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Species Abundance and Environmental Association of Nemacheilidae in South-Central Bhutan

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Abstract

Nemacheilids are freshwater fishes distributed mainly in the South–central regions of Bhutan and environmental variables seems to have significant effect on their distribution, abundance, and diversity. However, existing knowledge and information about nemacheilid's association with its environment is limited in Bhutan. This study aimed to assess nemacheilid's abundance and determine its association with environmental variables in the South–central Bhutan. The study was conducted in the three major river basins; Punatsangchhu, Mangdechhu and Amochhu, which comprises of Dagana, Sarpang, Tsirang, Samtse and Trongsa district. The data were collected from each stream using systematic random sampling, with samples collected at 100 m intervals spanning up to 500 m long. A total of 56 plots were surveyed from which nemacheilids were found in 22 plots. Eleven species were identified in three genera. The mean relative abundance of *Schistura beavani* was the highest in Dagana (9.5), *Paracanthocobitis* cf. *botia* in Sarpang (18.8), and *S. devdevi* in Tsirang (35.0) and Samtse (15.3). The lowest mean relative abundance of *P.* cf. *abutwebi* was in Dagana (3.3), *Aborichthys* sp.2 in Sarpang (6.8), *S. scaturigina* in Tsirang (5.0) and *Aborichthys* sp.4 in Samtse (2.1). No nemacheilids were found in Trongsa. The canonical correspondence analysis (CCA) showed a significant association between species abundance and 11 environmental variables (p = 0.01, Monte Carlo test with permutation of 999). Elevation and temperature were the most influential variables followed by total hardness, electrical conductivity, turbidity, pH, total dissolved solids, salinity, ammonia, dissolved oxygen and chloride with lesser importance. It is recommended to explore additional variables, sites and anthropogenic activities to further elucidate the dynamics of nemacheilids abundance and formulate effective conservation strategies.

Keywords: Abundance, Bhutan, CCA, environmental variables, Nemacheilidae

Introduction

Interaction of environmental variables in landscapes with biotic factors can result in environmental variations, which play a crucial role in shaping the distribution pattern of freshwater fishes within river ecosystem (Beamish and Plongsesthee, 2015). Studies have shown that environmental variables, along with other habitat features, are significant contributors towards species diversity and abundance (Beamish *et al*., 2008; Suvarnaraksha *et al*., 2012; Giacomazzo *et al.,* 2023). Growth and distribution of fish fauna is influenced by the physico-chemical attributes of an ecosystem (Tobor, 1992; Lawal *et al.,* 2023; Roy and Saikia, 2023) making it important to observe their interaction with the biotic factors. Tem-

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perature, dissolved oxygen (DO) and pH are some of the commonly used physico-chemical parameters to assess relation with species diversity and its abundance (Negi and Mamgain, 2013; Ghimire and Koju, 2021; Mariu *et al.,* 2023) beacause these parameters are fundamental for basic metabolic processes, growth and reproduction, contributing as significant factors in their survival (Abowei *et al*., 2010; Haque *et al.,* 2023).

Nemacheilid loaches are commonly found in the fast flowing streams with occasional occurrences in various environments such as large rivers and caves, exhibiting its highest diversity in Southeast Asia, boasting approximately 260 described species (Kottelat, 2013; 2020). Ongoing study continues to unveil new species and genera within this family with the majority of these new species being documented and described in works by Kottelat (2000, 2001, 2023) and Freyhof and Serov (2001). Nemacheilidae species have been recorded in various parts of Bhutan, particularly in the warmer regions (Gurung and Thoni, 2015).

Nemacheilidae species are commonly traded as ornamental fishes particularly in Northeast India (Ganguly *et al.,* 2018). However, overexploitation of these ornamental fishes has led to unsustainable practices in management of such fishes (Dash *et al*., 2023). Although ornamental fish farming is not widely practiced in Bhutan, nemacheilids as ornamental fishes could have significant future potential. The potential ornamental fish trade might lead to overexploitation in wild population and could affect vulnerable Nemacheilidae species in Bhutan. Additionally, the existing knowledge and research on the ecological interaction of nemacheilids in the country is limited, which represent a significant gap in our understanding of these species and their roles in freshwater ecosystems. Therefore, this study focus to study the association between environmental variables and nemacheilids in order to guide in understanding the fundamental elements of nemacheilids' ecological interaction, identifying critical habitats, and assisting in formulating effective conservation strategies.

Materials and Method

Study area

The study area encompasses five districts of the three major river basins (Punatsangchhu, Mangdechhu and Amochhu basins). Amochhu is a trans-boundary river which originates from the Tibet (an autonomous region of China) and flows through the western districts of Haa and Samtse, ultimately flowing into India. Additionally, Bindu Khola was also explored in Samtse which connects with Jaldhaka river, originating from the southeastern Sikkim (National Center for Hydrology and Metorology [NCHM], 2020). Punatsangchhu originates from Gasa which flows through Tsirang, Sarpang and Wangdue Phodrang districts and drains into India (NCHM, 2017). Mangdechhu is one of four major sub-basins of Manas basin which originates near Gangkhar Puensum and flows into Zhemgang via Trongsa district (NCHM, 2017). Subtropical and warm broad-leaved forests are predominant in the region with the exception of Trongsa with elevation ranging from 100 – 1000 m and 1000 – 2000 m above sea level respectively (Department of Forests & Park Services [DoFPS], 2022). Cool broad-leaved forest is predominant in Trongsa. Subtropical forests experience rainfall of 2,500 – 5,000 mm while warm broad-leaved forests have rainfall of 2,300 – 4,000 mm (DoFPS, 2022). This region is generally characterized by hot and humid summer and dry and cold winter (DoFPS, 2022). Plots were sampled following Gurung and Thoni (2015) where Nemacheilidae fishes have been reported in these river systems.

Sampling method

A simple random sampling was used in selecting rivers for each district. Considering the confluence of each stream with the main rivers as the starting point, sampling stretches of about 500 m were considered for sampling. A total of 56 plots (Dagana, 14 plots; Sarpang, 10

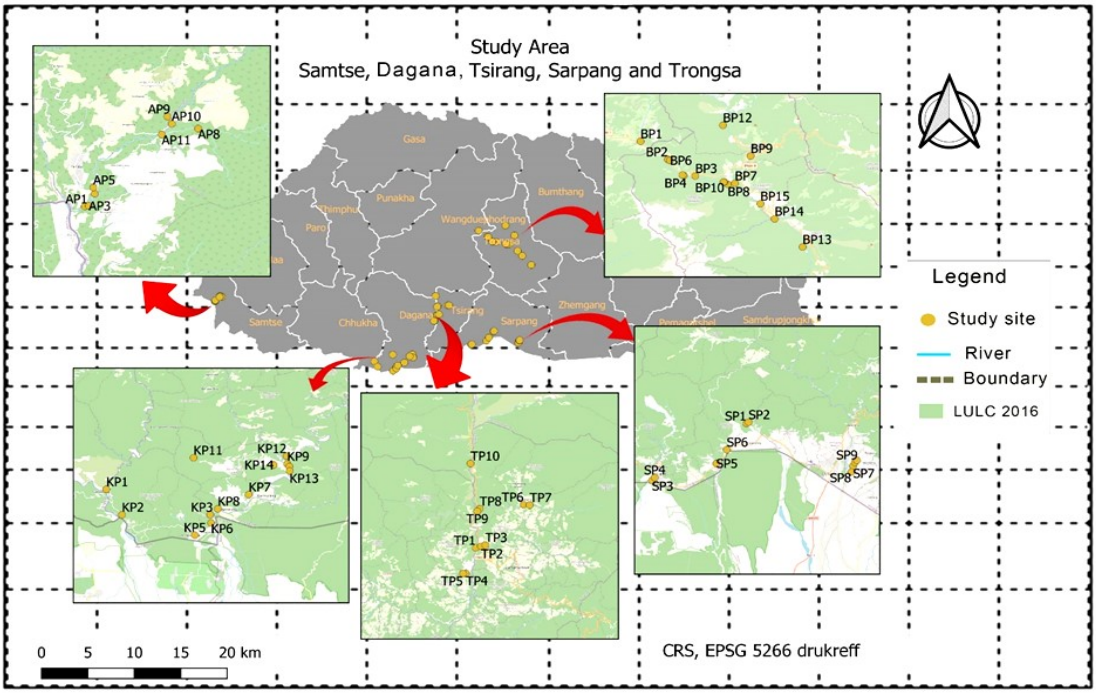


Figure 1. Map showing study plots in respective districts with rivers.

plots; Tsirang, 10 plots; Samtse, 8 plots; Trongsa, 15 plots) were surveyed while inaccessible streams were not surveyed due to lack of accessible routes to reach these locations. Within the 500 m stretch, five plots were selected with an interval of 100 m. Each selected plot was 20 m long where the presence of nemacheilids was recorded and collected. Since the nemacheilids are cryptic in behavior, samples were collected using electro fishing device and temporary water diversion. The captured fishes were released back in the water after identification and enumeration of the species. Species which could not be identified in the field were fixed in 10% formalin and preserved in 70% ethanol for subsequent identification. Prior to fixing, the specimens were euthanized using 0.04% clove oil. Identification keys and visuals provided in Jayaram (1981, 2010) and Gurung and Thoni (2015) were referred for fish identification. Unconfirmed specimens were designated as 'sp.1, sp.2 and so on.

Environmental variables

Parameters that were measured on site included pH, electrical conductivity (µS/cm), temperature (°C), dissolved oxygen (ppm), and total dissolved solids (mg/L) which were measured by ProDSS multiparameter digital water quality meter. Elevation (m) was recorded by Garmin eTREX 20 (± <10m). Chloride, ammonia, and total hardness were measured using methods adopted by American Public Health Association ([APHA], 2017). Salinity was calibrated using salinity meter and turbidity by Nephelometric method at the Soil Air and Water Testing (SWAT) laboratory of the College of Natural Resources (CNR), Royal University of Bhutan (RUB). Sampling was conducted during autumn season from September to November, 2022.

Data analysis

Total relative abundances of each species for each district were calculated using a conversion factor obtained by dividing the total abundance estimate of each species by the total number of fish caught. Species diversity was calculated using the Shannon diversity index (Shannon and Weaver, 1949):

$$H' = -\sum_{n=1}^{n} (pi * ln\ pi)$$

Where H' represents the diversity index, p_i is the proportion of each species in the sample and ln *pi* is the natural logarithm of this proportion. Smith and Wilson's (1996) Evar was used to calculate the species evenness as it is one of the most appropriate evenness indices for general use:

$$E_{var} = 1 - 2/\pi \arctan\left\{\sum_{s=1}^{S}\left(\ln(x_s) - \sum_{t=1}^{S}\ln(x_t)/S\right)^2 /S\right\}$$

Where x_s is the abundance of the s^{th} species, S is the number of species in the sample, and arctan provides an angle in radians. Canonical correspondence analysis (CCA) was used to find the association between species abundance and environmental variables using R version 4.3.2. Plots which had no nemacheilid species were removed from the analysis.

Results and Discussion

Species abundance and composition

The study recorded a total of 281 nemacheilid individuals from 22 plots belonging to three genera (*Aborichthys* Chaudhuri 1913, *Schistura* McClelland 1838 and *Paracanthocobitis* Grant 2007) and 11 species (*Aborichthys elongatus* Hora 1921, *Paracanthocobitis* cf. *botia*, *Schistura beavani* (Günther 1868), *Paracanthocobitis* cf. *abutwebi*, *Aborichthys boutanensis* (McClelland 1842), *Schistura devdevi* Hora 1935, *Schistura scaturigina* McClelland 1839, *Aborichthys* sp.1, *Aborichthys* sp.2, *Aborichthys* sp.3 and *Aborichthys* sp.4) (Table 1). Earlier studies have recorded four genera of nemacheilids from the family Nemacheilidae (Gurung and Thoni, 2015). However, the genus *Triplophysa* Rendahl 1933 was not detected in the current study. It is exclusively found at higher elevations (Wang *et al*., 2015) making their presence very unlikely in lower-altitude environments.

The plots from Dagana comprised of *Aborichthys elongatus, Paracanthocobitis* cf. *botia, Schistura beavani, P. abutwebi*, *S. scaturigina* and *Aborichthys.* sp.1. Of the 11 species recorded in this study, most species were present in Sarpang except *Aborichthys* sp.3 and *Aborichthys* sp.4. Tsirang plots had two species (*S. devdevi* and *S. scaturigina*) while Samtse plots had four species (*A. elongatus, S. devdevi, Aborichthys* sp.3 and *Aborichthys* sp.4). Varied comparative mean relative abundances were examined in four districts where *S. beavani* was more abundant in Dagana, *Paracanthocobitis* cf. *botia* in Sarpang, and *S. devdevi* in Tsirang and Samtse (Table 1). The least mean relative abundant species were *Paracanthocobitis* cf. *abutwebi* in Dagana, *Aborichthys* sp.2 in Sarpang, *S. scaturigina* in Tsirang and *Aborichthys* sp.4 in Samtse. All the 15 sampling plots from Trongsa did not have nemacheilids, which could be attributed to the presence of *Salmo trutta* Linnaeus 1758 (Brown trout), which is a predatory species. Brown trout was commonly found in all the 15 sampling plots in Trongsa and this fish is an invasive species (Jan *et al*., 2023; Lowe *et al*., 2000) which might have predated on the native aquatic species.

Incidence of occurrence

Nemacheilids were present in about 39% of the 56 plots of the five districts (Table 3). Incidence of occurrence for *A. elongatus* was the highest in Sarpang (60%) followed by Dagana (46%) and Samtse (25%). Occurrence of *Paracanthocobitis* cf. *botia* was the highest in Sarpang (60%) followed by Dagana (8%) and were absent in other districts. *Schistura devdevi* was the highest in Sarpang (40%) and Tsirang (40%) followed by Samtse (25%). Sarpang had the highest occurrence incidences of *S. beavani* (40%), *S. devdevi* (40%), *S. scaturigina* (40%), *Aborichthys* sp.1 (40%) and *Aborichthys* sp.2 (40%). Likewise, *Aborichthys* sp.3 and *Aborichthys* sp.4 occurred in Samtse. *Aborichthys* sp.2 were present in Sarpang (40%). Studies in certain parts of India, Nepal, and Pakistan found Nemacheilidae to be the second most abundant fish family following Cyprinidae (Hasan *et al*., 2015; Khatri *et al*., 2020; Baidya, 2022). Changlu *et al*. (2021) reported the presence of *Aborichthys* and *Schistura* species from Manas basin which is a continuation of Mangdechhu and other larger tributaries downstream. However, no species from the family were found in Trongsa in this study, which probably induces that the species recorded are predominantly found in the warm water region. This could also be one of the reasons as to why nemacheilids were not observed in Trongsa due to cooler temperature range in comparison to other districts included in this study.

Environmental variables

Environmental conditions varied significantly among different districts. Mean turbidity was highest (11.8 ± 16.0 NTU) in Dagana and lowest in Sarpang (3.0 ± 1.3 NTU) (Figure 2). The heavy rainfall was experienced during the time of data collection in Dagana which probably explains high water turbidity from the sample (Korman *et al.,* 2021).

The average chloride concentration was the highest (70.6 ± 27.6 mg/L) in Dagana and the lowest (35.2 ± 21.9 mg/L) in Trongsa. Chloride was found to be on higher range in Dagana and Tsirang as compared to other districts of this study. Elevated levels of chloride concentration can indicate that the river is polluted, which can impact the physiological processes of organisms (Pal and Chakraborty, 2017). However, the chloride concentration in the study was significantly below the maximum permissible limit (below 250 mg/L) of the World Health Organization ([WHO], 2022) indicating occurrence of safe environment for the fishes. However, the average concentration of ammonia was highest (0.12 ± 0.05 mg/L) in Trongsa and lowest in Tsirang (0.02 ± 0.01 mg/L). Beamish *et al*. (2008) observed that loaches are rarely present when ammonia levels exceed 0.05 mg/L. However, in cases of low ambient condition of ammonia, it has no significant influence on species abundance (p = 0.12). In Trongsa, the average ammonia concentration was high (>0.05 mg/L) which can be attributed to visible anthropo-

Table 1. Range and mean relative abundance of Nemacheilidae species in rivers of Dagana, Sarpang, Tsirang and Samtse. SD indicates the standard deviation of mean relative abundance.

Species	Dagana (13 plots)		Sarpang (10 plots)		Tsirang (10 plots)		Samtse (8 plots)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
A. elongates	0 –8	9.5 ± 16.4	0 – 3	12.6 ±	0	0	0 – 4	4.2 ± 8.3
P. cf. botia	0–5	4.8 ± 17.3	0 – 4	18.8 ±	0	0	0	0
S. beavani	0 –	18.6 ±	0 – 3	8.5 ± 11.2	0	0	0	0
P. cf. abutwebi	0 – 5	3.3 ± 7.0	0 – 5	11.9 ±	0	0	0	0
A. boutanensis	0	0	0 – 3	8.5 ± 10.0	0	0	0	0
S. devdevi	0	0	0 – 5	18.1 ±	0 – 5	35.0 ±	0 – 8	15.3 ± 31.9
S. scaturigina	0 – 5	3.8 ± 9.6	0 – 3	7.9 ± 10.8	0 – 2	5.0 ± 15.8	0	0
Aborichthys sp.1	0 –	6.1± 12.3	0 – 3	7.0 ± 10.0	0	0	0	0
Aborichthys sp.2	0	0	0– 3	6.8 ± 9.5	0	0	0	0
Aborichthys sp.3	0	0	0	0	0	0	0 – 5	3.5 ± 9.8
Aborichthys sp.4	0	0	0	0	0	0	0 – 3	2.1 ± 5.9

genic activities including hydropower dam construction in the area (Alla and Liu, 2021).

The mean temperature was the highest in Sarpang (25.8 ± 1.5°C) and the lowest in Trongsa (11.5 ± 1.9°C). Temperature is known to be a critical factor in managing species diversity as it has direct affiliation with the metabolism of the fishes (Oberdorff *et al*., 1995). Nemacheilids were found in temperatures ranging from 17.6-25.8°C similar to the range observed in prior studies by Suvarnaraksha *et al*. (2012) and Beamish and Plongsesthee (2015). The highest (8.5 ± 0.5) mean pH was recorded in Trongsa and lowest (7.4 ± 0.3) in Dagana. Teleosts species can be found in small but critical pH range from 4.0-5.0 or up to 9.0-10.0 (Parra and Baldisserotto, 2007).

Table 2. Diversity indices for four districts for 11 species.

District	*S*	*E*	*H'*
Dagana	6	0.59	1.46
Sarpang	9	0.95	2.15
Tsirang	2	0.54	0.39
Samtse	4	0.81	1.21

Note: *S*=Richness, *E*=Evenness, *H'*=Shannon's diversity index

The pH measured was under suitable range (7.3-9.9) supporting ideal water quality.

Salinity was the highest (86.7 ± 36.4 ppm) in Sarpang and the lowest (15.9 ± 2.1ppm) in Samtse. Salinity significantly influences the growth and density of aquatic organism's population (Jamabo, 2008). The increased salinity reduces swimming activities such as searching behavior, potentially disrupting the ecological processes of their habitat (Leite *et al*., 2022). Three plots in Dagana exhibited slightly high levels of salinity which prompts further investigation into potential contributing factors, particularly anthropogenic influences in the surrounding vicinity. These elevated salinity levels may be indicative of various human activities, which need in-depth examination to identify specific sources and potential environmental implications.

Decline in dissolved oxygen concentration in rivers is mainly attributed to higher temperature (Tongnunui *et al*., 2023). However, the average dissolved oxygen concentration across all districts was high, with Tsirang recording the highest concentration (8.95 ± 0.6 ppm) and lowest at Samtse (6.3 ± 1.4 ppm).

The mean electrical conductivity was the highest in Sarpang (168.4 ± 69.6 µS/cm) and the lowest in Samste (31.5 ± 4.6 µS/cm). The highest mean total hardness was recorded in Trongsa (236.8 ± 47.3 mg/L) and the lowest in Samtse (49.7 ± 17.8 mg/L). Extreme levels of hardness are known to have negative impacts on the growth and embryos of fishes (Swain *et al*., 2020). Although the concentration of total hardness in Trongsa is below the permissible limit of 500 mg/L (WHO, 2022), absence of nemacheilids from Trongsa could suggest lower tolerance for total hardness.

The mean total dissolved solids was the highest in Sarpang (77.2 ± 32.4 mg/L) and the lowest in Samtse (14.1 ± 1.9 mg/L). Elevated concentrations of suspended particles in the rivers has the potential to disrupt its natural ecological water system (Adjovu *et al*., 2023) and hence, previous studies have reported changes in total dissolved solids to have negative effect on fishes (Ostrand and Wilde, 2004; Mueller *et al*., 2017). However, the levels of total dissolved solids from all sampling plots fall below the permissible limit (500 mg/L) (National Environment Commission [NEC], 2020) indicating favourable condition for nemacheilids.

Elevation ranged from 109-2460 m with the lowest at Dagana and the greatest at Trongsa. However, nemacheilids were present in elevation ranging from 109 m (Dagana) to

1019 m (Samtse).

Diversity of nemacheilids

Of the 11 species recorded across four districts, Sarpang (S = 9) and Dagana (S = 6) had the largest number of species. Two species were found in Tsirang and four species in Samtse, however, no species were found in Trongsa (Table 2). Species diversity was the highest in Sarpang (H' = 2.15) and the lowest in Tsirang (H' = 0.39) (Table 2). Tsirang recorded only species from the genus *Schistura* while Sarpang consisted species from all the three genera.

Sarpang exhibited the highest evenness (E = 0.95) while the lowest evenness score was exhibited by Tsirang (E = 0.54) (Table 2). High evenness value seems to suggest balanced distribution of nemacheilids with minimal dominant species. However, Tsirang and Dagana exhibited low evenness score suggesting imbalanced distribution of the species. The uneven distribution of the species can be attributed to the presence of dominant species of *Schistura devdevi* in Tsirang and *Schistura beavani* in Dagana. Additionally, variation in physico-chemical parameters affects the water quality, which in turn affects the distribution patterns of fishes and other aquatic organisms (Hasan *et al*., 2015). The average concentration of turbidity for Dagana (11.8 NTU) and Tsirang (5.9 NTU) was slightly above the permissible limits of NEC, 2020 and WHO, 2022 which may have influenced the distribution of the nemacheilids. Other factors such as competition for resources, presence and absence of predators, and human disturbances may also have influenced their ecological structure resulting in variation in their diversity and evenness.

Environmental association with nemacheilid abundance

Canonical correspondence analysis (CCA) of environmental parameters with the abundance of nemacheilids significantly correlated with 11 environmental factors (p = 0.01, Monte Carlo test with permutation of 999). Axis 1 and 2 of CCA analysis explains 25.7% and 15.3% of the variation of data, respectively. Axis 1 was positively correlated to pH, chloride, and elevation (Table 4). Turbidity, electrical conductivity, ammonia, and total hardness correlated positively on second axis while

Table 3. Incidence of occurrence (%) for each species for each districts and overall plots.

Species	Incidence of occurrence %					
	Dagana (N=13)	Sarpang (N=10)	Tsirang (N=10)	Samtse (N=8)	Trongsa (N=15)	56 plots
Aborichthys elongatus	46	60	0	25	0	25
Paracanthocobitis cf. *botia*	8	60	0	0	0	13
Schistura beavani	38	40	0	0	0	16
Paracanthocobitis cf. *abutwebi*	23	50	0	0	0	14
Aborichthys boutanensis	0	50	0	0	0	9
Schistura devdevi	0	40	40	25	0	18
Schistura scaturigina	15	40	10	0	0	13
Aborichthys sp.1	23	40	0	0	0	13
Aborichhthys sp.2	0	40	0	0	0	7
Aborichthys sp.3	0	0	0	12.5	0	2
Aborichthys sp.4	0	0	0	12.5	0	2

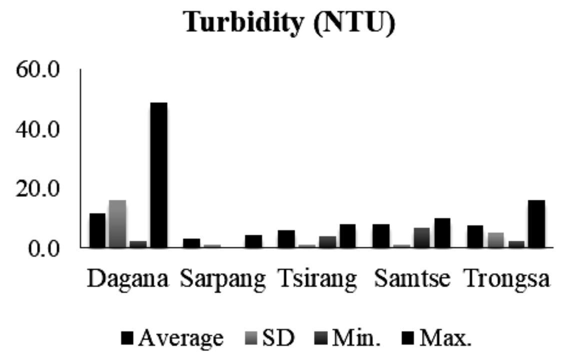
Table 4. Canonical correspondence analysis coefficients for environmental variables for 22 plots

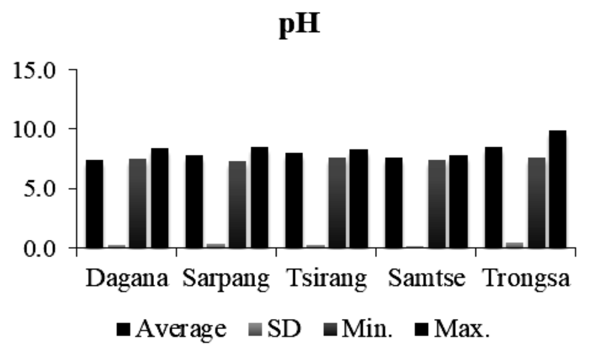
Variables	Axis 1	Axis 2
Turbidity (NTU)	-0.108	0.352
pH	0.103	0.339
Temperature (°C)	-0.732	-0.092
Electrical conductivity (µS/cm)	-0.392	0.038
Dissolved oxygen (ppm)	-0.286	-0.008
Total dissolved solids (mg/L)	-0.335	-0.015
Salinity (ppm)	-0.334	-0.015
Chloride (mg/L)	0.048	0.236
Ammonia (mg/L)	-0.314	0.251
Total hardness (mg/L)	-0.392	0.300
Elevation (m)	0.951	-0.186

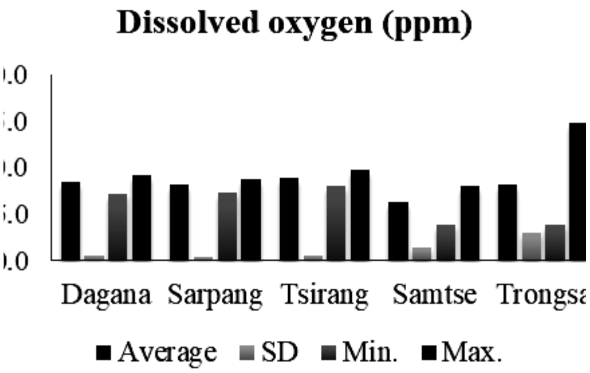
temperature, dissolved oxygen, total dissolved solids, and salinity illustrated negative gradient on Axis 1 (Figure 3). The CCA ordination indicates the relative environmental preference of the species. The measure of magnitude is quantified by the length of the vector.

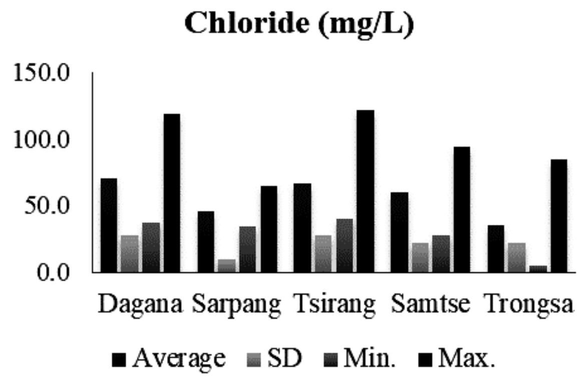
The ordination showed species distribution in three groups; group one included *Aborichthys elongatus*, *Paracanthocobitis* cf. *abutwebi*, *Aborichthys* sp.1, *Schistura scaturigina* and *S. beavani*, group two included *S. devdevi*, *Aborichthys* sp.3 and *Aborichthys* sp.4 and, group three included *A. boutanensis*, *Paracanthocobitis* cf. *botia* and *Aborichthys* sp.2. Habitat of species in group one, on average, was characterized by the highest turbidity, electrical conductivity, total dissolved solvents, salinity, chloride, ammonia, total hardness, the lowest dissolved oxygen, and elevation. Group two species habitat, on average, was characterized by highest temperature, lowest turbidity, pH, dissolved oxygen, chloride, moderate electrical conductivity, total dissolved solvents, salinity, ammonia, total hardness, and elevation. Group three species habitat, on average, had the highest elevation, dissolved oxygen, and similar pH range as group one habitat; lowest temperature, electrical conductivity, total dissolved solvents, salinity, ammonia and total hardness, and moderate chloride and turbidity.

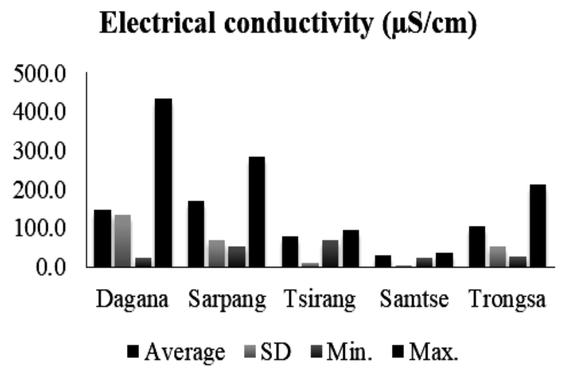
The CCA biplot indicated *Aborichthys* sp.4 to have strong negative association with elevation while *Aborichthys* sp.1, *Schistura scaturigina* and *S. beavani* have opposite association. *S. scaturigina* and *S. beavani* had weak negative association with total hardness and ammonia and, to varying degrees, with turbidity (Figure 3). The loaches in Bhutan have been recorded mainly in the warmer regions ranging from South to the central region (Gurung and Thoni, 2015). Beamish and Plongsesthee (2015) and Tongnunui *et al*. (2016) conducted similar studies where lower elevation had significance in the species abundance and diversity indicating that loach species prefer warmer regions with the exception of some genera not present in this study. Absence of nemacheilid species in Trongsa could be attributed to higher elevation and colder temperatures. However, Changlu *et al*. (2021) have reported presence of *Schistura* species in Manas basin which suggests the need to explore the region during different seasons. The absence of nemacheilids could also be attributed to the presence of predators. *Aborichthys* sp.1 had weak negative association with electrical conductivity, dissolved oxygen, total dissolved solids and salinity. Similar studies by Dwivedi *et al*. (2016) and Satpathy *et al*. (2021) corroborated with the result of this study which suggests the species to be more sensitive to low levels of electrical conductivity and total dissolved solids. *A. boutanensis*, *Paracanthocobitis* cf. *botia* and *Aborichthys* sp.2. were negatively associated with pH and chloride. This is corroborated in similar study by Rajbanshi *et al*. (2021) for nemacheilids. *Paracanthocobitis.* cf. *abutwebi* had high negative association with temperature while *S. devdevi* had opposite association. *A. elongatus* had weak association with environmental variables suggesting its adaptability to thrive in broad conditions.

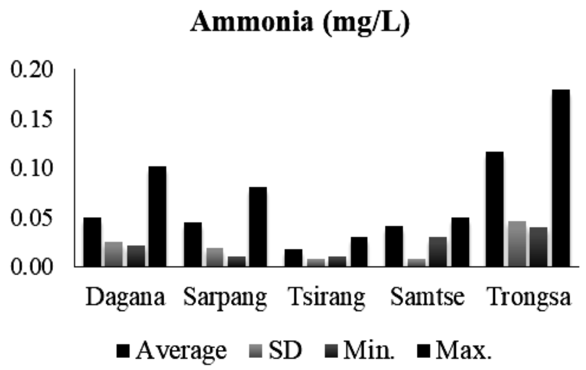


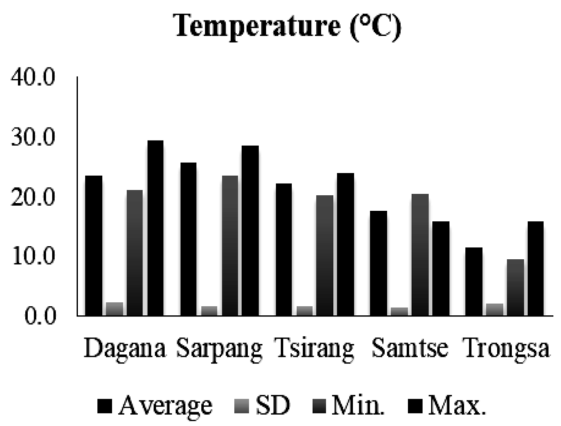


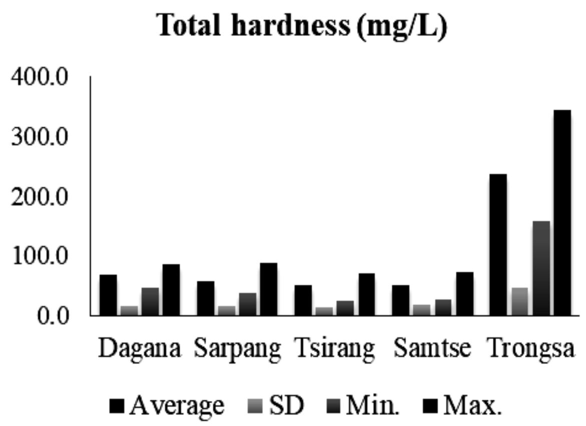












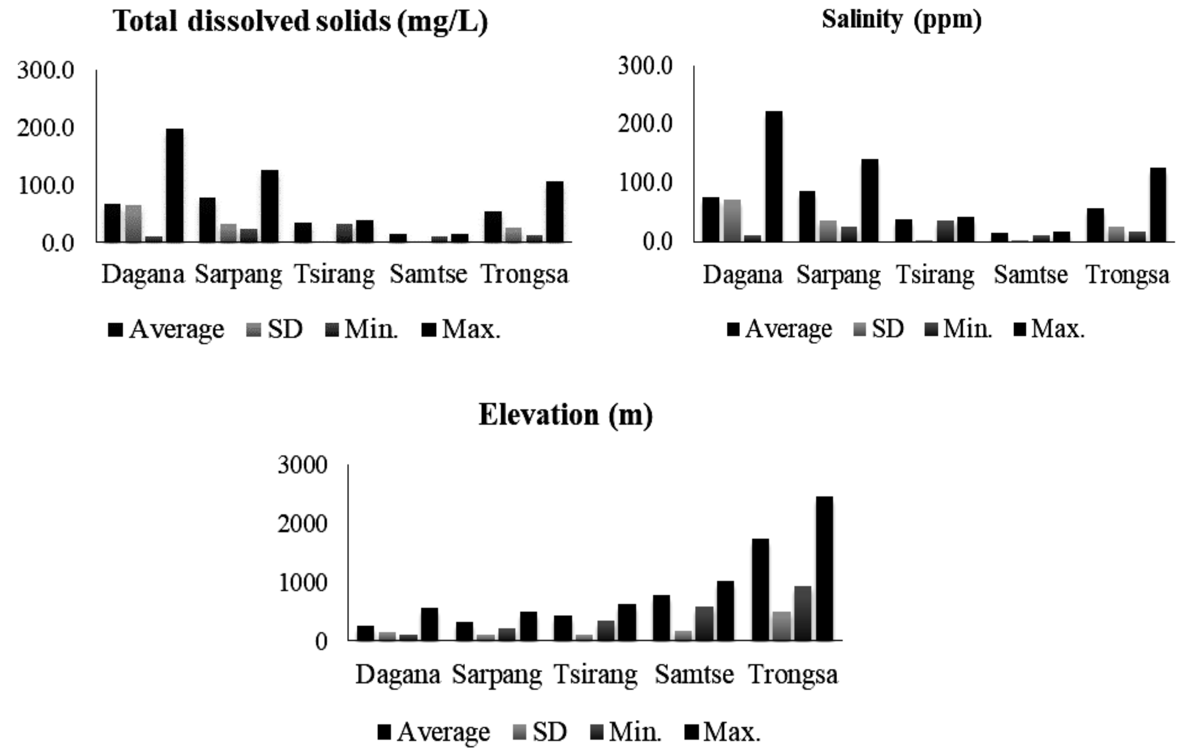


Figure 2. Average, standard deviation (SD) and range of 11 physico-chemical parameters for 56

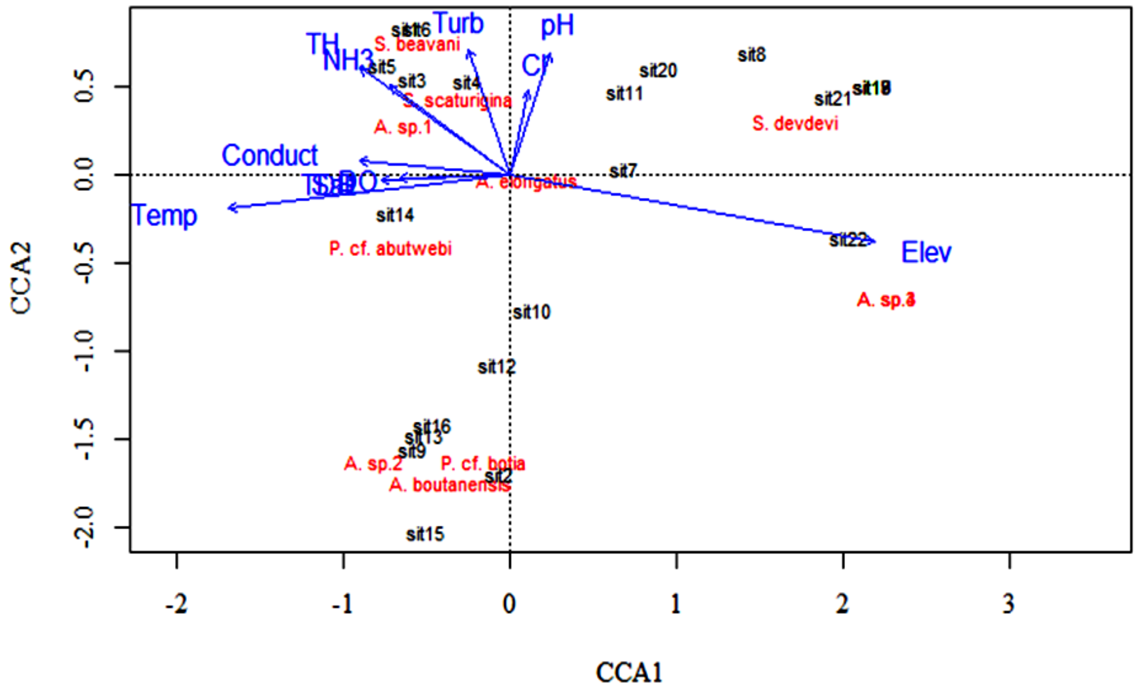


Figure 3. Distribution of fish species with respect to significant environmental variables across five districts of Bhutan.

Conclusion

Nemacheilidae species diversity and abundance were found high mainly in the warmer regions with lower elevation range. The observed strong negative association with elevation observed in CCA in multiple groups underscores the influence of altitude on the distribution patterns of these species. The identified clusters, each representing a unique ecological niche, contribute to our understanding of the complex interactions between environmental factors and fish species in the region. Turbidity, ammonia, total hardness, electrical conductivity, salinity, pH, chloride, and total dissolved solids were of lesser but important factors affecting species abundance. Specific environmental parameters that serve as key indicators for the abundance of nemacheilids can provide information which is crucial for monitoring and assessing the health of aquatic ecosystems in Bhutan, guiding policymakers and resource managers in their efforts to preserve biodiversity. It is essential to acknowledge the inherent complexity of ecological systems, and factors beyond those considered in this study which may contribute to variations in species abundance. Due to the elusive nature and habitat preferences of the species, nemacheilids in Bhutan are not widely distributed, which is a challenge in their collection. Therefore, future research study should explore additional study areas, and environmental variables not included in this study, and employ more nuanced methodologies to further elucidate the intricate interactions shaping the abundance dynamics of nemacheilids.

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