Impact of anthropogenic activity on habitat of Zebra fish (*Danio devario*) in River Nimmi of Western Himalaya, Uttarakhand, India

Bipin Kumar Sati [1], Madhu Thapliyal [2] ✉ and Ashish Thapliyal[1]

Abstract

Uttarakhand is on fast growth path. Several hydro-electric power projects, etc., are being constructed leading to increase in demand of raw material for construction which is obtained by mining of river beds. River Nimmi is one tributary of river Yamuna, where river bed mining is rampant. This study investigated the impact of anthropogenic activity on the habitat of zebra fish that is known to survive in small pools of water for a long period of time. The Shannon-Weiner diversity index of all the study sites ranged from 1.361 to 1.566, Simpson index of dominance ranged from 0.6444 to 0.6805 and Simpson index of diversity (1-d) range was between 0.3422 to 0.42. Various physicochemical parameters of water from different sites did not show significant variation. Population of *Daniodevario* is falling rapidly which was recorded 129 in August 2012 to only in lower 40s in 2013 at site 2. This decline of population at site 2, that has maximum diversity, is mostly due to vanishing side pool due to anthropogenic activity. Our study shows that there is an urgent need to pay attention to the problem of habitat loss due to anthopogenicacitivty in river beds of Uttarakhand.

Keyword: Zebra fish, Anthropogenic activity, River bed mining, construction, habitat, Uttarakhand

Introduction

India is one of the 17 mega biodiversity countries of the world.The diverse geography and climatic conditions have carved varied ecological habitats like forests, grasslands, wetlands, coastal, marine and desert ecosystems that harbors and sustains biodiversity. (Pande and Arora 2014) Uttarakhand region, with its lofty mountain peaks of the Himalaya and its glaciers, is the origin of several rivers – e.g. the Ganges and the Yamuna. Each of these rivers has several big tributaries. The winding river valleys and mountain slopes of this region are home to variety of flora and fauna (Joshi 2004).

In the decade that followed the grant of statehood to Uttarakhand in the year 2000, the development activities in the states took off. Infrastructure and real estate development, triggered by the cash flow from tourism and the real estate moguls, have led to a phenomenal increase in demand for energy and construction related material. The energy demand skyroketed due to increase in growth of industrial units and population. To meet this demand, several hydroelectric power (HEP) projects have either been already constructed or are in the process of being constructed. A report of IIT Roorkee suggests that there is a potential of 22000 MW energy (IIT Report, 2011) in Uttarakhand& because of this the state has also been nick named as "Urja Pradesh". The hysterical drive for energy using HEP has been so hyped that the state authorities have planned to construct several dams on each of the rivers in Uttarakhand. To harness the maximum energy, these are 5 commissioned hydro-electric power projects, 13 hydro-electric power projects are in construction phase and 41 have been planned in Garhwal region. All this activity is within a radius of 200 km, upstream from Rishikesh on river Ganges (source- www.sandrp org.in, Fig.1) to achieve the energy potential target. Besides hydroelectric power projects, several road construction projects have also been completed or are underway. All these anthropogenic activity is already happening and some groups correlate construction of roads by blasting rocks in hills, construction of several hydroelectric power projects and mining in river beds to alterations in the fragile Himalayan environment (Saha2014). Hydro – electric power project are a major source income to Uttarakhand Tehri Hydropower Development Corporation (THDC) alone earned revenue of 5953 million during the year 2013-2014 and hence

Author's Address
[1]Department of Biotechnology, GEU, Dehradun, Uttarakhand
[2]Department of Zoology, Pt. LMS Govt. PG College, Rishikesh, Uttarakhand
E-mail:madhuthapliyal@gmail.com

these HEP's are a lucrative options. (THDC India report 2013-2014). Besides HEP projects, several roads, housing complexes, etc. are also being constructed.

The increase in demand for construction related material has led to indiscriminate mining of river beds throughout the state. The operations & extent of finance involved in all these activities including river bed mining is huge. With respect to mining of river bed, all sort of heavy equipment used in construction is used to load various types of vehicles with construction material like sand and stones (Fig-2a, 2b). As per a report on forest department of Uttarakhand, it is suggested that, in the year 2012, the state government generated a profit of Rs 300 -350 crore from the sites of river bed mining, which was spread to a total of 1608 hectare of land across all districts (Chakravarty 2013). The current profits of revenue generated from mining in current time are estimated over 500 crore. Besides legal mining of river beds, illegal mining is also wide spread. The finances in this segment are unknown but thought to be couple fold higher than the government revenue. One of the victims of this indiscriminate mining of natural resources is the habitat and ecological environment where these activity happen. Habitat is crucial for biodiversity where different species occupy different niches. Some of these species are important indicators of habitat disturbance. Ones such species that we investigated during the present study is Danio commonly known as Zebra fish. This fish is well known to survive in small pools of water for a long period time and is a native of streams of Uttarakhand as reported elswhere (Sterba 1962; Talwar and Jhingran 1991; Jayaram 1999; Daniels 2002). Zebra fish is a small shoaling, omnivorous, cyprinid fish. It is native to inland streams and rivers of India and is widely distributed in shallow, slow-flowing waters on the Indian subcontinent. It belongs to the minnow family (Cyprinidae) of order Cypriniformes (Spence *et al.* 2008). Zebra fishcan tolrate a wide range of temperature ranging from 6 ℃ (degree centigrade) to 38 ℃ and are relatively tolerant of human disturbance and are able to survive and reproduce well in altered habitats (Boisen *et al.* 2003; Engeszer *et al.* 2007; Mayden *et al.* 2007; Spence *et al.* 2008). Zebra fish is an important model on which several research are carried out by

large number of labs & researchers but the wild type zebra fish is found only in few geographical locations like north eastern part of Indiaand few locations in western Himalayan region including Uttarakhand region of India. In thepresent study, we used zebra fish as an indicator species for assessing impact of anthropogenic activity on river Nimmi, a tributary of river Yamuna, located in district Dehradun of Uttarakhand. This stream flows through the Pondha area of Dehradun. This area has seen a huge increase in construction activity due to upcoming new Universities, Schools and Colleges. Besides, individuals of Pondha area have also started construction on private property to provide the necessary accommodation to the increasing number of students. Hence this area was appropriate for investigation activity on habited of fish species. There are several reports that just in list zebra fish species in Uttarkhand and western Himalaya, but our report is among the first report, to be best of our knowledge, investigating impact of anthropogenic activity on habitat of zebra fishin Himalayan region of Uttarakhand.

Materials and Method
Study area
River Nimmi is a tributary of Yamuna, it originates in the foothill of Mussourie and merge into the Yamuna. The present study was carried out in a part of river Nimmi in district Dehradun of Uttarakhand. For establishing sampling location, the entire length of river Nimmi was scanned. A total of 8 locations were initially surveyed (Fig 3a & 3b). From these sites, we selected 3 sites (latitude 29'26'' 31'28" N longitude 77'49". 82'6" E) that were approximately 2 km apart.

Fish Population assessment
Fish population assessment was done every month using three pass technique, during morning hours between 6 to 10 AM, at all the three sites and different habitats (pool, run, and riffle, rapid). Sampling was done with the help of scoop net with mesh size 1.8mm to 4mm. All fish samples were identified on the basis of their morphological characters. Fish captured were released in the same habitat without any harm.

Identification of *Danio* species
To identify *Danio* species, fin sample was preserved in 95% ethanol before DNA

extraction(Fig 4) The fish was not harmed and only a small parts of the fin was clipped for sample. Genomic DNA was isolated from fin tissue by proteinase K digestion followed by a standard phenol–chloroform method. A specific segment of cytochrome Oxidase unit 1 (COI- 655 bp) was used as a molecular marker for identification of species. Polymerase Chain Reaction (PCR) for COI segment was performed as per Ward *et al.* 2005 total reaction volume of 50 μLwas constituted containing 20–50 ng template DNA, 5 μL of 10× PCR buffer, 5 μL of MgCl$_2$ (25 mmol/L), 1 μL of dNTP(10 mmol/L),10 pmol/of eachprimer FF:TTCTCCACCAACCACAARGAYATGGT(whereR=A/G,=YC/T)FR:CACCTCAGGGTGTCCGA

ARAAYCARAA (where R=A/G, =Y=C/T) and 2.5 units of *Taq* DNA polymerase (Thermo Fisher). PCR was performed on Eppendorf Mastercycler 5533 (Eppendorf, Germany). After PCR the samples was checked on 1.5% of Agarose gel. The PCR sample were stored and purified with Exonuclease 1 with and shrimp alkaline phosphatase (EXO1-SAP). The amplified fragment were sequenced and the sequences were analyzed by using sequence scape software v2.7 (Applied Bio system 3130 genetic analyzers). The name of the fish, collection code, sequence, trace file was then uploaded to National center for Biotechnology Information(NCBI) GenBank.

Fig1– Extent of anthropogenic activity specially construction of hydro–electro power projects in Garhwal Himalaya various projects planned or already build on Ganges and Yamuna or their tributaries. Map are also shown in the picture besides various other hydro–electric projects (source-sandrp.in)

Fig 2a– The figures showing heavy vehicles on River Yamuna / Nimmi basin Dehradun Uttarakhand, India Photo courtesy B. Sati

Fig 2b– Anthropogenic activity showing mining in river beds of Uttarakhand state
(Photo courtesy Samir Mehta)

Fig 3a– location of site as shown by Google map (from site 1 –site 8)
Fig 3b– major collection sites 1, site 2 and site 3 selected for sampling (located on Google map).

Fig 4– Photograph of *Zebra fish (Danio sp)* specimen sampled from site 2. This
specimen was identified as *Danio devario* by using mitochondrial COI marker
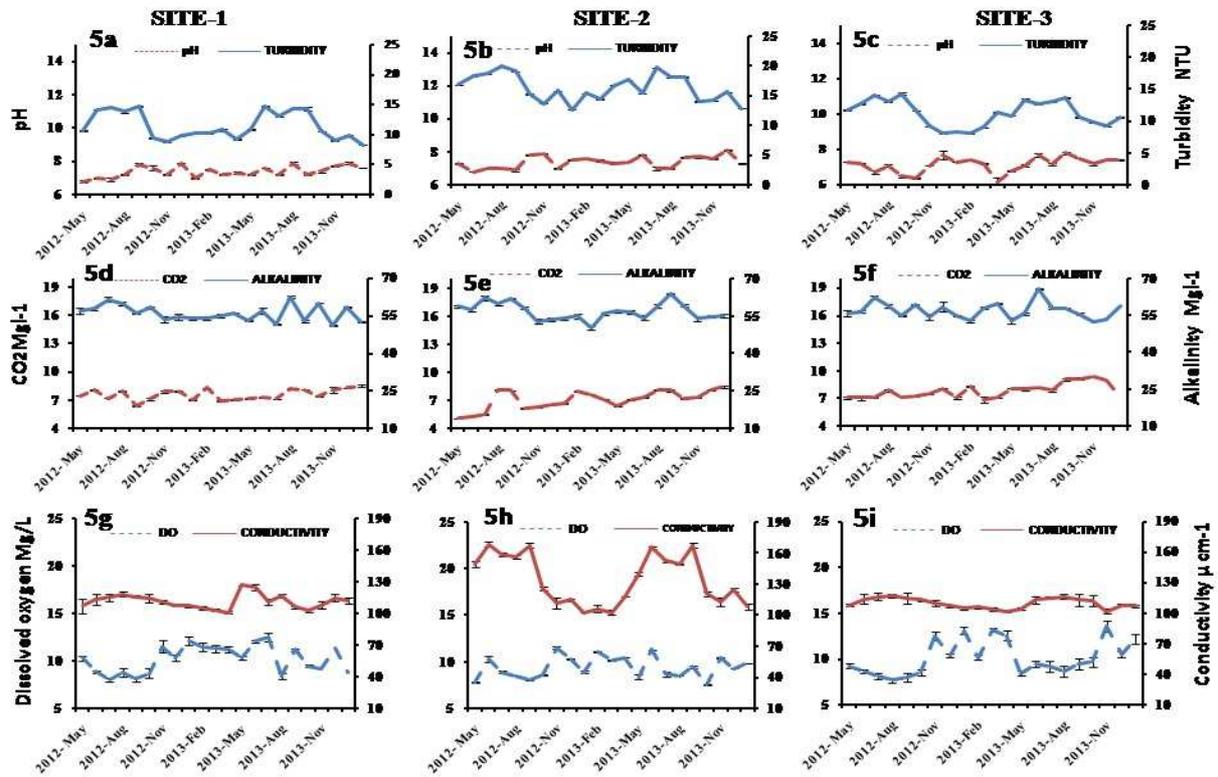(Gene bank accession number KR809737, KR809738)

Fig 5- Represent Two year data of physico chemical parameter from site 1,2,3 of river Nimmi. No major alteration of physico chemical parameters in river during the entire period of study. 5a, 5b and 5c are shows the variation of pH and turbidity parameters at site 1, 2 and 3 . Fig 5d, 5e and 5f showing the CO_2 and alkalinity in site 1,2,3 respectively during study time. Fig 5g, 5h and 5i showing the DO and conductivity at site 1,2,3 during study time period in river Nimmi respectively. 5h showing drastic changes in condutivitity on site 2.
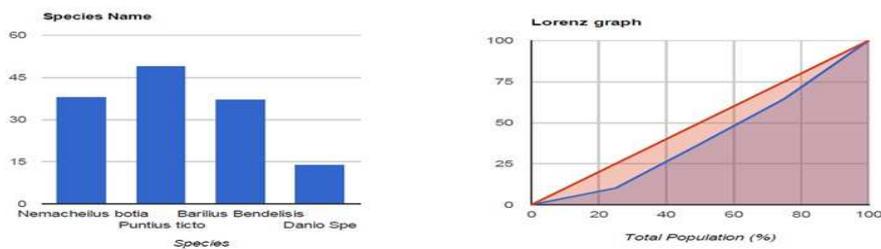

Fig 6- Fish species abundance and distribution in River Nimmi, Dehradun ,Uttarakhand.

Water Quality Analysis

The physicochemical parameters of water (pH, DO, CO_2, Alkalinity, Conductivity, and Turbidity) were determined as per standard protocols of APHA, 1995 and Trivedi and Goel 1984. Water was collected from 20 cm depth (at fixed times every day, as per APHA) from three study sites in the first week of every month for entire duration of study (May 2012 to January 2014).Details of the methods are follows:

Dissolved Oxygen (DO)

For dissolved oxygen, we used winkler's method. In short, we collected water sample and filled it in a BOD bottle and analyzed the sample at site. For determining DO content, we added 1 ml of $MnSO_4$followed by 1 ml of alkali-iodide-azide reagent. After the formation of a brown manganese hydroxide pricipate, we added 1 ml concentrated H_2SO_4. Then we mixed the contents by inverting several times until the pricipitate dissolved completely. Finally, we titrated 201 ml of this mixture in bottle with standard $Na_2S_2O_3$ content was calculated using the formula:
DO mg/L = V x M/0.025
Where
V = ml of thiosulphate solution used
M = molarity of thiosulphate titrant

Free CO_2

The free CO_2 in the water was estimated by titrating the sample using a strong alkali of pH 8.3. The analysis was made on the spot. 8-10 drops of neutralized Phenolphthalein indicator were added to 100 ml of water sample taken in an Erlenmeyer's flask, and titrated with N/44 Sodium hydroxide (NaOH) until a pink color appears. The volume of titrant was noted down and free CO_2 was calculated as follows and expressed as mgL^{-1}
Free CO_2 (mgl^{-1})=(ml × N) × of titrant 1000 ×44/ml of sample.

Alkalinity

To the determine alkalinity, 100 ml of sample was taken in an Erlenmeyers flask and 8 to 10 drops of phenolphthalein indicator were added to the turning the sample of water pink. This was titrated with 0.1 HCL till the disappearance of the colour.Following calculation was done to find out the the carbonate or phenolphthalein, Alkalinity.

Alkalinity was the expressed as mg/L using the formula :

Phenolphthalein Alkalinity (mgL^{-1})=(A × N) of HCL × 1000 × 50 ml of sample .

Conductivity and Turbidity

Electrical conductivity and turbidity were measured by using a field kit (systronics – model no- 460 E) on the sampling site. The meter was pre-calibrated in lab using specified standards.

Diversity index

Shannon –Wiener index

Species diversity was calculated following Shannon-wiener index (H-index, Shannon, *et al.* 1963: Simpson, *et al.* 1949) which depends on both the number of species present and abundance of each species using following formula:
$H=-\sum Pi \log_2 Pi$
Where
H= Shannon-Wiener index and Pi=ni/N
ni= Number of individuals of each species in the sample.
N=Total Number of individuals of all species in the sample.

Simpson's Diversity Indices

Simpson's diversity index is a measure of diversity. In ecology, it is often used to quantify the biodiversity of habitat. It takes into account the number of species present, as well as the abundance of each species
Simpson's index of diversity 1– D
Simpson's Index of Dominance**:** was calculated by using the formula:
$D=\sum Ni (Ni-1) /N (N-1)$
Where
Ni = The total number of individuals of a Particular species.
N = The total number of individuals of all Species.

Results and Discussion

In our present study a total of 387 fishes were sampled from river Nimmi. All the water quality parameters measured at three sampling sites in the river Nimmi were within the same range and do not show any significant statistical difference during the same period of study. Results of physico-chemical parameters revealed trends displayed in

Table 2.Surface water temperature ranged from a minimum of 6.1±0.21⁰C, maximum of 26.03±0.25⁰C during study periods for site 1, 2 and 3 respectively Fig 5 .

Table 1.Fish species abundance and biodiversity indices of River Nimmi, Dehradun, Uttarakhand.

Biodiversity indices	Site-1	Site-2	Site-3
Shannon-Weiner index (H)	1.566	1.361	1.541
Simpson's index of dominance (D)	0.6805	0.6444	0.6596
Simpson's index of diversity (1-D)	0.3422	0.42	0.3536

The pH values ranged from a minimum of 6.2 to 7.9 maximum. The minimum Conductivity recorded was 101.12±1.0µ/mhos/cm and the maximum value recorded was 169.12±2.3µ/mhos/cm, during study periods. The value of DO fluctuated from minimum 7.61±0.57 mg/l to a maximum value of 13.63±0.61 mg/l. Lowest free $CO_2$was 5.1±0.01 mg/l and the highest free CO_2 content was 9.5±0.33 mg/l. The alkalinity ranged from 50.3 mg/l to 62.5mg/l . The minimum turbidity was recorded 8.1±0.04NTU and the maximum was 19.9±0.07NTU. These values did not differ significantly statistically and our results show that the parameters physico-chemical parameters measured were almost same throughout the river at all three sites.

Fish population assessment study reveal that river Nimmi has four fish species belonging to four genera and two families. These fishes were from *Nemacheilidae* and *Cyprinidae* family and formed the dominant group. As far as the abundance of the species is concerned, species like, *Puntius ticto, Barilius bandilius and Nemacheilus botia*, were most common recorded during the study period. *Danio species* was identified a *Danio devario* NCBI GenBank accession number KR809737, KR809738.*Danio devario* was found in only at site II.*Danio devario* was present only at site II and its population showed drastic decline from 129 to lower 40's. We repeated the sampling of site II in 2014-15 and observed less than 10 fishes of *Danio devario* (Fig. 6).

Diversity index were calculated and are represented in Table- 1. Shannon- Weinerdiversity index (H) of the fish population at site 1 was 1.566 followed by 1.541 at site-3 and lowest of 1.361 at site-2. The simpson dominance index (D) value at site-1 was 0.6805, at site-3 it was 0.6596 while at site -2 it was 0.6444. Value of D is between 0 and 1, where 0 represents infinity diversity and 1 means no diversity. In our study the simpson index of diversity (1-D) for site- 1 is 0.3422, for site- 2 it is 0.42 and for site-3 it is 0.3536 (Table-1).The abundance, habitat of fishes of river Nimmi during the richness is high in the site-1 and site-3 in comparison to site -2. The presence & absence of various fish species at different sites in Table-3

Table: 2 Two year data physico - chemical parameter (Max-Mim) from site 1, 2, 3 of river Nimmi. No major alteration of physico –chemical parameters in river during the entire of study.

	TEMP SURFACE (°C)		pH		ALKALINITY (mg l⁻¹)		CO_2 (mg l⁻¹)		DO (mg l⁻¹)		CONDUCTIVIY µ S cm⁻¹		TURBIDITY (NTU)	
	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
Site -1	25.2± 0.29	8± 0.24	7.9± 0.02	6.8± 0.05	62.5± 0.43	51.2± 0.36	8.5± 0.13	6.9± 0.03	12.5± 0.43	8.1± 0.22	127± 0.1	101.12±1.0	14.8± 0.09	8.3±0.07
Site -2	26.03 ±0.25	6.1± 0.21	7.9± 0.02	6.8± 0.01	64.2± 0.25	50.3± 0.84	8.5± 0.13	5.1± 0.01	11.53 ±0.13	7.61 ±0.57	169.12±2.3	103± 0.08	19.9± 0.07	12.7± 0.06
Site -3	25.2± 0.29	8.2± 1.23	7.8± 0.01	6.2± 0.22	65.8± 0.43	52.32 ±1.22	9.5± 0.33	7.1± 0.15	13.63 ±0.61	7.7± 0.31	117.3 ±2.12	102.22±1.3	14.3± 0.17	8.1±0.04

As statistical analysis was one using origin 9.1 software (Origin lab corporation, Northampton, MA-USA) : All data were collected in the triplicates and mean along with standard error of mean (SEM) error with standard deviation was

calculated to create the final data table, drawgraphs, and carry out statistical analysis. In our present study, we investigated the impact of anthropogenic activity on pattern of species distribution and their habitat in river Nimmi. At site 1, most common

fish species were *Puntius ticto, Nemacheilus botia, Barilius bendelisis* andat site-2, fish species observed were *Puntius ticto, Nemacheilus botia, Barilius bendelisis & Danio devario* whileatsite-3 *Puntius ticto, Nemacheilus botia, Barilius bendelisis* were observed. Running water of the river usually had *Puntius ticto, Nemacheilus botia, Barilius bendelisis* while *Danio devario* was only found in the shallow pools located in site 2 only. These side pools also occasionally had *Puntius ticto* species.The severity of illegal river bed mining can be imagined by the fact that this occur all over Uttarakhand. The state of Uttarakhand has been on a path of fast growth with various projects including roads, a large number of hydropower projects, etc are underway and for this, one of the counstruction material comes from river bed mining. This mining is leading to vulnerabilities of species in these habitats and this aspact of activity has been completely ignored. Illegal riverbed

mining is so destructive and rampant that Swami Nigmanand gave up his life fasting to stop it just two years ago (Thakkar, 2013). According to the Geological Survey of India (GSI), Uttarakhand riverbed mining causes several alterations to the physical characteristics of both a river and riverbed. These can severely impact the ecological equilibrium of a river and damage plants, animal habitats (Edward and Jeffery 2009). Variation in species diversity in different sampling sites indicated that specific habitats occupied by specific species are being altered and hence the biological communities there will be lost. Nimmi River is one of the tributaries of Yamuna, where river bed mining is prominent. The mining is continuously on-going in several river beds, even after the intervention of Government and Administration and these has been entiresite damage to the same of the habitats

Table:3 Species distribution and richness, according to the sampling site river Nimmi (2012-2014)

Species Name	Latitude and longitude	Site- 1	Site- 2	Site- 3
Nemacheilusbotia	(**Site-1**) 30'22'' 49.10" N, 77'58". 49.20" E	+	+	+
Puntiusticto	(**Site-3**) 30'22'' 55.30" N, 77'58". 49.33" E	+	+	+
Bariliusbendelisis	(**Site-3**) 30'22'' 55.30" N, 77'58". 49.33" E	+	-	+
Daniodevario	(**Site-2**) 30'23'' 09.11" N, 77'58". 58.74" E	-	+	-

(-) Absent, (+) Present

The diversity of fish species was assessed at all three sites using standard species diversity indices . The data summarized in the Table-1 point out to the fact that the diversity is maximum at site 2. Site 2 has a area where the valley is very broad and there are several side pools. These pools have a trickle of water flowing through them and they get flooded only during rainy season or during heavy rains. This fragmentation of habited into running water and side pools provides good opportunity for *Danio devario*. to thrive. It is well known that *Danio devario* prefers these side pools and slow flowing water; hence they are a dominant species in the shallow pools at the site 2. These side pools are almost nonexistent at site 1 or site 3 and *Danio devario* not found there. But maximum river bed mining occurs at site 2 (fig 2a , 2b) At this site water is diverted from one side to another and the mechanized motor vehicle and mining vehicle

usually dig up the sides first or travel over them. Zebra fish is well distributed in north eastern part of India, but very few population clusters have been reported from Uttarakhand. All these habitat in Uttarakhand are in the river bed mining zone It has to be understood that the side pools are in fact a very specific neiche for *Danio devario* and if the current level of activity is maintained, this habitat and the native and endemic species will be lost soon.To get a better description of fish diversity, a measure of species richness and evenness of their distribution were undertaken in all sites. In nature the value of Simpson's Index of Dominance (D) ranges between 0 and 1. With this, index 0 represents infinite diversity and 1, no diversity. The bigger the d value, the smaller the diversity. The Simpson's index of diversity (1- D) represents the probability that two individual fish randomly selected from a sample will belong to different

species. The value of this index also ranges between 0 and 1, the greater the value, the greater the sample diversity. The Simpsons reciprocal index (1/D), the value of the index starts from 1 representing a community with one species. The higher the value, the greater the diversity. The maximum value here 4 representing the total number of species encountered in this study. The diversity index clearly shows thats site 2 has the maximum diversity as there are two different habitats available there. But our findings clearly points out to the fact that the anthropogenic activity and the alteration in habitat of at site 2 has changed that habitat of zebra fish in Nimmi River of Uttarakhand. The habitat is almost lost and this is only one of the cases. Immediate attention has to be paid to this problem of habitat distruction due to anthorphogenic activity and necessary remedial steps needs to be taken urgently.

Acknowledgement

References

APHA (American Public Health Association), 1995. Standard methods for examination of water and wastewater, *American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington, DC, USA.*

Boisen, A.J.Z., Amstrup, J., Novak, I., and Grosell, M., 2003. Sodium and chloride transport in soft water and hard water acclimated zebra fish (*Danio rerio*). *Biochimicaet Biophysica Acta*.1618207-218.

Chakravartty, A., 2013. *Undone by rampant mining illegal buildings*, www.org.in/news 41450.

Daniels, R.J.R., 2002 *Freshwater fishes of peninsula India*. Universities Press, Hyderabad.

Edward, F., Roseman, Jeffrey, S., 2009. Review of fish diversity in the Lake Huron basin *Aquatic Ecosystem Health & Management*. 12 11–22.

Engeszer, R.L., Patterson, A., and Rao, D., 2007. Parichy. Zebrafish in the Wild: A Review of *Natural History and New Notes from the Field. Zebra fish*. 4 21-40.

Indian Institute of Technology, IIT Roorkee., 2011. Assessment of Cumulative Impacts of Hydroelectric projects in Alaknanda-Bhagirathi basins. Report prepared by AHEC, IIT, Roorkee. Submitted to the Ministry of Environment and Forests, Govt. of India.

Jayaram, K. C., 1999. The freshwater fishes of the Indian region. *Narendra Publishing House, Delhi.*

Joshi, S.C., 2004. Uttaranchal Environment & Development, *Gyanodaya Prakashan Nainital Uttrakhanchal 316.*

Mayden, R.K., Tang, K.L., Conway, K.W., Freyhof, J., Chamberlain, S., Haskins, ML., Schneider, L., Sudkamp, M., Wood, R.M., Agnew, M., Bufalino, A., Sulaiman, Z., Miya, M.,and Saitoh, K., He, S., 2007. Phylogenetic Relationships of Danio within the Order Cypriniformes: a framework for comparative and evolutionary studies of a model species. Journal of Experimental Zoology Part B, *Molecular and Developmental Evolution*. 308642–654.

Saha, S., 2014. A tale of two Rivers, Archi Design Perspective research, *Article & features* July - August www.archidesignperspective.com.

Shannon, C.E. and Wiener, W., 1963. *The mathematical theory of Communication University Illinois press*, Urbana 36.

Simpson, E.H., 1949. *Measurement of Diversity Nature* 163-688.

Spence, R.G., Gerlach, C., Lawrence, C. and Smith., 2008. The behaviour and ecology of the zebra fish, *Danio rerio*. *Biological Reviews*. 83 13-34.

Sterba, G., 1962. Freshwater Fishes of the World. *London Vista Books* 878.

Pande, H. K, and Arora, S. India's fifth national report., 2014. To the convention on biological diversity *Ministry of Environment and Forests Government of India* .

Talwar, P. K. and Jhingran, A. G., 1991. Inland fishes of India and adjacent *countries*. *Oxford & I. B. H. Publishing, Calcutta*.

Thakkar, H. (2013). www.international rivers.org/blogs/257/india%E2%80%99s-himalayan-floods-a-man-made-disaster.

THDC, *26 Annual report*. 2013-2014.

Trivedi, R.K., and Goel, P.K. 1984. Chemical and Biological Methods for Water Pollution Studies, *Environmental Publication*, India.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcode *Australia's fish species*. 360 1847– 1857.