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Microhabitat utilisation and coexistence strategies of sucker fish *Garra mullya* (Sykes, 1839) and zodiac loach *Mesonoemacheilus triangularis* (Day, 1865) in a wooded hill stream of Southern Kerala, India

Ruby Thomas[1]* and Raju Thomas K.[2]

[1]*Department of Zoology, St. Berchmans College (Autonomous), Changanassery- 686 101, Kerala, India*

[2]*Department of Zoology, Mar Thoma College, Tiruvalla-689 103, Kerala, India*

Abstract

The habitat requirements of fishes vary with their biological and physiological processes. In this study conducted from March 2019 to February 2020, we focused on *Garra mullya* (Sykes, 1839) and *Mesonoemacheilus triangularis* (Day, 1865) in a wooded hill stream of the Pamba river basin in southern Kerala, India. These species were selected due to their ecological significance and prevalence within the freshwater ecosystems of the region. Our investigation aimed to identify and quantify microhabitat availability, utilisation patterns, and niche partitioning mechanisms influenced by trophic requirements, morphological and physiological characteristics, and stream biochemical conditions. Our focus was on fifteen environmental descriptors and attributes that operate on the microhabitat scale. Principal Component Analysis (PCA) and Pearson correlation were employed to reveal the habitat selectivity and association of species with multiple environmental variables at the focal point position of each observed fish. Microhabitat selection in *G. mullya* and *M. triangularis* was driven by trophic requirements, morphological and physiological characteristics of the fish, and biochemical conditions of the stream. *G. mullya* inhabited cobble and rubble-dominated microhabitats and favoured closer proximity to shelter and banks. *M. triangularis*, on the other hand, preferred gravel-filled microhabitats and was found to use locations near banks and shelters, more frequently. The coexistence of *G. mullya* and *M. triangularis* along the water column can be attributed to resource allocation and substrate variability.

Keywords: Benthic rheophiles, Coexistence, Microhabitat preference, Resource partitioning, Substrate variability

Introduction

The aquatic ecosystems of Kerala boast a rich and diverse array of ichthyofaunal communities and serve as critical breeding and feeding grounds for numerous fish species. Understanding the relationships between various fish species and their environments is essential for managing human activities impacting these ecosystems. The taxonomic composition, distribution, abundance, and species richness of fish are influenced by the complexity of their aquatic habitats. The ecological importance and unique biodiversity of the wooded hill streams of southern Kerala cannot be overstated. The Pamba river system, in particular, is a key habitat for many endemic fish species, including several popular for cultivation and ornamental purposes. Radhakrishnan (2006) identified 174 fish species in Kerala's rivers, with 55 species found specifically in the Pamba river. Numerous studies have documented ichthyofaunal diversity in the major streams and rivers of southern Kerala, including work by Bijukumar & Sushama (2001), Jameela & Ramachandran (2009), Swapna (2009), Jancy & Jobiraj (2017), Arunkumar & Arunachalam (2018), Sheeja (2018), Salu & Ambili (2019), and Sojomon (2022).

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* Author for correspondence: rubythomas92@gmail.com

The ichthyofaunal community chooses its habitat based on the physical structure and multiple ecological aspects of an aquatic environment. Habitat selection is a behavioural process influenced by the geomorphology of streams, climate, quality of the water, the extent of predation, and the level of competition to ensure individual fitness (Fretwell & Lucas 1969). Coexistence is related to the division of resources or the separation of occupied niches. At smaller scales, such as the microhabitat level, where individual behaviour is influenced by environmental factors, niche segregation is anticipated to occur (Morris 1996). The most effective way to use resources that might allow coexistence between ecologically similar species in a shared space is thought to be spatial partitioning. However, recent research has shown that niche partitioning in animal populations is driven by spatial (Jessopp et al. 2020) and temporal (Watabe et al. 2022) variations. A species' ability to survive under particular environmental conditions determines how widely distributed it is in space.

Microhabitat studies examine the resources available in relation to that are being used, particularly the location of fish in the water column (Mazzoni et al. 2011). Studies of microhabitats are important in aquatic environments because they shed light on fish behaviour, community structure, and species restoration. The study of fish microhabitats in temperate and tropical aquatic systems was mainly based on qualitative observation and descriptive data (Gorman & Karr 1978; Baltz et al. 1993; Johnson & Douglass 2009; Kanno et al. 2012; Choi et al. 2020). During development projects in aquatic ecosystems, the microhabitat needs of specific fish species can be used to prioritise habitat improvement measures and conservation (Bond & Lake 2003; Pinto et al. 2006; Boavida et al. 2010).

The hill stream teleosts *Garra mullya* (Sykes, 1839) and *Mesonoemacheilus triangularis* (Day, 1865) have evolved to thrive in rapidly flowing water environments. *M. triangularis* possess a cylindrical body with small and compact fins which facilitate their living amongst pebbles and shingles in swift running waters (Menon 1989). While *G. mullya* maintains its position in a strong current with the help of its suctorial disc and horizontally positioned paired fins. The proboscis aids in reducing the force and speed of rushing torrents. *G. mullya* and *M. triangularis* are belonging to the order Cypriniformes in the ichthyofaunal families Cyprinidae and Nemacheilidae, respectively (Talwar & Jhingran 1991).

G. mullya and *M. triangularis* were chosen for their ecological importance and prevalence in local freshwater habitats, making them suitable subjects for understanding microhabitat preferences and ecological interactions within this specific stream environment. The selected stream within the Pamba river basin was chosen based on its representative characteristics and accessibility, offering a focused context to study these species' habitat preferences and niche dynamics.

In the present study, we aimed to achieve the following objectives: to quantify the microhabitat availability and utilisation by *G. mullya* and *M. triangularis* from the wooded hill streams of the Western Ghats in southern Kerala; to reveal the niche partitioning mechanisms in *G. mullya* and *M. triangularis* by analysing the ecological factors that facilitate their coexistence within the same habitat; and to support future decision-making processes to effectively improve habitat management and conservation efforts by providing data and insights on critical habitat needs and potential threats to these species.

Material and methods

Study area

The study was conducted in a hill stream locally known as Chorakakki, located in the Ranni forest division of Kerala, southern India (Figure 1). The stream is located at an altitude of 163 meters AMSL and coordinates of 9.304° N and 77.054° E, in the Western Ghats. The perennial stream eventually empties into the Pamba river and has rapids, glides, pools, riffles, runs, and falls as mesohabitat units. The area is characterised by an herbaceous riparian buffer and shows no signs of human activity.

Sampling and microhabitat monitoring strategy

Sampling and habitat inventory studies were carried out monthly from March 2019 to February 2020 in a single, consistent 200 m stream section. This consistent locality was chosen to ensure comprehensive data collection and minimise variability. Target fish samples of *G. mullya* and *M. triangularis* were gathered using scoop nets and cast nets. Not all caught specimens were preserved; a representative sample was selected for detailed analysis. Specifically, 5 specimens of *G. mullya* and *M. triangularis* were preserved each month. These preserved specimens were stored in 10% Formalin for subsequent stomach content analysis. The abundance of each species was also recorded. The standard manuals of Talwar & Jhingran (1991), and Jayaram (1999) were used to confirm the identity of the preserved specimens. Concurrent with the sampling of fish, an investigation into the availability and use of microhabitats was conducted. Microhabitat availability was measured using a 900 cm^2 quadrat and a ten-meter transect line across the 200 m study stretch. The depth of the water column, current velocity, and the stream bed substrate were determined at random locations.

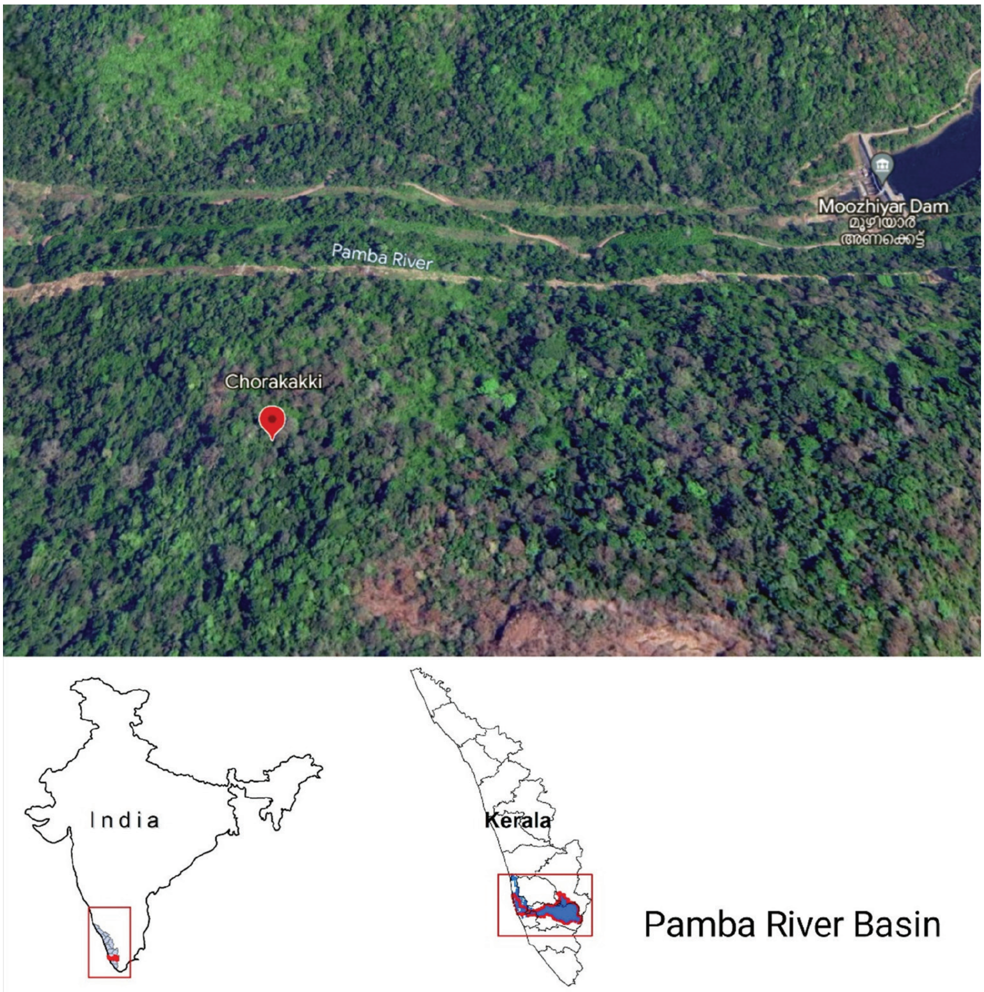

Figure 1. Map showing the Pamba River basin with an indication of the surveyed hill stream Chorakakki, in southern Kerala, India

In order to determine which microhabitats are predominantly exploited by the target individuals, daylight stream surveys were conducted. After observing the location of an undisturbed fish (focal point position), seven microhabitat descriptors consisting of average current velocity, focal point velocity, water column depth, distance from substratum (straightedge, nearest cm), distance from the shelter, the identity of all species within 20 cm of the specimen (ie., nearest neighbour), and the percentage composition of seven substratum categories (bedrock, boulder, rubble, gravel, sand, silt, debris) in a 900 cm^2 quadrat directly below the fish were estimated. Using a measuring tape, the distance to the closest bank and shelter was calculated perpendicular to the flow by measuring from the surface location above the focal point position of the fish. Utilising a meter scale, the distance from the substratum is determined. Focal point velocity, specifically, was calculated as the average velocity of water at the focal point position. This measurement was obtained using a current meter positioned to measure water velocity directly in contact with the fish. The baseline method for the assessment of microhabitat availability and its use by the ichthyofaunal community was obtained from Grossman & Freeman (1987). To ascertain the role of food habits in microhabitat partitioning of target fish species, direct observation of the fish in their natural habitat was combined with stomach content analysis of the preserved specimens. The Points (Numerical) method developed by Swynnerton & Worthington (1940), which takes into account the majority of the food items, was the one used for the analysis. The size of the food item is taken into account during random classification, and the stomach contents of each subject were totalled. The sum of the points for each food item is used to show the percentage makeup of the diet.

At the sampling point, the following environmental descriptors were measured and analysed: water column depth/stream depth, water column width/stream width, riparian shade/canopy cover, water temperature, pH, total dissolved solids (TDS), conductivity, total hardness, dissolved oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), light intensity, air temperature, wind speed, and current velocity. A graduated wading rod and a measuring tape were used to measure the width and depth of the stream, respectively. For measuring current velocity, a pygmy current meter was used. The pH and conductivity of the stream water were measured using a pen-style pH meter and conductivity meter, respectively. TDS and water temperature were estimated using a digital water tester (AP-1). Methods of the American Public Health Association (2005) were followed for estimating BOD, COD, DO, and Total hardness. On-site light intensity was measured using a digital lux meter (FULCRUM-FC1010B); atmospheric temperature and wind speed measurements were made using an anemometer (BENETECH-GM816). To calculate the amount of riparian shade, present along the stream reach, a spherical densiometer was used.

Data analyses

The role of environmental variables in the microhabitat preference of target fish species was evaluated by using a Principal Component Analysis (PCA). A set of 15 environmental descriptors mentioned above, along with the abundance of each species were chosen for PCA. Pearson correlation analysis was conducted to examine the associations between variables for each fish species. The results were visualised using a corrplot, also known as a correlation matrix. The utilisation and partition of microhabitat descriptors by the individual fishes were visualised graphically with the help of the ggplot2 package (Wickham 2016). Boxplots were generated to depict the distribution and variability of each ecological descriptor across identified microhabitat types. These boxplots illustrate key statistical measures such as the median, quartiles, and outliers, offering insights into the spatial preferences and utilisation patterns of the target fish species. All the analyses were performed using the R software, version 4.2.2 (R Core Team 2022). Graphical representations of focal point velocity, substrate preference, and diet composition were created using Microsoft Excel, ensuring clear visualisation and analysis of these key aspects of the study.

Results

The evaluation of the microhabitat availability in the hill stream identified a variety of habitats, each with its own set of environmental characteristics (Table 1). In addition to the primary species of interest, *Garra mullya* and *Mesonoemacheilus triangularis*, several other fish species were observed and occasionally caught during the investigation. These included *Haludaria fasciata*, *Barilius* spp., *Devario* spp., *Rasbora daniconius*, *Amblypharyngodon* sp., *Bhavania australis*, *Homaloptera montana*, *Travancoria jonesi*, *Schistura denisonii*, *Mystus* spp., *Glyptothorax* sp., and *Channa gachua*. Each species occupies specific microhabitats such as pools, riffles, and runs, contributing to the overall biodiversity and ecological dynamics of the surveyed stream.

The hill stream's greatest width, 8.27 m, was measured during periods of intense downpours. The deepest measured water level was 1.2 m. The substrate composition in the study stream was mainly formed of bedrock, boulder, cobble, pebble, gravel, sand and silt. The sucker fish *G. mullya* and the loach *M. triangularis* tended to be frequent in pools and riffles along the stream channel. The pool formed the mesohabitat unit with the greatest abundance. The Zodiac loach *M. triangularis* was observed to be exploiting larger distances from the shelter (mean= 68.85, median= 40.8) and bank (mean= 110.35, median= 100) when compared to *G. mullya* (mean= 54.80, median=39.5; mean= 106.5, median= 100). The two species all exhibited close proximity to the stream substratum (Figure 2). Both species also occupied areas with moderate to low focal point velocities (Figure 3).

The substrate's composition significantly impacted the distribution of *G. mullya* and *M. triangularis* along the stream channel. The two species had different preferences for the substrate (Figure 4). *Mesonoemacheilus* occupied microhabitats dominated by gravel (52%), cobble & rubble (26%), and boulder (17%). Sand (4%), silt (0.8), and debris (0.2%) were less preferred by the loach community. While *Garra* was over-represented in areas with cobble & rubble (73%), followed by smaller amounts of boulder (12%), gravel (7%), and bedrock (5%). The benthic inhabitants have

Table 1 Habitat/Microhabitat availability in the wooded hill stream of southern Kerala, India

Variable	Habitat/microhabitat availability
Habitat types	Falls, rapids, cascades, riffles, pools, glides and run
Stream width	6.17±1.8 m (8.2 m maximum)
Stream depth	0.71±0.28 m (1.2 m maximum)
Substrate types	Bedrock, boulder, cobble, pebble, gravel, sand and silt
Instream cover	Deep pool, boulder edge, overhangs, aquatic macrophytes and leaf litter
Bank stability	Good
Riparian shade	39.71±9.99%

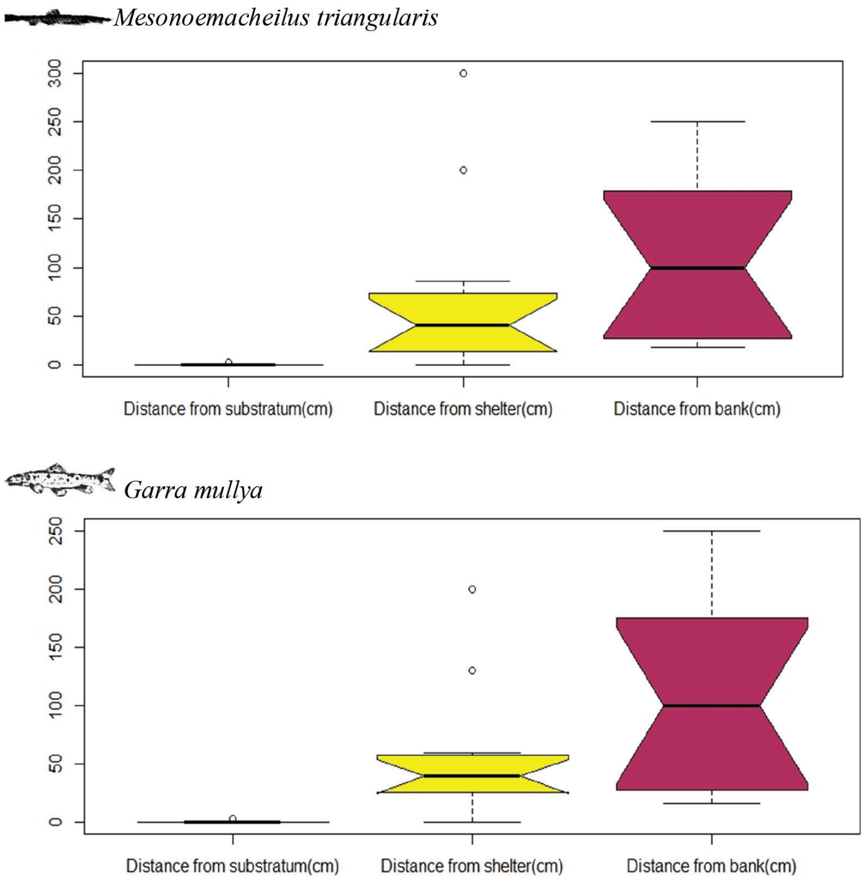

Figure 2. Boxplot of the ecological descriptors calculated for each microhabitat type (distance from substratum, shelter, and bank) preferred by *Mesonoemacheilus triangularis* and *Garra mullya* in the wooded hill stream of southern Kerala, India [The whiskers hit the highest and lowest values within 95% of the distribution for each descriptor, the box contains 50% of the data, and the thick horizontal line depicts the distribution's median. Single data points outside of the 95% range are represented by open circles]

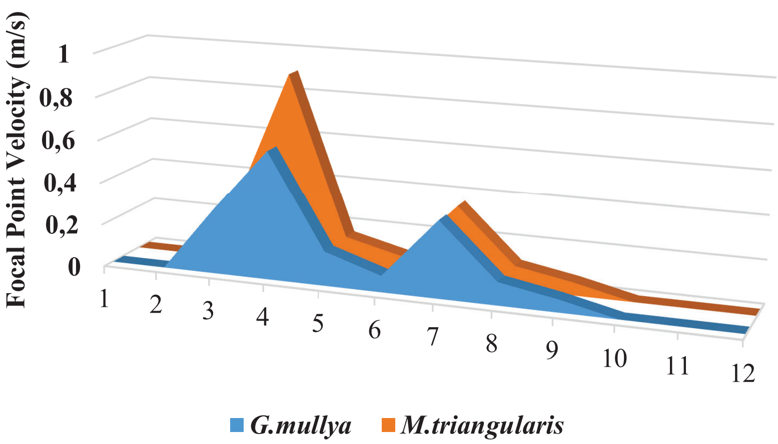

Figure 3. Focal point velocity preferred by *Mesonoemacheilus triangularis* and *Garra mullya* in the wooded hill stream of southern Kerala, India

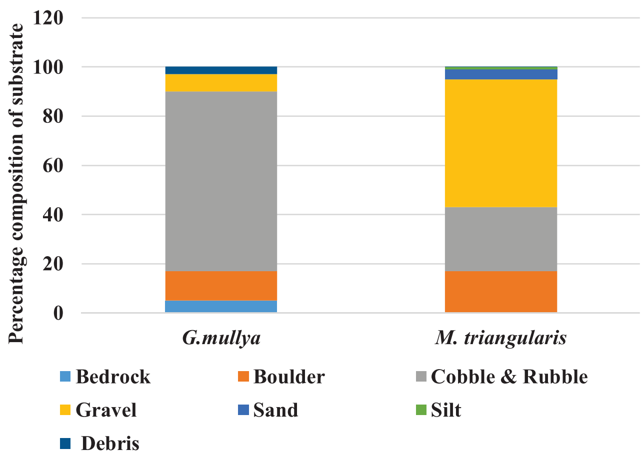

Figure 4. Association of *Garra mullya* and *Mesonoemacheilus triangularis* individuals with substrate categories in the wooded hill stream of southern Kerala, India

always coexisted in the water column, and an analysis of the contents of their guts revealed that the species divide up the available food sources in the stream ecosystem (Figure 5). Detritus made up the majority of the food item consumed by *G. mullya*, accounting for 37% of its total weight, followed by algae (33%), soil/sand particles (22%), and plant debris (8%), in that order. In contrast, *M. triangularis* primarily favoured insects (42%) and shrimps (38%) in the stream ecosystem. These fishes' diverse eating habits and the abundance of food resources in the surveyed stream sustained their dominance and coexistence. Direct observation of *G. mullya* in its natural environment showed that the fish is a typical "scraper," scraping algae from the hard substrate. Furthermore, *Garra* was sustained by the debris deposits in the stream channel.

According to PCA results, the first (PC1) and second (PC2) principal components explained 39.08% and 16.36% of the variances, respectively. PCA biplot (Figure 6) revealed that *G. mullya* was most often associated with water temperature, while *M. triangularis* was significantly associated with wind speed.

The correlation analysis further explained the individual species had a high correlation with some measured environmental descriptors (Figure 7). The Zodiac loach *M. triangularis* was positively influenced by wind speed, conductivity, and TDS (r =0.43 each). *Garra mullya* exhibited a strong positive correlation with air temperature (r = 0.71) and water temperature (r =0.51).

Total hardness was the only variable which showed a negative association with the sucker fish *Garra mullya* (r = -0.23) in the study stream. During the stream surveys, individuals of *G. mullya* primarily co-occurred with *M. triangularis*. *Barilius* sp. was occasionally associated with this benthic guild along the stream channel.

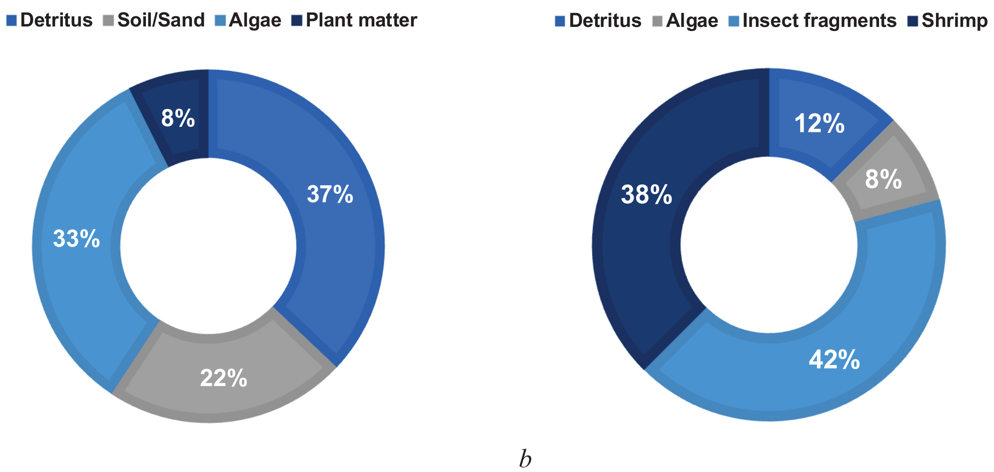

Figure 5. Diet composition of (a) *Garra mullya* and (b) *Mesonoemacheilus triangularis* in the wooded hill stream of southern Kerala, India

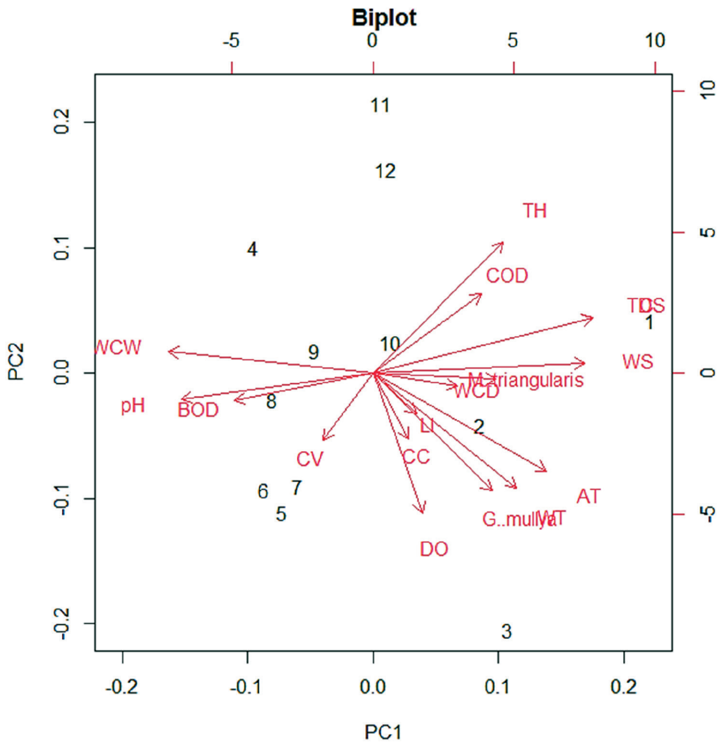

Figure 6. PCA Biplot showing the relationship between *Garra mullya* and *Mesonoemacheilus triangularis* individuals with the environmental descriptors in the wooded hill stream of southern Kerala, India [Abbreviations used in PCA Biplot are Water Column Depth-WCD, Water Column Width-WCW, Canopy Cover-CC, Water Temperature-WT, Total Dissolved Solids-TDS, Conductivity-C, Total Hardness-TH, Dissolved Oxygen-DO, Biological Oxygen Demand-BOD, Chemical Oxygen Demand-COD, Light Intensity-LI, Air Temperature-AT, Wind Speed-WS, Current Velocity-CV]

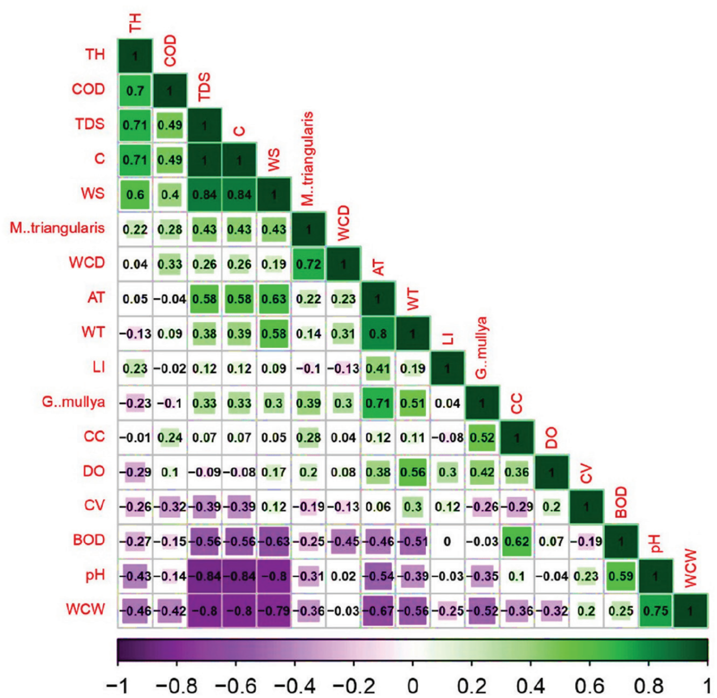

Figure 7. Correlation matrix showing the association between *Garra mullya* and *Mesonoemacheilus triangularis* individuals with the selected environmental descriptors in the wooded hill stream of southern Kerala, India [The matrix is based on Pearson correlation analysis, with positive correlations represented in green and negative correlations in blue]

Discussion

This study highlights the distinct microhabitat preferences and niche partitioning mechanisms of *G. mullya* and *M. triangularis* in a wooded hill stream of southern Kerala. These preferences are influenced by the trophic requirements, morphological and physiological characteristics of the fish, and the biochemical conditions of the stream. Environmental heterogeneity plays a crucial role in preserving biodiversity because it allows different species to take advantage of distinct resources and microhabitats, which in turn supports a thriving and varied aquatic community (Tamme et al. 2010). On a microhabitat scale, *G. mullya* and *M. triangularis* showed synchronised dislodgement in the investigated hill stream. *G. mullya* occupied microhabitats dominated by cobble & rubble and preferred a smaller distance from shelter and bank. Active individuals of *M. triangularis* selected microhabitats with gravel and registered with higher use of positions close to shelter and bank. However both the species exhibited a strong association with pool mesohabitat use, low to moderate velocity, and extremely close proximity to the substrate. Wootton (1990) and Mazzoni et al. (2011) suggested the role of morphological and physiological characteristics (size, form, and position of fins) in the habitat selection of fishes. The analysed *G. mullya* and *M. triangularis* are benthic rheophiles adapted to live in fast-flowing waters. Lujan & Conway (2015) noticed that: (i) oral adhesive disc and dorsoventral depression found in the *Garra* prevents displacements by moving out of the water column and on or into bottom substrates, which also maximises the accessibility to algal resources on a solid substrate (ii) the non-scraping benthic rheophiles in the loach family (eg. *M. triangularis*) largely escape the flow by living and foraging mostly within interstitial spaces between or under rocks, and retaining or exaggerating an anguilliform body shape (iii) they occupy boundary layer (where water velocity approach zero due to frictional drag of the stone on water) in the fast-flowing habitats. The results of this research showed that both species preferred a focal point velocity range of 0-0.9 cm and avoided headwater torrents. These adaptations and availability of coarse-grained substratum contributed to the dominance of *G. mullya* and *M. triangularis* in the respective hill stream.

The present study further revealed that habitat selection is influenced by several environmental descriptors. A similar observation was also made by Vlach et al. (2005). Substrate variability is another descriptor which causes differences among habitats and the distribution of ichthyofauna (Inoue & Nunokawa 2002). The substrate stability and variability, debris deposits, and surrounding vegetation in the respective stream environment supported a rich variety of aquatic invertebrates. As per the gut content analysis, the majority of the food that *G. mullya* ingested comprised detritus and algae. The *M. triangularis* preferred mainly insects and shrimps. It is therefore obvious that the resources were fairly distributed among the fish in their stream environment. Selvanathan & Wesley (2000) reported *M. triangularis* as a non-selective feeder primarily consuming insects and detritus. Analysis of food habits in *G. mullya* from the Western Ghats revealed that the fish feeds mainly on algae and detritus (Joseph & Wesley 2000). Resource allocation can be seen as the main driver behind the coexistence of *G. mullya* and *M. triangularis*. These results support those of Westrelin et al. (2022), who demonstrated that species coexistence in stream assemblages is influenced by habitat division. Members of various habitat-use guilds shared available trophic resources and water column areas among themselves as well. According to Yu & Lee (2005), the success of fishes in locating food may be linked to their differences in habitat use. Thus, the loach and sucker fish population in the Western Ghats' torrential stream is less competitive due to resource partitioning and variability in microhabitat descriptors.

The one-year study period may not have allowed for the entire seasonal change of environmental variables or habitat use. Furthermore, even though we concentrated on fifteen environmental variables, niche partitioning and habitat selection may potentially be influenced by other unmeasured factors. In order to have a thorough knowledge of how microhabitat utilisation and niche partitioning change over time, future research should expand the study to include several years. Further research on environmental variables and their interactions may yield more profound understanding of the ecological needs and adaptations of these species. Incorporating more sympatric species into the research could aid in clarifying more general patterns of resource utilisation and community organisation within these environments.

In conclusion, the results of the present study indicate that *G. mullya* and *M. triangularis* are associated preferably with specific microhabitat variables. The microhabitat information can contribute to an understanding of the environmental choices and trends in the stream fish assemblage in the Western Ghats streams of the Pamba river in Kerala. Studies on habitat scale give information to select habitat improvement strategies taken into account during development efforts and are crucial for conserving this local ichthyofauna. Based on our results, this locality within the research basin emerges as pivotal for the maintenance of populations of both *G. mullya* and *M. triangularis*. Therefore, safeguarding and protecting these habitats are imperative for ensuring the long-term conservation of these species and their ecological integrity.

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