Exploring Freshwater Fish Biodiversity in Bhutan through Species Distribution Models: A Case Study on Snowtrout (Cyprinidae: *Schizothorax spp*.)

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Abstract

Conservation and biodiversity management are focal points for the United Nations Convention on Biological Diversity (CBD). However, monitoring agencies in participating nations that supply data are now severely hampered by political/economic constraints, with "data-deficient" species as a result. This issue will only grow as Himalayan glaciers wane, monsoons shift/diminish, and available freshwaters decline due to climate change. Habitat for cold-water native fishes will shrink while non-natives pre-adapted to warmer, disturbed habitats sharply increase. Effects will be most apparent in countries like Bhutan, where legislation exists to actively conserve/protect biodiversity yet with freshwater fishes recognizably data-deficient, a management issue that must be quickly adjusted, given rapid climate change. Yet, what are the best strategies to accomplish this? And how might they be implemented/sustained as climate change intensifies?

This study demonstrates a potential approach by deriving a Species Distribution Model (SDM) for two putative and three unidentified Bhutanese snowtrout (Cyprinidae; *Schizothorax spp*.), using limited occurrence/distribution data collected by National Research & Development Centre for Riverine & Lake Fisheries (NRDCR&LF, MoAF, Haa). Two influential niche parameters were identified: Seasonal precipitation and annual temperature range, both actively being revised by climate change. Surprisingly, preferred snowtrout habitat is broadly distributed across mid-elevations of Bhutan, supporting the presence of recognized but "unidentified" forms (i.e., data-deficient). Additional long-term monitoring is needed to compensate for these deficiencies and to manage snowtrout as climate-driven impacts alter riverine flows. Both are immediate requirements for the conservation of Bhutan's freshwater biodiversity.

Keywords: Climate change, ecological niche, GIS, management, monitoring

Introduction

The United Nations Convention on Biological Diversity (CBD) was implemented in 1993 to sustain, manage, and globally conserve biological diversity and its genetic resources (https://www.un.org/en/observances/biological-diversity-day/convention). However, the baseline data for the CBD are derived from ongoing monitoring programs in participating nations (N=194, excluding the U.S.), yet this collaboration has been severely constrained by political and economic issues. The end-result is best understood by accessing global metrics that track species-level declines: Freshwater vertebrate species have undergone an 84% reduction over a 40-year span, relative to those found in terrestrial and marine systems (WWF Living Planet Report

: https://livingplanet.panda.org/en-us/; WWF The World's Forgotten Fishes: https://www.worldwildlife.org/publications/the-world-s-forgotten-fishes). This statistic is even more disconcerting when evaluated in a geographic context: Freshwater systems occupy <1% of our global surface, yet they support 12% of all described species and ~33% of all vertebrates (Strayer & Dudgeon 2010; Garcia-Moreno et al. 2014). These data clearly underscore both the importance and the vulnerability of global freshwater ecosystems, despite their relatively reduced global presence (Román-Palacios et al. 2022).

Climate change and regional biodiversity

Climate change has been identified as the most debilitating of the 12 "newly emerging freshwater biodiversity threats" (Reid et al. 2019), with negative impacts only increasing as global ecosystems continue to fragment and disappear (Barbarossa et al. 2020). In addition, of the 31 ecological processes identified as supporting freshwater ecosystems, 23 (74%) are identified as being steadily and consistently eroded by climate change (Scheffers et al. 2016). These include major shifts in species-distributions, and rapid changes in their phenology (i.e., the timing of seasonal life history events in relation to climate; Woods et al. 2021). Increasing temperatures will also promote the spread of invasive generalist fishes with elevated thermal tolerances, whereas temperature-intolerant native species will be forced further upstream into cooler habitats, or temporarily congregate (albeit briefly) within localized areas such as springs or heavily shaded pools that temporarily possess more moderate temperatures (Hayden et al. 2017).

These global considerations also translate to localized regions, such as the Eastern Himalaya, which extends from the Koshi Valley of Nepal eastward to Yunnan, China. The biodiversity within these inland aquatic ecosystems is not only diverse but also of great importance to regional livelihoods and economies (Yao et al. 2012). In addition, the Eastern Himalaya is home to ~10% of the global population (i.e., 2010 estimate) with dramatic population growth predicted over the next 40 years: Bangladesh increasing by 55%; India 50%; Nepal 46%; and Bhutan 40% (Allen et al. 2010, p.10). Rapid growth, coupled with ongoing climate change, will also accelerate the demand for natural resources, particularly freshwater. This will undoubtedly impact aquatic biodiversity, and by default, its conservation and management. Biodiversity hotspots have been identified in the Eastern Himalaya and include the following: Nepali and Indian terai (marshy land between the Himalayan foothills and its plains); the Sikkim/ Darjeeling hills (northeastern India); southeast Tibet (Xizang, China); the entirety of Bhutan; as well as western Myanmar (Allen et al. 2010).

Freshwater biodiversity and "data-deficiencies"

Unfortunately, ~33% of global freshwater taxa (N=1,073, fishes=520) have been categorized by the International Union for Conservation of Nature (IUCN) as being "… data-deficient" (i.e., insufficient information available to diagnose distributions, abundances, or conservation status) (Fig. 1, top; https://www.worldwildlife.org/publications/the-world-s-forgotten-fishes;). This translates to the Eastern Himalaya as well, where N=161 fishes are data-deficient (= "DD;" Fig. 1, bottom). Why is this important? Previously, DD_species were not categorized on the Red List due to the risk associated with their misclassification. This resulted in the development of conservation priorities that were regionally biased (i.e., more unclassified DD_species in some regions as opposed to others). When researchers evaluated the IUCN DD_species database using machine-learning algorithms, 17% (N = 7,699) were identified as having a high probability of extinction (Borgelt et al., 2022), indicating DD_species are considerably more threatened than those deemed data-sufficient.

Limited data, coupled with the inherent complexities of freshwater systems, force participating CBD nations to continually adjust their conservation strategies, particularly as they relate to climate-driven fluctuations in species distributions. This means resident agencies must either re-establish anew their previous endeavors, or instead initiate new programs that quantify distributions and population trends of native biodiversity, as well as diagnose their threats, both ongoing and anticipated (Allen et al. 2010, p.68). Of the 62 valid *Schizothorax* taxa (Froese & Pauly 2019), ~48% (N = 30) have now been assigned conservation status (Fig. 2, top; IUCN Red List = https://www.iucnredlist.org/search/list?taxonomies=103279&searchType=species) (IUCN conservation listings defined = https://portals.iucn.org/library/sites/library/files/documents/RL-2001-001-2nd.pdf). Importantly, threatened species now represent a compilation of three different categories: Vulnerable, endangered, and critically endangered. The conservation status of snowtrout (Fig. 2, bottom) has been summarized in the following percentages: 27% (N = 8) are "DD"; 37% (N = 11) are "LC" (least concern: With a lower risk of extinction); 7% (N = 02) are "NT" [near threatened: Close to (or would be) threatened without ongoing conservation measures]; 10% (N = 03) are "VU" (vulnerable: Likely to become endangered unless survival and reproduction are elevated); 3.3% (N = 01) are "EN" (endangered: At risk of extinction due either to reduced numbers or rapidly changing environmental and/or predation pressures); 13.3% (N = 04) are "CR" (critically endangered: Numbers have (or will) decrease by 80% within three generations, with an extremely high risk of extinction in the wild); and 3.3% (N = 01) are "EX" (extinct: No surviving individuals).

Adjustments for "data-deficiency"

One method for monitoring programs to adjust for data-deficiencies is by developing "species distribution models" (SDMs) that incorporate what is known of the contemporary and/or historic locations for a biodiversity element within a given region. These data, in turn, allow range extensions/condensations to be predicted, as derived from niche preferences (i.e., those species-specific environmental requirements that allow populations to survive in specialized habitats; Soberón & Nakamura 2009; Pyron 2016). This approach is particularly well-suited to delineate habitats of poorly known species for which data are recognizably sparse.

Here, we apply an SDM to identify environmental preferences and potential habitat for a data-deficient group of freshwater fish species (snowtrout = *Schizothorax sp*p. complex), endemic to the high-elevation habitats of the Himalaya. Our focus is specifically on Bhutan, a recognized biodiversity hotspot in the Eastern Himalaya. Our SDM predicts the potential range of snowtrout based on contemporary Bhutanese records but does so independent of their taxonomic identification to species. We use the SDM to quantify how persistence of snowtrout changes along environmental gradients, and to derive predictions for future distributional changes based on a fluctuating climate. This is particularly important in connectivity-limited freshwater ecosystems where contemporary environmental gradients may be altered or even reversed due to localized hydropower development and/or gravel/sand dredging (Wangchuk & Tshering 2022).

Materials and methods

The study area

Bhutan, a designated biodiversity hotspot in the Eastern Himalaya (Allen et al. 2010; Fig. 4), is topographically situated between two major politico-economic powers, China to the north and India to the south, east, and west.

It is a predominantly mountainous country with its highest peak > 7500 meters above sea level (masl). Geographically, it extends from 88–92°N, 27–29°E and encompasses five major and two minor river basins which traverse steep slopes with high velocity (Fig. 4). All rivers flow southward and eventually join the Brahmaputra River in India. The total length of these rivers and their tributaries = ~7200 km (https://www.fao.org/3/L8853E/L8853E02.htm.

Snowtrout as a case study

Snowtrout (*Schizothorax spp*.; Fig. 3) is a genus of freshwater fish within the Family Cyprinidae (subfamily Cyprinini, Tribe Schizothoracini; Yang et al. 2015). It is monophyletic (i.e., descended from a single common ancestor, to include all taxa originating from it, as well as the ancestor itself; Slobodian & Pastana 2022). It contains 62 valid species (Froese & Pauly 2019) distributed across deserts, lakes, and rivers, and spanning the Qinghai-Tibetan Plateau (QTP), the Himalaya, as well as those snow-fed tributaries of Central and Southeast Asia. Snowtrout species are largely omnivorous/herbivorous, often using cartilaginous jaws to scrape algae and biofilm from stones and rocks. It is a predominantly cold-water taxon (i.e., thermal- and oxygen-level requirements that parallel trout, mahseer, and numerous smaller-bodied genera; Sarma et al. 2018).

Morphologically, snowtrout is characterized by the following attributes: An inferior mouth, a cartilaginous covering on the lower jaw, two pair of oral barbels, three rows of pharyngeal teeth, and the last undivided dorsal fin ray being bony and serrated posteriorly. Scales are very reduced, an attribute shared with other high-elevation fishes (Families Cobitidae and Salmonidae). Snowtrout (and Schizothoracines in general) are remarkably similar in general morphology and consequently are often quite difficult to distinguish one from another, such that field identification can be problematic, particularly when early life history stages are involved (Barat et al. 2012a; Qi et al. 2012; Mir et al. 2013; Tang et al., 2019).

In Bhutan, three unidentified lineages have been recorded, as well as two putative species: *S. progastus* (IUCN: https://www.iucnredlist.org/species/168256/6470549), and *S. richardsonii* (IUCN: https://www.iucnredlist.org/species/166525/174786567#errata) (NRCR&LF 2017, pp. 63–68). We elected to develop our snowtrout SDM as a generalized rather than species-specific model, given the potential ambiguity associated with field identifications, compounded by data-deficiency (specifically the absence of specific sampling locations that can statistically support predictions regarding stream niches). Taxonomic uncertainties surrounding identification and distribution of Bhutanese snowtrout also impinge upon freshwater conservation and management, a topic discussed later in the manuscript.

Species distribution models (SDMs)

SDMs are employed to broadly predict potential locations across habitats and do so by combining species occurrences (historic as well as contemporary) with environmental data (Elith & Leathwick 2009; Fois et al. 2018). Large-scale SDMs will be utilized to predict changes in fish distributions and (eventually) community composition, particularly as temperatures elevate in the coming decades. Efforts to ensure the accuracy and reliability of these models are an ongoing endeavor (Araújo et al. 2019), yet contemporary results demonstrate that SDMs are easily capable of not only identifying potential climate change impacts on global aquatic ecosystems, but also identifying how distributions will shift as a result (Perrin et al. 2022).

Snowtrout occurrence data

The raw data for this study were derived from fishes sampled in 2014–15 by the National Research Center for Riverine and Lake Fisheries (NRCR&LF, Haa, Bhutan) (NRCR&LF 2017). Those surveys quantified species-composition and distributions of 55 fish species collected at 50 different river locations in Bhutan. From these data, we classified snowtrout as either being present at a particular sampling site (N = 36; 72%) or absent (N = 14; 28%). The sample size for "presence" is 61% greater than that for "absence," and this offers a potential source of bias for the development of the model. To avoid this, we equilibrated our data by creating random pseudo-absence points (N = 22) to match the total for presence. Further, while doing so, we also implemented an inverse distance weighting method to adjust for the sampling density of our presence points. Some data are in close geographic proximity to one another, and this may also be problematic in a statistical sense. We thus employed Moran's I-statistic to test for spatial autocorrelation amongst our points (i.e., the association among values of a variable strictly attributable to their close relative proximity on a 2-dimensional surface which, in turn, deviates from the statistical assumption that observations are independent of one another; Getis 2008).

Data acquisition

We downloaded raster layers at 30s resolution for 19 bioclimatic variables (plus elevation) from WorldClim V.2.1 (http://worldclim.com/version2). These included monthly temperature and precipitation values (i.e., minimum, maximum, and averages), as recorded from 1970–2000. Slope and flow direction were additional layers derived from the elevational map. We rescaled the latter layers at 30s to match the resolution of our previous layers. A total of 22 environmental variables were collected and subsequently adjusted to accommodate the spatial boundaries of Bhutan. Hydrologic data for streams and rivers, based on elevational data, were also derived from Hydro Sheds (http://www.hydrosheds.org). The shapefile for the map of Bhutan was acquired from The Humanitarian Data Exchange (https://data.humdata.org/), which was then converted from feature to raster layer, and rescaled at 30s resolution using ARC Pro (https://pro.arcgis.com/en/pro-app/latest/get-started/get-started.htm).

Developing the species distribution model

The SDM, also known as a suitability habitat model, was created using the optimum ranges for each selected variable. A simple binary method was applied that assigned to each cell of the raster layer either a "1" (= values suitable for snowtrout) or a "0" (= not suitable). This process was then repeated for all layers. After the reclassification process was completed, a Boolean interaction operation was employed to combine all the layers, using program Model Builder. The results of our simple binary (1/0) methodology in which potential riverine habitats were scored according to their suitability for snowtrout, are provided in Table 1. This process was then repeated for all layers, with variables ranked according to their importance by AIC scores (Table 2). The overall AIC score of our SDM was 62.02, which explained ~60+% of the variability in our dataset.

Results and discussion

IUCN conservation status for snowtrout

Of the snowtrout compiled by the IUCN, ~30% fell within a combined "threatened/extinct" category, ~27% were "data-deficient" (Total = 57%; Fig. 2), while ~43% were of "least concern/near threatened." However, the remaining valid snowtrout species (52%; N = 32) were recorded as "not evaluated" (NE: Not yet assessed by the IUCN). This is our largest snowtrout category by far, and while its size agrees with the large estimated number of species now in existence (~8,700,000, ±1,300,000;https://www.calacademy.org/explore-science/how-many-species-on-earth), it also demonstrates how little is known about snowtrout natural history.

Snowtrout species distribution model

Despite the many unknown aspects surrounding snowtrout, we were able to accurately characterize its preferred habitat in Bhutanese drainages and identify suitable locations for occupancy. However, to accomplish this, our model could not be "overfit" in a statistical sense (i.e., when training data are an exact fit to the model, which then cannot be used to accurately evaluate data not yet collected, thus defeating the purpose of the model; https://www.ibm.com/cloud/learn/overfitting).

Overfitting most often occurs when data exhibit multicollinearity [i.e., two (or more) predictors are highly correlated in a regression context, thus making it difficult not only to determine the effect each has on the response variable, but also which should then be included in the model; https://www.jmp.com/en_us/statistics-knowledge-portal/what-is-multiple-regression/multicollinearity.html].

Prior to developing our SDM, we evaluated our predictor variables for multicollinearity by implementing Spearman correlation coefficient analysis, and subsequently selected seven (of 22 variables) for inclusion in the model (critical value > 0.85; Sharma el al. 2021a). These included: Geographic variables (elevation, slope); bioclimatic parameters (precipitation of driest month, isothermality, precipitation seasonality, temperature annual range); as well as a single hydrologic variable. Three of the seven (i.e., Seasonal precipitation, annual range of temperature, flow direction) demonstrated elevated AIC scores, whereas the remaining four were less influential (Table 2).

The SDM visualizes where suitable snowtrout habitat would be found in Bhutan and did so based on preferred locations within the optimum environmental range (Fig. 5), as derived from our binary selection criteria (Model Builder methodology; Fig. 6). The resulting map displays suitable habitat for snowtrout, based on the SDM (Fig. 7). Although snowtrout are widely distributed across Bhutan (red circles, Fig. 7), locality absences (i.e., sampling sites without snowtrout; yellow circles, Fig. 7) are also broadly distributed. Some absences seemingly fall outside the Bhutanese drainage pattern and represent pseudo-absence points randomly selected to equalize the number of absent sites relative to presence.

Snowtrout distribution in Bhutan: Elevation

Adaptations (i.e., specific signatures in the genome) have been identified in snowtrout (and Schizothoracines in general) which allow these species to not only exist but also thrive within high elevation habitats (Tong et al. 2017; Zhang et al. 2017). Considerable niche plasticity is thus promoted in snowtrout, with several species occupying high-elevation niches (*S. richardsonii*: Sharma et al. 2021b; *S. oconnori*: Guo et al. 2016; *S. waltoni*: Guo et al. 2019; *S. macrophthalmus*, *S. nepalensis*, *S. raraensis*: Regmi et al. 2021: *S. wangchiachii*: Ye et al. 2011). Other species have successfully subdivided the mid-range elevations into different niches as well (Li et al. 2009 Appendix 2; Regmi et al. 2021; Chafin et al. 2021), although the preponderance of fish biodiversity in Bhutan is found at lower elevations (Wangchuk & Tshering 2022).

Our results indicate snowtrout are distributed from 53–5000 masl in Bhutan, reflecting its diverse adaptations to steep slopes and rapidly-flowing rivers. In addition, preferred snowtrout habitat fell within a wide-range of mid-elevation tributaries across Bhutan. This is a positive and encouraging result that corresponds with a previous SDM evaluation for *S. richardsonii*, extensively distributed across the Himalaya (Sharma et al. 2021a), as well as with a broad range of minimum/maximum elevations previously tabulated for snowtrout (Li et al. 2009, Appendix 2).

Given that snowtrout is recognized as a high-elevation fish (Regmi et al. 2021; Sharma et al. 2021a). it was somewhat surprising to find its occurrence at very low elevations in Bhutan (i.e., < 1000m), Several scenarios might explain this phenomenon. Temperature profiles in low-elevation streams might not be consistent, but instead fluctuate seasonally with flows. In this sense, cooler water from upper elevation tributaries might impact downstream reaches during or immediately following monsoonal floods, with snowtrout accompanying these flows (NRCR&LF 2017). Alternatively, cryptic, but currently unrecognized species of snowtrout might segregate along lower elevational clines, again reflecting the diversity of elevational ranges displayed by snowtrout (Li et al. 2009, Appendix 2). The latter also tacitly supports the existence of those "unidentified" and thus "data-deficient" snowtrout found in Bhutan (i.e., NRCR&LF 2017, pp. 63–68).

Snowtrout distribution in Bhutan: Temperature

Water temperature is s an important factor regarding the geographic distributions and local occurrences of snowtrout, which is recognized as a stenothermic, cold-water species (i.e., capable of sustaining but slight temperature variation). It has an upper tolerance of ~20°C and is active in Himalaya streams at 0°C during December/January. But as temperatures drop precipitously during winter months, snowtrout will migrate to lower elevations (as noted above), where they often comprise a sizeable component of the fishery catch in larger rivers and tributaries. For example, *S. richardsonii* thrives in snowmelt water (≤5°C) but has difficulty with water temperature ≥20°C (Barat et al. 2012b).

Our results also highlight the potential for snowtrout to expand its range upwards in elevation as habitats in the mid-to-low-elevations are lost due to climate change. However, this situation is potentially constraining as well. With ongoing climate change, temperatures in streams will initially contract on their trailing edges (= lower elevation). Thus, Himalayan cold-water specialists such as snowtrout with a limited thermal tolerance (Sharma et al. 2021a) will be forced upward in elevation to compensate, at least until high-elevation glaciers eliminates favorable habitat. This ultimately establishes a higher elevation "habitat squeeze" for such species (per Davis et al. 2015; Sharma et al. 2021a), where available habitat effectively disappears.

By the same token, local extinctions can also occur frequently, particularly as the habitat contracts at the "warm edge" of the distribution (i.e., lower latitudes and elevations for Bhutanese snowtrout). This phenomenon is global in nature, being broadly similar across climatic zones, clades, and habitats, yet its occurrence is significantly greater in the following groups: Tropical versus temperate species (55%–39%), animals versus plants (50%–39%), and within freshwater habitats relative to those terrestrial and marine (74–46%–51%) (Wiens 2016). Local extinctions are thus predicted to be common in snowtrout, given its habitat propensity and its resulting squeeze at both termini of its range [i.e., colder (upper elevation) and warmer (lower elevation)].

Temperature also impacts spawning in snowtrout (Sehgal 1999; Sharma & Mehta 2010; Joshi et al. 2016; Sarma et al. 2018). In streams of Kashmir (India), for example, *S. richardsonii* and *S. longipinnis* spawn during May–June at 10–17°C. In the Sutlej River (eastern-most tributary of the Indus River, Himachal Pradesh and Punjab, India), *S. richardsonii* will migrate upstream in March to spawn in tributaries at 17.5–21.5°C. In addition, some schizothoracines will engage in multiple spawning events, as determined by the occurrence of optimal temperatures and flow rates for egg deposition. For example, two separate spawning seasons are recorded for *S. richardsonii* in the Balkhila River (Uttarakhand, India). Here, relatively large eggs (3–4 mm diameter) are deposited in shallow pools (50–70 cm depth) and adhere to the substrate until hatching.

Snowtrout distribution in Bhutan: Invasives

Cold-water exotic fishes, particularly brown trout (*Salmo trutta*), represent another challenge for snowtrout when found in sympatry (i.e., within the same geographic area). Brown trout are recognized by the IUCN as one of the "100 worst global invasive alien species" (https://portals.iucn.org/library/sites/library/files/documents/2000-126.pdf). Aggressive brown trout negatively impacts snowtrout when both are sympatric, and actively predated upon juveniles. Snowtrout has consequently evolved several life-history modifications that have accelerated its demography when sympatric with brown trout: Maturing at a much smaller total length, developing elevated fecundity, yet with eggs demonstrably smaller in diameter than normal due to the diminished volume of stored yolk (Sharma et al. 2021b).

Surprisingly, brown trout has also adapted its life-history to cope with warmer waters, and has done so by narrowing its dietary niche, feeding at a higher trophic level, and elevating its physiological efficiencies such that energy is more efficiently converted into higher trophic level production (O'Gorman et al. 2016). Despite this, warm-water populations of brown trout can only be sustained over longer periods by either returning to colder water for reproduction, or by recruiting new individuals from cold-water populations. However, a reduction in cold-water habitat due to climate change will be much more detrimental to endemic snowtrout, in that it lacks the capacity (per brown trout) to adapt physiologically and/or trophically to warming temperatures.

Brown trout was first introduced into Bhutan circa-1930 (Petr 1999), with two hatcheries (Haa and Wangchutaba) producing ~20,000 trout fingerling/year. Stockings of brown trout subsequently established viable, self-replenishing stocks in Haa, Thimphu, Paro, and some tributaries of the Sankosh and Manas rivers (Mo, Ho, Mangdi, and Chamkhar). Stocking was discontinued in 1983, on the assumption that it suppressed indigenous cold-water fishes, such as *S. progastus*. Remaining brown trout populations are either legacies from the last stockings (primarily high-elevation lakes, some of which were once stocked), or those that now exist as self-reproducing remnants. Anecdotal evidence suggests that brown trout subsisted well in some lakes, fell into poor condition in others, and disappeared from a few.

Taxonomy, conservation, and management of snowtrout

Global biodiversity is severely impacted by a rapidly changing climate (IUCN: https://www.iucn.org/theme/climate-change), with numerous, negative results. These include "indirect" effects (i.e., reduction/elimination of habitat, promotion of invasive species, enhancement of wildlife trade), as well as "direct" effects (i.e., diminished genetic diversity, augmented hybridization, accelerated extinctions). The latter are manifested primarily within the most basic of taxonomic categories, the species (defined as the largest gene pool possible under natural conditions;https://evolution.berkeley.edu/evolution-101/speciation/defining-a-species/). The formal recognition of a species as a taxonomic entity has obvious scientific importance, as it allows the formulation of conservation measures that can sustain its existence (IUCN Red List: https://www.iucn.org/theme/species/our-work/iucn-red-list-threatened-species).

However, the grouping of individuals into species also underscores a potential conflict between conservation and systematics [e.g., the taxonomic category of a "species" is not a fixed and authoritative entity, but rather, a hypothetical grouping potentially modifiable and/or replaceable at some future time (Sites & Marshall 2003)]. Species-designations thus represent "scientific uncertainty," in that they can be combined, renamed, or even eliminated as taxonomic entities through re-interpretations of older data and/or derivation of newer data through advanced technologies.

The same holds true for biodiversity at the infraspecific level [i.e., individuals that cluster at the species-population interface, previously termed as a "subspecies," a "race," or a "variety" (Sullivan et al. 2014), although a greater number of more precise terms are now available (Mussmann et al. 2020)]. The only difference is that "species" is a formal and thus recognizable unit (i.e., the lowest taxonomic category), whereas infraspecific groupings are not (although they are often interpreted erroneously as such; Reydon & Kunz 2021).

Snowtrout taxonomy within the Himalaya
These considerations have direct application for snowtrout in the Himalaya, and here we focus on two species of particular interest in Bhutan: *S. progastus* and *S. richardsonii* (NRCR&LF 2017, pp. 63–68). Both are distributed along elevational gradients within tributaries of the Ganges and Brahmaputra rivers, with *S. richardsonii* trophically classified as a substrate-scraper with a "blunt-nosed" body shape that inhabits higher-elevation reaches, whereas *S. progastus* is a carnivore-omnivore with a "pointed-nose" body shape found downstream within mid-level tributary reaches (Edds 1993; Singh et al. 1995; Molur & Walker 1998; Shrestha 1999; Shrestha & Edds 2012; NRCR&LF 2017).

In the Gandaki and Karnali rivers of Nepal (Central Himalaya), both species group by elevation, irrespective of their river-of-origin (i.e., upstream = *S. richardsonii*; downstream = *S. progastus*). Both similarly diverge along an elevational gradient in the Koshi River of Nepal (Eastern Himalaya) but differ morphologically from conspecifics in the Gandaki and Karnali rivers (consistent with mtDNA data; Regmi et al. 2021). To further disentangle this situation, genome-wide single nucleotide polymorphisms (SNPs) were derived from these samples using next-generation sequencing, with Bhutanese samples included as well (i.e., *S. progastus*, *S. cf. oconori*, but not *S. richardsonii*; here, the abbreviation "cf." indicates the specimen is in the genus *Schizothorax*, and believed to be *S. oconnori*, but actual species-level identification has yet to be verified) (Chafin et al. 2021).

These results indicated that *S. richardsonii* and *S. progastus* have indeed diverged from one another along an elevational gradient in Nepal (per morphological analyses; Regmi et al. 2021), but both share a common origin rather than each evolving independently, as previously expected given the geographic isolation of the drainages. However, each became genetically homogeneous due to historic hybridization within-drainage, with their hybrids then separating along an elevational gradient, much like the parental forms. This was also observed in Bhutanese *S. progastus* and *S. cf. oconnori*, although much less pronounced.

Thus, the evolution of Himalayan snowtrout varies according to temporal scale: Isolation over geologic time (by drainage vicariance), followed subsequently by altitudinal specialization (by adaptation). This has acted to contravene the homogenizing effects of interspecific hybridization. Importantly, this diversity is not sufficiently represented by current taxonomy, which provides yet another example of the previously mentioned conflict between systematics and conservation.

Conclusions and recommendations
To successfully comply with expectations of the U.N. Convention on Biological Diversity (CBD, participating countries must establish two components: (1) Ongoing monitoring programs that provide real-time data on distributions, movements, and threats to native freshwater fishes; and (2) Freshwater conservation policies ratified by the legislative and executive branches of government (Wangmo et al. 2022). Long-term freshwater fish monitoring will provide a wealth of data that is clearly needed in Bhutan, not just for conservation in the strict sense, but also as a capacity-building agenda, with external universities collaboratively training young, resident scientists.

These endeavors will not only sustain freshwater biodiversity, but also support associated livelihoods of constituent citizens who rely heavily upon fully functional wetland ecosystems and the ecosystem services they provide (i.e., defined as those “provisioning,” “regulating,” “supporting,” and “cultural” benefits obtained from nature; Millennium Ecosystem Assessment 2005).

Although monitoring data will accumulate slowly, there are pre-emptive approaches that can be temporarily invoked to coalesce species and their frequented habitats, both of which are rapidly being shifted by ongoing climate change. Here, we demonstrate such an approach by employing species delimitation modeling (SDM) to define and identify habitat for a supra-specific grouping of freshwater fish (snowtrout, *Schizothorax*), a genus composed of several 'data deficient' species in Bhutan. Our assumption is that snowtrout species share similar 'generalized' habitat requirements, and thus modeling those conditions for the genus can serve as a surrogate for species yet to be confirmed within Bhutan.

Author contributions
All authors contributed to the conceptualization and design of the study. KW, MRD, and MED assisted in collecting samples in the field. KW and ST coordinated and/or supervised fieldwork. SW performed bioinformatic analyses. SW and MED generated figures. SW drafted the initial manuscript. All authors contributed to revising the manuscript, and subsequently approved the final products for submission.

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Tables:

Table.1 Optimal ranges (partial response curves) for the Bhutanese snowtrout (*Schizothorax spp.*) species distribution model (SDM). Suitable range values are assigned “1” while the remainder are assigned “0.” Ppt = Precipitation; Temp = Temperature; Ann = Annual.

Variable	Binary assignment
Ppt driest month	3–16 = 1/ rest = 0
Slope	1–23 = 1/ rest = 0
Elevation	53–2800 = 1/ rest = 0
Flow direction	46–54 =1/ rest = 0
Temp Ann Range	17.5–33.6 = 1/ rest = 0
Ppt by Season	72–120 = 1 rest = 0

Table. 2 Seven (of 22) environmental variables included in the Bhutanese snowtrout (*Schizothorax spp*.) species delimitation model (SDM). Data are based upon their individual Akaike Information Criterion (AIC) scores, as produced from univariate generalized linear models. Ppt = Precipitation; Temp = Temperature; Ann = Annual.

Variable	AIC
Ppt driest month	88.58
Slope	93.25
Elevation	93.48
Isothermality	94.42
Flow direction	101.4
Temp Ann Range	100.6
Ppt by Season	100.8

Figure Headings:

Figure 1 (Top). Global summary of conservation classifications by taxonomy from the Red List, International Union for Conservation of Nature (IUCN) (Allen et al. 2010, Table 6.2, p.69). (Bottom) Proportion (%) of species in each global IUCN Red List Category within the Eastern Himalaya. (Allen et al. 2010, Table 6.1, p.69). EX=Extinct; EW=Extinct in Wild; CR=Critically Endangered; EN=Endangered; VU=Vulnerable; NT=Near Threatened; LC=Least Concern; DD=Data Deficient.

Figure 2 (Top). Designated categories of the International Union for Conservation of Nature (IUCN) Red List. (Bottom) Compilation of Red List categories (as %) for *Schizothorax* (snowtrout: Family Cyprinidae; https://www.iucnredlist.org/search?taxonomies=103279&searchType=species).

Figure 3. *Shizothorax sp. cf richardsonii* (i.e., potentially *S. richardsonii*) captured at the confluence of the Berti Chhu and Mangde Chuu, Bhutan.

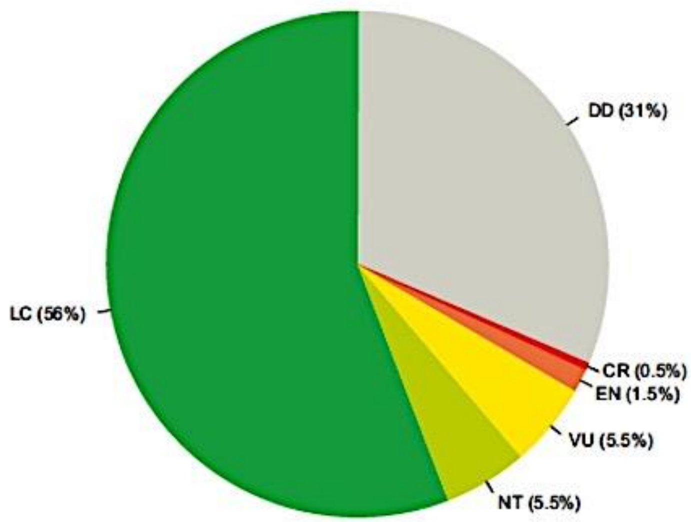
Figure 4. Map depicting the elevational gradient of riverine systems within Bhutan (Eastern Himalaya) that drain south into the Brahmaputra River (India). The Qinghai-Tibet Plateau (QTP: People's Republic of China) lies to the north, Sikkim (India) to the west, Arunachal Pradesh (India) to the east, with West Bengal (India) to the southwest, and Assam (India) to the southeast.

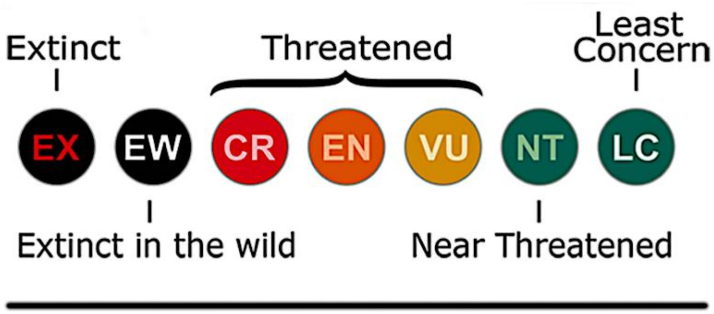
Figure 5. Partial response curves for variables (N=6) generated from a preliminary generalized linear model (GLM) analysis of 22 environmental variables selected for potential inclusion into the species delimitation model (SDM) for Bhutanese snowtrout (*Schizothorax spp*.).

Figure 6. Reclassified variables (per Table 2) recombined via Boolean interaction in Program ModelBuilder to yield a depiction of suitable aquatic habitat for Bhutanese snowtrout (*Schizothorax spp*.).

Figure 7. Topographic output for the species distribution model (SDM) depicting favorable riverine habitat for Bhutanese snowtrout (*Schizothorax spp*.). The red and yellow dots indicate presence and absence, respectively, of snowtrout as derived from contemporary and historic samples. The blue lines represent suitable habitat for snowtrout.

	Global Red List Category	Number of species
	Extinct	0
	Extinct in the Wild	0
Threatened categories	Critically Endangered	5
	Endangered	15
	Vulnerable	50
	Near Threatened	46
	Least Concern	263
	Data Deficient	141
	Total	**520**

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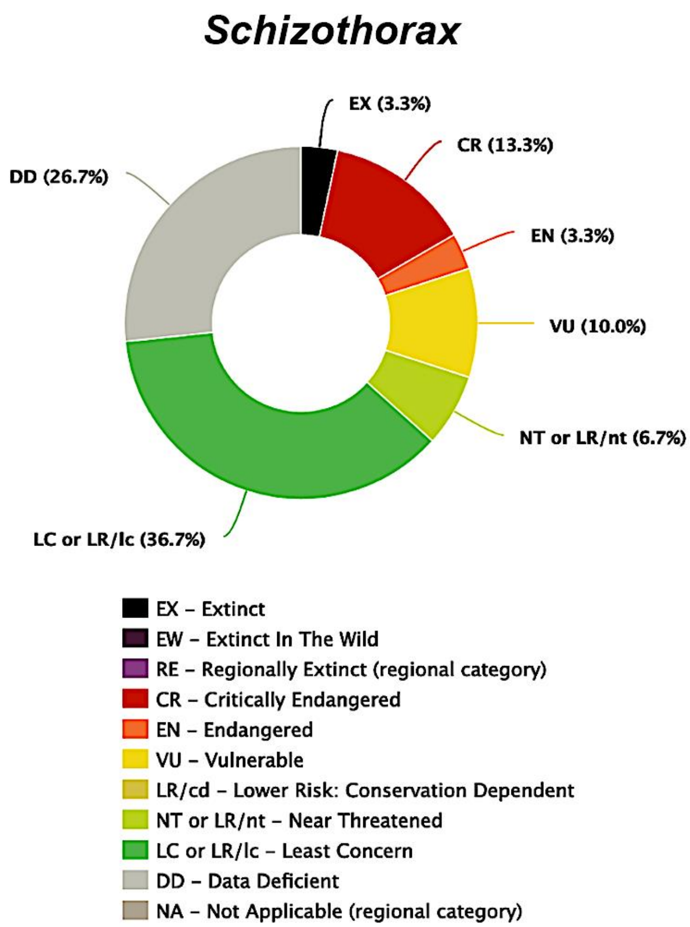
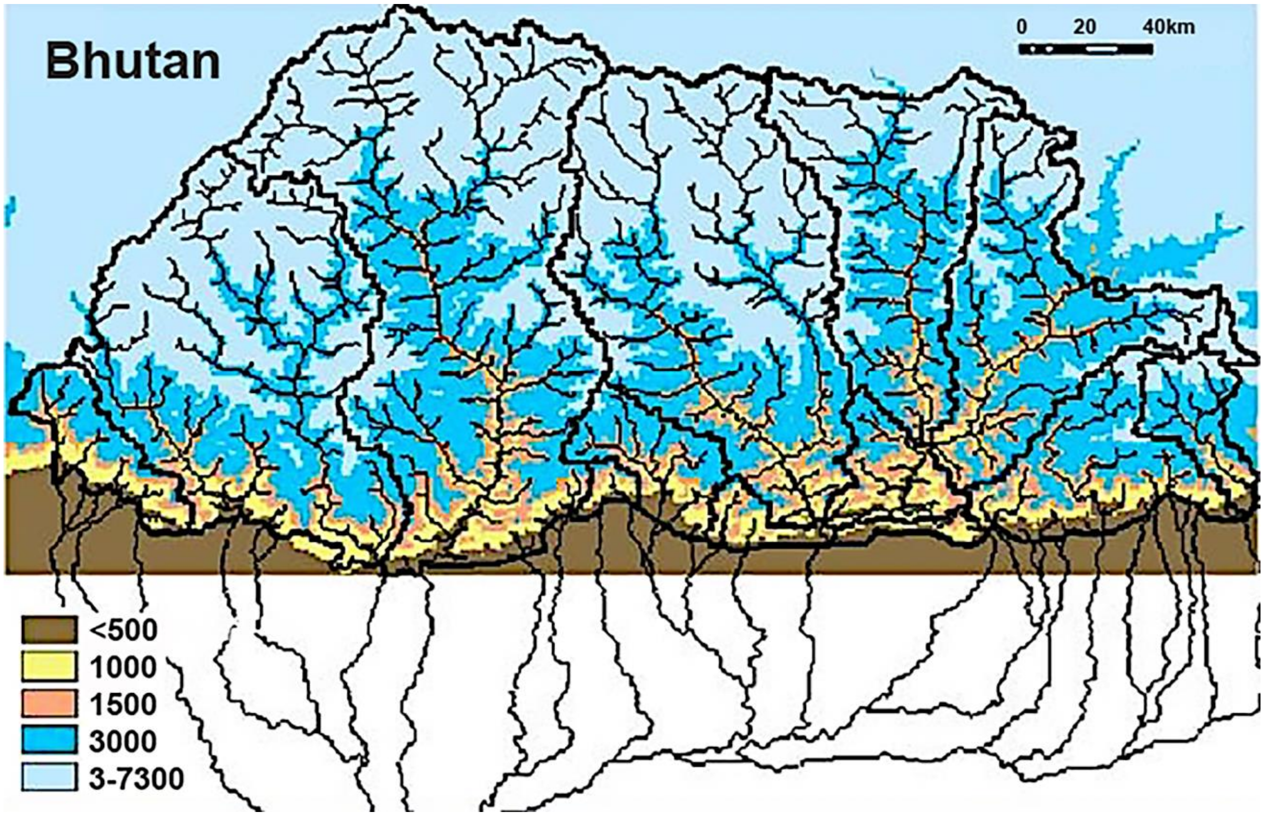
Threatened
Least Concern
EX
EW
CR
EN
VU
NT
LC
Extinct in the wild
Near Threatened


Schizothorax
EX (3.3%)
CR (13.3%)
EN (3.3%)
VU (10.0%)
NT or LR/nt (6.7%)
LC or LR/lc (36.7%)
DD (26.7%)
EX – Extinct
EW – Extinct In The Wild
RE – Regionally Extinct (regional category)
CR – Critically Endangered
EN – Endangered
VU – Vulnerable
LR/cd – Lower Risk: Conservation Dependent
NT or LR/nt – Near Threatened
LC or LR/lc – Least Concern
DD – Data Deficient
NA – Not Applicable (regional category)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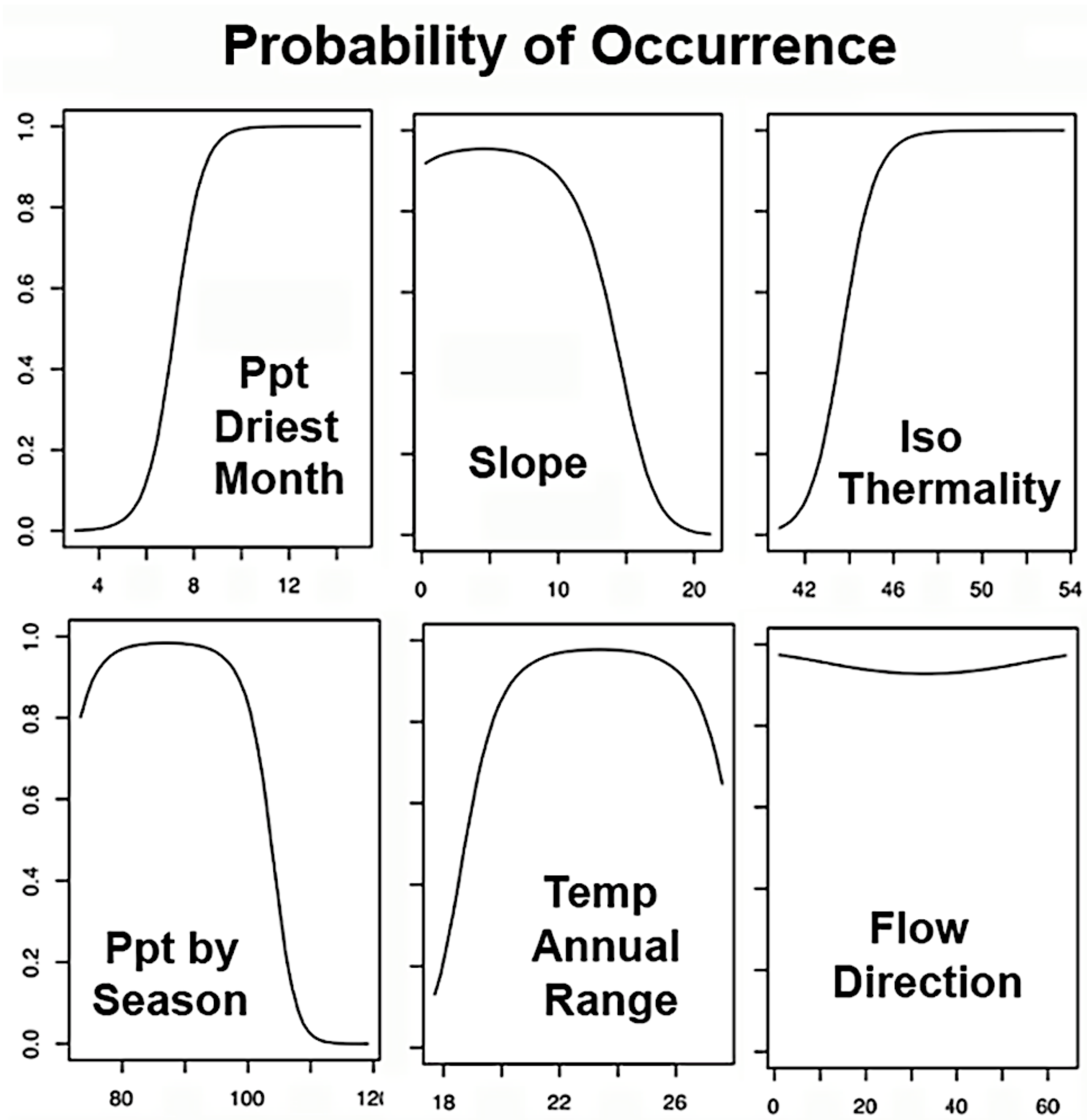
Probability of Occurrence
Ppt Driest Month
Slope
Iso Thermality
Ppt by Season
Temp Annual Range
Flow Direction
0.0
0.2
0.4
0.6
0.8
1.0
4
8
12
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42
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80
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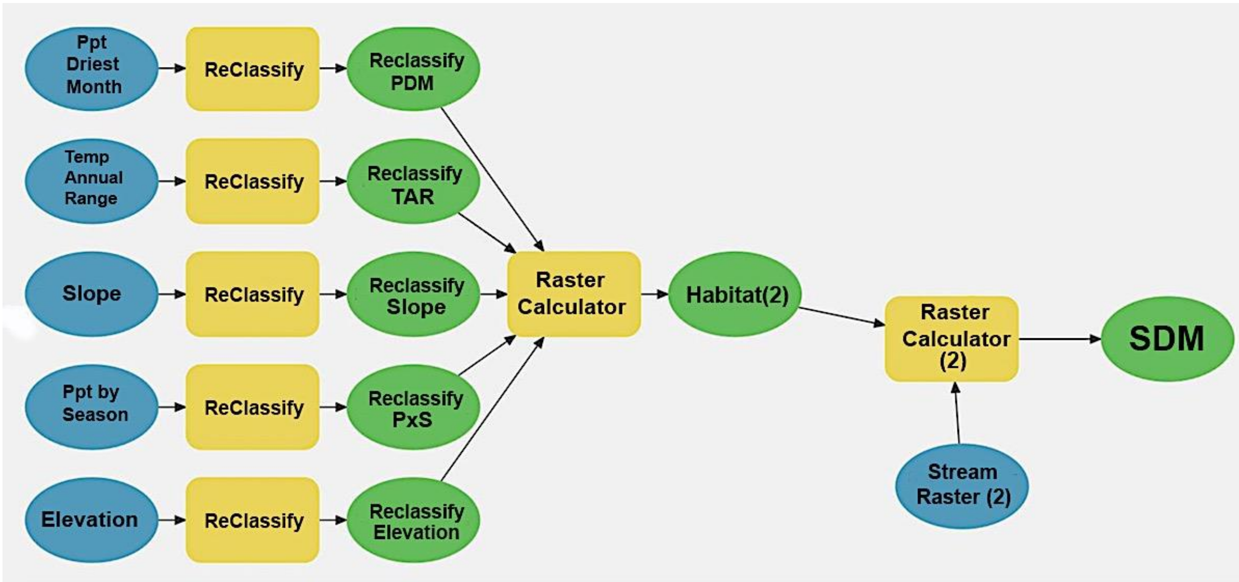
Ppt
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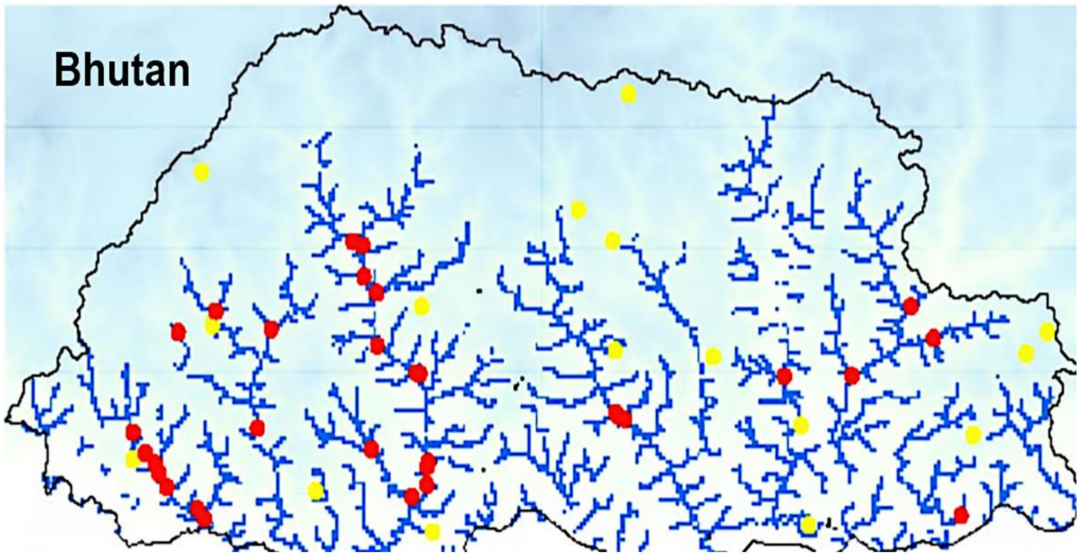
Bhutan