between taxa evenness and agriculture zone for monsoon (R^2 = 0.112) and post monsoon (R^2 = 0.001) season (Figure 7). Similarly, there was a weak association between taxa richness and distance to agriculture during monsoon (R^2 = 0.054) and post monsoon (R^2 = 0.054) (Figure 8).

This shows that the agriculture zone has lesser influence on the diversity index of macroinvertebrates for monsoon as well as post monsoon seasons (Figure 9). According to Stubbington *et al*. (2009), there is a significant impact on lake due to change in land use, although the abundance of certain species is said to increase, the diversity and species richness are said to be decreasing (Efitre *et al*., 2001).

Lake water quality

The overall water quality assessment using Nepal Lake Biotic Index (NLBI) indicated that the lake was heavily polluted (lake water quality class (LWQC) = 3.15) (Table 3). The poor water quality could be due to stagnant water with small volume inflow and outflow. Moreover, the lake receives significant amount of inorganic surface runoff from surrounding paddy field during monsoon. The nutrient inflow enrich water making fit for surface algae bloom (Chatzinikolaou and Lazaridou, 2007; Benetti and Garrido, 2010). Leaf litters and vegetative materials such as twigs and logs were observed inside the lake from surrounding vegetation cover (forest east and west zone), which also aid in making water high in productivity (Hepp and Santos, 2009; Dorji, 2014). In such scenario, less macro -invertebrate thrives in the lake which in turn is likely to affect habitat quality.

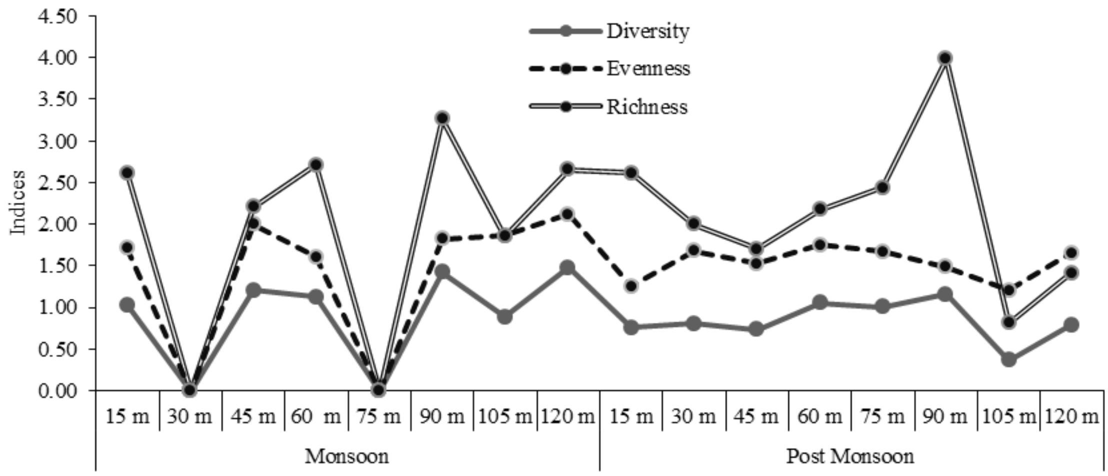


Figure 9: Shannon Wiener Diversity index and distance to agriculture zone

Table 3: Transformation scale of NLBI to LWQC degree of pollution and colour code

NLBI	*LWQC*	*Degree of pollution*	*Colour code*
6.10–10.00	High	None to minimal	Blue
4.91–6.09	Good	Slightly	Green
4.00–4.90	Fair	Moderately	Yellow
2.00–3.99	Poor	Heavily	Orange
0.00–1.99	Bad	Extremely	Red

Conclusions

Anthropogenic land disturbances have significant effect on functioning of aquatic ecosystem. Macro-invertebrate in lentic water system plays important role in maintaining the condition and structure of lake by decomposing organic matter deposited in the lake. In this study, assessment of macro-invertebrate diversity and water quality in Adha Lake was carried out as it inhabits endangered animals and has socio-cultural significance. Eight orders and twenty families of macro -invertebrates were identified in Adha Lake. *Chironomidae* family was the most dominant taxa in all the major zones followed by *Baetidae* family. Families represented with less diversity were *Acrididae, Aeshnidae, Tabanidae, Hydrophilidae, Libellulidae, Simuliidae* and *Culicidae.* There was no significant difference in diversity index between monsoon and post monsoon season ($p > .05$), however there was relatively high taxa richness in catchment zone compared to other zones. Also, associations of diversity and richness with physiochemical parameters were not significant. However, there were significant differences between the physiochemical parameters of the two seasons, $p < .05$. The surrounding land use had negative influence on water quality and the water quality as per the NLBI assessment was poor and heavily polluted. Therefore, if priority is given to support the conservation of White-bellied heron's habitat in and around the lake, then it is critical to manage and restore the water quality of the lake.

The study is first of its kind in exploring the limnology of low altitude lake in Bhutan, thus, this study may be only used as baseline information to study low altitude lakes in chirpine and broadleaved forest zones. Since the study was conducted for two seasons only, there is a need to conduct the study for all the four seasons of a year. Besides HKH-bios index and NLBI scoring system, a comprehensive study of macro-invertebrate to develop similar index for Bhutan is essential.

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References

Alagoa, K.J. and Aleleye-Wokoma, I.P. (2012). Human Induced Variations of Selected Physico- chemical Parameters of Taylor Creek in the Niger Delta, Bayelsa State, Nigeria, *Resources and Environment*. 2(2): 45-50. doi: 10.5923/j.re.20120202.07

Benetti, C.J. and Garrido, J. (2010). The influence of stream habitat and water quality on water beetles assemblages in two rivers in northwest Spain, *Vieet Milieu.* 60(1): 53-63.

Bosquet, Y. (1990). *Beetles associated with stored products in Canada: an identification guide.* Agriculture Canada.

Brraich, O.S. and Kaur, R. (2017). Temporal composition and distribution of benthic macro- invertebrates in wetlands, *Current Science*. 112(1): 116-125. DOI: 10.18520/cs/v112/i01/ 116-125.

Chatzinikolaou, Y. and Lazaridou, M. (2007). Identification of the self-purification stretches of the Pinios River, Central Greece, *Mediterranean Marine Science*. 8(2): 19-32.

Choate, P.M. (1999). Introduction to the identification of beetles (Coleoptera), Dichotomous Keys to some Families of Florida Coleoptera, *Entomo Mont Mag*. 102: 156-162.

Clenaghan, C., Giller, P.S., Ohalloran, J. and Hernan, R. (1998). Stream macroinvertebrate communities in a conifer-afforested catchment in Ireland: relationships to physico-chemical and biotic factors, *Freshwater Biology*. 40(1): 175-193. DOI: 10.1046/j.1365-2427.1998.00330.x.

Coffman, W.P. and FerringtonJr, L.C. (1996). Chironomidae, *An introduction to the aquatic insects of North America*. 3: 635-754.

Dhakal, S. (2006). Study on Physiochemical Parameters and Benthic Macroinvertebrates of Balkhu Khola in Kathmandu Valley, Central Nepal: Management of water, wastewater and environment, Challenges for the developing countries, Kathmandu. Nepal.

Dorji. T. (2014). *Macroinvertebrates diversity in response to environmental variables in headwater streams* (Project Report). Royal University of Bhutan: Thimphu.

Dunlop, J., McGregor, G. and Horrigan, N. (2005). *Potential impacts of salinity and turbidity in riverine ecosystems*. Queensland. ISBN: 1 74172 0788

Efitre, J., Chapman, L.J. and Makanga, B. (2001). The inshore benthic macroinvertebrates of lake Nabugabo, Uganda: seasonal and spatial patterns, *African Zoology.* doi:org/10.1080/ 15627020.2001.11657139.

Fernandez-Diaz, M. and Benetti, C. (2008).*Influence of iron and nitrate concentration in water on aquatic Coleoptera community structure: application to the Avia river.* Spain: Ourense.

Foote, B. (1987). Chironomidae, *Immature Insects*. 762-764.

Hartmann, A. (2006). *Key to Odonata (Dragonflies and Damselflies) of the HKH Region*. Presented in Regional Capacity Building Workshop on the Macro-Invertebrates' Taxonomy and Systematics for Evaluating for the Ecological Status of Rivers in the Hindu Kush-Himalayan (HKH) Region.

Hepp, L.U. and Santos, S. (2009). Benthic communities of streams related to different land uses in a hydrographic basin in southern Brazil, *Environmental monitoring and Assessment*. 157(1-4): 305-318. DOI:

10.1007/s10661-008-0536-7.
Jigme Singye Wangchuck Nation Park. (2014). *Jigme Singye Wangchuck National Park: Biodiversity action plan.* Department of Forest and Park Services, Ministry of Agriculture and Forest: Royal Government of Bhutan.

Kasangaki, A., Chapman, L.J. and Balirwa, J. (2008). Land use and the ecology of benthic macro- invertebrate assemblages of high-altitude rainforest streams in Uganda, *Freshwater Biology*. 53(4): 681-697. doi:10.1111/j.1365-2427.2007.01925.x.

Korte, T., Baki, A.B.M., Ofenböck, T., Moog, O., Sharma, S. and Hering, D. (2010). Assessing river ecological quality using benthic macroinvertebrates in the Hindu Kush-Himalayan region. *Hydrobiologia*. 651(1): 59-76.

McGinley, M. (2014). Species richness. <http://:www.eoearth.org>. Accessed on March 15, 2016.

Millennium Ecosystem Assessment. (2005). *Ecosystems and Human Well-being: Wetlands and Water Synthesis*. Washington, DC: World Resources Institute.

Morihara, D.K. and McCafferty, W.P. (1979). The Baetis larvae of North America (Ephemero- ptera: Baetidae), *Transactions of the American Entomological Society.* 105(2): 139-221.

Negi, R.K. and Mamgain, S. (2013). Seasonal variation of benthic macro invertebrates from Tons River of Garhwal Himalaya Uttarakhand, *Pakistan Journal of Biological Sciences*. 16(22): 1510. DOI:10.3923/pjbs.2013.1510.1516.

Nesemann, H., Shah, R.D.T. and Shah, D.N. (2011). Key to the larval stages of common Odonata of Hindu Kush Himalaya, with short notes on habitats and ecology, *Journal of threatened Taxa*. 3(9): 2045-2060. doi: 10.11609/JoTT.02759.2045-60.

Pescador, M.L., Rasmussen, A.K., Richard, B.A. and McCarron, E. (2000). *A guide to the stoneflies (Plecoptera) of Florida*. State of Florida, Department of Environmental Protection, Division of Water Resource Management.

Shah, R.D.T., Shah, D.N. and Nesemann, H. (2011). Development of a macroinvertebrate-based Nepal Lake Biotic Index (NLBI): an applied method for assessing the ecological quality of lakes and reservoirs in Nepal, *International Journal of Hydrology Science and Technology*. 1(2): 125-146. doi: 10.1504/IJHST.2011.040744.

Stubbington, R., Wood, P.J. and Boulton, A.J. (2009). Low flow controls on benthic and hyporheic macroinvertebrate assemblages during supra-seasonal drought, *Hydrological Processes*. 23(15): 2252-2263. doi: 10.1002/hyp.7290.

Sutherland, A.B., Meyer, J.L. and Gardiner, E.P. (2002). Effects of land cover on sediment regime and fish assemblage structure in four southern Appalachian streams, *Freshwater Biology*. 47(9): 1791-1805. doi: 10.1046/j.1365-2427.2002.00927.x.

Wilson, E. (1992). *The diversity of life.* New York: W.W. Norton and Company INC.

Yuan, L.L. (2004). Assigning macroinvertebrate tolerance classifications using generalized additive models, *Freshwater Biology*. 49(5): 662-677. doi: 10.1111/j.1365-2427.2004. 01206.x.

Zinabu, G.M. (2002). The effects of wet and dry seasons on concentrations of solutes and phytoplankton biomass in seven Ethiopian rift-valley lakes, *Limnologica-Ecology and Management of Inland Waters*. 32(2): 169 -179. doi: http://dx.doi.org/10.1016/S0075-9511(02)80006-8.