# FISH SPECIES RICHNESS AND ITS RELATIONSHIP WITH ELEVATION IN THE EASTERN HIMALAYAS OF BHUTAN

#### KARMA WANGCHUK\* AND SINGYE TSHERING

National Research & Development Centre for Riverine & Lake Fisheries, Ha, Department of Livestock, Ministry of Agriculture & Forests, Royal Government of Bhutan

\*Author for correspondence: karmaw@moaf.gov.bt

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**ABSTRACT:** Bhutan located within the Eastern Himalayas is defined by steep and high mountains with elevations ranging from 200 - 7,570 meters above sea level. The region also serves as a stronghold of endemism and hotspot for biodiversity. Fishes in the montane region, are at an elevated risk of climate change impact due to their specific adaptations to local conditions. The situation is further exacerbated as water resources in the region are being developed through numerous anthropogenetic activities. Thus, understanding the factors responsible for establishment and maintenance of rich fish biodiversity is crucial for prioritizing conservation efforts. We examined the relationship between patterns of fish species richness and elevation within Bhutan major river systems. Data from 59 randomly selected stations ranging from small headwater streams to large rivers across five major and two minor drainages were examined. We found that fish species richness showed strong negative association with elevation, suggesting that more species are encountered in lower elevation streams/reaches and fewer are encountered with increasing elevation. Our study therefore sheds light on the need to prioritize conservation and management measures for aquatic biodiversity in Bhutan particularly, in protecting lower elevation reaches with higher diversity.

Keywords: Aquatic ecosystems; conservation; elevation gradient; longitudinal structure; species richness

#### **1. INTRODUCTION**

The spatial variation of biodiversity and the factors responsible for maintaining it is foundational to ecology (Gaston 2000). Spatial patterns concerning variation of species richness (i.e., the number of species) and assemblages (i.e., composition of species) are important indicators of ecosystem processes and function (Lek et al. 2005) and may reveal underlying issues with the overall health of aquatic ecosystems (Gou et al. 2015). For example, high species richness may indicate a flourishing and unimpacted ecosystem, while low species richness may suggest perturbation or stress caused by 'natural' or anthropogenic sources. By surveying communities and assessing spatial patterns in biodiversity, we can gain insight into the processes governing our ecosystems and better prioritize conservation and management efforts, e.g., sampling, monitoring, planning, protection (Zbinden et al. 2022a). Effective management and conservation of biodiversity, thus relies on proper understanding of the factors that shape the patterns of biodiversity (Lopez-Delgado et al. 2020) and how they change at varying spatial and temporal scales (Allen and Starr 1982; Zbinden 2020; Zbinden et al. 2022b).

Mountain ecosystems have rich biodiversity and steep environmental gradients within a small spatial scale, and thus are ideal for studying spatial variation in biodiversity. Variation in species richness along elevational gradients of mountain ecosystems have been well documented across a wide range of taxa (Lomolino 2011). Several hypotheses have been proposed to explain these elevation patterns to include climate (McCain 2007), space (Changbae et al. 2013), historical factors (Feng et al. 2016), and distribution (Pan et al. 2019). The climate hypothesis factoring temperature and water seem to be the most widely supported explanation in terms of both latitudinal and elevational patterns of species richness (Rhode 1992). Hawkins et al. (2003) concluded that water and energy and their interaction provided a strong explanation for the diversity gradients observed in plant and animal globally. However, most studies examining patterns of species richness have focused on plants (Grytnes 2003), mammals (McCain 2004), birds (McCain 2009), with very little work on fishes (Oberdorff et al. 1995 and Bhatt et al. 2012). Further, studies on freshwater fishes from mountain rivers including Bhutan are either largely lacking or incomplete, a situation that looms large due to inherent difficulties of conducting systematic sampling across the remote and logistically challenging terrains (Barman et al. 2018).

Bhutan is located within the Eastern Himalayas -a component of Qinghai-Tibetan Plateau (QTP) and serves as a stronghold of endemism and hotspot for biodiversity (Myers et al. 2000). Montane fish, in particular, are at a higher risk of climate change impact due to their specific natural histories that reflect adaptations to local conditions such as water temperature and flow regime (Comte & Grenouilet 2015). Thus, understanding the factors responsible for the establishment and maintenance of rich fish diversity is crucial for prioritizing conservation efforts (Favre et al. 2015; Poff 1997). This is particularly important as Bhutan's water resources are being developed through numerous anthropogenic activities that include agriculture, hydropower, tourism, and small-scale industry (WWF-Bhutan 2016). Our study therefore assumes relevance particularly in terms of identifying and predicting regions of high species diversity for focused conservation and management.

In this study, we examine the relationships between elevation and fish species richness. Similar studies have been conducted by Bhatt et al. 2012, examining patterns of fish species richness along Teesta River (east of Bhutan). Our study involving five major and two minor river basins in Bhutan provides a more comprehensive examination in terms of geographical breadth. More importantly, our results add to the relatively sparse data that is available on patterns of fish diversity and assemblages in the region. Specifically, we aimed to test the following

hypotheses: (i) fish species richness would be influenced by elevation; and (ii) fish species richness would be negatively associated with elevation. We expect species richness to be higher in downstream lower elevation streams compared to the headwaters, thereby necessitating wildlife and conservation managers to prioritize conservation and management measures for aquatic biodiversity in lower elevation reaches with higher diversity.

# 2. MATERIALS AND METHODS

#### 2.1 Study area

Bhutan is located within the Eastern Himalayas and shares its western, southern, and eastern boundaries with northern India. Its water resources are spread across five major and two minor river basins that in combination represent 7,200 km of waterways (Liu et al. 2012). Topographically, Bhutan is primarily defined with steep and high mountains ranging from 200 meters above sea levels in the southern foothills to above 7,500 meters in the high mountains. These mountains present logistical challenges in carrying out systematic fish sampling (Barman et al. 2018). As a result, data on fish diversity within Bhutan did not exist or was largely incomplete until recently (NRCRLF 2013; NRDCRLF 2020).

In this regard, The National Research & Development Centre for Riverine & Lake Fisheries, under the Department of Livestock carried out an assessment of fish biodiversity in Bhutan's major river basins in 2013. The study was carried out in two phases: phase one included three river basins (and tributaries) in western Bhutan (Amochhu, Wangchhu & Punastangchhu); and phase two included the remaining river basins in eastern Bhutan (Aiechhu, Manas and Nyera-Amarichhu). Data used in our study was acquired from 59 randomly selected stations (see Figure 1) across five major and two minor drainages. These sites represent reaches from small headwater streams and tributaries to large rivers in the lower mainstem. Sampled stream reaches were 1,000 meters in length.

Turbidity varied from clear to murky, cobble filled reaches in the headwaters to lower gradient muddy



**Figure 1:** This map shows the location of 59 fish sampling sites across Bhutan. Sites were selected randomly depending on accessibility. Points on the map are represented by different colors that correspond to respective river basins. Fish species richness (i.e., the number of unique species present) was recorded at each site.

tributaries, often with large woody debris, downstream. Elevation gradient ranged from 108 - 2,750 meters above sea level.

## 2.2 Sampling

Fish were collected from wadeable streams between 2013 and 2017. All sampling operations were carried out diurnally from 07:00 AM till 05:00 PM. Fish were collected using several gear types: cast nets, seine nets, drag nets, hand nets, minnow traps, angling, and electroshocker. Samples were preserved immediately in 10% formalin and later identified to species in the laboratory.

At each sampled location, two environmental parameters and geographic coordinates were logged. A hand-held GPS locator (Garmin Ltd.) was used to record latitude, longitude, and elevation of sampling points. Temperature was measured using a standard mercury-in-glass thermometer. Stream order was assigned using simple binary criteria with main stems being assigned '1' and all tributaries/headstreams as '2'.

# 2.3 Statistical analysis

All statistical analyses were performed using R Statistical Software (R Core Team 2020) and R Studio (version 4.0.2). We wanted to examine the relationship between patterns of species richness with four environmental predictor variables. The relationship between species richness and predictor variables (latitude, longitude, elevation, and temperature) was tested with multiple regression. Multiple regression is a statistical method used to analyze relationship between a single dependent variable and multiple independent variables. In our case, the analysis allows us to examine the strength of the relationship between species richness and four environmental variables (Table 2) as well as the importance of each of the predictors to the relationship.

Prior to analysis, necessary statistical assumptions of normality, linearity, non-collinearity, and homoscedasticity were examined using visual analysis and appropriate statistical examinations. More specifically, we examined residual plots, box plots, and scatter plot matrices, for normality, homoscedasticity, and independence. Additionally, Shapiro's test for normality, Levene's test for homogeneity of variance, and Durbin Watson's test for independence were performed. The overall fit of the model and relative effect of each predictor (environmental variables) to total variance in species richness was determined using Akaike information criterion (AIC) and  $R^2$  values. AIC was used for assessing model fit as it helps identify models that best explain the observed variation in data while at the same avoiding overfitting.

Latitude and temperature were strongly co-related with elevation and were therefore dropped from the final analysis. No transformations were required. Longitude was excluded from the study after preliminary analysis showed no effect on species richness. Temperature and latitude were also dropped from the final analyses due to high collinearity with elevation.

# **3 RESULTS AND DISCUSSION**

Shipiro-Wilk's Test for normality did not show evidence of non-normality (p value: 0.4736 >0.05). This was also supported by visual analysis of box plots (see Figure 2). Levene's test for homogeneity of variance did not show evidence of heteroscedascity (p value: 0.4382 > 0.05). Values for species richness and elevation ranged from 2 -20 (mean: 8.23, SD: 5.39) and 108 – 2750 (mean: 691.28, SD: 648.21) (see Table 1). Species richness was strongly negatively associated with elevation indicating fish diversity decreases with elevation (see Figure 3). This was supported by the candidate models involving elevation. AIC ranked the elevation and stream type model as the best fitting candidate model, although all three top ranked models, which included elevation were functionally identical (Delta AIC < 3) (see Table 4). Models that included elevation in the analysis, explained relatively the same amount of variance in species richness ( $R^2$ : 0.43 – 0.46). The  $R^2$  value of the best model (Table 5) suggested that 46% of the variance in species richness in our study region was explained by elevation and stream type

together. Although elevation appears to be the primary driver of variance in fish species richness across the study sites in Bhutan.

In aquatic ecosystems, spatial patterns of fish species richness and assemblage composition are important indicators of ecosystem processes and function (Lek et al. 2005). Effective management and conservation of aquatic biodiversity therefore rely on proper understanding of the processes and functions that shape the patterns of species richness and assemblages (Lopez-Delgado et al. 2020), and more importantly, how they are affected by environmental factors at varying spatial and temporal scale (Allen and Starr 1982). We examined patterns of species richness within major river basins of Bhutan along two environmental parameters. Fish species richness showed strong negative association with elevation, suggesting that fish species richness is higher in lower elevation streams/reaches and gradually declines with increasing elevation.

Bhutan's water resources include small headwater streams in the north to large rivers in the south (WWF-Bhutan 2016). Headwaters differ from large rivers in terms of primary source energy input, habitat size, current velocity, habitat heterogeneity and temporal variability. Larger rivers at lower elevation tend to have higher habitat heterogeneity, volume, and temporal stability (Jackson et al. 2001; Mathews 1998; Zbinden 2017), allowing exploitation by a broader range of taxon (Oberdorff et al. 1999). Bhatt et al (2012) pointed out that water discharge is the main driver of fish species richness in the Himalayan rivers. True to this pattern, rivers in Bhutan swell towards lower elevation with increased water discharge and basin area. Headwaters at higher elevation provide limited resources and niches to be occupied by a broader set of taxa. This explains the pattern of lower species richness in downstream reaches. Additionally, water temperature is also known to play a critical role in determining fish diversity in freshwater habitat. Rivers in lower elevation in the Himalayas (including Bhutan) exhibit higher temporal stability compared to the headwaters, due largely to its buffering capacity inherent to increased water volume. This is crucial especially for the physiologically less tolerant group of taxa such as fish (poikilotherms). Our results are

Site	Species Richness	Latitude	Longitude	Elevation	Stream Type
Above Damchhu	2	27.12163	89.53770	1898	1
Amo Chhu	20	26.87934	89.37129	200	1
Bandar Bir	4	26.73625	89.85718	110	2
Baso Chhu	4	27.34163	89.91437	969	2
Berti Chhu	6	27.16303	90.66344	588	2
Bhur Khola	17	26.92541	90.39614	384	2
Bibigang Chhu	7	27.15630	90.66784	581	2
Budi Chhu	2	27.03194	90.07428	327	2
Changchey Chhu	5	26.96990	90.06999	297	2
Daga Chhu	2	26.95119	90.00059	398	2
Dakpai Chhu	4	27.14614	90.69164	527	2
Dang Chhu	1	27.48311	89.91274	1220	2
Dara Chhu	2	27.06572	89.89563	1050	2
Diglai	5	26.88853	91.74867	397	2
Dik Chhu	8	27.26716	90.04756	695	2
Dimalari	8	26.86312	91.59767	559	2
Dol Khola	6	26.90404	90.35409	368	1
Dorti Khola	18	26.86597	89.37716	191	2
Dungsam Chhu	7	26.81914	91.48347	258	2
Dungsam Chhu - I	13	26 81358	91 49529	210	2
Dungsam Chhu - U	13	26.81914	91 48343	250	2
Gomri Chhu - lower	5	20.01714	91 57764	1028	1
Guniteawa	2	27,51012	89 32516	2480	1
Haa Chhu	2	27.31012	80 28883	2480	1
Homa Doban	2	27.37093	90.00620	2700	1
Kalikhola	10	26.30304	80.83280	165	2
Kama Chhu	19	20.72201	00.03209	103	1
Kanie Chilu Khaini Khala	10	27.20947	90.03390	272	2
Khaimi Khoia	10	20.89138	90.01221	2/2	2
Khatsadrap Chhu	2	27.41121	89.36830	2429	2
Kirigang Chhu	16	26.96600	90.85723	216	2
Labrang	17	20.73208	89.89093	132	2
Lingden Margar adhart	/	27.05544	89.18505	598	2
Marangdhut	19	26.86284	90.97910	131	2
Martang Chhu	3	20.87030	91.30013	324	2
MoChnu	2	27.61978	89.83794	1235	1
Mori Chhu	5	27.25957	91.19422	546	1
Morongang Chhu	9	26.94220	90.87059	198	2
Nye Chhu	8	26.96461	89.25250	404	1
Nyıchula	1	26.95056	89.99774	496	1
Nyra Ama Ri	11	26.92831	91.67455	417	2
Nyra Ama Ri - U	12	27.00325	91.55209	837	2
Paa Chhu	19	26.90629	89.34871	301	1
Paro Chhu	3	27.43426	89.40758	2277	1
Pho Chhu	2	27.60904	89.86912	1288	1
Piping Chhu	12	26.75964	89.74235	226	2
Raidak	9	26.74236	89.73786	155	1
Richanglu	4	26.92844	91.67451	400	2
Rindigang Chhu	8	27.00509	90.83593	282	2
Sam Chhu	10	27.02680	89.21587	654	1
Sarpang Khola	15	26.88302	90.27122	363	1
Sheti Bagar	13	26.90928	90.03387	274	2
Sunkosh	17	27.01975	90.07088	326	1
Taklai	11	26.86028	90.54674	233	1
Thimphu Chhu	2	27.38568	89.58056	2195	1
Tinkuchhu	6	27.22045	90.15187	907	1
Toebrong Chhu	5	27.52636	89.87232	1232	2
Unari Chhu	9	27.21044	91.20301	570	2
Wang Chhu	5	27.03991	89.58894	1410	1
Vongri ahhu	5	27 21041	91 20795	506	1

consistent with earlier studies by Bhatt et al. (2012) and Fu et al. (2004) on fish diversity in the neighboring river basins in the Himalayas. Similar to our results, earlier studies found diversity of freshwater fishes generally declines with increasing elevation. On the contrary, our results are different from the studies on conducted on tree distribution (Acharya et al. 2011), bird diversity (Acharya et al. 2011), and diversity of vascular plants (Liang et al. 2020) along the elevation gradient of the Himalayan region. Unlike in fish, the species richness patterns in these taxonomic

**Table 2:** Environmental variable considered

 for building of regression models of fish

 species richness

Temperature	Water temperature (° C)
Latitude	Decimal degrees
Longitude	Decimal degrees
Stream type	Stream type (tributaries vs main channel)
Elevation	Meters above sea level
Richness	Total number of fish species

groups peaked at intermediate elevations. These contrasting results in patterns of species richness in taxonomic groups inhabiting terrestrial and aquatic ecosystems, suggest the two are likely to be different. Thus, our study provides support to the view that delineated effort must be made with regards to conservation and management aquatic ecosystem.



Figure 2: Boxplots summarizing distribution of species richness against four environmental variables.



**Figure 3**: Scatter plot examining linearity between species richness and environmental variables. Species richness shows strong collinearity with latitude and elevation and weak collinearity with longitude and stream type.

Table 3: A correlation matrix between the predictor variables indicated that
some predictors were strongly correlated. Note the high correlation between
Elevation and Latitude, Latitude and Temperature

	Latitude	Elevation	Temperature	Longitude	Stream type
Latitude	1.000	0.843	-0.631	-0.221	-0.246
Elevation		1.000	-0.769	-0.248	-0.298
Temperature			1.000	0.307	0.408
Longitude				1.000	0.372
Stream type					1.000

## **Table 4. Model Ranking**

Model	Predictor Variables	Κ	Number of Variables	AICc	Delta AICc	R-squared	LL
Model 3	Elev, Stream type	4	2	336.16	0.00	0.47	-163.71
Model 2	Temp, Elev, Stream type	5	3	336.21	0.05	0.49	-162.54
Model 1	Lat, Temp, Elev, Stream type	6	4	337.90	1.74	0.50	-162.14
Model 4	Temp, Lat, Stream type	5	3	339.33	3.17	0.46	-164.10

Coefficients	Estimate	SE	T value	Pr(> t )
(Intercept)	40.6612	5.0304	8.083	5.66e-11***
Elevation	-4.7612	0.6727	-7.078	2.57e-09***
Stream type	-1.8248	1.1140	-1.638	0.107
and the second	<ul> <li>International Content (1997)</li> </ul>	the second strength of the	00, S. 40, S.	to and the second second

Multiple R-squared: 0.4734, Adjusted R-squared: 0.4546

P-value: 0.000000016

### 4 CONCLUSIONS & RECOMMENDATION

Identifying patterns of variations in fish species richness and making predictions across the landscape can help guide strategies for management and conservation of aquatic biodiversity. Our study examining the patterns of fish species richness across environmental factors in Bhutan, showed that elevation has the strongest influence on fish species richness. This provides an important reference for prioritizing conservation and management paradigms. Particularly, data study could help from this concentrate anthropogenic developments in reaches with lower fish diversity at the same time protecting those with higher diversity as reserves can be a significant conservation pursuit. Hydropower plants along with other river regulation activities have significant impact on aquatic ecosystems (e.g., reduction in discharge, alteration in flow regime, habitat fragmentation and barriers to fish migration) Pandit and Grumbine (2012). In Bhutan, several hydropower plants are being developed, with plans for many more in the future. Therefore, we recommend that information coming out of studies much like ours be used in planning, design and execution of dams across such biodiversity gradient. Our study also posits that factors affecting species richness in terrestrial and aquatic ecosystem are likely to be different, thus strongly advocating the need to adopt calibrated measure for management and conservation of aquatic resources.

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